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En vue de l'obtention du diplôme de Doctorat Es-Sciences
En Sciences Vétérinaires

Thème :

Contribution à l'étude des souches de *Coxiella burnetii* isolées chez l'Homme et différentes espèces animales en Algérie.

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Liste des abréviations :

ACCM2: Axenic Acidified Cysteine Citrate Medium **2**

aCL: anti-Cardiolipin

AUS: Abdominal Ultrasonography

CD4: cluster Differenciation 4

CD8: Cluster Differenciation 8

CDC : Centers of Disease Control and prevention.

CFT: Complement Fixation Test

CMR: Chloroform Methanol Residue

CSF: Cerebrospinal fluid

DNA: Deoxyribonucleic Acid

EDTA : Acide ethylene-Diamine-tetra-Acetic

EEAI: Early Endosomal marker protein

ELISA: Enzyme Linked Immunosorbent Assay

EPH: Entreprise Public Hospitalière.

ESR: Erythrocyte Sedimentation Rate

IAP: Integrin Associated Protein

IFA: Immunofluorescence Assay

IFI: Immuno-fluoréscence Indirecte

IFN$_\gamma$: Interferon $_\gamma$

IgA : Immunoglobuline A

IgG: Immunoglbuline G

IgM : Immunoglobuline M

IHU : Institut Hospitalo-Universitaire

LC3 : Microtubule-associated protein Light-Chain 3

LCV: Large-Cell Variant

LPS: Lipopolysaccharide

MLVA: Multilocus Variable-number Analysis

MRI: Magnetic Resonance Imaging

MST: Multispacer Sequence Typing

NAME: Numerical Atmospheric-dispersing Modelling Environment

PBS : Phosphate –Buffered Saline

PCR: Polymerase-Chain-Reaction

pH : Potential Hydrogen

QFS: Post-Q Fever Fatigue Syndrome

RNA: Ribonucleic Acid

SCV: Small-Cell Variant

SNP: Single Nucleotide Polymorphism

TESSy: The Europrean Surveillance Sytem

TLR4: Toll-like Receptor 4

UK: United Kingdom

USA : United States of America

USAF: United State Air Force

VCC: Vacuole Containing *Coxiella*

VHD: Valvular Heart Disease

VNTR: Variable-Number Tandem Repeat

WHA: **Third World Health Assembly**

WHO: World Health Organization

SOMMAIRE

Résumé.

Dans le but d'étudier la fièvre Q chez l'Homme et différentes espèces animales en Algérie, nous avons procédé aux trois volets :

En premier volet, nous avons étudié l'impact de l'infection par *Coxiella burnetii* lors d'avortements spontanés fébriles chez les femmes, en utilisant une méthode sérologique (Immunofluorescence Indirecte -**IFI**) et une méthode moléculaire (**q PCR**), sur deux services obstétrique-gynécologie dans deux hôpitaux à Alger, EPH HACENE BADI (Ex BELFORT) et EPH ZERALDA, pour la période allant d'Avril 2014 jusqu'à Novembre 2015. Parmi les **725** femmes incluses (un groupe de cas **380** femmes ayant subi un avortement spontané fébrile et un groupe témoin comprenait **345** femmes qui ont accouché sans autres infections ou complications). Des anticorps contre *Coxiella burnetii* ont été détectés par **IFI** chez trois **(03, 0.79%)** patientes ; tous les échantillons du groupe témoin étaient négatifs. Par ailleurs, seulement quatre (**04, 1.05%**) échantillons placentaires appartenant le groupe de cas sont revenus avec **qPCR** positive pour **IS1111** et **IS30a** également.

En deuxième volet, nous avons travaillé au centre national des maladies infectieuses de l'hôpital **EL-HADI FLICI, Ex ELKETTAR** à Alger**,** ente la période Aout-Octobre 2017**,** afin d'étudier l'impact de *Coxiella burnetii* sur la fièvre prolongée non-spécifique. Un total de **140** patients (**70** patients dans un groupe témoin et **70** patients dans un groupe de cas), ont été évalués pour l'identification de *Coxiella burnetii* par sérologie **IFI** et **q PCR**. La sérologie par **IFI** dans le groupe de cas est revenue positive pour **03** des **70** sérums (**4,30 %**), alors que tous les sérums appartenant au groupe témoin sont revenus négatifs. Nos résultats moléculaires, montrent un seul (**01/70, 1,42 %**) sang total est revenu positif en q PCR pour *Coxiella burnetii* appartenant au groupe de cas, alors que tous les patients du groupe témoin avaient q PCR négative.

Finalement, afin d'étudier la fièvre Q chez les animaux, nous avons démontré la présence moléculaire de *Coxiella burnetii* dans des échantillons de nature différente chez les bovins, les ovins, les chiens et les chats, provenant des élevages bovins et ovins situés dans le nord-est de l'Algérie, et des ruminants abattus dans l'abattoir d'Alger, ainsi que des chiens et chats errants de la fourrière canine d'**El HARRACH**-Alger. À cet égard, un total de **599** échantillons ont été prélevés pendant la période allant de Mars-Octobre 2017, dans divers échantillons de sang, de placenta, de foie, de rate et d'utérus. Nos résultats q PCR ont montré que sur **344** échantillons de sang total, seulement **15 (4,36 %)** étaient positifs pour *Coxiella burnetii*, alors que seulement

06 (2,35 %) échantillons positifs sur un total de **255** échantillons d'organes collectés. Chez les bovins, **03 (4%)** échantillons positifs ont été trouvés dans des échantillons de sang et de foie dans chacun d'eux. Au niveau des fermes au Nord-Est algérien, **01 (1,19%)** échantillon de sang de mouton a donné un résultat positif q PCR, et **03 (8,57%)** échantillons de placenta étaient positifs. A la fourrière canine d'Alger, **08 (10%)** et **03 (5%)** échantillons de sang ont montré des q PCR positives pour *Coxiella burnetii* chez les chiens et les chats respectivement. En outre, le génotypage **MST** a montré que le **MST 33** a été génotypé dans des échantillons de sang des bovins et des ovins, ainsi que dans des échantillons de placenta des ovins. Alors que, le sang total des chiens et de chats, a donné **MST 21**. De plus, le **MST 20** a été détectée dans des échantillons de foie de bovins.

Mots clés : *Avortement, Chiens et chats, Coxiella burnetii, Fièvre prolongée, Fièvre Q Algérie, IFI, qPCR , Ruminants, Zoonoses.*

Abstract.

In order to study Q fever in humans and different animal species in Algeria, we carried out the three parts:

In the first part, we studied the impact of *Coxiella burnetii* infection during febrile spontaneous abortions in women, using a serological method **IFA** and a molecular method **q PCR**, on two obstetrics and gynaecology departments in two hospitals in Algiers, EPH HACENE BADI (Ex BELFORT) and EPH ZERALDA, between April 2014 and November 2015. Of the **725** women included (a case group of **380** women with febrile spontaneous abortion and a control group included **345** women who gave birth without further infections or complications). Antibodies against *Coxiella burnetii* were detected by **IFA** in three **(03, 0.79%)** patients; all samples in the control group were negative. In addition, only four **(04, 1.05%)** placental samples belonging to the case group returned with **q PCR** positive for IS1111 and IS30a also.

In the second part, we worked at the National Centre for Infectious Diseases, **EL-HADI FLICI** Hospital, Ex **ELKETTAR**, in Algiers, between August-October 2017, to study the impact of *Coxiella burnetii* on non-specific prolonged fever. A total of 140 patients (**70** patients in a

control group and **70** patients in a case group) were evaluated for the identification of *Coxiella burnetii* by **IFA** serology and **q PCR**. **IFA** serology in the case group returned positive for **03** of the **70** sera **(4.30%),** while all sera in the control group returned negative. Our molecular results show only one (**01/70, 1.42%**) whole blood returned positive for **q PCR** for *Coxiella burnetii* in the case group, while all patients in the control group had **q PCR** negative.

Finally, in order to study Q fever in animals, we demonstrated the molecular presence of *Coxiella burnetii* in samples of a different nature in cattle, sheep, dogs and cats from cattle and sheep farms in Northeastern Algeria, and ruminants slaughtered in Algiers, as well as stray dogs and cats from the El HARRACH –Algiers- canine pound. A total of **599** samples were collected between March-October 2017 from various blood, placenta, liver, spleen and uterus samples. Our **q PCR** results showed that out of **344** whole blood samples, only **15 (4.36%)** were positive for *Coxiella burnetii*, while only **06 (2.35%)** were positive out of a total of **255** organ samples collected. In cattle, **03 (4%)** positive samples were found in blood and liver samples in each of them. At the farm level in Northeastern Algeria, **01 (1.19%)** sheep blood sample tested positive **q PCR**, and **03 (8.57%)** placenta samples were positive. At the Algiers dog pound, **08 (10%)** and **03 (5%)** blood samples showed positive **q PCR** q for *Coxiella burnetii* in dogs and cats respectively. In addition, **MST genotyping** showed that **MST 33** was genotyped in blood samples from cattle and sheep, as well as in placenta samples from sheep. While, the whole blood of dogs and cats, gave **MST 21**. In addition, **MST 20** was detected in cattle liver samples.

Key words: *Abortion, Coxiella burnetii, Dogs and Cats, IFI, Prolonged Fever, Q Fever Algeria, qPCR, Ruminants, Zoonosis.*

الملخص

لأجل دراسة مرض الحمى س عند الإنسان و بعض الفصائل الحيوانية في الجزائر، قمنا بدراسة تشمل ثلاثة محاور:

في المحور الأول، قمنا بدراسة تأثير الإصابة بكوكسيلا بورنيتي على الإجهاض التلقائي الصحوب بالحمى عند النساء الحوامل، و قد إستعملنا التحليل المصلي على طريقة الإشعاع المناعي الغير مباشر، و أيضا دراسة جزيئية جينية، و قد قمنا بدراستنا هته على مستوى مصلحتي أمراض النساء و الولادة على مستوى مستشفيين بالعاصمة الجزائر، الأول مستشفة حسن بادي (بالفور سابقا) و الثاني مستشفى زرالدة، في الفترة الممتدة بين أفريل 2014 و نوفمبر 2015. بين 725 مرأة اللواتي كن قيد دراستنا (في مجموعتين؛ الأولى تجريبية تضم 380 إمرأة تعرضت لإجهاض تلقائي محموم، و مجموعة ثانية شاهدة بها 345 إمرأة ذات ولادة

طبيعية). النتائج أظهرت أن الأجسام المضادة لكوكسيلا بورنيتي قد وجدت عند 03 عينات مصل (0.79 %) من المجموعة التجريبية، و باقي المجموعة الشاهدة أعطت نتائج سلبية. فيما يخص التحليل الجزيئي الجيني، فقط 04 عينات مشيمية (1.05 %) من المجموعة التجريبية كانت موجبة لكوكسيلا بورنيتي للمورثتين الخاصيتيت أس 1111 و أس 30 أ.

في المحور الثاني، قمنا بدراستنا على مستوى المركز الوطني للأمراض التعفنية، مستشفى الهادي فليسي (القطار سابقا) بالعاصمة، في الفترة الممتدة بين شهري أوت و أكتوبر 2017, بغية دراسة العلاقة بين الإصابة بكوكسيلا بورنيتي و أعراض الحمى المطولة الملازمة لها.قمنا بجمع 140 عينة دم من 140 مريض (مجموعة شاهدة مكونة من 70 مريض، و مجموعة تجريبية بها أيضا 70 مريض). عينات المجموعتين تم فحصها بتقنتي تحليل المصل الإشعاعي الغير المباشر و أيضا التحليل الجزيئي الجيني. المجموعة التجريبية أظهرت 03 عينات إيجابية من أصل 70 عن طريق التحليل الإشعاعي الغير مباشر (4.3 %) بينما كل عينات المجموعة الشاهدة كانت سلبية. التحليل الجزيئي الجيني أظهر إيجابية عينة واحدة من أصل 70 عينة للمجموعة التجريبية (1.42%).

في المحور الثالث و بهدف دراسة الحمى س عند الفصائل الحيوانية، قمنا بأخذعيمات دم و بعض الأعضاء (كبد، طحال، رحم) من مجترات (بقر و غنم) على مستوى مذبح الحراش، و عينات دم و مشيمة من مزارع مجترات من شرق و شمال الجزائر ؛ كما قمنا بأخذ عينات دم على مستوى محجرة الكلاب و القطط الضالة بالحراش ، هذا العمل كان بين شهري مارس و أكتوبر 2017. إجمالا قمنا بتجميع 599 عينة من الفصائل الحيوانية المذكورة سابقا. نتائج التحليل الجزيئي الجيني أظهرت 15 عينة دموية إيجابية من أصل 344 عينة (4.36 %) لكوكسيلا بورنيتي، بينما فقط 06 عينات من أصل 255 عينة أعضاء (2.35 %) كانت إيجابية لكوكسيلا بورنيتي. عند فصيلة الأبقار، 03 عينات (4%) كانت إيجابية منها دموية و كبدية. على كستوى مزارع المجترات شرق و شمال الجزائر، عينة واحدة (1.19 %) و 03 عينات مشيمية غنمية (8.57%) كانت إيجابية أيضا. على مستوى محجرة الكلاب و القطط الضالة بالحراش، النتائج أظهرت 10% و 5 % من العينات الدموية للكلاب و القطط كانت إيجابية على التوالي لكوكسيلا بورنيتي. و من جهة أخرى، التصنيف الجيني النمطي أظهر أن النمط 33 موجود في عينات الدم لكل من فصيلتي الأبقار و الأغنام، و أيضا في عينات المشيمة الغنمية، بينما دم الكلاب و القطط أظهر النمط الجيني 21, و أخيرا النمط الجيني وجد عند عينات الكبد البقرية.

الكلمات الدالة:

الإجهاض. كلاب وقطط. مجترات .كوكسيلا بورنيتي. الحمى الدائمة .الحمى س الجزائر. التحليل المصلي الإشعاعي الغير المباشر. التحليلي الجزيئي الجيني. أمراض متنقلة من الحيوان للإنسان .

Introduction générale.

Indéniablement, les maladies infectieuses restent la sphère la plus importante durant ces dernières décennies. L'équilibre entre les anciennes et les nouvelles infections n'a jamais basculé d'un côté. Constamment, de nouvelles maladies infectieuses apparaissent, et des anciennes émergent. Dans ce contexte, **75%** des maladies infectieuses ré-émergentes sont des zoonoses. Ce fait pourrait être lié à la relation très sensible entre hôte-pathogène, qui connaissent plusieurs changements phénotypiques et génotypiques, ainsi acquérir de nouvelles caractéristiques et surviennent dans des environnements ou des véhicules alimentaires imprévus. Tous ces changements peuvent être causés par les changements climatiques et leurs impacts environnementaux sur la biodiversité qui a affecté le monde entier.

La Fièvre Q, cette zoonose, depuis sa découverte, a connu une attention particulière partout où elle a été décelée. Les nouvelles avancées et connaissances sur *Coxiella burnetii*, l'agent causal de la maladie, lui a donné plus d'importance. La manifestation clinique variable, son cycle intracellulaire, ainsi que sa plasticité génomique, mettent les diagnosticiens en débat permanent. Au fil du temps et des lieux, les épidémies de fièvre Q ont incité les pays concernés à développer des mesures de contrôle et de prévention pour faire face à ses répercussions potentielles sur la santé publique, l'élevage et l'économie. En **2003**, le **CDC** l'a classée comme agent bioterroriste de catégorie B, ce qui a écoulé beaucoup d'encre sur *Coxiella burnetii*, et ça a élevé la barrière de l'épidémio-surveillance dans tous les pays du monde.

Dans les pays africains, conscients du danger potentiel de la fièvre Q pour la santé publique et des lacunes importantes dans les connaissances actuelles sur la maladie, les études sur l'agent causal de la fièvre Q ont pris une nouvelle dimension, où l'on constate une augmentation des résultats obtenus à la suite de travaux de recherche sur le sujet, ainsi que des collaborations entre les différents acteurs du secteur de la santé afin de détecter les réservoirs et sources de contamination par la fièvre Q, et en savoir plus sur l'agent pathogène.

L'infection à *Coxiella burnetii* est détectée chez l'homme et chez un large éventail d'espèces animales en Afrique, mais la séroprévalence varie considérablement selon les espèces et les lieux. En Algérie, comme dans d'autres pays africains, la séroprévalence de la fièvre Q chez l'homme et l'animal est mal connue en raison du manque d'outils de diagnostic et de la mauvaise gestion des élevages. En outre, il y a un manque flagrant de travaux de recherche relatifs à l'infection de *Coxiella burnetii* chez l'animal ou l'homme. Toutefois, des efforts de recherche ont été menés en Algérie sur la fièvre Q animale, entre autre les ovins, les dromadaires ainsi que sur les tiques, où ils ont identifié la présence de *Coxiella burnetii* sérologiquement et moléculairement dans les sérums animaux, et sur les tiques respectivement [10.14.15].

En Mai **2010**, un jeune médecin vétérinaire algérois, durant son expérimentation en post graduation en Algérie, il a contracté une infection, d'où la nécessité de son hospitalisation pendant des mois pour un tableau clinique atypique ; une fièvre prolongée, hépatite granulomateuse, cytolyse hépatique, arthro-myalgie, des éruptions cutanées et altération de l'état général. Défaut de connaissances et manque de moyens de diagnostic, le jeune patient a dû quitter l'hôpital sans diagnostic, il a été transporté à l'étranger, où ils ont pu diagnostiquer une fièvre Q chronique, ce passage à la chronicité l'a payé le patient par une vie condamnée et thérapie et séquelles à long terme ; ce jeune médecin vétérinaire est moi, ***Ghaoui Hichem***.

A l'issue de mon épreuve personnelle avec cette maladie, la fièvre Q, en **2012,** après une longue discussion avec mon directeur de thèse**,** nous avons conclu le manque flagrant des travaux qui ont été faits sur la fièvre Q en Algérie, nous avons jugé judicieux de tracer un travail de recherche sur *Coxiella burnetii* l'agent causal de la fièvre Q en Algérie, afin de mettre en évidence les différents réservoirs et les sources de contaminations de la bactérie, et surtout de sensibiliser les acteurs de la santé public afin pouvoir poser un diagnostic précoce de la fièvre et établir un traitement nécessaire adéquat à temps.

Notre manuscrit s'articule sur quatre (**04**) parties, **une première** est consacrée à une revue de la littérature de la fièvre Q et son agent causal *Coxiella burnetii*, **une deuxième** partie traitant l'étude de *Coxiella burnetii* l'agent causale de la fièvre Q chez l'Homme en Algérie, **une troisième** partie visant à identifier les réservoirs et les sources de contaminations en Algérie, avec leurs bases génétiques. **Une quatrième** partie englobe les conclusions et perspectives issues de notre travail. A la fin une partie annexes consacrée aux travaux de recherches qui ont été faits en parallèle durant notre étude, et aussi les manifestations scientifiques dont nous avons participé.

Partie I :

Revue de la littérature

Avant-propos.

Dans cette revue, nous nous sommes focalisés sur la distribution locale et temporelle de la fièvre Q, mettant en évidence les facteurs de risque impliqués et les différences variables dans chaque épisode, en étudiant également son expansion sur le critère du développement ou non des pays affectés. De plus, une meilleure connaissance de l'aspect microbien de *Coxiella burnetii* peut nous apporter des réponses qui nous permettront d'éclairer les connaissances et les pistes de recherche sur la fièvre Q.

Coxiella burnetii est l'agent pathogène de la fièvre Q qui reste une zoonose mondiale à l'exception de la Nouvelle Zélande. Le cycle naturel de cette bactérie n'inclut pas les humains, qui sont considérés comme des hôtes accidentels. Le véritable réservoir est large et comprend des mammifères, des oiseaux et des arthropodes, principalement des tiques. La fièvre Q est le plus souvent contractée après l'inhalation par l'homme de particules de poussières infectées, et aussi en manipulant des tissus animaux infectés, tels que les urines, les fèces ou les produits de parturition. La transmission de personne à personne est rare, mais elle a été documentée. Cependant, *Coxiella burnetii* a été identifié dans le sperme d'hommes infectés, ce qui a entraîné la transmission sexuelle du pathogène. L'infection aiguë ou chronique à *Coxiella burnetii* présente un large éventail de manifestations cliniques ; environ **50 %** de toutes les infections à *Coxiella burnetii* sont asymptomatiques et peuvent également entraîner une pyrexie inexpliquée qui rend le diagnostic difficile pour les spécialistes des maladies infectieuses. Après inhalation, les symptômes peuvent se développer après **10 à 90** jours, et cette durée dépond de la dose infectante. *Coxiella burnetii* a été repéré là où il a été recherché. En raison des enquêtes épidémiologiques menées dans la plupart des pays développés, des images claires des facteurs d'exposition, le cycle de vie des hôtes-réservoirs est amplement décrit, d'où une incidence

généralement assez faible. Par ailleurs, dans la plupart des pays en voie de développement, les outils de diagnostic de la fièvre Q ne sont pas bien disponibles, de sorte que son impact global sur la santé publique a été largement sous-estimé. De ce fait, nous n'avons pas de profil épidémiologique clair de la fièvre Q dans ces pays. En conséquence, les foyers enregistrés et leurs études sont assez rares, à l'exception de quelques études récentes menées dans certains pays qui visaient à identifier les réservoirs et les sources de contamination de *Coxiella burnetii* chez l'homme.

Review:

Between Livestock's and Humans, Q Fever

Disease is emerging at low noise

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Review Article

Between Livestock's and Humans, Q Fever Disease is Emerging at Low Noise

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Abstract

Coxiella burnetii is the pathogenic agent of the Q fever which remains a worldwide zoonotic disease. The natural cycle of this bacterium is not reported to include humans, who are considered incidental hosts. The true reservoir is wide and includes mammals, birds and arthropods mainly tick. Q fever is most often contracted after human inhalation of infected dusts particles, handle infected animal tissues, such as urine, faeces or birth products. Person-to-Person transmission is rare but it has been documented. However, *Coxiella burnetii* has been identified in the semen of infected males, and this has resulted in sexual transmission of the pathogen. Acute or chronic *Coxiella burnetii* infection exhibits a wide spectrum of clinical manifestations; roughly 50% of all infections with *Coxiella burnetii* are asymptomatic, and can lead also to an unexplained pyrexia that makes diagnosis difficult for infectious disease specialists. Following inhalation, symptoms can develop after 10 to 90 days, depending on the dose. *Coxiella burnetii* has been flagged where it has been searched for. Due to the epidemiological surveys in most developed countries, clear pictures about exposure factors, hosts-reservoirs life cycle are amply described, hence its incidence is generally quite low.

Keywords: Q Fever; Zoonosis; *Coxiella burnetii*; Outbreaks; Description; Bio-Threat

Abbreviations

CDC: Centers of Disease Control and Prevention; USA: United States of America; VNTR: Variable-Number Tandem Repeat; MLVA: Multilocus Variable-Number Analysis; MST: Multispacer Sequence Typing; SNP: Single Nucleotide Polymorphism; UK: United Kingdom; NAME: Numerical Atmospheric-dispersing Modelling Environment; DNA: Deoxyribonucleic Acid; RNA: Ribonucleic Acid; TESSy: The Europrean Surveillance System; IFA: Immunofluorescence Assay; PCR: Polymerase-Chain-Reaction; WHA: Third World Health Assembly; USAF: United State Air Force; WHO: World Health Organization; IgG: Immunoglbuline G; IgM: Immunoglobuline M; IgA : Immunoglobuline A; ELISA: Enzyme Linked Immunosorbent Assay; CFT: Complement Fixation Test; pH: Potential Hydrogen; LCV: Large-Cell Variant; SCV: Small-Cell Variant; ACCM2: Axenic Acidified Cysteine Citrate Medium 2; LPS: Lipopolysaccharide; VCC: Vacuole Containing Coxiella; LC3: Microtubule-Associated Protein Light-Chain 3; EEAI: Early Endosomal Marker Protein; IAP: Integrin Associated Protein; TLR4: Toll-like Receptor 4; CD4: Cluster Differentiation 4; CD8: Cluster Differentiation 8; aCL: anti-Cardiolipin; VHD: Valvular Heart Disease; CSF: Cerebrospinal Fluid; MRI: Magnetic Resonance Imaging; AUS: Abdominal Ultrasonography; ESR: Erythrocyte Sedimentation Rate; QFS: Post-Q Fever Fatigue Syndrome; EDTA: Acid Ethylene-Diamine-Tetra-Acetic; IHU : Institut Hospitalo-Universitaire; PBS : Phosphate –Buffered Saline; IFN_{γ}: $Interferon_{\gamma}$; CMR: Chloroform Methanol Residue.

Introduction

Undeniably, infectious diseases remain the sphere the most important in the recent decades. The balance between old and new infections, has never tilted to one side. Constantly, new infections diseases appear, and the old ones emerge. Through the studied past, 75% of the re-emerging infectious diseases are zoonosis. This fact may be linked to the high sensitive relationship between Host-Pathogen, which are knowing several phenotypical and genotypic changes, thus acquiring new characteristics and arise in unexpected environments or food vehicles. All these changes, may be caused by climate shifts, and their environmental impacts on biodiversity that has affected the howl world.

Q Fever, zoonosis disease, since it discovery, it attracted a special attention where ever it was flagged. New advances and knowledge's about *Coxiella burnetii* the causative agent of the disease, gave it more importance. The variable clinical manifestation, intracellular cycle life, and also to its genomic plasticity, put the diagnosticians in constant debate. Over time, and places, Q Fever outbreaks have prompted the countries concerned to develop control and preventive measures to deal with its potential repercussions on public health, livestock and economy. In 2003, the CDC, classified it as bioterrorism agent category B, this allowed a lot of ink to flow on *Coxiella burnetii*, and raises the barrier of epidemiosurveillance across all countries of the world.

In this review, we focused on the local and temporal distribution of Q fever, highlighting the risk factors involved in each episode, and the variable differences between each one, also studying its expansion on the development criterion or not of the affected countries. Additionally, knowing more the microbial aspect of *Coxiella burnetii*, may give us answers that will illuminate the knowledge and research paths on Q Fever.

Q fever outbreaks, in time and place.

History and first description of *Coxiella burnetii*

Firstly, in the **1930**s, the causative agent of Q fever was described simultaneously in two near concurrent incidences in two different continents; Queensland, Australia and in Montana, USA [1]. After the august **1935** incident of undiagnosed febrile illness among abattoir workers in Brisbane, Queensland; Edward Derrick was assigned to investigate the cause of this epidemic [1-3], which led him to name the disease "Q" fever in reference to the first letter of English word "Query" meaning "Question" until fuller knowledge should allow a better name [4]. Consequently, Derrick failed to identify the infectious agent of this disease, but he was able to transmit the fever to guinea pigs in blood and urine from infected patients. In the meantime, he concluded wrongly that the etiologic agent was a "virus". However, Burnet and Freeman also indicated the Rickettsia-like properties in smears from the spleen of infected mice [5]. Meanwhile, on the other side of the pacific in Montana, USA, coincidentally to research on Rocky Mountain Spotted Fever, an unknown tick infectious agent, passing through filters, infecting the guinea pigs, Gram-negative, and produced unexpected clinical signs, it was named The Nine Mile Agent. Rolla Dyer, Being the Director of the National Institutes of Health, USA, after a laboratory-acquired infection by the new agent, this step led him to report a breakthrough and confirmed that the Australian and American groups were investigating the same organism. It was proposed to rename the organism; *Coxiella burnetii* to credit both groups for their pioneering research into this newly identified infectious agent [6-8].

Worldwide Q fever outbreaks mapping

Coxiella burnetii has been flagged where it has been searched for. Due to the epidemiological surveys in most developed countries, clear pictures about exposure factors, hosts-reservoirs life cycle are amply described, hence its incidence is generally quite low. In developing countries, information on endemicity is generally scare and limited to seroprevalence studies in exposed populations or case reports. This presumably reflects misdiagnosis, rather than lower incidence [9]. Most reports of Q fever outbreaks are from rural areas and rare associated directly or indirectly with farms or farm animals [10,11]. Nevertheless, urban outbreaks have been described after exposure to slaughterhouses [12,13], animal research laboratories, parturient cats, contaminated straw, and following wind-borne spread of *Coxiella burnetii* from farmlands [14]. In some Urban outbreaks, the source of infection was never determined [15].

Q fever in developed countries

Australia

After the first descriptive investigations of the causative agent of Q fever, Australia has been known as the cradle of *Coxiella burnetii.* In 1930s, following the acute onset of a distinct clinical entity among the abattoir employees and dairy farmers in Brisbane, tens of cases of a Query fever have been reported. Subsequently, Derrick's investigations showed that it was the first Q fever outbreak ever registered in history, where 09 cases have been confirmed by guinea pigs transmission. Thereby, an occupational aspect has been attributed to this first outbreak. Since, Australia has experienced several outbreaks and episodes of sporadic cases of Q fever, consequently, it is considered one of the countries with highest rates of the Q fever in the world; 1.9 cases/100 000 populations, which is the mean annual national rate between 2004-2013. In addition, more than 50 cases/100 000 populations, have been reported in South-West Queensland and north-West New South Wales [16]. In 2015, in New South Wales, an atypical outbreak of Q fever affecting low-risk residents of a remote rural town, presenting different historic profile of Q fever notifications from this region, where the hypotheses were based around the inhalation of aerosols or dust contaminated, or also transmission via ticks due to the increased activity of Kangaroo in and around town [18]. In other side, Victoria's 10 year mean annual rate is 0.5 cases/100 000 populations [16]. Nonetheless, a current review published in 2018, included 10 years' retrospective study (1994-2003) of human acute Q fever in Victoria, confirmed a total of 659 cases of acute Q fever, allowing decreased rate for 4.2% per annum; among others, abattoir workers and related occupations rate decreased to 10.9% per annum, whereas those among dairy farmer's rate to 14.9% per annum, this improvement may result from the success of the vaccination's pro-

gram of high-risk works in Australia [16]. A recent Q fever outbreak (2012-2014), was linked to an intensive goat and sheep dairy farm in Victoria, where a seroprevalence of 15% of non-pregnant milking goats were reported, confirming an active infection for different animal species; consequently, seventeen employees and one family member were confirmed with Q fever over a 28-month period. In this outbreak, the genotyping of the causative *Coxiella burnetii* was identical in both human and goat, that clearly defines the source of this outbreak [17].

United States

In the 1930's, United states was among the first countries which identified the causative agent of Q fever disease *Coxiella burnetii;* by studying a pathogen that can be transmitted via ticks in Rocky Mountain Spotted Fever. In March 1946, an explosive outbreak of illnesses happened among Stock Handlers and Slaughterhouse Workers in Amarillo, Texas. In order to better investigate the serological profile of Q fever disease; Derrick and Cox considered wise to use a serological test which being of great assistance in retrospective diagnosis. The observations showed that the Weil-Felix test gives negative evidence in Q fever, however the Complement Fixation provides an adequate serological profile. A total of 55 sera were collected from patients infected in this outbreak, and tested by the complement fixation method, it appeared that 49 were Q fever positive, and the titers were low as a rule during the first week of illness. Subsequently the titers generally reached and maintained high levels at least for a few weeks [19]. To better characterize Q fever epidemiology in the United States, an extensive review was realized between 1946 and 1986. Published reports of national disease surveillance, individual cases, outbreak investigations, and serologic surveys were reviewed, where a variety of diagnostic tests were used to detect antibodies to *Coxiella burnetii*, which varied in their diagnostic sensitivity and specificity, and their criteria for defining a positive result. In national surveillance reports from 1948-1978, a total of 1168 human cases were reported, with a mean of 38.9 cases/year, the cases were reported from almost every state but in California the cases were highest. Otherwise, the human cases mean decreased to 28.5 cases/year between 1978-1986. Among human cases, livestock handlers had a significantly higher prevalence of antibodies to *Coxiella burnetii* than did persons with no known risk. In this review, animal studies showed that goats had a significantly higher average seroprevalence (41.6%) than sheep (16.5%) or cattle (3.4%). Evidence of antibody to *Coxiella burnetii* was reported also among various wild-animal species, including coyotes, foxes, rodents, skunks, raccoons, rabbits, deer, and birds [20]. In 2006, Q fever endocarditis based on an extremely high antibody titers against *Coxiella burnetii* phase I antigen despite treatment by Doxycycline with recurrent fever for 14 months, was followed for 10 years with 31 years old farmer from West Virginia, having a history of congenital heart disease, including dextrocardia, a double-outlet right ventricle, a ventricular septal defect and sever pulmonary stenosis. This case represents the longest follow-up period for a patient with chronic Q fever in the United States [21]. In 2011, a multistate Human Q fever outbreak was reported. The episode began in April 2011, when an abortion storm took place among goat-farm in Washington, where 14 aborted cases (28%) were described by the farm's owners, and *Coxiella burnetii* positive goat placental specimens were collected. One month later, a patient with flu-like symptoms tested positive for Q fever in Washington state and described similar symptoms in other household members. Days after, multiple cases of Q fever were reported in Montana. Twenty-one (21) human cases registered in both Washington and Montana states, were linked to visitors exposed to direct contact with goat new born originated from a single farm in Grant County, Washington where the abortion storm occurred one month earlier. In addition, Interviews with the WA Farm A owners, and review of their sales records, led to an expanded list of epidemiologically linked farms. Goats sold by from WA Farm A were traced to 20 other farms in 14 counties across three states (Washington, Montana, and Oregon). The owners of 17 total farms (13 in Washington, including WA Farm A; three in Montana; one in Oregon) were contacted and agreed to participate in the outbreak investigation [22]. In October 2015, a rare Q fever outbreak hit the United States, when five American medical tourists came down with an unusual illness after travelling to Germany for a controversial treatment involving injections with sheep cells which aims to improve their health and vitality. *Coxiella burnetii* was identified as the causative agent of this outbreak. The treatment is not permitted in the United States. The five New York residents received the "live cell therapy" in May 2015. About a week later, they developed fever, fatigue and other symptoms in favour of *Coxiella burnetii* infection. Three of them were recovered but two still experiencing symptoms more than 9 months later [23]. Recently, Pettey., *et al*. reported A case of Q fever after liver transplantation, this case highlights the need to include Q fever in the differential diagnosis for fever of unknown origin in solid organ transplant hosts [205].

Netherlands

Since 2007, one of the largest reported outbreak of Q fever in humans ever reported in the literatures, occurred in the Netherlands, involving 4026 cases, at least 14 of these patients, nearly all of them with severe underlying conditions, have died. Epidemiological investigations identified small ruminants as source, especially goat [24-26]. The question which is getting a lot of attention and it's still hanging; in this developed countries, how such a historical epidemic can occur? And that so many cases are reported? In order to answer to this question, we have to clarify that three factorial axes could be incriminated: First, the increasing number of goats in highly populated areas noting the close distance between livestock farms and dwelling place; more than 140000 received

goats had been enrolled from 2000 to 2009, therefore the goat farms increased from 33 to 58 farms which greatly accentuated the risk of goat-human infection. Second, environmental factors could be charged too, in 2007 the Netherlands has known a large dry period without rain, this climate could promote the transmission of *Coxiella burnetii* from infected farms [27]. Third, possible introduction of a more virulent strain of *Coxiella burnetii*, in this manner, the central veterinary institute has reported that one Multilocus Variable-number tandem-repeat Analysis (MLVA) type prevails on many dairy goat farms in the southern part of the Netherlands [26,27]. Moreover, the lack of contact between the veterinary sector and the human health sector, it has had an impact on the communication of information about the Q fever outbreak in animal environment, which has not been communicated in time. In December 2009, it was decided to start culling more than 50 000 pregnant goats on infected farms, this decision has had an important impact, but unfortunately these interventions were issued too late [28].

France

In France, from 1985 to 2009, 3727 patients had acute Q fever (one third female patients), where the yearly distribution of acute Q fever showed a continuous increase. Q fever was diagnosed more often in southern France, where the French National Reference Center (NRC) is situated (Marseille). This increase incidence suggests several interpretations, one of them, could be the improved diagnostic capability caused by development and availability of commercial diagnostic test [29]. However, in France, some investigations on the Q fever outbreaks have focused on another risk factor in *Coxiella burnetii* transmission; including the influence of weather conditions on the spread and escalation of Q fever cases, namely wind frequencies according to the seasons and its changes. The Mistral is a corridor wind, from northwest to north, which concerns the northern part of the western Mediterranean basin. It can blow at more than 100 km/h in the plain, especially in the lower Rhône valley.

Studies have been carried out to investigate the link between the mistral wind and outbreaks of Q fever in the Bouches-du-Rhones region, particularly in Marseille, Aix en Provence and the Martigues region. Between 1990 and 2003, two major studies have been conducted. The first was realized from 1990 to 1995 in Martigues regions, small town in the western Marseille, it extends along the banks of the Etang de Berre and the Caronte Canal. This small town, apparently has more cases of Q fever than the surrounding towns, subsequently it was judicious to investigate the Q fever epidemiologic situation of Martigues. Because infectious particles containing *Coxiella burnetii* can easily be transported by the wind, and the fact that a large number of sheep are located windward of the study area, we wanted to determine whether wind direction and strength, as well as sheep breeding, could be significantly associated with the seasonal distribution of cases that occur in the study area. In the study's period, 289 patients had been admitted with an active Q fever, with a high incidence from the Etang de Berre region with 35.4/100 000 inhabitants, where the Mistral (> 8m/s), which blows on the local steppe where more than 70 000 sheep are breed in open fields. The results showed that the seasonal distribution of the cases with that of the Mistral in the areas study, shows a clear correlation between this wind and the number of Q fever cases, Consequently, there is a highly endemic area close to Marseille, which constitutes a significant public health threat to the population [30]. In the second study, in the Marseille region, authors hypothesized that the norther wind (The Mistral) that blows over a slaughterhouse which is used 1 day each year by Muslim population of Marseille for the traditional sheep feat "Aid El-kebir", it also blows towards two shelters which are 2 till 6 Kilometres away from this slaughterhouse; thus the Mistral may involve in spreading of *Coxiella burnetii*. From 1999 to 2003, a total of 668 homeless were recruited, especially during the year period when the slaughterhouse was used in Sheep Feast, in 04 years, the strength of the Mistral measured as a mean of the daily recorded was 30.37 Km/h (1999: 12 days, 36.8 Km/h; 2000: 2 days, 28.4 Km/h; 2001: 1 day, 26.2 Km/h; 2002: 6 days, 30.1 Km/h). Results showed that *Coxiella burnetii* IgG phase II antibodies were found in 27 of 668 (4.04%) in the two shelters. To conclude, homeless were likely exposed to *Coxiella burnetii* in shelters during the month that followed the "Aid El-Kebir", where the Mistral wind playing a critical role in this outbreak [13]. In addition, another study suggested the aerosols contamination by *Coxiella burnetii*, between 01 April and 26 June 1996, an outbreak of Q fever with 29 cases of acute Q fever, was observed among the inhabitants of Briançon, a town located in the French region of the Hautes Alpes, where goats, cattle, and sheep are bred in this area, but the main farms are located far from the town itself. Located in the town of Briançon, a slaughterhouse whose major activity of slaughtering was usually conducted between February and April, especially in the period before Easter (lamb meat is traditionally eaten during the Catholic Easter meal), this slaughterhouse was incriminated as source of spreading of *Coxiella burnetii*. The wind spreading was suspected in this outbreak, but they were unable to formally demonstrate the role of the heliport. One question remains: Why did this outbreak occur during the year 1996? This epidemic probably followed a Q fever outbreak among the sheep, as has been described by Dupuis., *et al*. in a Swiss Alpine valley [32]. A longitudinal follow-up study of a naturally infected sheep flock was performed by Joulie., *et al.* in 2017, in order to investigate relationships between seropositivity and bacterial shedding in the vaginal mucus, describe the kinetics of antibodies, including responses to vaccination, monitor maternal antibodies in ewe lambs, and compare serological results in

milk and serum samples. They reported that some dairy females did not seroconvert although they shed *Coxiella burnetii* in their vaginal mucus or despite vaccination. Overall, antibody levels in adult females were found to remain stable over time, with exceptions during the mating and lambing periods. Maternal antibodies decreased during the first month after birth. Interestingly, antibody levels in milk were correlated with those in serum [204].

United Kingdom

According to the Q fever's historic in the United Kingdom, we can deduce that most cases of Q fever in UK are sporadic. Q fever cases number had a tendency to increase considerably from 1970 till 1995, reaching a stable incidence ranging from 0.15 to 0.35 cases/100 000 inhabitants per year [33]. For the same period, outbreaks reported in the literature, in different parts of UK, showed the professional aspect of *Coxiella burnetii* contamination, within laboratories staff, postal workers, and experimental research staff [33]. In addition, we can halt on the largest outbreak in UK occurred in Solihull 1989, in which the cases were associated with windborne spread from farmland to an urban area [34,35]. South Wales in September 2002, during the renovation of cardboard manufacturing plant, likely the potential contamination by *Coxiella burnetii* of the straw board in walls and ceilings disturbed by this workshop, was associated to a Q fever outbreak, where 95 employees and subcontractors were serologically positive for an acute infection [36]. Furthermore, in summer 2006, a widespread outbreak recorded in Scotland, occurred at a rural co-located slaughterhouse and cutting plant. The outbreak's investigations reinforced the rejection of the null hypothesis of no association between inhalation of aerosols contaminated with fomites from the sheep lairage and testing positive for Q fever. The onset went off when the Scottish Public Health Department received notification of respiratory and gastrointestinal symptoms in employees of the plant, at that time, the plant was dealing with the slaughter, boning and dispatching of approximately 1650 cattle and 5000 to 10 000 sheep per week. Consequently, 110 confirmed from 179 suspected cases were reported [37].

"We believed that sharing and applying different techniques and information between different fields of research is of paramount importance for successful outbreak investigation", that was the slogan of a multi-sectoral team that contributed with close collaboration and information exchange, within veterinary, human health, and meteorological agencies and local authorities on outbreak investigations which occurred in Cheltenham. In June 2007, The Gloucestershire Health Protection Team, reported 30 Q fever confirmed cases living in the town of Cheltenham where no cases had been reported in this area in the previous three years. Telephone survey was conducted to identify risk practices at local farms. In the meantime, and for the first often, the Numerical Atmospheric-dispersing Modelling Environment "NAME" was used in order to identify whether air from the identified sheep in nearby; the modelling showed that air from all the three farms was carried over Cheltenham in the estimated risk period. According to the previous outbreaks, 2% to 5% of those infected may be hospitalised; extrapolating from the cases we identified retrospectively through 15 hospital admission, suggests that possibly up to 500 people may have been infected (with the asymptomatic Q fever form) [38].

NAME: Numerical Atmospheric-dispersion Modelling Environment

The areas of highest dosage (pink, orange and yellow) covers Cheltenham town centre. A black diamond marks the location of the farm. Red dots mark the addresses of cases resident in Cheltenham. A black line illustrates the outer limit of the built up areas in Cheltenham. The filled black circle marks the town centre which

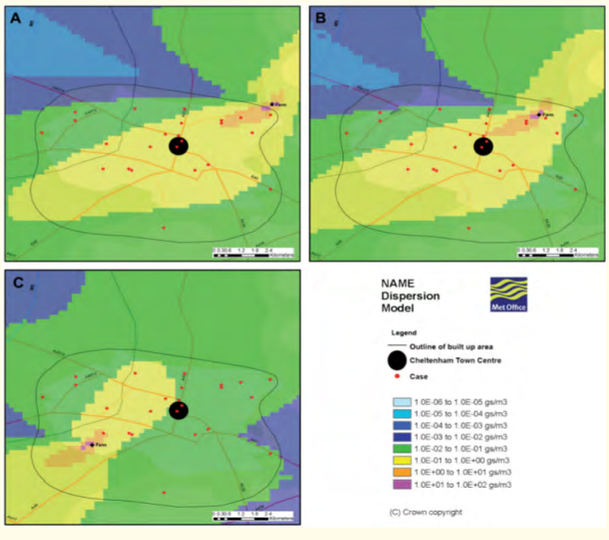

Figure 1: NAME air dosage maps obtained by modelling a continuous release from the high risk farms (A, B, and C) for the time period 23 April-7 May 2007. (Reprinted from Wallensten *et al,* 2010)

Q fever in other developed countries

In Switzerland, a central Europe country, Q fever registers a low endemicity, with an incidence of 0.15 cases/100 000 inhabitants per year, corresponding to 10-12 cases per year. Since 1999, reports of human cases to public health authorities were no longer mandatory, consequently its epidemiology is now largely unknown. In addition to the large Swiss outbreak of Q fever occurred in 1983,

with 415 human cases were reported, another outbreak had occurred in 2012, in Terrace vineyards of Lavaux, where 10 acute Q fever human cases were diagnosed, and the investigations showed that the epidemiological source was the sheep flocks which gave 43% of seropositivity in this region [39]. In the same continent, in 2016 an unusual outbreak of Q fever took place in manufacturers of hoists and chains in Spain, in this epidemic episode, no apparent occupational-associated risk was noted, and 08 employees were detected with an acute Q fever. The widespread distribution of *Coxiella burnetii* DNA in dust samples collected from the plant facilities, suggests that the infection had occurred inside this factory, and the most probable vehicle for the bacteria entering the factory was the worker's boots which were worn inside the infected goat farms and also in the factory [40]. The Canary Islands (Spain) are considered an endemic territory, with a high prevalence in both humans and livestock. A study conducted by Bolanos-Rivero., *et al.* in 2017, aiming to Detect *Coxiella burnetii* DNA in peridomestic and wild animals and Ticks in Canary Island. They found eight rodents (8%) and two rabbits (1.5%) were found to be positive, with the spleen being the most affected organ, and also 6.1% of the processed ticks distributed between those removed from livestock (11.3%), domestic dogs (6.9%), and from wild animals (6%) [203].

Slovakia, in 1952, the first outbreak of Q fever in was occurred among agricultural workers, the source of infection was a sheep flock imported from Romania. More recently, in September 1992, the largest outbreak of human Q fever in Slovakia began as a result of imports of 1181 goats from Bulgaria. Within the next two months, several abortions occured which resulted in two outbreaks. During the first one, 11 humans who were in direct contact with animals were infected [200]. The second epidemic, however, involved as many as 113 human cases. Epidemiological investigations revealed that the source of infection was a contaminated aerosol in a local pub that arose from the clothing of farm workers who witnessed the abortion of one of the goat shortly before [201].

In Turkey, as an Asian country, Q fever has not shaken the Turkish people as a new infection; in 1953, Payzin described it as follows: "The occurrence of the infection among human beings animals throughout Turkey suggests that is not a New disease in this country, and the disease has been known to the Turkish people as an animal infection, under the name 'ESKI HASTALIK' which means 'Old disease' [41]. Thereby, the first outbreak of the Q fever was reported in 1948in Akasaray province, where 21 human cases were diagnosed. Recently in 2000s, precisely in 2002, an outbreak of Q fever was reported near the Black Sea region in Northern Turkey, with 46 human cases, and most of them from Tokat province [42]. In Turkey, as in other parts of the world, the results of seropositivity significantly differ in terms of the regions and study groups. In 2008, Kilic found a seroprevalence of 32.3% in people residing in Urban Turkish area [43]. A series of Turkish studies conducted between 2006 and 2011 with high risk group, showed that a seroprevalence of 65.9% in slaughterhouse workers, 42.9% in Butchers, 32.8% in farmer, 30.6% in veterinarians, 32% in veterinary technicians, 28.5% in animal lovers [43-45]. Despite these evidences, the number of studies conducted to investigate the seroprevalence of *Coxiella burnetii* in Turkey over the last few years is very limited. In Eastern turkey, Erzincam Province, 2017, an overall seroprevalence of *Coxiella burnetii* was found to be 8.7% in rural and non-rural residents, with significant risk factors including raising cattle and exposure to infected animals or their birth products [46].

In China, the disease was initially reported in 1950 and in between 1989–2013, there have been 29 reports on Q fever in this country. Studies' results showed that the overall prevalence of *Coxiella burnetii* infections in the reports is 10% in humans, 15% in cattle and 12% in goats. Cattle and goats had the highest seroprevalence of all the domestic animals studied and a wide variety of ticks were found to be infected. Mice were also commonly infected and had high copy numbers of *Coxiella burnetii* DNA, suggesting they might be important in the epidemiology of Q fever in China [48].

In December 2016, The Europrean Surveillance Sysytem "TESSy" reported that 27 EU/EEA countries provided information on Q fever in humans, reporting a total of 851 cases, 824 of which were confirmed (96.2%). In the same report, six countries reported zero cases in 2015 ((Estonia, Iceland, Lithuania, Malta, Poland and Slovakia). Paradoxically, in Poland, a study published in 2015 was carried out in order to have a prevalence of *Coxiella burnetii* in humans occupationally exposed to animals, where 46 human cases were confirmed serologically (IFA), within 10 samples presented the presence of specific sequences of *Coxiella burnetii* DNA BY using the real-time PCR. These results confirmed the presence of Q fever in Poland, thing has been denied by TESSy's report [47,49]. The question stills hanging, is the TESSy reports are based only on the countries reports? Do countries take into account the results of research that has been carried out? It's just to clarify the reliability of reports given by the TESSy. According to the TESSy 2016 report, the number of cases reported in 2015 is higher than during the years 2011 to 2014. The number of cases dropped in 2012 to increase again in the following years. Between 2012 and 2015, the number of confirmed cases increased by 56%. In 2015, the notification rate was 0.19 cases per 100 000 populations, which is similar than in 2011 but higher than in 2012, 2013 and 2014. From 2011 to 2015, the notification rate was varying between 0.12 and 0.19 cases per 100 000 populations. On the following table (Table 1), numbers and rates of confirmed Q fever cases in some European countries between 2011 and 2015 [49].

Country	2011		2012		2013		2014		2015	
	Confirmed cases		Confirmed cases		Confirmed cases		Confirmed cases		Confirmed cases	
	Number	Rate	Number	Rate	Number	Rate	Number	Rate	Number	Rate
Belgium	6	0.1	18	0.2	5	0.0	4	0.0	8	0.1
Estonia	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
France	228	0.4	5	0.0	158	0.2	209	0.3	250	0.4
Germany	285	0.4	198	0.2	114	0.1	238	0.3	311	0.4
Netherlands	80	0.5	63	0.4	20	0.1	26	0.2	20	0.1
Poland	0	0.0	0	0.0	0	0.0	1	0.0	0	0.0
Spain	33	-	58	-	75	-	77	-	97	-
United Kingdom	43	0.1	12	0.0	46	0.1	60	0.1	21	0.0

Table 1: Number and rate per 100 000 of confirmed Q fever cases by country and year, EU/EEA, 2011–2015 (adapted from The Europrean Surveillance Sysytem, 2016).

Q fever in developing countries

In most developing countries, Q fever diagnostic tools are not well available, consequently its overall impact on public health was widely underestimated. Otherwise, we haven't a clear epidemiological profile of Q fever in these countries. As a result, registered outbreaks and their studies are quite rare, except recent studies occurred in some countries which aimed to identify reservoirs and contamination sources of *Coxiella burnetii* to humans.

First Q fever clinical cases and outbreaks in Africa

Since 1947, the Q fever has caused a major epidemic in Africa countries. In Southern Morocco, a focus of Q fever was discovered by Blanc in 1947 [50], then the first isolation of *Coxiella burnetii* (old *Rickettsia burnetii*) was done in Congo from three human subjects, where the organism was also isolated from cows' and goats' milk, dog and cattle ticks, and human body lice [52].

In 1950, the Third World Health Assembly (WHA), aware of the potential danger of Q fever to public health and of the large gaps in the existing knowledge of the disease, passed a resolution calling for a preliminary study of its prevalence throughout the world. A program of epidemiological research and surveys was accordingly encouraged by the WHA in 33 countries. In 1951, in three Northern African countries, Algeria, Morocco and Libya. Throughout Morocco, Q fever outbreaks were assigned in six towns with 38% human infection and 55% goats, 45% cows and 38% sheep. In addition, 02 human clinical cases and 22 human cases among men of the United State Air Force (USAF) stationed in Tripoli, were reported in Algeria and Libya respectively [52]. In 1952, Halawani and colobarotors, reported 11 positive human cases from 77 in Egypt. Moreover, the WHO survey revealed 27 positive goat's sera from 230, 9 positive goats and sheep from a total of 929 in Tunisia [51]. In 1953, Giroud., *et al.* state in the slaughter-houses in Douala, Cameroons, 16 positive sera from 113 healthy workers [53]. More recently in 1955, Kaplan reported human cases of Q fever in nine African countries, from Morocco to South Africa, suggesting that the infection was widespread in that continent [52]. In 1957 and 1958, others outbreaks have been reported in Batna and in Tlemcen in Algeria [54].

Recent Q fever in Africa

At the twilight of the last century and at the early of the 21st one, studies on the causative agent of Q fever have taken on a new dimension in African countries, where we note an increase in the results obtained following research work in the subject, as well as collaborations between the various actors in the health sector in order to detect reservoirs and sources of Q fever contamination, and know more about the pathogen. *Coxiella burnetii* infection is detected in humans and in a wide range of animal species across Africa, but seroprevalence varies widely by species and location.

In 1995, seroprevalence studies of Q fever conducted by Raoult., *et al.* showed the highest seropositivity rates in Mali, Burkinafaso, Nigeria and Central African Republic, which are countries with the highest density of domestic ruminants [55]. In addition, 1% of patients in Casablanca and 18% in Fez in Morocco had reactive antibodies phase II to *Coxiella burnetii* [56]. In 2003, Schelling states the seroprevalence rate in human of 1% in Chad, and 80% of camels seropositive in Egypt [57]. Three years earlier, Potasman published the fact that Q fever has been reported in travellers returning from a safari tour [58]. In Sousse hospital, in Tunisia, 26% of blood donors were seropositive for acute Q fever in 2008. At the same year Letaief reported 21 acute Q fever cases among patients hospitalized for acute fever illness in Tunisia [59]. Simultaneously, in Ghana rural Ashanti region, 17% of two-year-olds of population study were seropositive to *Coxiella burnetii* [60]. In addition to be-

ing sources for disease transmission to humans, *Coxiella burnetii* in animals can decrease livestock productivity which can have socio-economic and indirect health effects on humans, especially among livestock-keeping populations in resource-limited settings [61].

In an agropastoral region of Algeria, seroprevalence rates of 15% with peaks up 30% in villages where the disease is hyper-endemic, have been observed in Eastern Algeria in 2009 [54]. The causative aspect was the close contact with infected animal and their products, as consumption of unpasteurized raw milk which was incriminated in pathogen shedding in bovine milk with 22% of raw milk in Egypt in 2009 [62]. From 2010 to 2011; many studies have been conducted, where thy mentioned 4% and 32% cattle seroprevalence in Nigeria and Cameroons respectively [63,64]. In addition, both in Senegal and Tanzania, seroprevalence of Q fever was 24.5% and 5% of the population study correspondingly [65-67], with febrile illness and sever pneumonia cases. Moreover, in most African countries, seroprevalence rates are elevated in domestic ruminants, surveys in cattle showed rates ranging from 4% in Senegal to 33% in Nigeria and 18% in Ghana [68]. In 2012, different countries investigated Q fever animal contamination sources, mainly goat, sheep and cattle. In Egypt and Sudan, the seroprevalence in Goat was nearly similar with 24%, otherwise the sheep seroprevalence was higher in Egypt with 33%, this could be due to the intensive breeding of sheep and goats which are widespread in, even to the human close contact with these species [69,70]. Consequently, the *Coxiella burnetii* infection may spread in human, which could explain the different Q fever seroprevalence rates that varying across countries. Schelling., *et al*. in 2012, state a seroprevalence of 16% in Egyptian patients [71]. Moreover, in 2013 Crump., *et al.* conducted an investigation in cohort in Tanzania of severely ill febrile patients where they found 26.2% zoonosis, among which 30% where due to Q fever infection [72]. Concurrently, in rural clinic in western Kenya, a seroprevalence survey on banked sera of febrile patients, who were diagnosed for an acute lower respiratory infection, being found to have acute Q fever with 3% from a total of 30.9% as a global rate, reported Knobel., *et al* [73]. In rural regions of most of these countries, human households are in close vicinity to domestic ruminants, making transmission easier than it is elsewhere, as a result, Ratmanov detected *Coxiella burnetii* DNA in 2% to 22% of household samples in rural Senegal [74].

Aiming to study *Coxiella burnetii* in febrile patients in rural and urban Africa, in 2014 Angelakis., *et al*, worked for blood samples from febrile and non-febrile patients from six African countries and from France were investigated retrospectively for Q fever infection by molecular assays targeting the IS1111 and IS30A spacers. Results showed that no cases were found in Morocco, Tunisia and Mali with 00/48, 00/84 and 00/400 samples respectively. However, 6 positive q PCR for both IS1111 and IS30A spacers were found in Senegal from a total of 511 blood samples. In Oran, Algeria, they found one patient infected with *Coxiella burnetii* who presented asthenia, respiratory symptoms and suffered from a persistent fever and myalgia for 6 days. The installation of point-of-care laboratories in rural Africa can be a very effective tool for studying the epidemiology of many infectious diseases [65]. Vanderburg published in 2014 a Systematic Review dealing the epidemiology of *Coxiella burnetii* Infection in Africa. In a part, he described a human cohorts comprising individuals with infective endocarditis in Sousse and Sfax, Tunisia, as well as Algiers, Algeria, have demonstrated *Coxiella burnetii* as the causative pathogen in 1–3% of cases [61]. He concluded that *Coxiella burnetii* has been implicated as a cause of livestock abortion and could be responsible for substantial economic burdens, but more rigorous studies are required to determine this and other sequelae of disease in animals. Secondly he judges that risk factors for human exposure to Q fever are poorly understood, but a more detailed understanding of how human exposure in different communities is linked with animal infection patterns and animal husbandry practices is clearly needed [61]. As in Nigeria, *Coxiella burnetii* has been detected in up to 60% of cattle milk samples, which is considered as source of human contamination [61].

In Algeria, Khaled in 2016, conducted a study in order to identify the positive sources of Q fever in Algeria; where he found a seroprevalence of 14.1% among small ruminant's flocks, and he determined the shedder flocks of *Coxiella burnetii* via the vaginal swab with 21.3% q PCR positive [76]. Whereas, Bessas and Aouadi in the same year, reported the presence of *Coxiella burnetii* by q PCR in spleen dog (0.80%) and blood/ticks of small ruminant (4.73%) respectively [75,77]. These results could lead to the various sources of *Coxiella burnetii* contamination in Algeria. More recently, in 2017, Benaissa studied the causative agent of Q fever in the dromedary camel (*Camelus dromedarius*) population in Algeria, where he found that antibodies to *Coxiella burnetii* were found in 71.2% of all camels investigated and the true prevalence was calculated as 71.1% [Dromedary Algeria], these result prove that even in southern Algeria where the highest temperature are registered in Algeria (up 58 °C) *Coxiella burnetii* could escape and infect one of the highest immune system in animal world, thus the pathogen's high resistance to high temperatures in the outside environment could be demonstrated [78]. Abushahba., *et al.* in 2017, between August 2016 and January 2017, in El Minya –Egypt, calculated a seroprevalence of Coxiella burnetii IgG antibodies, with 25.68%, 28.20%, and 25.71% in sheep, goat, and humans respectively, which clearly points out that Q Fever may be emerging in this area [210]. A few *Coxiella burnetii* genotypes (including genotypes 2,6,16,19,30,35,36 and 52) have been characterized in Africa, mainly in ticks; only genotypes 19 and 35 have been detected so far in human [66,79].

Q Fever in others developing countries

Q fever presence in the Middle Easter areas is being reported from Syria [80], in 2000, when Bottieau reported that a Belgian patient developed Q fever after a journey in Syria, thus *Coxiella burnetii* infection was diagnosed because of the presence of granulomas with a central vacuole in a bone marrow biopsy. Furthermore, Faix in 2005, described Q fever outbreak occurred in 22 (58%) of 38 US-Marines deployed to Iraq, all patients presented Fever, while respiratory symptoms were found in 76%, and gastrointestinal symptoms in 53% were, dust and exposure to animals and ticks were the main risk factors incriminated [82].

In Iran, the first clinical case of acute Q fever in human was reported in 1952 [83]. Then positive cases of infection with *Coxiella burnetii* in cattle, sheep, and goats had been reported as 7%, 3.2% and 1.7% respectively in 1976; after this year, the disease was forgotten in Iran and no human cases were reported. In 2011, a cross-sectional study was conducted in different regions of the Sistan va Baluchestan province, in Iran, a total of 190 sera were collected from butchers and slaughterhouse workers; phase I and II of Q fever were found 18.1% and 14.4%, respectively; Additionally, a significant relationship was found between seropositivity of Q fever and camel slaughtering [81]. Kayedi in 2014, found 45 sheep among 330 ones (13.64%) tested positive for IgG for Q fever and 23 animals were border-line (6.97%) [84]. Moreover, in 2017, Mobarez states the overall seroprevalence of IgG phase I and phase II antibodies of Q fever in human between 2005-2016 was 19.8% and 32.86% respectively [85].

In Lebanon, information about the presence of *Coxiella burnetii* infection is scanty and only related to a survey performed in the last century when Garadebian recorded *Coxiella burnetii* between both sick and healthy people in 1956 [86]. Last year, in 2018, for the first time in Lebanon, Dabaja studied the human seroprevalence of Q fever, where 421 human sera from 05 different Lebanese provinces, the sera were screening for IgG phase II antibodies against *Coxiella burnetii* Enzyme Linked Immunosorbent Assay (ELISA) Kit and IFA. Results showed that 38.70% were estimated positive samples by ELISA, and 37% by IFA test. This results were different across the 05 Lebanese province, this may be due to the presence of high density of livestock production and of major agricultural areas in Akkar and Bekaa provinces [87]. In North-western Palestine, the average annual incidence of Q fever between 1998-2004, was 0.6 cases/100 000 cases (20 to 70 cases per year) [88]. In this country a few outbreaks were reported, with the majority occurring in rural or adjacent areas following outbreaks of Q fever in livestock, and all were relatively limited in scale. In June 2005, An unusual outbreak Q fever its magnitude and place of occurrence in boarding high School in North-western Palestine was reported by Amitai, this outbreak has caused the infection of 103 students and 05 employees of the High School.

In India, the data in most of the published reports on the prevalence of *Coxiella burnetii* infection in humans and animals are based on results from sero-surveys employing capillary agglutination and complement fixation tests, aside from those in the few reports on the isolation of these agent. In 2008, Vaidya conducted a comparison of different diagnostic tool for Q fever disease in humans with spontaneous abortions. He worked for a total of 368 samples (placental bits, vaginal swabs, sera…) collected from 74 women with spontaneous abortions, using the q PCR targeting IS1111, and IFA test, results were for 25.68% IFA positive, and for 21.62%. These results testify that *Coxiella burnetii* may be incriminated in spontaneous abortions in women [89].

In the Latin America, thorough examination of the literature in the 04 languages (English, French, Spanish, and Portuguese) testify that studies' results of Q fever disease are frightening although Q fever is present worldwide [90]. According to the available literature, 07 (Belize, Costa Rica, Guatemala, Guyana, Honduras, Paraguay, Suriname) Latin America countries have never reported any cases of Q fever throughout all the past century till nowadays [90]. Otherwise, in Colombia, Mexico, and Brazil, studies' results showed several seroprevalence studies in exposed populations, where some of ones are based on the febrile and the respiratory aspect of Q fever disease. In addition, there are no publications on Q fever in the Amazon region, except in and Ecuador French Guiana. In this later, which is a French oversea territory located on the Northeastern coast of South America, about 90% its 84 000 Km^2 surface is covered by the Amazonian rainforest. Coxiella burnetii was first described in 1955 in French Guiana, but the real interest arose throughout the years. Studies found an annual incidence of 37 cases/100 000 persons between 1996-2000, up to 150 cases/100 000 persons in 2005 [91], and 17.5 cases/100 000 persons between 2008-2011 [92]. Moreover, groups at risk are not clearly defined. The main risk factor for Coxiella burnetii infections are working in construction/public works, living near bats, wild mammals, or the forest, levelling work and gardening [93].

After this great dive into the history of Q fever outbreaks around the world, in time and place, we were able to establish a map (Figure 2), on which we distributed chronologically the different Q fever outbreaks mentioned above, based on the colors-years ratio, aiming to facilitate the monitoring and memorization of these episodes over time and in places.

Microbiological aspect

Bacterium

The pathogen agent of Q fever disease, *Coxiella burnetii*, is belonging to the gender of *Coxiella*, which is placed in the domain of *Bacteria*, Phylum of *Proteobacteria*, Class of *Gammaproteobacteria*, Order of *Legionellae*, family of *Coxiellaceae* [94]. *Coxiella burnetii*

Figure 2: Q Fever outbreaks across the world, in time and place (personal synthesis).

has a cell wall similar to that of Gram-negative bacteria. However, this small coccobacillus (0.2 to 0.4 µm wide and 0.4 to 1 µm long) is not stainable with the Gram technique. The Gimenez method is used to stain *Coxiella burnetii* isolated in culture or directly in clinical samples [95]. The estimated doubling time of the bacterium is between 20 and 45 h in *in vitro* cell culture [96]. It is an intracellular pathogen, replicating in eukaryotic cells, targeting macrophages (lymphocytes, lymphatic nodes, spleen, liver, lungs….), monocytes blood circulating [97], trophoblasts [98]. Its existence in Free-Living Amoebae was also described [99,88], thus in Murine adipocytes [100]. The bacterium actively participates in the genesis of intracellular vacuole which acquiring phagolysosome-like characteristics, such as an acidic pH, acid hydrolysates, and cationic peptides, getting several strategies for adaptation to this exceptionally stressful environment [101]. First, *Coxiella burnetii* genome is still discovering and surprising. Its genes encode an important number of basic proteins that are probably involved in the buffering of the acidic environment of the phagolysosome-like vacuole. Also, four sodium-proton exchangers and transporters for osmo-protectants are found allowing this bacterium to confront osmotic and oxidative stresses [94]. *Coxiella burnetii* division mode is complicated and characterized by 2 morphologic forms corresponding to biphasic development cycle. The large-cell variant (LCV) of the bacterium is an exponentially replicating form, whereas the small-cell variant (SCV) is a stationary non-replicating form [102]. SCVs are small rods (0.2 to 0.5 µm long) characterized by condensed chromatin, a thick envelope, and an unusual internal membrane system. LCVs have a larger size (>0.5 µm), a dispersed chromatin, and an envelope similar to that of classical Gramnegative bacteria. SCVs are typical of the stationary phase and poorly active metabolically. They are observed after prolonged culture (21 days) in Vero cells and in axenic acidified cysteine citrate medium 2 (ACCM2) [103]. SCVs are stable in the environment and are highly resistant to osmotic, mechanical, chemical, heat, and desiccation stresses [104]. The transcriptome analysis of the SCV has revealed upregulated genes involved in the oxidative stress response, cell wall remodelling, and arginine acquisition. Also, SCVs show an unusually high number of cross-links in their peptidoglycan, which probably are involved in their exceptional environmental resistance [102].

The LCV transforms into the SCV, which is the spore-like form of *Coxiella burnetii*. In this form, the bacterium is highly resistant to environmental stress [105]. The resistance allows *Coxiella burnetii* to survive in the environment while keeping its infectivity [105]. Subsequently, they can survive for 7 to 10 months on wool at ambient temperature, for more than 1 month on fresh meat, and for more than 40 months in milk [96]. The high virulence of *Coxiella burnetii*, the possibility of its aerosolization, and its environmental stability and have led the U.S. Centers for Disease Control and Prevention (CDCP) to classify this bacterium as a category B biological threat agent. A bioterrorism attack with this pathogen, although not associated with the high death rates observed for Class A agent, could cause significant disability and possibly long-term consequences due to persistent infection in the population.

Phase's variation. Like several other Gram-negative species, *Coxiella burnetii* displays antigenic variation similar to smooth-rough variation, which is related to changes in lipopolysaccharide (LPS) layer [106]. Highly virulent phase I, phenotypically expresses a full-length and smoothed LPS, are able to replicate in immunocompetent hosts. While phase II, less virulent, unable to replicate in immunocompetent hosts, phenotypically carries a rough LPS [105,107,108]. The situation is complicated further by phase I and phase II forms. Phase variants display different LPS lengths with phase I organisms producing a full-lengh LPS with O antigen sugars, and phase II organisms producing a truncated LPS without O antigen [109]. LPS is demonstrated in both the *Coxiella burnetii* LCV and SCV, although presence of LPS is mainly associated with the SCV [110]. When mixed population of *Coxiella burnetii*'s phase I and phase II, is injected to an immunocompetent host (animal model), phase II bacteria, unable to infect cells, are eliminated, this led getting a homogeneous population of phase I bacteria [111]. During serial passage in cell culture, phase I *Coxiella burnetii* can convert into phase II [112]. Both LPS phenotypes can be distinguished via phase-specific antibodies. Phase I antibodies are directed against the full-length LPS of phase I, whereas phase II antibodies are directed against common surface proteins [113]. These surface proteins are also present in the surface of phase I *Coxiella burnetii*, but seem to be shielded by the long phase I LPS [105]. This antigenic variation is very important in serology and supports the diagnosis

for the differentiation between acute and chronic Q fever in humans [114,115].

Intracellular cycle and virulence's factors

Histopathological analysis on *Coxiella burnetii* has identified monocytes and macrophages as the primary infection sites, but epithelial and endothelial cell infection has also been evident [97,117]. During infection, *Coxiella burnetii* is attached to macrophages by $\alpha_v\beta_3$ Integrin, which triggers phagocytosis of the bacterium by an Actin-dependent mechanism [118]. The bacterium will then divert phagocytosis to its advantage. The nascent vacuole containing *Coxiella* (VCC) acquires the RAB5 GTPase as soon as 5 minutes after internalization. This GTPase stimulates the fusion of the VCC with early endosomes, resulting in acidification up to pH 5.4 and acquisition of the early endosomal marker protein, EEA1, which is characteristic of phagosomal development normal. However, unlike phagosomes, the VCC also acquires autophagosomal markers LC3 (Microtubule-associated protein Light-Chain 3). The maturation of the VCC causes it to lose RAB5 and EEA1 and acquire RAB7 GTPase and the membrane glycoprotein associated with the lysosome, LAMP1, 40 to 60 minutes after internalisation. This results in acidification up to a pH of 5. These phenomena are also characteristic of normal phagosomal development. Two hours after internalization, lysosomal enzymes, including Cathepsin D, are beginning to accumulate in the VCC and the pH drops further to about 4.5. This process is significantly delayed compared to the normal phagolysosomal acquisition of Cathepsin D. The delay in the development of the VCC would seem to allow the conversion of small variants into large ones (SCV to LCV). From 8h to 2 days after internalization, the VCC expands to occupy an increasingly large space in the cytoplasm of the host cell. This process is dependent on protein synthesis by the bacteria and involves the recruitment of GTPase RHO and RAB1B to the VCC membrane. The RHO GTPase is probably involved in the maintenance of this large vacuole, while the recruitment of RAB1B from the endoplasmic reticulum appears to facilitate acquisition of additional membranes to create this spacious CSS [119].

Phase II internalization is more efficient, resulting in better multiplication, thus explaining why phase II bacteria grow more rapidly than phase I, resulting in a shift from phase I to phase II in the laboratory [120].

More precisely, we note some differences between phase I and phase II in both pathway internalization and surviving in phagosomes. In phase I bacteria' attachment, it's involving $\alpha_v\beta_3$ Integrin, Integrin Associated Protein (IAP), and Toll-like Receptor 4. However, attachment of the phase II is mediated without TLR4 [120-122]. In addition, phase II *Coxiella burnetii* maturation, involve the Rab7, and acquiring Cathepsin D. Finally, phase I *Coxiella burnetii* bacteria are internalized and survive intracellular killing, whereas phase II bacteria are efficiently phagocytized and then killed in macrophages [120]. They have found that the addition of IgG antibodies from patients with endocarditis promotes the creation of large vacuoles. This finding may explain why large vacuoles *in vivo* are seen only during chronic infection [120].

Pathogenesis and immune response to *Coxiella burnetii*

In human infection is usually acquired from aerosols but infection by ingestion of contaminated dairy products is also possible. In animals too, the portal of entry is the oropharynx. The organism is highly infectious, with the infectious dose being as low as one organism [123]. Following inhalation, the organism must first invade before subsequently causing systemic infection. The alveolar macrophage has been proposed as the primary target. It has been suggested that one factor contributing to the attenuation of phase II forms versus phase I forms is the nature of the interaction between the organism with host cells [109].

After primary multiplication in the regional lymph nodes, an ensuing bacteraemia lasts for 5-7 days and the organism then localizes in the mammary glands and the placental of pregnant animals [106].

Immune control of *Coxiella burnetii* is T-cell dependent but does not lead to its eradication [122]. *Coxiella burnetii* can be found in apparently cured people, as well as in the dental pulp of experimentally infected and apparently cured guinea pigs [122,124]. *Coxiella burnetii* DNA can also be found in circulating monocytes or bone marrow of people infected months or years earlier [120]. Haematogenous spread results in the organism infection the liver, spleen, bone marrow, the reproductive tract and other organs**.** This is followed by the formation of granulomatous lesions in the liver and bone marrow and the development of endocarditis involving the aortic and mitral valve [106]**.** These granulomas are made possibly by the migration of monocytes through the vascular endothelium. A lipid vacuola forms the centre of the typical Q fever granuloma, it's surrounded by fibrinoid ring [33]. Few, if any isolated bacteria can be found in granuloma during the acute phase. TLR4 has a role in granuloma formation, since knouk-out mice deficient for this receptor have decreased number of these granuloma [122]. Specific immunoglobulins are secreted following infection. IgG is mainly directed against phase II antigen, whereas IgM is directed against both phase I and II cells [33]. Monocytes from convalescent patients are able to kill *Coxiella burnetii*. TLR4 modulates the cytokine (interferon γ and tumour necrosis factor) response following acute infection [120].

During chronic Q fever the immune response is ineffective, and also may be harmful, causing leucocytoclastic vasculitis and glomerulonephritis. *Coxiella burnetii* continues multiply despite high concentrations of all three classes of antibodies (IgG, IgM and IgA) to phase I and phase II bacteria. Lymphocytes counts and CD4-to-CD8 ratio are lowed [2]. Organ biopsies do not show granulomas but large vacuoles containing *Coxiella burnetii* can be detected in infected tissues such as heart valves and liver, and also in aneurysms. Monocytes from these patients are not able to kill *Coxiella burnetii* [31], and do not migrate through the endothelium [120].

In mice, both humoral and cellular immune responses are important in limiting the infection. Macrophages and other mononuclear cells are believed to be the major target cells during *Coxiella burnetii* infection [125]. T-cells are suggested to be critical for clearance of *Coxiella burnetii* after infection. B-cells are important for the prevention of tissues damage [108]. Antibodies can be detected as early as 14 days' post-inoculation for anti-*Coxiella burnetii* phase II antibodies and 21 days in the case of anti-*Coxiella burnetii* phase I antibodies. This is comparable to what was observed in goats. Upon infection, goats generate a phase I and phase II specific IgM and IgG response. After two weeks' post-infection, a strong phase II specific IgM and IgG antibody response can be detected while a less pronounced IgM anti-phase I response is present as well. IgG anti-phase I antibodies start to rise at 6 weeks' post-infection [105]. This information can help in the diagnosis of Q fever, understanding herd dynamics and will be helpful in improving vaccines.

Little is known about the pathogenesis of Q fever in domestic animals. Under laboratory conditions, *Coxiella burnetii* inoculation of both guinea pigs and mice results in systemic infection, including pneumonia, hepatitis and splenomegaly [126]**.** The severity of pathological changes depends on the strain. Splenomegaly is thought to be an indicator for the virulence of *Coxiella burnetii* strains in guinea pigs and mice [126]. Also the inoculation route seems to influence pathogenesis. In mice, intranasal inoculation is mainly associated with pneumonia, whereas intraperitoneal inoculation is mainly associated with hepato-spenomegaly [105]**.** In inoculated pregnant mice, *Coxiella burnetii* is abundantly present in both the foetal and maternal parts of the placenta [127]. In goats this is different [128]. In pregnant goats, the trophoblast of the allantochorion are target cells of *Coxiella burnetii* which multiplication occurs. *Coxiella burnetii* antigens are barely detected in adjacent maternal parts of the placenta. Sanchez in 2006, reported that in other maternal organs, *Coxiella burnetii* DNA, but no viable bacteria, can be found at some time points during pregnancy [129]. Recent research showed that infection route does not influence the pathogenesis and that *Coxiella burnetii* is not excreted during pregnancy [128]. This hampers the detection of infected pregnant goats. Recently, in 2017, Pellerin., *et al.* studied the Attachment of *Coxiella burnetii* to the *zona pellucida* of *in vitro* produced goat embryos, they clearly demonstrated that *Coxiella burnetii,* after *in vitro* infection at 10^9 *Coxiella*/ml, stick strongly to the external part of the *zona pellucida* of *in vitro* without deap penetration; also *Coxiella burnetii* DNA was detected in all the eight batches of infected embryos after the 10 successive washing [130].

Clinical presentations

The clinical presentation of Q fever, including the proportion of people presenting with pneumonia and/or hepatitis, or other typical or atypical Q fever clinical signs, varies greatly from series to series and depends on the geographic origin of the infection. For example, pneumonia is more common than hepatitis in eastern Canada, while in southern Spain pneumonia is rare, and hepatitis is very common. In southern France, pneumonia is common and hepatitis very common [120].

Primary infection

Wide diversity of clinical symptoms is noted in *Coxiella burnetii* primary infection. The incubation period for the primary infection before the onset of symptoms can last from 2 to 3 weeks, and it depends on the size of the inoculum. In a large proportion of patients, the primary infection can be asymptomatic [120]**.** In other cases, pneumonia, hepatitis, or flu-like syndrome can be observed. The determinants of the symptomatology in *Coxiella burnetii* primary infection depend on host factors and on the strain involved. Age and sex ratio, have marked the stakes in the Netherlands epidemic, with symptomatic patients being significantly older and more often men than asymptomatic patients [131]. Children are also less frequently symptomatic than adults [132].

Clinical expressions of Q fever are often subclinical or extremely mild. For instance, during a Q fever outbreak in Switzerland in 1987, of the 415 patients diagnosed with Q fever, 224 were seropositive but asymptomatic (54%) and only 2% of those affected were hospitalized.

Acute infection

During acute Q fever, the incubation is ranging from 14 to 39 days, and its expression depends widely to hosts factors. Thereby, the clinical signs vary greatly from patient to patient, where the immune-compromising patients express the disease more than others. In the Netherlands, the recent acute Q fever outbreak showed a mortality rate of 1.2% within approximately 1 month after hos-

pitalization of patients. All lethal cases suffered severe underlying medical conditions [133]. The notification criteria established in The Netherlands in 2010, for the laboratory diagnosis of acute Q fever, is as following: a positive *Coxiella burnetii* specific PCR, IgM phase II antibodies, and fourfold increase of the IgG phase II antibodies titre. Distinctively, four major presentations are detailed. These are as follow;

Isolated febrile syndrome

In acute Q fever with isolated febrile syndrome (flu-like illness), additionally to the sudden onset of high fever (high grade fever, 40°C) which is the predominant sign, other symptom can be established, as myalgia and headache, mostly retro-orbital. The fever can last for more than 15 days [120,134]. In Spain, 21% of Q fever episodes presented febrile syndrome lasting for more than one week and less than three weeks [135], also in the recent Netherlands Q fever outbreak, it was the most frequent sign among the affected patient. This nonspecific presentation is misleading and supports the fact that clinicians should include screening for *C. burnetii* primary infection in the presence of an isolated fever of unknown origin [104].

Respiratory illness

Atypical pneumonia is one of the most commonly recognized forms of acute Q fever, which we find in 15%, 82%, 33%, 22% of patients with immunosuppression, fever, headaches, and myalgia respectively [120,135]. The duration of symptoms varies from 10 to 90 days. Moreover, the prevalence of pneumonia during the primary infection is highly variable. It's considered as the major manifestation of acute Q fever in Spain, Canada, Nova Scotia, Switzerland, and Cayenne. In this later country, in French Guiana, *Coxiella burnetii* MST 17 is responsible for the highest rate of community-acquired pneumonia in the world (around 40%) [92]. Patients generally present non-productive cough, minimal auscultatory abnormalitie, but some patient present with acute respiratory distress, also nonspecific on the chest radiograph [135].

Hepatitis

In Q fever endemic countries, hepatitis is more frequent than pneumonia. Such as France, Spain, Portugal, Taiwan [104]. Patients presenting 2%, 98%, 61% and 44% of immunosuppression, fever, headaches, and myalgia respectively, presenting also hepatitis [120]. Three major forms of hepatitis may be encountered: an infectious hepatitis-like form of hepatitis with heapatomegaly but seldom with jaundice, clinically asymptomatic hepatitis, and prolonged fever of unknown origin with characteristics granulomas on liver biopsy [135].

Other accompanying findings are anorexia, vomiting, and sometimes diarrhea and painful hepatomegaly [92,104], also hyperbilirubinemia which was found in 37% of cases in Taiwan [136]. In developing countries where coinfection with viral hepatitis is high, clinical manifestations of *Coxiella burnetii* hepatitis do not seem to be more severe. Fatal cases due to hepatic insufficiency are very rare and have been reported in a child, or in patients with cancer or alcoholism [104].

Other acute Q fever manifestation

Other clinical aspects of acute Q fever may occur, and its manifestation depends of the strain's virulence and host's immunity. In a case of a 41-year-old male, from USA, complaining of body aches, fever, nausea, malaise, bilateral knee pain, and vomiting. The Clinical examination revealed a notable erythematous blanching rash all over his body, positive serologic testing for *Coxiella burnetii* was confirmed, additionally the skin biopsy of the rash lesion, showed neutrophilic inflammatory destruction of small vessels with extravasation of red cells, nuclear dusting, and fibrinoid necrosis of the blood vessels, which remains specific for Leukocytoclastic vasculitis, this case confirms the variation of Q Fever expression from a case to another [208]. Basically, we can classify these Q fever manifestation as following:

- **Cardiac involvement:** Pericarditis and myocarditis, are each found in 1% of cases, while myocarditis is frequently fatal [120]. Furthermore, acute endocarditis was observed in Q fever primary infection associated with high level IgG antibodies anti-cardiolipin (aCL) without any valvular Heart Disease (VHD), typically, the vegetation's were localized in the aortic valve [104]. The pathophysiological scenario for this new entity would be that *C. burnetii* primary infection causes an explosive secretion of autoantibodies, including IgG aCL, causing autoimmune valvular lesions [104].

- **Neurological signs:** Apart from headache, which is a common sign in acute Q fever, revealing a possible neurological tropism of the bacterium. Consequently, aseptic meningitis and/or encephalitis, may occur in 0.2% to 1.3% of patients with Q fever, which are rarely accompanied by seizures and coma [135]. Cerebrospinal fluid (CSF) findings show lymphocytic meningitis. This neurological involvement could be caused by the immunological disorder following the bacterium invasion, resulting in sensory neuropathy or Guillain-Barre syndrome [137,138].

- **Dermatological signs:** Dermatological lesions are more common than generally thought, ranging from 1% to 9% of patients with acute Q fever, it consists mainly of transient punctiform rashes, maculopapular eruptions, vesicular exanthema, and more rarely, erythema nodosum [104,120,135].

- **Rare acute Q Fever clinical manifestations:** Many other acute Q fever presentations were found. Old rapports showed a typical "doughnut" or "fibrin ring" granuloma in biopsy specimens of bone marrow, during *Coxiella burnetii* primary infection, then a case of bone marrow necrosis was reported too [104]**.** In addition, uncommon manifestations of acute Q fever have been described, such as acute lymphadentis [139]**,** cholecystitis [140], haemolytic anaemia**,** pancreatitis, lymphadenopathy mimicking lymphoma, and splenic rupture [135].

Chronic infection

Chronic Q fever can develop from a primary infection in about 1% to 5% of patients, whom differ from those with acute disease in age, predisposing conditions, clinical and laboratory findings, and evolution. It was initially described as lasting for more than 6 months after the onset, and can become manifest years after the initial infection [105,135]. Typically, the heart is the most commonly involved organ, followed by arteries, bones, and liver. Clinical symptoms include non-specific fatigue, fever, weight loss, night sweats an hepato-splenomegaly as well as endocarditis in patients with underlying valvular damage or immunocompromised, (bicuspid aortic valve, minimal mitral insufficiency, mitral valve prolapse), 30% to 50% of them develop chronic endocarditis. Systematically, echocardiography in all patients with acute Q fever is recommended to exclude underlying cardiac lesions [120,141]. Clinically, the disease usually presents as a subacute or acute blood culture-negative endocarditis [135], and it's undistinguishable from acute endocarditis, since fever is frequently recurrent or absent, and vegetations may be difficult to detect by echocardiography [120]. Q fever endocarditis is the most frequently reported form of persistent *C. burnetii* infection in the literature, its prevalence ranging from 3% to 10% in England, France, Brazil, and Thailand [29,135,142,143]. In Africa, it represents from 1% to 3% of infective endocarditis in cohort studies [61]. However, its prevalence is probably underestimated in most developing countries, where microbiological tools for diagnosis are lacking. Other manifestations of chronic Q fever include infections of aneurysms or vascular draft, osteoarthritis and osteomyelitis [135].

In 2012, Wegdam-Blans., *et al*. [141], established the Dutch Q Fever Consensus Group, in order to classify and organize better chronic Q fever diagnosis. The Consensus has been classified into three categories by three "P", Proven chronic Q fever, Probable chronic Q fever, and Possible Chronic Q fever.

Proven chronic Q fever

Positive *Coxiella burnetii* PCR in blood or tissue or IFA titer of 1:1,024 for *Coxiella burnetii* phase I IgG, and Definite endocarditis according to the modified Duke criteria or Proven large-vessel or prosthetic infection by imaging studies (18 F-FDG PET, CT, MRI, or AUS).

Probable chronic Q fever

IFA titer of 1:1,024 for *Coxiella burnetii* phase I IgG and one or more of the following criteria: Valvulopathy not meeting the major criteria of the modified Duke criteria; Known aneurysm and/or vascular or cardiac valve prosthesis without signs of infection by means of TEE/TTE, 18 F-FDG PET, CT, MRI, or abdominal Doppler ultrasound; Suspected osteomyelitis or hepatitis as manifestation of chronic Q fever; Pregnancy; Symptoms and signs of chronic infection such as fever, weight loss, and night sweats, hepatosplenomegaly, persistent elevated ESR and CRP; Granulomatous tissue inflammation, proven by histological examination Immunocompromised state.

Possible chronic Q fever

IFA titer of 1:1,024 for *Coxiella burnetii* phase I IgG without manifestations meeting the criteria proven or probable chronic Q fever. (MRI, magnetic resonance imaging; AUS, abdominal ultrasonography; ESR, erythrocyte sedimentation rate).

Both laboratory techniques (PCR, Coxiella burnetii phase I IgG titers) used in diagnosis of chronic Q Fever, have drawbacks; PCR on blood has a low sensitivity and the cut-off values of anti-Coxiella burnetii phase I to distinguish past from chronic infection, are still debated, and are either not sensitive or specific enough [209].

Infection during pregnancy

Both acute and chronic Q fever have been described during pregnancy. In mammals, *Coxiella burnetii* undergoes reactivation during pregnancy and thus is responsible for higher rates of abortion, prematurity, a low birth weight [135], likewise in humans, primary infection in pregnant women, whether or not symptomatic, may be followed by the same poor outcomes, mainly when *Coxiella burnetii* infection occurs during the first trimester [142,144]. The foetus may be infected during pregnancy. Furthermore, Q fever may become chronic after delivery and be associated with recurrent miscarriages [120]. *Coxiella burnetii* has been isolated from the placenta of a woman who became pregnant **2** years after an episode of acute infection. Clinically, Q fever disease during pregnancy is most often asymptomatic [145], which lead to complicate more the course of the disease with utero fetal death, placentitis, thrombocythopenia. After invasion of the pregnant uterus and initial localization in the placenta, active *Coxiella burnetii* infections may hypothetically spread to the foetus hematogenously or by the amniotic-oral route and thereby compromise the foetus [206]. In order to investigate *Coxiella burnetii* in the precolostral blood samples in stillborn calves, Freick., *et al.* in 2017, sampled 56 stillborn calves, they demonstrated the presence of 7.1% of positive samples

for Coxiella burnetii DNA, and 1.8% sera were positive too for anti-Coxiella antibodies by ELISA, these results prove the coxiellaemia and precolostral seroconversion occurred sporadically in stillborn calves from this endemically infected herds [207]. Seroprevalence studies in pregnant women show very variable rates in areas of endemicity: 0.15% in Marseille- Franc, 3.8% in Canada, and 4.6% in London, United Kingdom, 47% in Denmark, and 9% in the recent Netherlands outbreak [91,146-149]. In 2014, a meta-analysis of 136 cases and 7 population-based studies confirmed that seropositivity and untreated Q fever during pregnancy, are associated with foetal death, and antibiotic treatment prevents this complication [150]. Moreover, genotypes from different geographical areas could induce different rates of obstetrical complications, which was suggested by Angelakis., *et al*. in 2013, when they reported *Coxiella burnetii* strains harboring the QpDV plasmid were associated with an increased risk of abortion [151].

Post-Q Fever fatigue syndrome (QFS)

In other hand, we have to pay more attention to the QFS, which is another long-term presentation of Q fever. In QFS patients *Coxiella burnetii* cannot be detected, and antibodies levels against the bacteria are low or negligible, these findings are completely upset in chronic Q fever. Symptoms of QFS include prolonged fatigue, arthralgia, myalgia, blurred vision and enlarged painful lymph node [33].

Genomic aspects.

In 2003, Seshadri., *et al.* sequenced the whole genome of *Coxiella burnetii* Nine Mile strain, this strain was isolated from ticks in Montana in 1935 and its genome is composed of 1,995,275 pb [94]. In addition, in 2007, the genome of Henzerling strain RSA 331, was isolated from the blood of an infected patient in northern Italy in 1945 [152]. Then, 3 other strains were sequenced, these are "K" and "G" isolates of chronic human endocarditis and the "Dugway" isolate of rodents, naturally attenuated [153].

As an intracellular pathogen, the most important factor for natural selection of *Coxiella burnetii* could be the interaction with its specific hosts niches. Genome reduction and the presence of mobile genetic elements and virulence related peudo-genes throughout the genome are predicted to be specific genome manifestations of the obligate intracellular life cycle of this pathogen [154]. Hence, a comparison of *Coxiella burnetii* genome sequences with specific emphasize on genes involved in pathogen-host (cell) interactions or modulation thereof, many shed light on adaptation mechanisms of *Coxiella burnetii* to various host species [154]. Previous studies have shown that *Coxiella burnetii* isolates differed respect to their plasmid type (QpH1, QpRs, QpDG, and QpDV), and lipopolysaccharide profiles [155]. *Coxiella burnetii* strains with a different genotypic profile can infect a variable range of host species with a different efficiency. For example, the CbNL01 genotype strains are predominantly strains in goats and in humans, whereas the CbNL12 genotype strains are commonly found in cattle and hardly in goats and humans [156].

Coxiella burnetii genotyping

Genotyping of bacteria is a key tool in the understanding of the epidemiology of infectious diseases. With regard to a zoonosis like Q fever, it is of tremendous importance, helping to find the animal source of human outbreaks. Currently, the three main discriminant genotyping methods used are multiple- locus Variable-Number Tandem Repeat (VNTR) analysis (MLVA), Multispacer Sequence Typing (MST), and Single Nucleotide Polymorphism (SNP) genotyping.

VNTR-MLVA genotyping

VNTR-MLVA genotyping was established by Svraka., *et al.* who amplified VNTR sequences from 21 *Coxiella burnetii* isolates [157]. They identified five main clusters and nine MLVA types. Arricau-Bouvery., *et al*. then analyzed 42 isolates and found 36 MLVA types. They proposed using two panels of markers to have a better discriminatory power [158]. However, MLVA is based on the analysis of relatively unstable repetitive DNA elements and can produce results that are too discriminatory. Moreover, it significantly lacks inter-laboratory reproducibility [104].

MST genotyping and "geotyping"

MST genotyping was introduced by Glazunova., *et al*. who identified 10 highly variable spacers located between ORFs [155]. This typing method identified 30 different genotypes and three monophyletic groups among 173 *Coxiella burnetii* isolates. These groups were partially correlated with plasmid types. The first group contained strains with the QpDV or QpRS plasmid, the second contained only strains with the QpH1 plasmid, and the third group contained plasmidless strains or strains with QpH1. This method is very discriminant and has been used most frequently in different studies around the world. MST genotyping helps to trace the spread of *Coxiella burnetii* from one region to another and from animal reservoirs to humans. Some MSTs are present across the five continents, whereas others are very specific to epidemic situations [104].

SNP genotyping

SNP genotyping was developed by a Dutch team during the outbreak in the Netherlands to provide a method directly applicable to animal and human samples without the need for enrichment by a culture step [159]. Ten discriminatory SNPs were selected using five *Coxiella burnetii* whole-genome sequences available in GenBank. (RSA493, RSA331, CbuG_Q212, Cbuk_Q154, and Dugway) [104].

Diagnosis of Q fever disease

In order to diagnose the Q fever, and identify the presence or not of *Coxiella burnetii* in samples specimens, several methods and technics are involved; each one of them differ with its sensitivity, specificity, and the target searched (DNA, immunoglobulins….).

Nonspecific laboratory Q fever diagnosis

Non-specific methods can be discussed when looking for the repercussion and effect of the presence of the bacterium on the heamatopoietic systems, the alteration of other functions, and also the corresponding hormonal fluctuations. knowing that Q fever gives a typical profile on all affected functions and systems.

Acute Q fever

The leukocyte count in patients with acute Q fever is usually normal. However, 25% of patients have an elevated leukocyte count, ranging from 14 × 10^9 to 21 × 10^9/liter. The erythrocyte sedimentation rate may be elevated. Thrombocytopenia is noted in 25% of patients. Liver enzyme levels are elevated in as many as 85% of patients. The increase in transaminase levels is usually moderate, ranging from 2 to 10 times normal values. During an episode of prolonged fever, the association of a normal leukocyte count, thrombocytopenia, and elevated hepatic enzyme levels are evocative of Q fever. However, thrombocytosis (>400 × 10^9 liter) may be encountered during convalescence. Twenty percent of patients have an elevated creatine phosphokinase level. In Q fever meningoencephalitis, a mild lymphocytic pleiocytosis is frequently noted in the spinal fluid [135]. Furthermore, a variety of autoantibodies have been described in acute Q fever, including antimitochondrial antibodies, and anti-smooth muscle antibodies [135].

Chronic Q Fever

In Q fever endocarditis, the cell-mediated inflammatory response to *Coxiella burnetii* has an impact on clinical and biological manifestation. Such as, conventional blood cultures remain negative, usual inflammatory syndrome, anaemia, elevated erythrocyte sedimentation rate, and polyclonal hypergammaglobulinemia. The leukocyte count may be normal, increased, or decreased. Thrombocytopenia and elevated hepatic enzyme levels are commonly found. Renal involvement is common, characterized by an elevated creatinine level and microhematuria. Monoclonal immunoglobulins are rarely observed, whereas cryoglobulins are frequently found. Autoantibodies are also frequent in chronic Q fever, particularly rheumatoid factor, anti-smooth muscle, or antinuclear antibodies. Antimitochondrial antibodies, circulating anticoagulant antibodies, and a positive Coombs' test may also be observed [135].

Specific Q fever laboratory Diagnosis

Additionally, to the clinical manifestations, the presence of the desired pathogen can be identified, either by its DNA or by its antigen-antibody reaction. The specific diagnosis is depending on the predominant symptoms, samples nature, sample quantity, and also to its storage conditions.

Collection and storage of specimens

Coxiella burnetii is a very infectious disease. Thus, only biosafety level **3** laboratories and experienced personnel should be allowed to manipulate contaminated specimens and cultivate this microorganism from clinical samples. Several human specimens are suitable for the detection of *Coxiella burnetii*, but their availability depends on the clinical presentation. DNA amplification may be performed from blood, cerebrospinal fluid, bone marrow, cardiac valve biopsy, vascular aneurysm or graft, bone biopsy, or liver biopsy specimens; milk; placenta; foetal specimens in case of an abortion; and cell culture supernatants. Blood should be collected on EDTA or sodium citrate, and the leukocyte layer should be saved for the amplification. Solid specimens should be kept frozen at 280°C before testing [135].

Serology

In the presence of symptoms suggestive of *Coxiella burnetii* infection, serology is the first-line diagnostic technique. The immune response induces the production of anti-phase II and anti-phase I antibodies [160]. *Coxiella burnetii* phase II antigen is obtained after several passages in cell cultures or eggs, and anti-phase II antibodies are predominant during primary infection. *Coxiella burnetii* phase I antigen is obtained from the spleens of infected mice, and anti-phase I antibodies are associated with persistent infection [135]. The phase II antibodies are detectable 7 to 15 days after the onset of clinical symptoms and decrease thereafter within 3 to 6 months [160]. Antibodies are detectable by the third week after infection in 90% of patients [135]. For that reason, two serum samples (one from the acute phase and one from the convalescent phase) should be analyzed. Cutoffs for a positive serological titer can vary between countries. Generally, titers of phase II IgG of ≥200 and/or IgM of ≥50 are considered significant for the diagnosis of primary Q fever infection [135,161], and phase II IgG titers tend to be higher than phase I IgG titers during primary infection [160]. Independently of the symptomatology, residual IgG antibody titers may be detectable for years and even for life [104]. Elevated phase I IgG titers (IgG I titer of ≥1:800) are associated with persistent Q fever. Higher phase I IgG titers correlate with a higher positive predictive value (PPV) for the diagnosis of *Coxiella burnetii* endocarditis: a PPV of 37% was found for IgG I titers of 1≥:800, and this

reached 75% for IgG I titers of ≥1: 6,400 in a study from the reference centre in Marseille-France [29]. For that reason, investigation for persistent infection should be performed in the case of persistent high levels of phase I antibodies 6 months after completion of treatment.

Serology methods

Indirect immunofluorescence assay (IFA) is the reference method, but the complement fixation test (CFT) and ELISA are also used. Others techniques exist, such as Western blotting, microagglutination, and the indirect hemolysis test, but they remain anecdotal. To date, only IFA, CFT, and ELISA are commercially available. The advantage of ELISA is that it is easy to perform, interpretation is less subjective than for IFA and CFT, and automation is possible. This method is mentioned in the CDC case definition of acute and chronic Q fever [160]. The specificity, sensitivity, and positive predictive value vary according to the technique and the antigen used. Most reference laboratories have developed their own in-house immunofluorescence assay. In the reference centre in IHU, Marseille-France, screening is performed with phase II antigen on serum diluted at 1:50 and 1:100 to detect total immunoglobulins (IgT) directed against *Coxiella burnetii* antigens [162]. For all positive screenings with IgT titers of ≥1:100, quantification detection of antibodies for the subclasses IgG, IgM, and IgA for both phase I and phase II is performed. The titration of IgM and IgA is performed after removal of IgG using a rheumatoid factor absorbent to eliminate false-positive results due to interference with this protein. Moreover, the sera are diluted in phosphate-buffered saline with 3% non-fat powdered milk to saturate the antigenic site and avoid a nonspecific fixation of antibodies. Sensitivity was assessed at 58.4% and specificity at 100% [162]. For sera with titers inferior to these cutoffs, the serology should be repeated within 10 to 15 days to confirm or rule out the diagnosis.

Indirect immunofluorescence assay (IFA)

In human medicine, the IFA adapted as a micro-immunofluorescenec technique is the current method for the serodiagnosis of Q fever. Briefly, both phase I and phase II *Coxiella burnetii* antigens are used; phase II antigen is obtained by growing *Coxiella burnetii* Nine Mile reference strain in cell culture, while phase I antigen is obtained from the spleen of laboratory animals inoculated with phase II *Coxiella burnetii* in cell culture. A few phase I cells may still be present in the phase II population and can be selected and propagated within animals. Antigen is diluted, dropped on the wells of a glass microscope slide, allowed to dry, and fixed with acetone [163]. In addition, antigen-spot slide wells may be purchased from a supplier providing the phase II form, or phase I and II forms of *Coxiella burnetii*. These can be adapted by replacing the human conjugate by a conjugate adapted to the animal species. Twofold dilutions of the serum under test are placed on immunofluorescence slides with wells previously coated with one or two antigens. If specific antibodies are present, they are fixed by the antigen on the slide. The complex is then detected by examination with a fluorescence microscope following the addition of the fluorescent conjugate recognising the species-specific immunoglobulins [163].

IFA materials and reagents

Microscope equipped for the fluorescence, humidified incubator, washing basin. Slides suitable for the antigen are necessary. The latter may be either prepared in the laboratory or purchased from a supplier. The method described is adapted from the BioMérieux kit, and is given as an example. Ready-to-use slides contain 12 wells per slide, each of 7 mm diameter, coated with phase II antigen obtained from culture on Vero cells and can be stored at 4°C or -20°C. Concentrated fluorescent conjugate, to be diluted when required with Phosphate –Buffered Saline (PBS) + 1% Evans blue at the dilution recommended by the manufacturer. PBS, buffered glycerine, Evans dye 1% solution.

IFA test procedure

- Inactivate the sera under test for 30 minutes at 56°C, then dilute serially from 1/40 to 1/64 In PBS.
- Allow the previously antigen-coated slides to warm to room temperature. Do not touch the wells.
- Add 20 µl of each serum dilution to the wells. Add negative and positive control sera. To one well, add 20 µl of PBS to serve as antigen control.
- Incubate in humid chamber for 30 min at 37°C. Wash the slide twice with PBS for 10 minutes each. Rinse with distilled water and air dry.
- Add to the wells, including the controls, 20 µl of the conjugate directed against the appropriate species.

IFA results' interpretation

A positive reaction will consist of small brilliant points against a dark background. Verify that the conjugate by itself and negative control serum give a negative result (absence of small brilliants points). Nonspecific fluorescence usually takes the form of spots of irregular shape. The positive control must give the known titer with ± one dilution [163].

Molecular detection

The ability to detect and quantify *Coxiella burnetii* DNA by real-time PCR has dramatically enhanced diagnostic and study approaches. Several PCR-based assays have been developed for the detection of *Coxiella burnetii* in clinical samples. The first standard

PCR systems targeted sequences of different types of plasmids, the 16S-23S RNA, the superoxide dismutase gene, the *com1* gene or the IS*1111* repetitive elements in human or animal samples [66,104,164]. The detection limits of these different methods ranged from 10 to 10^2 bacteria. Also, nested PCR systems have been proposed, but these methods lack specificity [165,166]. Real-time PCR or quantitative PCR (qPCR) is a less time-consuming technique than PCR and has the advantage of quantifying the amount of bacteria in clinical samples. Thus, this method has become the most frequently used PCR system for diagnosis. The qPCR system targeting IS*1111* (a repetitive element which is present in about 20 copies in the *Coxiella burnetii* Nine Mile genome) is the most sensitive [104,167]. This qPCR can detect the bacterium in the sera of patients within the first 2 weeks of infection, when serology is not yet positive. It also allows detection of *Coxiella burnetii* DNA in the blood of patients with persistent *Coxiella burnetii* infection [168]. In the Netherlands, Schneeberger., *et al*. found *Coxiella burnetii* DNA in 10% of seronegative samples from patients with signs of primary infection, confirming the usefulness of this method in the first 2 weeks of infection [169].

Recently, we improved the sensitivity of the qPCR test targeting the *IS1111* gene by concentrating DNA extracted from clinical samples by lyophilization. The detection limit of *Coxiella burnetii* DNA was 100-fold lower in lyophilized sera (1 bacterium/ml) than in nonlyophilized sera (102 bacteria/ml). This strategy was tested in 73 sera from patients with primary *Coxiella burnetii* infection and 10 sera from endocarditis patients, in whom the IS*1111* qPCR performed under the usual conditions remained negative. In patients presenting with primary Q fever, we observed qPCR sensitivity gains of 44% for the seronegative sera and 30% for early seropositive sera after lyophilization. The sensitivity of qPCR was also higher in sera from patients with endocarditis, of whom 8/10 (80%) were positive after lyophilisation [170].

Culture

The isolation of *Coxiella burnetii* can be achieved from a wide range of clinical samples, including old samples if they have been stored at -80°C before cultivation. The shell vial technique is still the most frequently used method. A sample of 1 ml of the clinical specimen is inoculated on HEL cell monolayers in shell vials. The shell vials are then centrifuged (700 ×*g* at 20°C) for 1 h. Centrifugation allows better attachment and penetration of *Coxiella burnetii* inside cells. Infected cells are then incubated at 37°C in a 5% CO2-enriched atmosphere for 5 to 7 days. Gimenez or immunofluorescence staining is used for detection of the bacterium inside cells [104].

New diagnostic tools

Other new tools have shown their efficiency in the confirmation of Q fever diagnosis. Such as the Immuno-PCR is an interesting method, combining the amplification power of PCR with the specificity and versatility of ELISA, allowing an improvement in sensitivity, its specificity was evaluated at 92%. In addition, the detection of *Coxiella burnetii*-specific gamma interferon (IFN_γ) production has been proposed as a new diagnostic tool. The IFN_γ production assay is performed after *in vitro* stimulation of whole blood with antigens from the Q-vax vaccine or the inactivated Nine Mile strain. The measurement of IFN_γ production is then performed using ELISA [104].

A rapid bio-optical sensor, that transduces the presence of the target DNA based on binding-induced changes in the refractive index on the waveguide surface in a label-free and real-time manner, with isothermal DNA amplification, this new diagnostic tool offers a rapid (<20 min) one-step DNA Amplification/Detection method. The team researchers confirm the clinical sensitivity (>90%) of the bio-optical sensor, they tested it for detecting *Coxiella burnetii* in 11 formalid-fixed paraffin-embedded liver biopsy samples acute Q Fever hepatitis patients, and in 16 blood plasma samples from patients in which Q Fever is the cause of unknown origin [213].

Infections sources and excrétion routes

It is believed that *Coxiella burnetii* is ubiquitous in the environment as was shown recently in a 3 years' study across the USA [171], where geographically diverse areas, both agricultural and urban, were sampled. The organism has reservoirs in wide range of wild and domestic animals, including mammals, birds and arthropods [172], although the true extent of the reservoirs is unknown. Traditionally, Q fever has been related to livestock, of greatest relevance to human disease are domestic ruminants, which are the most common source of human infections [109], therefore livestock-related occupations have been deemed to be a risk factor [173]. In recent years in Europe, 90% of cases of Q fever listed have had as a source of contamination sheep and goat products [174]. *Coxiella burnetii* infection of livestock is termed coxiellosis, a chronic but often symptomless disease. The uterus and mammary glands are sites of chronic infection in females, and this is associated with abortions in goats and sheep, and infertility in cattle [109]. Pets including cats, rabbits, and dogs, have also been demonstrated to be potential sources of urban outbreaks. A study conducted in Canada, demonstrated that 6 to 20% of cats have anti-*Coxiella burnetii* antibodies. Consequently, cats are suspected as an important reservoir of *Coxiella burnetii* in urban areas. Wild rats have been suspected as an important reservoir in Great Britain [135].

Knowledge of the excretion of *Coxiella burnetii* from infected animals is crucial in understanding the transmission routes and risks of human infection. Mammals when infected, shed the desiccation-resistant organisms in urine, faeces, milk, and especially, birth products [135]. Reactivation of infection occurs in female mammals during pregnancy. At birth, in the placenta of infected animals, vast quantities of pathogen are found, with high concentrations of *Coxiella burnetii* are registered (up to 10^9 bacteria per g of tissue) [135]. However, recent experimental indicate that comparable numbers of *Coxiella burnetii* are also excreted during the birth of lively kids [128]. In goat herds, both in aborting and non-aborting goats, *Coxiella burnetii* DNA has been detected in faeces, vaginal mucus and/or milk [175]. In cattle, also variable excretion via faeces, vaginal mucus and milk has been reported, sometimes independent of an abortion history. Sixty-five per cent of cows seem to shed *Coxiella burnetii* by only one route, evenly distributed over the three routes. Cows that excrete *Coxiella burnetii* by all three routes seem scare [176].

Shedding *Coxiella burnetii* by the later routes coincides with its replication in epithelial (trophoblast) cells of the placenta, and those of the entry site (lung epithelium), also in the epithelial cells of gut and udder. Sobotta., *et al.* in 2017, showed that these cell lines in bovine hosts, exhibited different permissiveness for *Coxiella burnetii*, thus, the udder cells allowed the highest replication rates, the intestinal cells showed an enhanced susceptibility to invasion, and lung and placenta cells also internalized the bacteria [211].

Mediannikov in 2010, found that in rural Senegal, endemic areas, humans similar to other mammals may become chronic excretors of *Coxiella burnetii* via faeces and milk, the found rates of shedding are, however not high and may not be compared with those identified in domestic animal [66]. All these high pathogen concentrations, are released to the environment [109]. Therefore, Q fever is an occupational hazard. At greatest risk are persons in contact with farm animals, but also at risk are laboratory personnel who work with infected animals [135]. Farming can facilitate environmental spread, such transport of infected animals and the spreading of contaminated manure onto fields. As well as, with the environment stability of the organism, pose a difficulty in containing outbreaks, as has been noted in the recent outbreak in the Netherlands [177]. Tozer., *et al*. study's, in 2012, confirmed that *Coxiella burnetii* can be readily detected in the environment, where they elucidated PCR evidence in wildlife urine and faecal samples as well as soil and dust samples [178]. Q fever was previously associated with dry, dusty and windy conditions as illustrated in the French study by Tissot-Dupont., *et al*. [179]. Airborne propagation can take place over long distances. In Briançon, France, a study reported the role of helicopters in the spread of aerosols near a slaughterhouse [180]. Various epidemiological surveys estimate the distance of diffusion during human epidemics: 400 m in Germany, 5 km in the Netherlands, 18 km in the United Kingdom, and 40 km in France [179,181-183]. In France, these are the "Mistral" and the geography of the places which have been conducive to long-distance broadcasting. Bacteria can also be transported through channels other than air, such as manure spreading, for example [184]. Other factors, such as vegetation and soil moisture, also appear to play an important role in the bacterial dispersal [186]. In the Netherlands, a study has shown that the risk of infection was 30 times greater within a 2 km radius of the infection's source, with respect to a radius of 5 km, during an outbreak, in a geographical area without natural obstacle and in low rainfall conditions and strong winds [182]. However, a recent study in North Queensland by Harris., *et al.* 2013, demonstrated that there was clear correlation with rainfall, with the highest number of cases occurring 3 months after peak rainfall. It was postulated that increased rainfall attracted increased numbers of wildlife due to the increase in vegetation associated with wet season and the subsequent drier period potentially resulted in the aerosolization of the pathogen [187].

A rang of arthropods, including ticks, have been shown to be able to be colonized via ingestion of contaminated blood feeds. These ticks release significant quantities of *Coxiella burnetii* in their faeces. While experimental transmission between guinea pigs has been avhieved via tick bite [109], arthropod vectors are not considered essential to the natural cycle of infection in livestock that live closely contact with other infected animals. However, ticks may play an important role in transmission in the wild, for example between birds [109,172]. In addition, *Coxiella burnetii* has been isolated from ticks, particularly in the Kangaroo-habituating Ambylomma triguttatum [118]; these results support the suggestion that wildlife may be potential reservoirs for humans. Furthermore, Banazis., *et al.* in 2009, have shown that Australian wildlife carry *Coxiella burnetii*. The study highlights that 33.5% of western grey Kangaroo serum samples tested positive for *Coxiella burnetii* antibody-ELISA and 12.25% of the tested western grey kangaroos had positive *Coxiella burnetii* DNA detected in faecal samples [189].

The route of infection determines, in part, the minimum inoculum size, the severity of the disease, and the clinical manifestations [190]. In human beings, infection results from inhalation of contaminated aerosols (amniotic fluid, placenta, contaminated wool/dusts). Compared with aerosols, subcutaneous and intramuscular inoculation require a lower inoculum to cause disease. Aerosol exposure to *Coxiella burnetii* causes a variable proportion on infection in those exposed [190]. In experimental models in mice [191] and guinea pigs [192], it is clear that there is a link between the route of inoculation and the prominent histological lesions. The respiratory route is associated with pneumonia and the intraperitoneal route

with hepatitis [193]. These findings cannot be extrapolated to natural infection in human beings but show that the route of infection may be one of the factors influencing clinical presentation [120]. Otherwise, the ingestion of raw milk infected with *Coxiella burnetii*, although it causes seroconversion, has never been clearly associated with a clinical disease in humans [120,199]. The analysis of dairy products in France has demonstrated the presence of *Coxiella burnetii* DNA but no viable bacteria [167]. There is no formal evidence of a food transmission. Sexual transmission of Q fever has been suspected in humans, and demonstrated in the mouse [135]. Sporadic cases of human-to-human transmission following contact with an infected parturient woman have been reported and have been suspected to occur by direct aerosol transmission, resulting in congenital infections, via intradermal inoculation, and via blood transfusion [135]. An infectious dose of 1 to 10 bacteria has been estimated using animal models after observation of the serological response and/or fever, and/or lesions in organs; these values have been observed in inoculated infections intraperitoneal, which does not meet the conditions natural [192].

In the figure 3, we summarized the different infection sources, excretion's routes, and transmission mode between species.

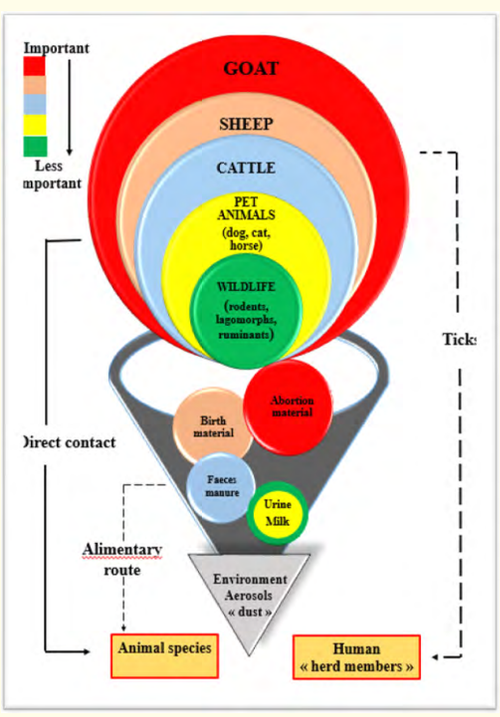

Figure 3: Transmission model for Q fever. An overview of the possible transmission routes of Coxiella burnetii from the animal reservoir to the human (and animal) hosts. (Adapted from Roest *et al,* 2013).

Bioterrorism threat

When considering microbes as weapons they can simplistically be divided into lethal agents an incapacitating agents. Lethal agents, such *Yersinia pestis* induce an acute disease with ahigh associated mortality rate. Incapacitating agents make people ill enough that they cannot carry on with normal life for a period of time, but ultimately most people will recover [109]. Because one single organism of *Coxiella burnetii* can cause disease in a susceptible person, this pathogen has been classified on category B as incapacitating bioterrorism agent [123,214].

Depending on the infective dose, *Coxiella burnetii* incubation period can be up to 3 weeks [4], with 4 days and 6 weeks representing the extremes [55]. Despite its low case-fatality rate, its ease of manufacture, its stability in the environment, and its ability to cause disease, *Coxiella burnetii* remains a high bioterrorism threat. Q fever is also part of military history, with some units having rates of over 30% during the Second World War [190].

Q fever treatment

Primary infection

While Q fever symptomatic primary infection, it is recommended to initiate antibiotic treatment using doxycycline (200 mg per day). In case of doxycycline intolerance, minocycline, clarithromycin (500 mg twice daily), fluoroquinolones (ofloxacin 200 mg three times a day or pefloxacin 400 mg twice a day), and co-trimoxazole (160 mg trimethoprim and 800 mg sulfamethoxazole twice daily) are alternatives [194,195].

Chronic infection (endocarditis)

Combination between the antibiotic treatment for *Coxiella burnetii* endocarditis doxycycline (200 mg/day) with hydroxychloroquine (200 mg 3 times/day) is useful. Hydroxychloroquine is necessary to raise the pH in the pseudolysosomal vacuole to restore doxycycline activity [196,197].

Measures' control and vaccination

Pasteurization and sterilization of milk remain the first step to take aiming to reduce the direct paths of contamination. when a livestock is suspected, limit the movement of personnel from one area to another, in order to limit the spread of the pathogen with tools and clothing contaminated by the bacteria, utensils and vehicles for animal transport must be disinfected and thoroughly washed down to avoid contamination of environment. Nevertheless, for the movement of animals from one sector to another, and especially to isolate sick subjects from clinically healthy ones, to facilitate epidemiological surveillance of the epidemic. In addition, the direction of dynamic winds must be discerned inside and outside livestock

buildings to avoid the dispersion of contaminated aerosols. A control radius of about 10 km around outbreaks; should be applied to estimate dispersion via wind and surrounding climatic conditions. A regular test must be carried out on sheep and goats in research institutions, and culling of seropositive animals should be considered. All equipment that was in contact with contaminated livestock, or their abortion products or excreta, must be destroyed, especially to avoid the potential risk of high resistance of *Coxiella burnetii* in the external environment and contaminated material. In THE uk, Health Protection Agency guidelines [215], suggest the use of 2% formaldehyde, 1% Lysol, 5% hydrogenperoxide, 70% ethanol or 5% chloroform for decontamination of surfaces, and spills of contaminated material should be dealt with immediately using hypochlorite (5000 p.p.m, available chlorine). However, they state that is impossible to decontaminate large areas of a potentially contaminated environment [109].

The ideal vaccine against *Coxiella burnetii* would be based on antigens with good immunogenic potential, but which do not cause side effects such as reactivation of the infection or local reactions. In humans and animals, 2 types of vaccines are used: Whole Cell (WC) cell-based vaccines of *Coxiella burnetii* either in phase I or phase II, and vaccines based on bacterial walls extracted with chloroform methanol (CMR, chloroform methanol residue) [198]. In human, several vaccine types were manufactured, as live attenuated vaccine, corpuscular vaccine, vaccine chemically treated with chloroform methanol, soluble vaccine. Otherwise, in animals, only one effective vaccine exists, it's composed of corpuscular antigens; as the Coxevac® vaccine has proven its effectiveness in the prevention of abortions as well as reduction excretion of the germ in goats and cattle [198].

A soluble antigen complex produced by extraction with trichloroacetic acid from the highly purified *Coxiella burnetii* phase I strain Nine Mile. Consequently, they could identify 39 bacterial proteins from which 12 were recognized as immunoreactive, thus a new vaccination approach may take place in the future [212].

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Conflict of Interest

None declared.

Bibliography

1. Kate D., *et al*. "The Epidemiology of Q Fever in England and Wales 2000-2015". *Veterinary Science* 4 (2017): 28.

2. Sabatier F., *et al*. "CD4+ T-cell lymphopenia in Q fever endocarditis". *Clinical and Diagnostic Laboratory Immunology* 4 (1997): 89-92.

3. Linda M., *et al*. "Chronic Q Fever in the Netherlands 5 Years after the Start of the Q Fever Epidemic: Results from the Dutch Chronic Q Fever Database". *Journal of Clinical Microbiology* 52-5 (2014): 637-1643.

4. EH Derrick. "Q Fever, a New Fever Entity: Clinical Features, Diagnosis and Laboratory Investigation". *Reviews of Infectious Diseases* 5-4 (1983): 790-800.

5. FM Burnet and Mavis Freeman. "Experimental Studies of the Virus "Q" Fever". *Reviews of Infectious Diseases* 5-4 (1983): 800-808.

6. Gordon E., *et al*. "A Filter-Passing Infectious Agent Isolated from Ticks". *PuBLIC Health Reports* 53-52 (1938): 2259-2276.

7. RE Dyer. "Human Infection". *PuBLIC Health Reports* 53-52 (1938): 2277-2282.

8. Joseph E and McDade. Historical aspects of Q fever. Q fever: the disease (1990): 5-21.

9. Loïc Epelboin., *et al*. "Q Fever in French Guiana: Tip of the Iceberg or Epidemiological Exception?". *Plos Neglected Tropical Diseases* 2016.

10. Parker NR., *et al*. "Q fever". *Lancet* 367 (2006): 679-688.

11. Arricau-Bouvery N and Rodolakis A. "Is Q fever an emerging or re-emerging zoonosis?". *Veterinary Research* 36 (2005): 327-349.

12. Armengaud A., *et al*. "Urban outbreak of Q fever, Briancon, France, to 1996". *Eurosurveillance* 2 (1997): 12-13.

13. Brouqui P., *et al*. "Q fever outbreak in homeless shelter". *Emerging Infectious Diseases* 2004 10: 1297-1299.

14. van Woerden HC., *et al*. "Q fever outbreak in industrial setting". *Emerging Infectious Diseases* 10 (2004): 1282-1289.

15. Steiner HA., *et al*. "Outbreak of Q fever among kitchen employees in an urban hospital". *European Journal of Clinical Microbiology and Infectious Diseases* 20 (2001): 898-900.

16. KA Bond., *et al*. "Review of 20 years of human acute Q fever notifications in Victoria 1994-2013". *Australian Veterinary Journal* 96-6 (2018): 223-230.

17. KA Bond., *et al*. "One Health approach to controlling a Q fever outbreak on an Australian goat farm". *Epidemiology and Infection* 144 (2016): 1129-1141.

18. Brett N Archer., *et al*. "Atypical outbreak of Q fever affecting low-risk residents of a remote rural town in New South Wales". CDI 42-2 (2017): E125-E132.

19. Jv Irons., *et al*. "Q Fever in The United States". *JAMA* 133-12 (1947): 819-820.

20. JH MCQuiston and JE. Childs. "Q Fever in Humans and Animals in the United States". *Vector Borne and Zoonotic Diseases* 2.3 (2002): 179-191.

21. Petros C., *et al*. "Chronic Q Fever in the United States". *Journal of Clinical Microbiology* (2006): 2283-2287.

22. Adam Bjork., *et al*. "First Reported Multistate Human Q Fever Outbreak in the United States 2011". *Vector-Borne and Zoonotic Diseases* 14 (2014).

23. Rare Q fever outbreak hits U.S medical tourists, 01/10/2015.

24. Honarmand H. "Q Fever: An old but still a poorly understood disease". *Interdisciplinary Perspectives on Infectious Diseases* (2012).

25. SN Shpynov., *et al*. "Comparison of genomes of Coxeilla burnetii strains using formal order analysis". *New Microbes and New Infections* 23 (2018): 86-92.

26. Roest HIJ., *et al*. "The Q fever epidemic in The Netherlands: history, onset, response and reflexion". *Epidemiology and Infection* 139 (2011): 1-12.

27. P Speelman. "The largest Q fever outbreak ever reported". *The Netherlans Journal of medicine* 68 (2010): 380-381.

28. Van Steenbergen JE., *et al*. "Een uitbraak van Q-koorts in Nederland - mogelijk verband met geiten". *Nederlands Tijdschrift voor Geneeskunde* 8 (2007): 151.

29. Diane Frankel., *et al*. "Q fever in France 1985-2009". *Ermerging Infectious Diseases* 17.3 (2011): 350-356.

30. Herve Tissot-Dupont., *et al*. "Hyperendemic Focus of Q Fever Related to Sheep and Wind". *American Journal of Epidemiology* 150-1(1999): 69-74.

31. Dellacasagrande J., *et al*. "Coxiella burnetii survives in monocytes from patients with Q fever endocarditis: involvement of tumour necrosis factor". *Infection and Immunity* 68 (2000): 160-164.

32. G Dupuis., *et al*. "An Important Outbreak of Human Q Fever in a Swiss Alpine Valley". *International Journal of Epidemiology* 16.2 (1987): 282-287.

33. M Maurin and D Raoult. "Q Fever". *Clinical Microbiology Reviews* 12.4 (1999): 518-553.

34. Smith DL., *et al*. "A large Q fever outbreak in the West Midlands: clinical aspects". *Respiratory Medicine* 87 (1993): 509-516.

35. Hawker., *et al*. "A large outbreak of Q fever in the West Midlands: windborne spread into a metropolitan area?". *Communicable disease and public health* 1 (1998): 180-187.

36. Hugo C., *et al*. "Q Fever Outbreak in Industrial Setting". *Emerging Infectious Diseases* 10.7 (2004): 1282-1289.

37. LE Wilson., *et al*. "Investigation of a Q Fever Outbreak in a Scottish Co-LocatedSlaughterhouse and Cutting Plant". *Zoonoses Public Health* 57 (2010): 493-498.

38. A Wallensten., *et al*. "Q fever outbreak in Cheltenham, United Kingdom, in 2007 and the use of dispersion modelling to investigate the possibility of airborne spread". *Surveillance and outbreak reports* (2010).

39. C. Bellini., *et al*. "Q fever outbreak in the terraced vineyards of Lavaux, Switzerland". *New Microbes and New Infections* 2.4 (2014): 93-99.

40. Hurtado., *et al*. "Enviromental sampling coupled with real-time PCR and genotyping to investigate the source of Q fever outbreak in a work setting". *Epidemiology and Infection* (2017).

41. Sabahattin Payzin, "Epidemiological Investigations on Q Fever in Turkey". *Bulletin of the World Health Organization* 9 (1953): 553-559.

42. Gozalan B., *et al*. "Is Q fever an emerging infection inTurkey?". La Revue de Santé de la Méditerranée orientale (2005): 384-391.

43. Hellenbrand W., *et al*. "Changing epidemiology of Q fever in Germany 1947-1999". *Emerging Infectious Diseases journal* 7.5 (2001): 789-796.

44. Berktas M., *et al*. "Seroprevalence of Coxiella burnetii antibodies in high risk groups in eastern Turkey". *Turkiye Klinikleri Journal of Medical Sciences* 3.1 (2011): 45-50.

45. Celebi B., *et al*. "Investigation of Q fever, Listeriosis, Toxoplasmosis and Cytic Echinococcosis seroprevalence in risk group". *Turk Hij Den Biyol Derg* 65.2 (2008): 67-73.

46. Aytekin Cikman., *et al*. "The seroprevalence of Coxiella burnetii in Erzincan, Turkey: Identification of the risk factors and their relationship with geographical featues". *Journal of Vector Borne Diseases* 54 (2017): 157-163.

47. Monika Szymanska-Czerwinska., *et al*. "Prevalence of Coxiella burnetii Infection in Humans Occupationally Exposed to Animals in Poland". *Vector-Borne and Zoonotic Diseases* 15.4 (2015): 261-267.

48. HS EL-Mahallawy., *et al*. "Q fever in China: a systematic review 1989-2013". *Epidemiology and Infection* 143 (2015): 673-681.

49. The Europrean Surveillance Sysytem "TESSy" (2016).

50. Blanc G., *et al*. A. Bull. Acad. Med (1947): 131-138.

51. Halawani E., El Dine KZ and El Fiki AYJ. *Egyptian Medical Association* 35 (1952): 339.

52. Martin M., *et al*. "The Geographeical Distribution of Q Fever". Bull. Wld Hlth Org 13(1955): 829-860.

53. Giroud P. *et al*. Bull. Acad. Mid 137 (1953): 29.

54. A Lacheheb and D Raoult. "Seroprevalence of Q-fever in Algeria". Clinical Microbiology and Infection (2009): 15-22.

55. Raoult D and Marrie T. "Q fever". *Clinical Infectious Diseases* 20 (1995): 489-495.

56. Meskini M., *et al*. "Seroepidemiology of Rickettsial infections in Morocco". *European Journal of Epidemiology* 11 (1995): 655-660.

57. Schelling E., *et al*. "Brucellosis and Q-fever seroprevalences of nomadic pastoralists and their livestock in Chad". *Preventive Veterinary Medicine* 61 (2003): 279 -293.

58. Potasman I., *et al*. "Outbreak of Q fever following a safari trip". *Clinical Infectious Disease* 30 (2000): 214 -215.

59. F Bellazreg N., *et al*. "Acute Q fever in hospitalised patients in Central Tunisia: report of 21 cases". *European Society of Clinical Microbiology and Infectious Diseases* 15 (2009): 138-139.

60. Kobbe R., *et al*. "Q fever in children, Ghana". *Emerging Infectious Diseases* 14 (2008): 344-346.

61. Sky Vanderburg., *et al*. "Epidemiology of Coxiella burnetii Infection in Africa: A OneHealth Systematic Review". *PLOS Neglected Tropical Diseases* 8.4 (2014): e2787.

62. Amin WF and Ahmed SO. "Detection of Coxiella burnetii in bovine milk samples using polymerase chain reaction". *Assiut Veterinary Medical Journal* 55 (2009): 23-31.

63. Kamga-Waladjo AR., *et al*. "Seroprevalence of Neospora caninum antibodies and its consequences for reproductive parameters in dairy cows from Dakar-Senegal, West Africa". *Tropical Animal Health and Production* 42 (2010): 953-959.

64. Scolamacchia F., *et al*. "Serological patterns of brucellosis, leptospirosis and Q fever in Bos indicus cattle in Cameroon". *PLoS One* 5 (2010): e8623.

65. Angelakis E., *et al*. "Coxiella burnetii-positive PCR in febrile patients in rural and urban Africa". *International Journal of Infectious Diseases* 28 (2014): 107-110.

66. Mediannikov O., *et al*. "Coxiella burnetii in humans and ticks in rural Senegal". *PLOS Neglected Tropical Diseases* 4 (2010): e654.

67. Prabhu M., *et al*. "Q fever, spotted fever group, and typhus group rickettsioses among hospitalized febrile patients in northern Tanzania". *Clinical Infectious Disease* 53 (2011): e8-e15.

68. Kamga-Waladjo AR., *et al*. "Seroprevalence of Neospora caninum antibodies and its consequences for reproductive parameters in dairy cows from Dakar-Senegal, West Africa". *Tropical Animal Health and Production* 42 (2010): 953-959.

69. Nahed HG and Khaled AAM. "Seroprevalence of Coxiella burnetii antibodiesamong farm animals and human contacts in Egypt". *Journal of American Science* 8 (2012): 619-621.

70. Hussien MO., *et al*. "Seroprevalence of Q fever in goats in the Sudan". *Veterinary World* 5 (2012): 394-397.

71. Ghoneim N and Abdel-Moein K. "Seroprevalence of Coxiella burnetii antibodies among farm animals and human contacts in Egypt". *Journal of American Science* 8 (2012): 619-621.

72. Crump JA., *et al*. "Etiology of severe non-malaria febrile illness in Northern Tanzania: a prospective cohort study". *PLOS Neglected Tropical Diseases* 7 (2013): e2324.

73. Knobel DL., *et al*. "Coxiella burnetii in humans, domestic ruminants, and ticks in rural western Kenya". *The American Journal of Tropical Medicine and Hygiene* 88.3 (2013): 513-58.

74. Ratmanov P., *et al*. "The correlation of Q fever and Coxiella burnetii DNA in household environments in rural Senegal". *Vector-Borne and Zoonotic Diseases* 13 (2013): 70-72.

75. Amina Bessas., *et al*. "Molecular evidence of vector-borne pathogens in dog and cat and their ectoparasites in Algiers, Algeria". *Comparative Immunology, Microbiology and Infectious Diseases* 45 (2016): 23-28.

76. H Khaled., *et al*. "Serological and Molecular evidence of Q fever among small ruminant flocks in Algeria". *Comparative Immunology, Microbiology and Infectious Diseases* 47 (2016): 19-25.

77. Atef Aouadi., *et al*. "Molecular evidence of tick-borne hemoprotozoan-parasites (Theileria ovis and Babesiaovis) and bacteria in ticks and blood from small ruminants in Northern Algeria". *Comparative Immunology, Microbiology and Infectious Diseases* 50 (2017): 34-39.

78. Mohammed H., *et al*. "Seroprevalence and risk factors for Coxiella burnetii, the causative agent of Q fever in the dromedary camel (Camelus dromedarius) population in Algeria". *Onderstepoort Journal of Veterinary Research* 84.1 (2017).

79. Sulyok KM., *et al*. "Identification of novel Coxiella burnetii genotypes from Ethiopian ticks". *PLoS One* 9 (2014): e113213.

80. Bottieau E., *et al*. "Q fever after a journey in Syria: A diagnosis suggested by bone marrow biopsy". *Acta Clinica Belgica* 55 (2000): 30-33.

81. Esmaeili S., *et al*. "seroprevalence of brucellosis, leptospirosis, and Q fever among butchers and slaughterhouse workers in South-Eastern Iran". *PLoS One* 11 (2016): e0144953.

82. Faix DJ., *et al*. "Outbreak of Q fever among US military in western Iraq". *Clinical Infectious Disease* 46 (2008): e65-e68.

83. Esmaeili., *et al*. "Seropepidemiological survey of Q fever and brucellosis in Kurdistan province western Iran". *Vector Borne Diseases* 14 (2014): 41-45.

84. Mohhamed Hassan Kayedi., *et al*. "Seroepidemiological study of Q fever in Lorestan province, western Iran 2014". *Iranian Journal of Microbiology* 9.4 (2017): 213-218.

85. Ashraf Mohabbati Mobarez., *et al*. "Seroprevalence of Q fever among human and animal in Iran a Systematic review and meta-analysis". *Plos Neglected Tropical Diseases* 11.4 (2017): e0005521.

86. Garabedian GA., *et al*. "Q fever in Lebanon (Middle East)". *American journal of hygiene* 63 (1956): 313-318.

87. Mayssaa Fawaz Dabaja., *et al*. "The First Serological Study of Q Fever in Humans in Lebanon". *Vector-Borne and Zoonotic Diseases* (2018).

88. Ziva Amitai., *et al*. "A Large Q Fever Outbreak in an Urban School in Central Israel". *Clinical Infectious Diseases* 50.11 (2010): 1433-1438.

89. VM Vaidya., *et al*. "Comparison of PCR, Immunofluorescence Assay and Pathogen Isolation for Diagnosis of Q Fever in Humans with Spontaneous Abortions". *Journal of Clinical Microbiology* (2008): 2038-2044.

90. Loïc Epelboin., *et al*. "Q Fever in French Guiana: Tip of the Iceberg or Epidemiological Exception?". *PLOS Neglected Tropical Diseases* (2016).

91. van der Hoek W., *et al*. "Antibodies against Coxiella burnetii and pregnancy outcome during the 2007-2008 Q fever outbreaks in The Netherlands". *BMC Infectious Diseases* 11 (2011): 44.

92. Edouard S., *et al*. "Comparison between emerging Q fever in French Guiana and endemic Q fever in Marseille, France". *The American Journal of Tropical Medicine and Hygiene* 90.5 (2014): 915-919.

93. Gardon J., *et al*. "Suburban transmission of Q fever in French Guiana: evidence of a wild reservoir". *The Journal of Infectious Diseases* 184.3 (2001): 278-284.

94. Seshadri R., *et al*. "Complete genome sequence of the Q-fever pathogen Coxiella burnetiid". *Proceedings of the National Academy of Sciences USA* 100 (2003): 5455-5460.

95. Gimenez DF. "Staining rickettsiae in yolk-sac cultures". *Stain Technology* 39 (1964): 135-140

96. Angelakis E and Raoult D. "Q fever". *Veterinary Microbiology* 140 (2010): 297-309.

97. Baca OG., *et al*. "Q fever and Coxiella burnetii: a model for host-parasite interactions". *Microbiology Reviews* 47 (1983): 127- 149.

98. Ben Amara., *et al*. "*Coxiella burnetii*, the agent of Q fever, replicates within trophoblasts and induces a unique transcriptional response". *PLoS One* 5 (2010): e15315.

99. La Scola B., *et al*. "Survival of Coxiella burnetii within free-living amoeba Acanthamoeba castellanii". *Clinical Microbiology and Infection* 7 (2001): 75-79.

100. Bechah Y., *et al*. "Persistence of Coxiella burnetii, the agent of Q fever, in murine adipose tissue". *PLoS One* 9 (2014): e97503.

101. Voth DE and Heinzen RA. "Lounging in a lysosome: the intracellular lifestyle of Coxiella burnetiid". *Cellular Microbiology* 9 (2007): 829-840.

102. Sandoz KM., *et al*. "Transcriptional profiling of Coxiella burnetii reveals extensive cell wall remodeling in the small cell variant developmental form". *PLoS One*? 11 (2016): e0149957.

103. Sandoz KM., *et al*. "Developmental transitions of Coxiella burnetii grown in axenic media". *Journal of Microbiological Methods* 96 (2014): 104 -110.

104. Carole Eldin., *et al*. "From Q Fever to Coxiella burnetii Infection: A Paradigm Change". *Clinical Microbiology Reviews* 30.1 (2017): 115-190.

105. Hendrik IJ Roest., *et al*. "Clinical microbiology of Coxiella burnetii and relevant aspects for the diagnosis abd control of the zoonotic disease Q fever". *Veterinary Quarterly* 33.3 (2013): 148-160.

106. Zerai Woldehiwet. "Q fever (coxiellosis): epidemiology and pathogenesis". *Research in Veterinary Science* (2004): 93-100.

107. Vishwanath S., *et al*. "Lipopolysaccharide phase variation determines the complement-mediated serum susceptibility of Coxiella burnetii". *Infection and Immunity* 56 (1988): 40-44.

108. Andoh M., *et al*. "T cells are essential for bacterial clearance, and gamma interferon, tumor necrosis factor alpha, and B cells are crucial for disease development in Coxiella burnetii infection in mice". *Immunology and Infections* 75 (2007): 3245-3255.

109. PCF Oyston and C Davies. "Q fever: the neglected biothreat agent". *Journal of Medical Microbiology* 60 (2011): 9-21.

110. Coleman SA., *et al*. "Proteome and antigen profiling of Coxiella burnetii development forms". *Immunology and Infections* 75 (2007): 290-298.

111. Narasaki CT., *et al*. "Lipopolysaccharide of Coxiella burnetiid". *Advances in Experimental Medicine and Biology* 984 (2012): 65-90.

112. Hotta A., *et al*. "Phase variation analysis of Coxiella burnetii during serial passage in cell culture by use of monoclonal antibodies". *Immunology and Infections* 70 (2002): 4747-4749.

113. Marrie TJ., *et al*. "Q fever areview and issues for the next century". *International Journal of Antimicrobial Agents* 8 (1997): 145-161.

114. Million, M., *et al*. "Q fever: current diagnosis and treatment options". *Médecine et Maladies Infectieuses* 39 (2009): 82-94.

115. Kampschreur LM., *et al*. "Chronic Q fever diagnosis -consensus guideline versus expert opinion". *Emerging Infectious Diseases* 21 (2015): 1183-1188.

116. Charles W., *et al*. "A human time dose response model for Q fever". *Epidemics* (2017).

117. Russell-Lodrigue, K., *et al*. "Clinical and pathological changes in a guinea pig aerosol challenge model of acute Q fever". *Infection and Immunity* 7411 (2006): 6085-6091.

118. Baca OG., *et al*. "Entry of Coxiella burnetii into host cells". *Acta Virologica* 37 (1993): 143-155.

119. Van Schaik EJ., *et al*. "Molecular pathogenesis of the obligate intracellular bacterium Coxiella burnetiid". *Nature Reviews Microbiology* 11 (2013): 561-573.

120. D Raoult., *et al*. "Natural history and pathophysiology of Q fever". *Lancet Infectious Diseases* 52 (2005): 19-226.

121. Capo C., *et al*. "Subversion of monocyte functions by Coxiella burnetii: impairment of the cross-talk between vαβ3 integrin and CR3". *The Journal of Immunology* 163 (1999): 6078-85.

122. Honstettre A., *et al*. "Lipopolysaccharide from Coxiella burnetii is involved in bacterial phagocytosis, filamentous actin reorganization, and inflammatory responses through Toll-like receptor 4". *The Journal of Immunology* 172 (2004): 3695-703.

123. McQuiston JH., *et al*. "Q fever". *Journal of the American Veterinary Medical Association* 221 (2002): 796-799.

124. Aboudharam G., *et al*. "Culture of *burnetiifrom* the dental pulp of experimentally infected guinea pigs". *Microbial Pathogenesis* 36 (2004): 349-350.

125. Shannon JG and Heinzen RA. "Adaptive immunity to the obligate intracellular pathogen Coxiella burnetiid". *Immunologic Research* 43 (2009): 138-148.

126. Russell-Lodrigue KE., *et al*. "Coxiella burnetii isolates cause genogroup specific virulence in mouse and guinea pigs moldels of acute Q fever". *Immunology and Infections* 77 (2009): 5640-5650.

127. Baumgartner W and Bachmann S. "Histological and immunecytochemical characterization of Coxiella burnetii associated lesions in the murine uterus and placenta". *Immunology and Infections* 3.60 (1992): 5232-5241.

128. Roest HIJ., *et al*. "Q fever in pregnant goats: pathogenesis and excretion of Coxiella burnetii". *PLoS One* 7 (2012): 14.

129. Sanchez J., *et al*. "Experimental Coxiella burnetii infcetion in pregnant goats: a histhopathological and immunohistochemical study". *Journal of Comparative Pathology* 135 (2006): 108-115.

130. JL Pellerin., *et al*. "Attachment of Coxiella burnetii to the zona pellucida of in vitro produced goat Embryos". *Theriogenology, an iternational journal of animal production* 2017.

131. van Loenhout JAF., *et al*." Severely impaired health status of non-notified Q fever patients leads to an underestimation of the true burden of disease". *Epidemiology and Infection* 143 (2015): 2580 -2587.

132. Maltezou HC and Raoult D. "Q fever in children". *Lancet Infectious Diseases* 2 (2002): 686-691.

133. Kampschreur LM., *et al*. "Acute Q fever related in hospital mortality in the Netherlands". *Netherlands Journal of Medicine* 68 (2010): 408-413.

134. Marrie TJ. "Q fever pneumonia". *Infectious Disease Clinics of North America* 24 (2010): 27- 41.

135. Pierre-Edourad Fournier., *et al*. "Diagnosis pf Q Fever". *Journal of Clinical Microbiology* 36.7 (1998): 1823-1834.

136. Chang K., *et al*. "Acute hepatitis with or without jaundice: a predominant presentation of acute Q fever in southern Taiwan". *Journal of Microbiology, Immunology and Infection* 37 (2004): 103-108.

137. Skiba V and Barner KC. "Central nervous system manifestations of Q fever responsive to steroids". *Military Medicine* 174 (2009): 857- 859.

138. Rustscheff S. "Q fever as a cause of pure sensory polyneuropathy— the six-year itch: a follow-up of an indigenous Swedish case". *Scand The Journal of Infectious Diseases* 37 (2005): 949-950.

139. Melenotte C., *et al*. "B-cell non-Hodgkin lymphoma linked to Coxiella burnetiid". *Blood* 127 (2016): 113-121.

140. Figtree M., *et al*. "Q fever cholecystitis in an unvaccinated butcher diagnosed by gallbladder polymerase chain reaction". *Vector-Borne and Zoonotic Diseases* 10 (2010): 421-423.

141. Wegdam-Blans MC., *et al*. "Chronic Q fever: review of the literature and a proposal of new diagnostic criteria". *Journal of Infection* 64 (2012): 247-259.

142. Watt G., *et al*. "Infective endocarditis in northeastern Thailand". *Emerging Infectious Diseases* 20 (2014): 473- 476.

143. Correia MG., *et al*. "Diagnosis of blood culture-negative endocarditis and clinical comparison between blood culture-negative and blood culturepositive cases". *Infection* 44 (2016): 459-466.

144. Carcopino X., *et al*. "Q fever duringpregnancy: a cause of poor and maternal outcome". *Annals of the New York Academy of Sciences* 1166 (2009): 79-89.

145. Tissot-Dupont H., *et al*. "Role of sex, age, previous valve lesion, and pregnancy in the clinical expression and outcome of Q fever after a large outbreak". *Clinical Infectious Disease* 44 (2007): 232-237.

146. Rey D., *et al*. "Seroprevalence of antibodies to Coxiella burnetti among pregnant women in South Eastern France". *European Journal of Obstetrics and Gynecology and Reproductive Biology* 93 (2000): 151-156.

147. Langley JM., *et al*. "Coxiella burnetii seropositivity in parturient women is associated with adverse pregnancy outcomes". *American Journal of Obstetrics and Gynecology* 189 (2003): 228 -232.

148. Baud D., *et al*. "Seroprevalence of Coxiella burnetii and Brucella abortus among pregnant women". *Clinical Microbiology and Infection* 15 (2009): 499 -501.

149. Nielsen SY., *et al*. "Prevalence of Coxiella burnetii in women exposed to livestock animals, Denmark 1996 to 2002". *Eurosurveillance* 18-28 (2013): 20528.

150. Million M., *et al*. "Revaluation of the risk of fetal death and malformation after Q fever". *Clinical Infectious Disease* 59 (2014): 256 -260.

151. Angelakis E., *et al*. "Q fever and pregnancy: disease, prevention, and strain specificity". *European Journal of Clinical Microbiology and Infectious Diseases* 32 (2013): 361-368.

152. CVI. Coxiella burnetii RSA 331. Craig Venter Institute. c. l., ed 2007.

153. Beare, P.A., *et al*. "Comparative genomics reveal extensive transposon-mediated genomic plasticity and diversity among potential effector proteins within the genus Coxiella". *Infection and Immunity* 77 (2009): 642-656.

154. Runa Kuley., *et al*. "Genome Plasticity and Polymorphisms in Critical Genes Correlate with Increased Virulence of Dutch Outbreak-Related Coxiella burnetiid". F*rnotiers in Microbiology* 8 (2017): 1526.

155. Olga Glazunova., *et al*. "Coxiella burnetii Genotyping". *Emerging Infectious Diseases journal* 11.8 (2005): 1211-1217.

156. Roest HIJ., *et al*. "Molecular epidemiology of Coxiella burnetii from ruminants in Q fever outbreak, The Netherlands". *Emerging Infectious Diseases* 17 (2001): 668-675.

157. Svraka S., *et al*. "Establishment of a genotyping scheme for Coxiella burnetiid". *FEMS Microbiology Letters* 254 (2006): 268 -274.

158. Arricau-Bouvery N., *et al*. "Molecular characterization of Coxiella burnetii isolates by infrequent restriction site-PCR and MLVA typing". *BMC Microbiology* 6 (2006): 38.

159. Huijsmans CJJ., *et al*. "Single-nucleotide-polymorphism genotyping of Coxiella burnetii during a Q fever outbreak in The Netherlands". *Applied and Environmental Microbiology* 77(2011): 2051-2057.

160. Anderson A., *et al*. "Diagnosis and management of Q fever—United States 2013: recommendations from CDC and the Q Fever Working group". *MMWR Recommendations and Reports* 62 (2013): 1-23.

161. Dupont HT., *et al*. "Q fever serology: cutoff determination for microimmunofluorescence". *Clinical and Diagnostic Laboratory Immunology* 1 (1994): 189 -196.

162. Imbert G and La Scola B. "Diagnosis of Q fever using indirect microimmunofluorescence". *Methods in Molecular Biology* 345 (2006): 197-202.

163. OIE Terrestrial Manual (2010).

164. Stein A., *et al*. "Study of the 16S-23S ribosomal DNA internal spacer of Coxiella burnetiid". *European Journal of Epidemiology* 13 (1997): 471- 475.

165. Fenollar F., *et al*. "Molecular detection of Coxiella burnetii in the sera of patients with Q fever endocarditis or vascular infection". *Journal of Clinical Microbiology* 42 (2004): 4919 -4924.

166. Fenollar F and Raoult D. "Molecular genetic methods for the diagnosis of fastidious microorganisms". APMIS 112 (2004): 785- 807.

167. Eldin C., *et al*. "Coxiella burnetii DNA, but not viable bacteria, in dairy products in France". *The American Journal of Tropical Medicine and Hygiene* 88 (2013): 765-769.

168. Rolain J-M and Raoult D. "Molecular detection of Coxiella burnetii in blood and sera during Q fever". *QJM* 98 (2005): 615-620.

169. Schneeberger PM., *et al*. "Real-time PCR with serum samples is indispensable for early diagnosis of acute Q fever". *Clinical and Vaccine Immunology* 17 (2010): 286 -290.

170. Edouard S and Raoult D. "Lyophilization to improve the sensitivity of qPCR for bacterial DNA detection in serum: The Q fever paradigm". *Journal of Medical Microbiology* 65 (2016): 462- 467.

171. Kersh GJ., *et al*. "Presence of Coxiella burnetii DNA in the environment of the United States 2006 to 2008". *Applied and Environmental Microbiology* 76 (2010): 4469-4475.

172. Raoult D. "Host factors in the severity of Q Fever". *Annals of the New York Academy of Sciences* 590 (1990): 33-38.

173. M Garner., *et al*. "A Review of Q Fever in Australia 1991-1994". *Australian and New Zealand Journal of Public Health* 21 (1997): 722-730.

174. EFSA Panel on Animal Health and Welfare (AHAW). "Scientific Opinion on Q Fever". *EFSA Journal* 8(5) (2010): 1595.

175. Roest HIJ., *et al*. "Q fever in 2008 in The Netherlands and the expectations of 2009". *Tijdschr Diergeneeskds* 134 (2009): 300-303.

176. Guatteo R., *et al*. "Shedding routes of Coxiella burnetii in dairy cows: implications for detection and control". *Veterinary Research* 37 (2006): 827-833.

177. Enserink, M. "Questions abound in Q Fever explosion in the Netherlands". *Science* 2010 327: 266-267.

178. Tozer, *et al*. "Potential animal and environmental sources of Q fever infection for humans in Queensland". *Zoonoses Public Health* 61 (2014): 105-112.

179. H Tissot-Dupont., *et al*. "Wind in November, Q Fever in December". *Emerging Infectious Diseases* 10 (2004): 1264-1269.

180. Carrieri MP., *et al*. "Investigation of a slaughterhouserelated outbreak of Q fever in the French Alps". *European Journal of Clinical Microbiology and Infectious Diseases* 21 (2002): 17-21.

181. Gilsdorf, A., *et al*. "Large Q fever outbreak due to sheep near residential areas, Germany 2005". *Epidemiology and Infection* 136 (2008): 1084-1087.

182. Schimmer, B., *et al*. "The use of a geographic information system to identify a dairy goat farm as the most likely source of an urban Q-fever outbreak". *BMC Infectious Diseases* 10 (2010): 69.

183. Hackert, VH., *et al*. "Q fever: single-point source outbreak with high attack rates and massive numbers of undetected infections across an entire region". *Clinical Infectious Diseases* 55 (2012): 1591-1599.

184. Berri M., *et al*. "Ovine manure used a garden fertiliser as a suspected source of human Q fever". *Veterinary Record* 153 (2003): 269-270.

185. Hermans, T., *et al*. "Landapplied goat manure as a source of human Q-fever in the Netherlands 2006-2010". *PLoS One* 9 (2014): e96607.

186. van der Hoek W., *et al*. "Q fever in the Netherlands: the role of local environmental conditions". *International Journal of Environmental Health Research* 21 (2011): 441-451.

187. P Harris, *et al*. "Acute Q fever in northern Queensland: variation in incidence related to rainfall and geographical location". *Epidemiology and Infection* 141-5 (2013): 1034-1038.

188. NRParker., *et al*. "Q Fever". *Lancet* 367 (2006): 679-688.

189. M. Benazis, *et al*. "A survey of Western Australian sheep, cattle and Kangaroos to determine the prevalence of Coxiella burnetiid". *Veterinary Microbiology* 143 (2010): 337-345.

190. Williams JC. Infectivity, virulence, and pathogenicity of Coxiellaburnetii for various hosts. In: Williams JC, Thompson HA, eds. Q fever: the biology of Coxiella burnetii. Boca Raton, Florida: CRC Press (1991): 21-71.

191. Marrie TJ., *et al*. "Route of infectiondetermines the clinical manifestations of acute Q fever". *The Journal of Infectious Diseases* 173 (1996): 484-487.

192. La Scola B., *et al*. "Pathologic changes during acute Qfever: influence of the route of infection and size in infected guinea pigs". *Infection and Immunity* 65 (1997): 2443-47.

193. Leone M., *et al*. "Effect of sex on Coxiella burnetii infection: protective role of 17_-estradiol". *The Journal of Infectious Diseases* 189 (2004): 339-45.

194. Gikas A., *et al*. "In vitro susceptibility of Coxiella burnetii to trovafloxacin in comparison with susceptibilities to pefloxacin, ciprofloxacin, ofloxacin, doxycycline, and clarithromycin". *Antimicrobial Agents and Chemotherapy* 42 (1998): 2747-2748.

195. Gikas A., *et al*. "In vitro susceptibility of Coxiella burnetii to linezolid in comparison with its susceptibilities to quinolones, doxycycline, and clarithromycin". *Antimicrobial Agents and Chemotherapy* 45 (2001): 3276 -3278.

196. Raoult D., *et al*. "Bactericidal effect of doxycycline associated with lysosomotropic agents on Coxiella burnetii in P388D1 cells". *Antimicrobial Agents and Chemotherapy* 34 (1990): 1512-1514.

197. Maurin M., *et al*. "Phagolysosomal alkalinization and the bactericidal effect of antibiotics: The Coxiella burnetii paradigm". *The Journal of Infectious Diseases* 166 (1992): 1097-1102.

198. Samira Boarbi., *et al*. "Coxiella burnetii, agent de la fièvre Q". *Canadian Journal of Microbiology* 62 (2016): 102-122.

199. Angelakis E., *et al*. "Q Fever". *Veterinary Microbiology* 140 (2010): 297-309.

200. Dorko E., *et al*. "Influence of the environ¬ment and occupational exposure on the occurrence of Q fever". *Central European Journal of Public Health* 20 (2012): 208-214.

201. Kováčová E., *et al*. "Suitability of various Cox¬iella burnetii antigen preparations for detection of serum antibodies by various test". *Acta Virologica* 42 (1998): 365-368.

202. G Flores-Ramírez., *et al*. "Protein composition of the phase I Coxiella burnetii soluble antigen prepared by extraction with trichloroacetic acid". *Acta virologica* 61 (2017): 361 - 368.

203. Margarita Bolanos-Rivero., *et al*. "Detection of Coxiella burnetii DNA in Peridomestic and Wild Animals and Ticks in an Endemic Region (Canary Islands, Spain)". *Vector-Borne and Zoonotic Diseases* (2017).

204. A Joulié., *et al*. "Coxiella burnetii circulation in a naturally infected flock of sheep: individual follow-up of antibodies in serum and milk". *American Society for Microbiology* (2017).

205. Lindsay A., *et al*. "A case of Q fever after liver transplantation". *Transplant Infectious Disease* (2017): e12737.

206. Agerholm JS. "Coxiella burnetii associated reproductive disorders in domestic animals—a critical review". *Acta Veterinaria Scandinavica* 55 (2013): 13.

207. Markus Freick., *et al*. "Detection of Coxiella burnetii DNA and anti-Coxiella burnetii IgG antibodies in precolostral blood samples of stillborn calves in an endemically infected Holstein dairy herd". *Folia Microbiologica* (2017).

208. Stephen S., *et al*. "Leukocytoclastic vasculitis presenting in association with Coxiella burnetii (Q fever) a case report". *Journal of Cutaneous Pathology* 45.1(2018): 71-73.

209. Kampschreur LM., *et al*. "Microbiological challenges in the diagnosis of chronic Q fever". *Clinical and Vaccine Immunology* 19 (2012): 787-790.

210. Mostafa F N., *et al*. "Cross-sectional study for determining the prevalence of Q fever in small ruminants and humans at El Minya Governorate, Egypt". *BMC Research Notes* 1.10 (2017): 538.

211. Katharina Sobotta., *et al*. "Permissiveness of bovine epithelial cells from lung,intestine, placenta and udder for infection with Coxiella burnetiid". *Veterinary Research* 48 (2017): 23.

212. G Flores-Ramírez., *et al*. "Protein composition of the phase I Coxiella burnetii soluble antigen prepared by extraction with trichloroacetic acid". *Acta virologica* 61 (2017): 361-368.

213. Bonhan Koo., *et al*. "A rapid bio-optical sensor for diagnosing Q fever in clinical specimens". *Journal of Biophotonics* 11.4 (2018): e201700167.

214. Centers for Disease Control and Prevention. Q fever. Atlanta: CDC 2003: CDC fact sheet.

215. www.hpa.org.uk/deliberate_accidental_releases/biological

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Partie II :

Contribution à l'étude de Coxiella burnetii l'agent causal de la fièvre Q chez l'Homme en Algérie.

Chapitre 1 :

Etude de l'aspect abortif de Coxiella burnetii chez les femmes enceintes sur L'Algérois.

Avant-propos.

En général, l'avortement spontané touche **10** à **20%** des grossesses, mais ses causes restent inconnues dans plus de **50%** des cas. Chez les animaux, la fièvre Q est associée à l'avortement épizootique chez les ongulés [11]. Chez l'homme, jusqu'à **90%** des femmes enceintes ont des anticorps suggérant une infection récente par *Coxiella burnetii* mais restent asymptomatiques [11]. Cependant, une infection symptomatique ou asymptomatique pendant la grossesse a été associée à des complications obstétricales, y compris des fausses couches, des accouchements prématurés et des décès fœtaux. La fièvre Q pendant la grossesse a été associée à de mauvaises issues obstétricales dans le sud de la France, au Canada, en Écosse et en Espagne, principalement lorsque la maladie est acquise au cours du premier trimestre [17.18]. Chez les femmes enceintes et les autres mammifères, la bactérie colonise et se multiplie dans l'utérus et le placenta, puis serait réactivée pendant les grossesses ultérieures. Lorsqu'une femme enceinte fait une infection asymptomatique, sa réactivation augmente le risque de passage pour une fièvre Q chronique, et la femme peut également connaître une issue défavorable de grossesse [19].

Nous avons étudié l'impact de l'infection par *Coxiella burnetii* lors d'avortements spontanés fébriles chez les femmes, en utilisant une méthode sérologique (Immunofluorescence Indirecte **-IFI**) et une méthode moléculaire (**qPCR**), sur deux services obstétrique-gynécologie (OB-GYN) dans deux hôpitaux à Alger (EPH HACENE BADI EX BELFORT, EPH ZERALDA) ? au cours de la période d'avril 2014 à novembre 2015.

Nous avons inclus dans le groupe de cas **380** femmes ayant subi un avortement spontané fébrile, tandis que le groupe témoin comprenait **345** femmes qui ont accouché par voie basse sans

complication. Parmi les **725** femmes incluses, des anticorps contre *Coxiella burnetii* ont été détectés par **IFI** chez trois **(03, 0.79%)** patientes ; tous les échantillons du groupe témoin étaient négatifs. Par ailleurs, seulement quatre (**04, 1.05%**) échantillons placentaires appartenant le groupe de cas sont revenus avec **qPCR** positive pour **IS1111** et **IS30a** également. Par conséquent. La comparaison de nos résultats obtenus avec la littérature, nous permettrait de suggérer un lien causal entre l'infection à *Coxiella burnetii* et l'avortement spontané fébrile dans les services d'OB-GYN à Alger.

Pour l'avenir, nous prévoyons de mener une autre étude avec un échantillonnage plus important et dans d'autres régions d'Algérie afin d'évaluer davantage la relation entre l'infection à *Coxiella burnetii* et les avortements spontanés fébriles.

Article 1:

Coxiella burnetii infection with women's febrile spontaneous abortion reported in Algiers.

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ORIGINAL ARTICLE

Coxiella burnetii infection with women's febrile spontaneous abortion reported in Algiers

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Abstract

We investigated Q fever infection in Febrile Spontaneous Abortions in women by using a serologic method (Immuno-Fluorescence Assay, IFA) and a molecular method (real-time quantitative PCR, qPCR) in Obstetric-Gynaecology (OB-GYN) services in two hospitals in Algiers. We included in the case group 380 women who experienced Febrile Spontaneous Abortion; the control group comprised 345 women who gave birth without any other infections or complications. Among the 725 women included, antibodies against *Coxiella burnetii* were detected by IFA in three (03) cases patients; all control group samples were IFA negative. In other hand, only four (04) placental samples among the case group came back with q PCR positive for IS1111 and IS30a too. A relationship between *C. burnetii* infection and febrile spontaneous abortion exists in OB-GYN services in Algiers.

Keywords: Immunofluorescence assay, Q fever, qPCR, real-time quantitative, PCR
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Introduction

Coxiella burnetii is the pathogenic agent of Q fever, which remains a worldwide zoonotic disease. The natural cycle of this bacterium is not reported to include humans, which are considered incidental hosts [1,2]. The true reservoir is wide and includes mammals, birds and arthropods, mainly ticks [1]. Q fever is usually an occupational disease, although isolated cases and outbreaks have been reported in people who have had indirect contact with infected animals [3]. Acute or chronic *C. burnetii* infection exhibits a wide spectrum of clinical manifestations; roughly 50% of all infections with *C. burnetii* are asymptomatic [4]. Acute Q fever typically arises from inhalation of aerosolized bacteria; rare but potentially severe chronic disease most commonly manifests as endocarditis [5].

In general, spontaneous abortion affects 10% to 20% of pregnancies, but its cause remains unknown in more than 50% of cases [3]. In animals, Q fever is associated with epizootic abortion in ungulates [6]. In humans, up to 90% of pregnant women have antibodies suggesting recent infection with *C. burnetii* but remain asymptomatic [7]. However, symptomatic or asymptomatic infection during pregnancy has been associated with obstetric complications, including miscarriage, preterm delivery and foetal death [8]. Q fever during pregnancy has been linked to poor obstetric outcomes in southern France, Canada, Scotland and Spain, primarily when the disease is acquired during the first trimester [9]. In pregnant women and other mammals, the bacteria will colonize and multiply in the uterus and placenta, then be reactivated during subsequent pregnancies [3]. When pregnant women have an asymptomatic infection with Q fever, its reactivation increases the risk of chronic Q fever, and the woman may also experience an adverse pregnancy outcome [10].

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The precise mechanisms by which the infection compromises pregnancy are largely unknown, but adverse pregnancy outcome has been reproduced in BALB/c mice in which infection followed by repeated pregnancies resulted in spontaneous abortion and perinatal death [10]. In humans, the role of Q fever during pregnancy has been recently questioned because of the discrepancy between the high risk of obstetric complications among women infected with Q fever in published case series and the absence of an increased risk of adverse pregnancy outcomes in population-based serologic studies [6].

Immunofluorescence assay (IFA) is the reference standard for the diagnosis of Q fever; it is based on detection of antibodies against two antigenic variations of *C. burnetii* lipopolysaccharide, phase I and phase II antigens [11].

In Algeria, little is known about Q fever because diagnostic tools are not readily available. As a result, few studies have been performed studying Q fever in Algeria, and we thus have no precise picture of this disease or its prevalence in this region. After the first cases reported in Algiers in 1948 by Portier et al. [12], outbreaks have been reported in Batna (in the French army in 1955 and 1957) and in Tlemcen (also in the army) [13]. In 1996 Lacheheb and Raoult [14] studied the seroprevalence of Q fever in a population of 729 patients from northeast Algeria; they found 113 positive sera by IFA, with a 15.5% seroprevalence. Furthermore, in order to study infective endocarditis caused by *C. burnetii,* Benslimani et al. [15] in 2005 studied cardiac valves and sera from patients with infective endocarditis and negative blood culture; only two of 61 serum samples were positive for *C. burnetii* antibodies by microimmunofluorescence, and all the cardiac valves came back negative by PCR.

To our knowledge, no study has been done on the abortion aspect of *C. burnetii* in Algeria in women. Thus, the aim of the present study was to investigate Q fever infection in febrile spontaneous abortions in women by using a serologic method (IFA) and a molecular method (real-time quantitative PCR, qPCR) in obstetric-gynaecology (OB-GYN) services in two hospitals in Algiers.

Materials and methods

Study design

In order to evaluate the abortive aspect of *C. burnetii* infection among women in Algiers, we considered it wise to focus our study on OB-GYN services which admit patients from rural areas where livestock of different animal species is widespread and where therefore a high level of contact with animals and their parturition products is reported. We recruited women in OB-GYN services from Hassen Badi Hospital (east of Algiers) and Zéralda Hospital (west of Algiers). The two hospitals receive pregnant women from neighbourhoods with cattle and sheep. Annually, each hospital admits approximately 8865 pregnant women, with 88% (7883) of them giving birth and 12% (982) experiencing miscarriage.

From April 2014 to November 2015, at the two hospitals, we admitted 18 640 pregnant women for delivery, including 2127 women experiencing spontaneous abortion (11.41% prevalence); of these, only women experiencing febrile spontaneous abortion were eligible to be included in the case group, where we sampled 380 women. A total of 345 women who gave birth without any other infections or complications were enrolled onto the study as the control group.

Inclusion criteria and case definitions

In order to select the patients for the case group, we found, by consulting with the obstetric emergency services unit, women who were likely to experience a febrile spontaneous abortion. All miscarriages were confirmed by ultrasound or pathologic examination. Functional signs included pelvic aches and fever (temperature >38.5°C). Physical signs included increased uterine volume and metrorrhagia found by speculum examination; open neck/trophoblastic neck found by manual vaginal examination; and trophoblastic detachment found by ultrasound. We thus selected case patients whose samples were likely to be positive for *C. burnetii* by serology and qPCR.

For the control group, we took into account all women who were admitted for physiologic vaginal delivery. In addition, they had contact with animals and/or came from rural areas. Women with a previous complicated obstetrics history or a complicated partum or other infection history were not included in the control group. Table 1 lists the characteristics of the case and control groups.

Ethics statement

All the women gave us permission to be included the study, including use of interview information and blood and placenta

TABLE 1. Patient information

Characteristic	Group	Variable			
No. of patients		**Age 20–30 years**		**Age 30–40 years**	
	Control	233		112	
	Case	244		136	
Gestational age		**4–8 weeks**	**9–13 weeks**	**4–8 weeks**	**9–13 weeks**
	Control	185	48	79	33
	Case	196	48	114	22
Animal contact		**Yes**	**No**	**Yes**	**No**
	Control	201	32	98	104
	Case	197	47	104	32
Living area		**Rural**	**Nonrural**	**Rural**	**Nonrural**
	Control	174	59	89	23
	Case	181	63	97	39
Abortion history		**Yes**	**No**	**Yes**	**No**
	Control	75	158	43	84
	Case	137	107	69	52

By using chi-square homogeneity test, we confirmed that the two patient groups included in this study were comparable and homogeneous.

samples. Clinical data were obtained by a standardized questionnaire that asked about clinical information, contact with animals and health history. These data were analysed retrospectively when the serologic analysis or molecular tests were positive.

Sample collection

We collected a total of 380 samples comprising placenta or sera samples from women experiencing febrile spontaneous abortion and 345 placenta or sera samples from women giving birth. The samples were collected aseptically in dry tubes and conserved at −20°C so they could be transported to the Emerging Tropical Infectious Diseases Research Unit at the Faculty of Medicine–Marseille for serology (IFA) and qPCR for *C. burnetii.*

Serologic assays

Serologic tests were performed using an indirect IFA, which is the reference method for the serodiagnosis of Q fever. We used reference strains *C. burnetii* Nine Mile I and Nine Mile II as antigens, and antigen preparation and purification were performed as described elsewhere [16].

Polymerase chain reaction

DNA was extracted by using a QIAamp Tissue Kit (Qiagen, Hilden, Germany), and qPCR was performed with a CFX96 thermocycler (Bio-Rad, Marnes-la-Coquette, France) using primers and probes specific for intergenic sequences *IS1111* and *IS30a* as previously described [17]. DNA from the *C. burnetii* Nine Mile II strain was used as positive control and sterile water was used as negative control.

Statistical analyses

In order to calculate the confidence interval and the significance level (p values) of the various results obtained, we used the Web Mediametrie application (http://www.mediametrie.fr/calculettes-mediametrie.php?id=intervalle) as well as the application of the Yates correction for the chi-square test, respectively. $p < 0.05$ was considered statistically significant; p values between 0.05 and 0.1 were considered nonsignificant.

Results

IFA serology

Among the 725 women included, antibodies against *C. burnetii* were detected by IFA in three case patients; all control group samples were IFA negative. Among the positive sera, the titres of antibodies to phase I and II *C. burnetii* antigens varied among immunoglobulin (Ig) G, IgM and IgA (Table 2).

Detection of *C. burnetii* by qPCR

qPCR was used for the detection of *C. burnetii* in placental samples by using *C. burnetii*–specific primers and a probe designed to amplify the *IS1111* gene, and confirmed by the second gene, *IS30a,* which remains highly *C. burnetii* species specific.

Four placental samples from 380 case subjects came back positive for both *IS1111* and *IS30a* genes. All samples from control subjects came back negative for these genes. The C_t values of the positive samples ranged from 29.13 to 32.97 (corresponding to 6.1 and 4.9 $\log_{10}$ DNA copies/mL). The *IS1111* and *IS30a* qPCR results are summarized in Table 3. Table 4 summarizes the case group's serologic and molecular results for *C. burnetii.*

In total, in this study we obtained six of 380 positive results for *C. burnetii* (IFA and/or qPCR) among the case group and no positive results among the control group; these results are statistically significant (p 0.0299). Using the application of the Yates correction for the chi-square test, significance was considered at $p \leq 0.05$.

Description of positive cases

Patient A, a 25-year-old housewife, was admitted to the OB-GYN service of Zéralda Hospital. She was from a south-western suburbs of Algiers, the Rahmania commune (Zéralda

TABLE 2. Acute and chronic titres of antibodies to *Coxiella burnetii* antigens

Group (*n* positive sera)	Total Ig screening[a]	Phase II antigen			Phase I antigen		
		IgG	IgM	IgA	IgG	IgM	IgA
Control (0)							
Case (3)							
A	Positive ≥100	1:200	1:25	1:200	0	0	1:50
B	Positive ≥100	1:200	1:200	1:800	0	1:50	0
C	Positive ≥100	1:200	1:100	1:200	0	0	0

[a]All sera were screened as first-line with total immunoglobulin (Ig). If serum is positive at 1/100 dilution, then antibodies present in this sample are differentially quantified (IgG, IgM, IgA).

TABLE 3. Placental sample results by real-time quantitative PCR

	IS1111 gene					*IS30a* gene				
Group	CB⁻	CB⁺	C_t values	Log_{10} DNA copies/mL	Positive control C_t	CB⁻	CB+	C_t values	Positive control C_t	Log_{10} DNA copies/mL
Control	345	0	—	—	—	345	0			
Case	376	4	31.3	5.4	25.72 = 7.1 log_{10} DNA copies/mL	376	4	31.9	26.37 = 6.9 log_{10} DNA copies/mL	5.2
			29.1	6.1				30.0		5.8
			32.7	5.0				32.9		4.9
			31.0	5.5				31.1		5.5

CB, *Coxiella burnetii*.

TABLE 4. Characteristics of pregnant women according *Coxiella burnetii* results for placental samples

	IFA, *n* (%), 95% CI		qPCR, *n* (%), 95% CI	
Characteristic	Positive (*n* = 3)	Negative (*n* = 377)	Positive (*n* = 4)	Negative (*n* = 376)
Age				
20–30 years	2 (0.82), −0.3 to 2	242 (99.18), 98 to 100.3	2 (0.82), −0.3 to 2	242 (99.18), 98 to 100.3
30–40 years	1 (0.74), −0.7 to 2.2	1135 (99.26), 97.8 to 100.7 p 0.60 NS*	2 (1.47), −0.6 to 3.5	134 (98.27), 96.1 to 100.5 p 0.94 NS*
Gestational age				
4–8 weeks	01 (0.32), −0.3 to 0.9,	309 (99.68), 99.1 to 100.3	03 (0.97), −0.1 to 2.1	307 (99.03), 97.9 to 100.1
9–13 weeks	02 (2.86), −1 to 6.8	68 (97.14), 93.2 to 101 p 0.16 NS*	02 (2.86), −1 to 6.8	68 (97.14), 93.2 to 101 p 0.50 NS*
Animal contact				
Yes	3 (1.00), −0.1 to 2.1	298 (99.00), 97.9 to 100.1	4 (1.33), 0 to 2.6	297 (98.67), 97.4 to 100
No	0 (0.00)	79 (100.00), 100 to 100 p 0.86 NS*	0 (0.00)	79 (100.00), 100 to 100 p 0.68 NS*
Residence				
Rural	2 (0.72), −0.3 to 1.7	276 (99.28), 98.3 to 100.3	3 (1.08), −0.1 to 2.3	275 (98.92),97.7 to 100.1
Nonrural	1 (0.98), −0.9 to 2.9	101 (99.02), 97.1 to 100.9 p 0.69 NS*	1 (0.98), −0.9 to 2.9	101 (99.02), 97.1 to 100.9 p 0.63 NS*
History of abortion				
Yes	2 (0.91), −0.3 to 2.2	219 (99.01), 97.7 to 100.3	3 (1.36), −0.2 to 2.9	218 (98.64), 97.1 to 100.2
No	1 (0.63), −0.6 to 1.9	158 (99.37), 98.1 to 100.6 p 0.87 NS*	1 (0.63), −0.6 to 1.9	158 (99.37),98.1 to 100.6 p 0.86 NS*

CI, confidence interval; IFA, immunofluorescence assay; NS, not significant; qPCR, real-time quantitative PCR.
*Level of significance when $p \leq 0.05$.

division). This commune is situated in a rural zone with livestock. The people who live in this environment are in permanent contact with animals, which is why they remain subject to different zoonotic diseases. This patient was admitted to the obstetrics emergency service while undergoing a spontaneous abortion without pathologic antecedents. It was her third pregnancy (G3). She had previously experienced one live birth (P1) and one abortion (A1). She was in the eighth week of pregnancy. Physical examination revealed a pale patient with high fever (temperature 39°C); she presented with pelvic aches, increasing uterus volume and metrorrhagia. According to the patient's history, she had drunk unpasteurized cow's milk, and she may have inhaled aerosols that came from stored cow's milk. This patient had IFA serology positive against *C. burnetii* phase II (IgG 1:200, IgM 1:25, IgA 1:200). Placental qPCR was negative for both *IS1111* and *IS30a*. The elevated level of antibodies against *C. burnetii* phase II indicated acute Q fever infection, which explains the high fever and infectious condition noted during her admission. It cannot be ruled out that this infection was the cause of abortion.

Patient B (G2 P1 A0) was a 27-year-old teacher admitted to the OB-GYN service of Zéralda Hospital. She came from the western suburbs of Algiers, from the Staouali commune (Zéralda division), which has cattle farms. She was in the 12th week of pregnancy and was admitted with a high fever (temperature 39.5°C) with skin rash and metrorrhagia. She ejected the abortion product 2 hours after admission. Patient history indicated that she had permanent contact with breeding cows, and she had felt feverish and tired during the pregnancy. This patient was the only case that was positive for *C. burnetii* by both IFA serology (IgG 1:200, IgM 1:200, IgA 1:800) and placental qPCR for *IS1111* and *IS30a* (C_t values: *IS1111,* 31.3 = 5.4 log_{10} DNA copies/mL, *IS30a,* 31.9 = 5.2 log_{10} DNA copies/mL) in which the correlation between IFA and qPCR matched well. Serology revealed an acute infection, which would explain the patient's rash, based on the physiopathogenesis of acute *C. burnetii* infection.

Cases C and D, aged 32 and 29 years, were a maid and a housewife, respectively, admitted to the OB-GYN service of Hassen Badi Hospital. The two came from the east region of Algiers, from the Chrarba commune (Eucalyptus division), where there are a large number of animal farms, especially poultry farms, so although these patients were living in nonrural housing, they had occasional animal contact. Patient C (G2 P0 A1) and patient D (G2 P1 A1) were in their 12th week of pregnancy. Physical examination revealed pale patients with a high fever (temperature 38.5°C). Both experienced a typical febrile abortion that took place 24 hours after admission.

Patient C had IFA serology positive for acute infection (IgG 1200, IgM 1100, IgA 1200) with placental qPCR negative for both *IS1111* and *IS30a*. Patient D's placental qPCR also came back positive for *IS1111* (C_t 29.13 = 6.1 $\log_{10}$ DNA copies/mL) and *IS30a* (C_t 30.0 = 5.8 $\log_{10}$ DNA copies/mL), but her serologic results were negative. Two different positive responses for the same clinical picture were evident, the interpretation of which is based on the variation of *C. burnetii* physiopathogenesis.

Patient E was a 34-year-old housewife (G3 P2 A0) admitted to the high-risk pregnancy service in Hassen Badi Hospital with a high fever (temperature 40°C) of a week's duration that did not respond to cefalexin 1 g at a rate of 3 g per day for 7 days. All haemocultures were negative for the usually tested germs. Hyperleukocytosis was estimated at 22 000/mm^3. The patient was ill during her admission until the foetus was expelled at 72 hours after admission. Subsequent testing including placental qPCR revealed *IS1111* (C_t 32.7 = 5 $\log_{10}$ DNA copies/mL) and *IS30a* (C_t 32.9 = 4.9 $\log_{10}$ DNA copies/mL); IFA serology was negative. This patient came from the El Harrach commune, 800 m away from the hospital, where she lived in nonrural housing. Her history indicated that she was in daily contact with a dog and domestic cats, which remained the only source of animal contamination. She experienced a peak in her fever on the day of spontaneous abortion.

Patient F was a 37-year-old housewife (G4 P2 A1) admitted to the OB-GYN service of Zéralda Hospital for a pneumopathy that occurred 3 weeks ago, which had remained undiagnosed and untreated. At admission, the patient was conscious and had a temperature of 38.5°C. She had a cough, sputum, dyspnoea, localized chest pain and tachycardia in addition to significant bleeding that ended by foetal expulsion. She lived in a rural area located in the commune of Douira, west of Algiers. The people of this region are known to consume raw cow's milk and homemade dairy products. Consumption of such products could be the source of infections caused by excretion of bacteria through animal milk, including *C. burnetii*. This patient's findings included positive placental qPCR for *IS1111* (C_t 31.0 = 5.5 $\log_{10}$ DNA copies/mL) and *IS30a* (C_t 31.1 = 5.5 $\log_{10}$ DNA copies/mL).

Fig. 1 shows the location of the two OB-GYN services studied and the distribution of positive cases on a map of Algiers.

Discussion

No Q fever epidemics have been recorded or verified clinically or biologically in Algeria, likely resulting in the sources of infection of *C. burnetii* being misunderstood. Our study is the first of its kind in Algeria to investigate the outcome of Q fever infection in pregnant women. We sought to learn the relationship between febrile spontaneous abortion and *C. burnetii* infection, hypothesizing an association between febrile spontaneous abortion and Q fever infection.

Our serologic findings indicated a seroprevalence of 0.79% (3/380) for IgG titres $\geq$1:200 phase II (Q fever acute infection) among the case group of women who experienced abortion, whereas women giving birth (control group) had negative serology (0/345). These results accord with those of Nielsen et al. [18] in Denmark, who found a 1.2% seroprevalence of acute Q fever infection among women who experienced

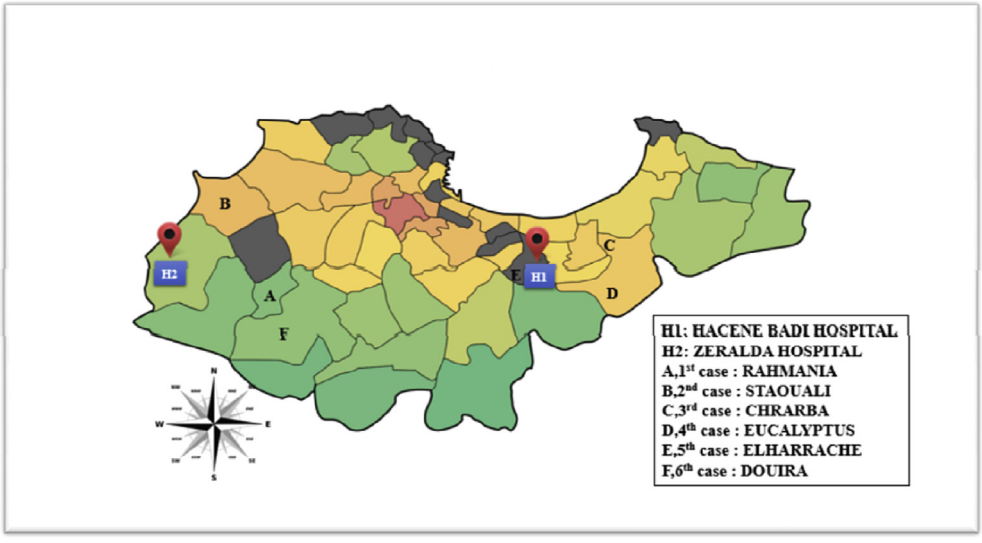

FIG. 1. Map illustrating two obstetric-gynaecology services and locations of positive cases in Algiers.

spontaneous abortion in the first semester of pregnancy, and who thus reported that no increased risk of adverse pregnancy outcome was found in women with verified exposure to *C. burnetii.* Moreover, another study reported a seroprevalence of 0.27% (2/738) in women who experienced spontaneous abortion in southeastern France [13].

Many previously reported findings could not be reproduced in the present study. Langley et al. [19] in 2003 reported that 3.8% (IFA positive) of parturient women in an endemic area had evidence of exposure to *C. burnetii* and that this exposure was associated with adverse pregnancy outcomes. However, Raoult et al. [20] and Quijada et al. [3] confirmed that Q fever acquired during pregnancy is a serious disease. Infection with *C. burnetii* in the first trimester frequently resulted in abortion. Raoult et al. identified 11 women in their first trimester, of whom seven (63.63%) experienced abortion. However, Quijada et al. reported an IFA-positive seroprevalence of 32.2% for women with spontaneous abortion (case group) and 23.3% in women who gave birth (control group); their study also reported that abortion history, rural housing, contact with cattle or sheep and cohabitation with pets were also associated with abortion. Concerning abortion history, McCaughey et al. [21] identified that women with a history of miscarriage or prematurity were more often seropositive than those without such a history (19.5% vs. 9.8%).

We found 1.05% qPCR-positive results for both *C. burnetii IS1111* and *IS30a* for the case group. However, previous results have shown that *C. burnetii* was not identified by qPCR or culture in the placentas investigated, with qPCR results negative for all placental samples [3,19,22]. These results are discordant with our findings; in our study, 1.05% placental samples were qPCR positive in the case group, which leads us to say that the association between febrile spontaneous abortion and the existence of *C. burnetii* in placentas are correlated. The study of Vaidya et al. [23] concluded that qPCR for placental samples among women with spontaneous abortion were 21.62% positive in 74 samples tested; this qPCR positivity explains the presence of *C. burnetii* in the placentas of women with abortion. We emphasize that there is a notable difference between our results (1.05%) and those of Vaidya et al. (21.62%), but in both studies results of qPCR of placental samples from spontaneous abortions were positive for *C. burnetii.*

Statistical analysis of the collected patient data and comparison with serologic and molecular results indicated that for duration of pregnancy, abortion history, rural housing and contact with animals, no significant differences were evident for the cases of febrile spontaneous abortion. In our study, most of the positive cases occurred in women who had had at least one abortion during their previous pregnancies, and they had had contact with animals or their parturition products where they lived; they were surrounded by animal farms, especially in the Algiers suburbs. Such an environment makes people vulnerable to infectious zoonosis.

Conclusions

Our study, which aimed to evaluate the relationship between spontaneous febrile abortion and infection with *C. burnetii* at two OB-GYN services in Algiers, is the first in this region to assess this aspect of *C. burnetii* infection. Our results and their comparison with literature allow us to say that a relationship between *C. burnetii* infection and febrile spontaneous abortion exists in OB-GYN services in Algiers. Looking ahead, we plan to carry out another study with a larger sample size and in other regions in Algeria in order to further assess the relationship between *C. burnetii* infection and febrile spontaneous abortions. Q fever causes a low-noise infection in OB-GYN services, for which an alarm bell must be sounded for every suspected *C. burnetii* infection.

Conflict of interest

None declared.

Acknowledgements

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References

[1] Mediannikov O, Fenollar F, Socolovschi C, Diatta G, Bassene H, et al. *Coxiella burnetii* in humans and ticks in rural Senegal. PLoS Negl Trop Dis 2010;4:654.

[2] Coste Mazeau P, Hantz S, Eyraud JL, Donadel L, Lacorre A, Rogez S, et al. Q fever and pregnancy: experience from the Limoges Regional University Hospital. Arch Gynecol Obstet 2016;294:233–8.

[3] Quijada SG, Terán BM, Murias PS, Anitua AA, Cermenõ JL, Frıas AB. Q fever and spontaneous abortion. Clin Microbiol Infect 2012;18: L533–8.

[4] Moodie CE, Thompson HA, Meltzer MI, Swerdlow DL. Prophylaxis after exposure to *Coxiella burnetii*. Emerg Infect Dis 2008;14:1558–66.

[5] Shpynov SN, Tarasevich IV, Skiba AA, Pozdnichenko NN, Gumenuk AS. Comparison of genomes of *Coxiella burnetii* strains using formal order analysis. New Microbes New Infect 2018;23:86–92.
[6] Million M, Roblot F, Carles D, D'Amato F, Protopopescu C, Carrieri MP, et al. Reevaluation of the risk of fetal death and malformation after Q fever. Clin Infect Dis 2014;59:256–60.
[7] Nielson SY, Molbak K, Henriksen TB, Krogfelt KA, Larsen CS, Villumsen S. Adverse pregnancy outcome and *Coxiella burnetii* antibodies in pregnant women, Denmark. Emerg Infect Dis 2014;20:925–31.
[8] Munster JM, Leenders AC, Hamilton CJ, Hak E, Aarnoudse JG, Timmer A. Placental histopathology after *Coxiella burnetii* infection during pregnancy. Placenta 2012;33:128–31.
[9] Angelakis E, Million M, D'Amato F, Rouli L, Richet H, Stein A, et al. Q fever and pregnancy: disease, prevention, and strain specificity. Eur J Clin Microbiol Infect Dis 2013;32:361–8.
[10] Nielsen SY, Hjøllund NH, Anderson NAM, Henriksen TB, Kantso B, Krogfelt KA, et al. Presence of antibodies against *Coxiella burnetii* and risk of spontaneous abortion: a nested case–control study. PLoS One 2012;7:e31909.
[11] Edouard S, Mahamat A, Demar M, Abboud P, Djossou F, Raoult D. Comparison between emerging Q fever in French Guiana and endemic Q fever in Marseille, France. Am J Trop Med Hyg 2014;90:915–9.
[12] Portier A, Vollenweider P, Grestle E. Sur un cas de Q fever (rickettsiose de Burnet-Derrick). Alger Medicale 1948;51:168–71.
[13] Rey D, Obadia Y, Tissot-Dupont H, Raoult D. Seroprevalence of antibodies to *Coxiella burnetii* among pregnant women in south eastern France. Eur J Obstet Gynecol Reprod Biol 2000;93:151–6.
[14] Lacheheb A, Raoult D. Seroprevalence of Q-fever in Algeria. Clin Microbiol Infect 2009;15(Suppl. 2):167–8.
[15] Benslimani A, Fenollar F, Lepidi H, Raoult D. Bacterial zoonoses and infective endocarditis, Algeria. Emerg Infect Dis 2005;11:216–24.
[16] Devine P. Diagnosis of Q fever. J Clin Microbiol 1998;36:3446.
[17] Fournier PE, Thuny F, Richet H, Lepidi H, Casalta JP, Arzouni JP, et al. Comprehensive diagnostic strategy for blood culture–negative endocarditis: a prospective study of 819 new cases. Clin Infect Dis 2010;51:131–40.
[18] Nielsen SY, Anderson NAM, Mølbak AK, Hjøllund NH, Kantsø B, Krogfelt KA, et al. No excess risk of adverse pregnancy outcomes among women with serological markers of previous infection with *Coxiella burnetii*: evidence from the Danish National Birth Cohort. BMC Infect Dis 2013;13:87.
[19] Langley JM, Marrie TJ, LeBlanc JC, Almudevar A, Resch L, Raoult D. *Coxiella burnetii* seropositivity in parturient women is associated with adverse pregnancy outcomes. Am J Obstet Gynecol 2003;189:228–32.
[20] Raoult D, Fenollar F, Stein A. Q fever during pregnancy. Arch Intern Med 2002;162:701–4.
[21] McCaughey C, McKenna J, McKenna C, Coyle PV, O'Neill HJ, Wyatt DE, et al. Human seroprevalence to *Coxiella burnetii* (Q fever) in Northern Ireland. Zoonoses Public Health 2008;55:189–94.
[22] Carcopino X, Raoult D, Boubli F, Stein A. Managing Q fever during pregnancy: the benefits of long-term cotrimoxazole therapy. Clin Infect Dis 2007;45:548–55.
[23] Vaidya MV, Malik SVS, Kaur S, Kumar S, Barbuddhe SB. Comparison of PCR, immunofluorescence assay and pathogen isolation for diagnosis of Q fever in humans with spontaneous abortions. J Clin Microbiol 2008;46:2038–44.

Chapitre 2 :

Coxiella burnetii et manifestations cliniques chez l'Homme en Algérie.

Avant-propos.

La fièvre Q est souvent sous-estimée et sous-déclarée en raison des signes cliniques non spécifiques, la sensibilisation insuffisante des médecins et des professionnels de la santé publique et la capacités de diagnostic qui restent limitées, en particulier dans les pays en voie de développement. Cette maladie a repris de l'importance en raison de l'augmentation des cas d'infections et de flambées épidémiques. Par conséquent, de nombreuses organisations telles que l'Organisation mondiale de la santé (**OMS**), les Nations Unies (**ONU**) et le Groupe australien l'ont définie comme une "infection d'importance croissante" (**OMS 2004**). Par ailleurs, la prévalence déclarée de la fièvre Q ne cesse d'augmenter en raison à la fois de la prévalence réelle et de l'intérêt accordé par les médecins et les épidémiologistes pour cette maladie.

En raison des symptômes non spécifiques de la fièvre Q et de manque de moyens diagnostiques qui ne sont pas largement disponibles en Algérie, les spécialistes des maladies infectieuses s'appuient uniquement sur le diagnostic clinique de la fièvre Q, ce qui les mettrait face à un challenge permanent. Pour ces raisons, nous n'avons pas d'image claire sur la prévalence réelle de la fièvre Q en Algérie. De ce fait, notre étude vise à détecter la présence de *Coxiella burnetii* chez des patients présentant un syndrome fébrile non spécifique, ayant présenté un tableau clinique en faveur d'une fièvre Q. Notre travail s'est déroulé au centre national des maladies infectieuses de l'hôpital **EL-HADI FLICI, Ex ELKETTAR,** à Alger. Un total de **140** patients (**70** patients dans un groupe témoin et **70** patients dans un groupe de cas) l'âge moyen est de **36±18** ans, varie de **5 à 72** ans, ont été évalués pour l'identification de *Coxiella burnetii* par sérologie **IFI** et **q PCR**. La sérologie par **IFI** dans le groupe de cas est revenue positive pour **03** des **70** sérums (**4,30 %**), alors que tous les sérums appartenant au groupe témoin sont revenus négatifs. Cette séroprévalence témoigne de la présence de *Coxiella burnetii* chez des malades

ayant une fièvre prolongée non spécifique en Algérie. D'après nos résultats moléculaires, parmi notre groupe de cas, un seul (**01/70, 1,42 %**) sang total est revenu positif en qPCR pour *Coxiella burnetii,* alors que tous les patients du groupe témoin avaient qPCR négative.

À la lumière des résultats obtenus dans cette étude et de leur discussion avec la littérature publiée, nous tenons à dire que la fièvre Q humaine existe toujours en Algérie chez les patients atteints d'une fièvre prolongée non spécifique, et elle demeure un risque majeur pour la santé publique. Il est également nécessaire de tirer la sonnette d'alarme à la tutelle afin d'améliorer fortement la sensibilisation à la santé publique et de mobiliser les différents acteurs impliqués pour faire face à cette maladie menaçante, ainsi que pour mieux identifier les sources de contamination et les voies d'excrétion du *Coxiella burnetii*. En perspectives, nous aimerions réaliser une autre étude avec un taux d'échantillonnage plus élevé et sur d'autres régions en Algérie afin de mettre en évidence une image plus claire de *Coxiella burnetii* en Algérie.

Article 2:

Q Fever in Nonspecific Febrile Illness Patients in Northern Algeria.

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Q Fever in Nonspecific Febrile Illness Patients in Northern Algeria

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Abstract

Our study aims to detect the presence of *Coxiella burnetii* in nonspecific febrile patients at the National Center of Infectious Diseases in Algeria, **EL-HADI FLICI Hospital**. A total of **140** patients (**70** patients in a control group and **70** patients in a cases group) the mean age is **36±18** range from **5 to 72**, were assessed for *Coxiella burnetii* identification by IFA serology and q PCR test. The IFA serology among the cases group came back positive for **03** out of **70** sera **(4.30 %),** whereas all sera belonging to the control group patients came back negative. This seroprevalence testifies the presence of *Coxiella burnetii* in nonspecific febrile illness patients in Algeria. According to our molecular findings, among our cases group, only one (**01/70, 1.42%**) whole blood *Coxiella burnetii* qPCR became positive, while all the control group patients had qPCR negative. In prospects, we would like to carry out another study with a higher sampling rate and on other regions in Algeria in order to highlight a calmer picture of *Coxiella burnetii* in Algeria.

Key words*: Coxiella burnetii, IFA Serology, Nonspecific Fever, Q Fever Algeria, qPCR, Seroprevalence.*

Introduction:

Coxiella burnetii is the causative agent of the Q fever disease which occurs worldwide, but most cases are reported from Australia, England and various Mediterranean countries, especially France **[2]**. Q fever is most often contracted after humans inhale infected dusts particles, handle infected animal tissues, such as urine, feces or birth products, or ingest milk contaminated with *Coxiella burnetii* **[28]**. Person-to-Person transmission is rare but it has been documented. For example, *Coxiella burnetii* has been identified in the semen of infected males, and this has resulted in sexual transmission of the pathogen **[1].**

This disease is asymptomatic in **60% [3]**, and can lead also to an unexplained pyrexia that makes diagnosis difficult for infectious disease specialists. Following inhalation, symptoms can develop after **10** to **90** days, depending on the dose. Lower doses often result in an asymptomatic outcome or mild cases characterized by a non-productive cough, fever and minimal abnormalities to normal breathing sounds. However, acute pneumonic Q fever could result in respiratory distress. The mortality rate ranges from **0.5** to **1.5% [1]**. When *Coxiella burnetii* infection occurs, the onset is usually abrupt with high and prolonged fever, severe headache, coughing, atypical pneumonia, myalgia and neurologic signs; the cutaneous signs are present in the acute form with up to **20%** of patients presented with transient punctiform rashes, purpuric or maculopapular eruptions **[3,4].** Symptomatic or asymptomatic Q fever, it may have different clinical forms; namely Self-limited febrile illness, acute Q fever and chronic Q fever **[5].** Although *Coxiella burnetii* infection can result from asymptomatic seroconversion to death, fever and pneumonia are the typical clinical manifestations, although other complications including hepatitis and endocarditis can also develop **[1]**.

The Q fever is often under ascertained and underreported because of their unspecific clinical signs, insufficient awareness by physicians and public health professionals and limited diagnostic capabilities, especially in developing countries **[6]**. This disease has regained

importance due the increasing cases of infections and outbreaks **[7]**. Consequently, many organisations such as the World Health Organisation (**WHO**), The United Nations (**UN**) and the Australian Group, defined it as an "Infection of Increasing Importance" (**WHO 2004**). Otherwise, the reported prevalence of Q fever is continuously increasing due to both true prevalence and improved quality interest of physicians and epidemiologists focusing on this disease **[8]**.

The Immunofluorescence Assay (IFA) is the state-of-the-art technique for the diagnosis of the Q fever, it's based on detection of antibodies against two antigenic variations of *Coxiella burnetii* lipopolysaccharide; Phase I & Phase II antigens **[9].**

In Algeria, Q fever exists as endemic disease which has been reported since **1948**. After the outbreaks which have been reported in Batna in **1957** and in Tlemcen in **1958 [10];** there have been no studies or prevalence of Q fever in humans in Algeria, except Dumas' study in **1984 [11]**, where the others studied the seroprevalence of slaughterhouse workers in Algiers. Then, Lachehab **[10],** published the results of his work aiming to estimate seroprevalence in a population limited to eastern Algeria. In addition, Benslimani in **2005 [12],** also worked on infective endocarditis in Algeria, where she highlighted the serological positivity of *Coxiella burnetii* on two patients with infective endocarditis. However, research efforts have been carried out in Algeria on animal Q fever, on sheep, camels as well as ticks; where they have identified the presence of *Coxiella burnetii* serologically in animal sera, and molecularly and on ticks **[13,14,15]**.

On account of the non-specific Q fever's symptoms and the lack of diagnostic means which are not widely available in Algeria, the infectious disease specialists rely on the clinical diagnosis of Q fever, thus it creates a diagnostic challenge. For that, we do not have a clear picture of the real proportions of the Q fever in Algeria. In light of these reasons, our study aims to detect the presence of *Coxiella burnetii* in patients who had a nonspecific febrile illness and have

presented a clinical picture in favour of infection by *Coxiella burnetii*; at the national centre of infectious diseases EL-HADI FLICI Hospital in Algiers.

Material & Methods

Study design

In order to identify the positive cases of Q fever in Algeria, we considered wise to focus our study at the National Reference Centre for Infectious Diseases in Algeria; named **ELHADI FLICI** hospital in Algiers (Algeria), which admits patients from all the Algerian departments. A case-control population based study was conducted between July and October **2017**; knowing that Algeria is a warm country of the Mediterranean basin, this period coincides with summer and autumn where vector-borne and tropical diseases are highest. A total of **140** patients were admitted to our hospital coming from different departments of Northern Algeria; **70** patients constituted the control group and the other **70** left patients constituted the cases group.

Inclusion criteria and cases definitions

In order to select the patients who best respond to the cases group, we considered only patients who were hospitalized for a nonspecific febrile illness associated or not with specific infectious causes, such as, HIV, hepatitis, tuberculosis, meningitis, dermohypodermitis, sepsis, malaria, brucellosis, organ infections including pneumonia and pyelonephritis. These patients may or may not have clinical signs in favour of *Coxiella burnetii* infection; such as a fever of unexplained origin with negative blood cultures, an acute respiratory pathology, granulomatous hepatitis, influenza syndrome, chills, arthralgia, myalgia, purpuric or maculopapular skin rash, an undiagnosed infectious syndrome. These patients were the subject of cases group which were formed of **70** patients.

Concerning the control group, we have carefully selected patients who have been at the emergency ward of the hospital, and have been diagnosed with pathologies other than infectious

diseases, and especially having a Blood Counting Formula without abnormalities; for a total of **70** patients were enrolled.

In parallel, for each patient (Cases group and Control group); we completed a questionnaire in order to have more information about them; such as epidemiological data, housing area (rural or not), contact with animals, profession, the notion of bite of the ticks. The mean age of the study population is **36±18**, range from **5** to **72**, **63** men and **77** women.

Ethics statement

All the patients gave us permission to include in the study, by interview information, blood samples. Clinical data were obtained through a standardized questionnaire with clinical information, contact with animal, health history. These data were analysed retrospectively when the serological analysis or molecular test were positives.

Sample's collection

A total of **140** samples comprising whole blood/sera from each patient (Control and cases group). The samples were collected aseptically in suitable tubes; the sera in dry tube and the whole blood in **EDTA** tubes (EDTA:Ethylenediaminetetraacetic acid). The samples were conserved at **-20°c** to handle them at the **URMITE** (Emerging Tropical Infectious Diseases Research Unit at the Institut Hospitalo-Universitaire (**IHU**) Marseille; for IFA serology and q PCR for *Coxiella burnetii*.

Serological assays

Serologic tests were performed using an indirect immunofluorescent antibody (IFA) assay, which is the reference method for the serodiagnosis of Q fever. We used reference strains *Coxiella burnetii* Nine Mile I and Nine Mile II as antigens, and antigen preparation and purification was performed as described **[29]**.

DNA extraction & Real time PCR

A total of **200** µL of DNA was extracted using the QIAamp Tissue Kit by QIAGEN-BioRobot EZ1, according to the manufacturer's instructions (Qiagen, Hilden, Germany). Extracted DNA was stored at -**20**°C under sterile conditions until it was used in PCR assays. Extracted DNA was used in qPCR amplifications to detect *Coxiella burnetii*. The final qPCR reaction mixture consisted of 5 µL DNA with 15 µL of mix from the Roche PCR Kit (Roche Diagnostics, Meylan, France). The PCR cycling parameters for the qPCR were 5 min at 95°C followed by 39 cycles each consisting of 5 sec of denaturation at 95°C and 30 sec of annealing at 60°C.

Statistical analyses:

In order to calculate the significance level (P values) of the various results obtained, we used the application of the Yates correction for Chi-square test. P-values less than 0.05 were considered statistically significant; and P-values between 0.05 and 0.1 were considered as Non-significant.

Results

IFA Serology

All sera belonging to the control group patients, came back negative for *Coxiella burnetii* tested by ImmunoFluorescence Assay (IFA), whereas among the cases group sera, only **03** out of **70** sera **(4.30 %)** came back positive for *Coxiella burnetii*. Based on the serology results, on the cases group, we notice that **03** patients from **70** patients **(4.28%)** are positive for phase II *Coxiella burnetii,* which suggests that these three patients had an old contact with *Coxiella burnetii.*

Detection of *Coxiella burnetii* by quantitative q PCR

q PCR was used for the detection of *Coxiella burnetii* in whole blood by employing *Coxiella burnetii* –specific primers and a probe designed to amplify the IS1111 gene and confirmed by the second gene IS30a which remains highly *Coxiella burnetii* -species specific.

Only one **(01)** whole blood sample from **140** came back positive to both IS1111 and IS30a genes, this positive samples belonged to the cases group. However, all the control's samples came back negative neither with IS111 nor IS30a. The Ct value of positive samples for them were 33.21, 33.74 for IS111 and IS30a respectively (Corresponding to **4.8** and **4.7 log10 number of DNA copies/mL**); and the Ct of positive control were 26.58, 27.02 for IS1111 and IS30a respectively (Corresponding to **6.8** and **6.7 log10 number of DNA copies/mL**). For that we had difficulties to be able to sequence and genotype these positives samples.

Table I, shows all the *Coxiella burnetii* IFA serology and IS1111; IS30a qPCR results obtained in this study.

The whole blood of the positive qPCR patient, goes also for positive *Coxiella burnetii* IFA serology; however, the two other positive IFA came back negative. Overall, we had 03/70 positive cases among the cases group *VS* 00 positive cases for the control group, which suggests that these results are statistically no significant **(NS)** with a p value of **0.258 (p=0.258)**. (Using application of the **Yates correction for Chi-square**; level signification (S) when **p** value is **≤0.05**).

Table I. *Coxiella burnetii* IFA Serology & IS1111, IS30a qPCR results for a control and a cases groups.

Groups	Positive sera	*Coxiella burnetii*			IS1111/ IS30a qPCR
		IgG	IgM	IgA	
Control group	00/70	-	-	-	00/70
Cases group	03/70 *NS p=0.258 (4 .30%)				01/70 (1.43%)
	N°1	100	00	00	n
	N°2	100	00	00	n
	N°3	200	100	200	Ct 33.21/33.74

n: negative. *All sera are screened as first-line with **Total **I**mmuno**g**lobulin. if the serum is positive at 1/100 dilution, then the antibodies present in this sample are differentially quantified (IgG, IgM, IgA). * level signification (S) when p value is ≤0.05*. NS: No significant.

Positive cases description

The three positive patients for *Coxiella burnetii*, present different clinical signs, which are summarized in table II. We find that unexplained febrile syndrome is a common sign between the three patients. In addition, two patients (First and Second cases) were diagnosed with meningeal syndrome. The third case had presented a significant algae syndrome, maculopapular rash without pruritus, hepatic hilar adenopathies, where also a visceral Leishmaniosis was diagnosed. The third patient presented an hyperleukocytosis, **CRP at 42 mg/L**, Thrombocytopenia at **89000 /mm³,** and a hepatic cytolysis at **5** times the normal; this disturbed

biological statute may explain the underlying infection with *Coxiella burnetii* beside the visceral leishmaniosis.

Table II. Epidemiologic data, clinical manifestations and biological findings of the positive *Coxiella burnetii* cases.

	First case CB IFA +/ qPCR-	**Second case CB IFA +/ qPCR-**	**Third case CB IFA +/ qPCR+**
Age/Sex	Girl,**08** years	Miss, **18** years	Miss, **19** years
Fever	Prolonged unexplained fever,	Prolonged unexplained fever	Prolonged unexplained fever
Diagnosis	Meningeal syndrome with brutal installation for 2 days,	Meningeal syndrome	Visceral Leishmaniosis,
Clinical signs	Arthralgia, Large joints, Purpuric rash, Asthenia,	Nothing special, Cerebral TDM: normal,	Significant algae syndrome, Maculopapular rash without pruritus, Hepatic hilar adenopathies,
Biological findings	Meningeal Lumbar puncture: -Cerebrospinal fluid clear, **1800/mm^3** elements, -Albumin = **0.52 g/l,** -Normal glucose level:**0.48 g/L.** CBC normal, **CRP < 6**,	Meningeal Lumbar puncture: - Cerebrospinal fluid clear, **700/mm^3** elements, -Albumin = **0. 94/l,** -Normal glucose level: **0.43 g/L**. **CRP < 6,**	**CBC** with hyperleucytosis, **CRP at 42 mg/L**, Thrombocytopenia at **89000 /mm^3,** Hepatic cytolysis at **5** times the normal,
Contact with animal	Cats, sheep, Dog.	Sheep, Cattle.	Goat, Sheep, Camels, Hare.
Evolution	Good.	Average.	evolution (exit hospital against medical advice).

Discussion

Northern Algeria is a hot and humid region, where vector-borne infectious diseases occur during the summer season. Q fever is an antropozoonosis which hasn't had enough attention in terms of studies and epidemiological surveillance in Algeria. Since **1948,** the Q fever has caused a major epidemic, then other outbreaks have been reported in Batna on **1957** and in Tlemcen in **1958 [10]**. Furthermore, there was no image or situation of human Q fever in said country, other than a few scattered studies that have been done; Dumas N,**1984**; Lachehab, **2009, [10,11]**.

Going from this unclear background, we thought it would be appropriate to conduct this study, which is focused on the identification of patients positive to *Coxiella burnetii* at the National Center of Infectious Diseases in Algiers; **ELHADI FLICI Hospital**, by serological and molecular identification of *Coxiella burnetii* the etiological agent of the Q fever disease.

A total of **140** patients were enrolled in this study, between July and October **2017**. This period includes Summer and Autumn where vectorial and tropical diseases rage. **One hundred forty (140)** sera and whole blood were assessed for serological and molecular identification of *Coxiella burnetii* infection. The seroprevalence of Q fever among the cases group was **4.3%,** and all the control group had a serology negative. These results prove the existence of *Coxiella burnetii* infection in the study population.

Despite being found throughout the world, the seroprevalence of *Coxiella burnetii* differs according the regions and occupations **[16,17]**.

Lachehab in **1996 [10]**, conducted a study in Eastern Algeria, then he reported that the population seroprevalence was estimated to be **16%**, confirming the endemic presence of Q fever in Algeria. These results were published in **2009**, where he also reported the results obtained by Dumas, **1984 [11]**; this study was conducted on slaughterhouse workers from Algiers, with **15%** seroprevalence; Lachehab reported also the unpublished seroprevalence of

14.19% given by TEBBAL in Eastern Algeria (Aures). This high prevalence may reflect the high number of sheep in Eastern Algeria. These seroprevalence are also comparable with ours, **4.30%** of seroprevalence could point to the presence of *Coxiella burnetii* in the Algerian northern region, and also may reflect the zoonotic aspect of Q fever, pointing out that northern Algeria is well known by the extension for various cattle, sheep and poultry farms; which remain major sources of *Coxiella burnetii* infection. The period of our study coincided with the summer season when vector-borne diseases are rampant, and also coincided with **Aid EL-AD'HA**, a Muslim religious feast, where Algerians would be in close contact with sheep, so zoonosis transmitted from sheep to humans can occur, that's why we have paid attention to the diagnosis of Q fever in this period in particular.

In Algeria's eastern region and, in Tunisia, others studies conducted among the febrile patients suspected to *Coxiella burnetii*; **Bellazreg 2009 [18]**, reported **21** cases of acute Q fever in hospitalised patients in central Tunisia. On the other hand, **Letaief 1995 [19]**; **Kaabia 2006 [20]**, reported seroprevalence by IFA of **26%** and **5.88%**, among blood donors and febrile patients respectively, in favour of acute Q fever. In Algeria's other western regions, in Morocco, **Meskini and Raoult, 1995 [21]**, reported seroprevalence by Western Immunoblotting for *Coxiella burnetii* phase II, of **1%** and **18.3%** in Casablanca, Fez respectively, for a total population study of **426** patients. In these regions seroprevalence remains fairly comparable to our seroprevalence or others previously obtained in Algeria. These findings confirm the presence and the dispersion of *Coxiella burnetii* over different northern Maghreb regions.

Furthermore, in Senegal, **Mediannikov** in **2010 [22],** reported a prevalence of **3.7%** and **24.8%** in patients of two villages in rural Senegal. Otherwise, **Angelakis** in **2014 [23]**, he had **2.2%** and **0.3%** seroprevalence in two other villages in Senegal. These results confirm that the distribution of *Coxiella burnetii* in a country would depend on environmental parameters and

factors, which may or may not favour infection, excretion and contamination of different animal species or Humans by *Coxiella burnetii*.

In Asia, similar studies have been carried out in Turkey, Lebanon, Iraq and Iran by **Cikman** (**2017**) **[7], Dabaja** (**2018**) **[24], Hamilton** (**2011**) **[25]** and **Mobarez** (**2017**) **[26]**, respectively. Seroprevalence varies from country to another, the highest was recorded in Iraq on US soldiers, **50%** of suspected Q fever patients had an acute infection. Seroprevalence in Lebanon and Iran, were very close; **37.05%** and **32.86%;** which explains the endemic aspect of *Coxiella burnetii* in these countries. Equally, Czerwinski, Poland 2015 **[27]**, reported *Coxiella burnetii* IFA seroprevalence of **31.12%** among farm-workers.

These different seroprevalences of *Coxiella burnetii* in countries located in different continents, allows us to say that the human infection status confirmed the worldwide high exposure of patients to *Coxiella burnetii*; which implicate the need for a strong improvement of public health awareness against this disease.

According to our molecular findings, among our cases group, only one (**01/70, 1.42%**) whole blood q PCR became positive, while all the control group patients had q PCR negative. A similar study was conducted in Madagascar by **Boone** in **2017 [6]**, where he reported no *Coxiella burnetii* q PCR positive among **1200** whole blood collected. Furthermore, **Czerwinski [27]**; reported **6.62%** q PCR for *Coxiella burnetii* among **151** whole blood patients. **Hamilton [25]**; found **9** positive IFA serology among **18** sera patients; within these **9** positive patients, only **6** became positive to *Coxiella burnetii*.

Concerning the animal Q fever, many studies were conducted in Algeria. **Khaled** in **2016 [14]**, conducted a study in order to identify the positive sources of Q fever in Algeria; where he found a seroprevalence of **14.1%** among small ruminant's flocks, and he determined the shedder flocks of *Coxiella burnetii* via the vaginal swab with **21.3%** qPCR positive. Whereas, **Bessas**

[13] and **Aouadi [15]** in the same year **(2016)**, reported the presence of *Coxiella burnetii* by qPCR in spleen dog **(0.80%)** and blood/ticks of small ruminant **(4.73%)** respectively. These results could lead to the various sources of *Coxiella burnetii* contamination in Algeria.

In light of the results obtained in this study, and their comparison with those obtained and discussed above, we wish to say that human Q fever still exists in Algeria at nonspecific febrile illness patients, and it remains a major public health risk. It is also necessary to ring the alarm bell at the guardianship in order to strongly improve public health awareness and mobilize the various public health actors to face this threatening disease, and also to better identify the sources of contamination and excretion routes of *Coxiella burnetii*; thus we will be able to prevent and treat this disease in optimal time. In prospects, we would like to carry out another study with a higher sampling rate and on other regions in Algeria in order to highlight a clearer picture of *Coxiella burnetii* in Algeria.

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Conflict of interests

The authors declare that they have no competing interests.

References

[1]. Oyston P.C.F and Davies C. Q fever: the neglected biothreat agent. Journal of Medical Microbiology. 2011; 60; 9-21.

[2]. Pavia C.S, McCalla C. Serologic detection of a rare case of Q fever in New York City having hepatic and unusual renal complications. Infection. 2010; 38; 325-329.

[3]. Raoult D, Marrie T. Q fever. Clin Infect Dis off Publ Infect Dis Soc Am. 1995; 20:489-95.

[4]. Raoult D, Tissot-Dupont H, Foucault C, Gouvernet J, Fournier PE, Bernit E, et al. Q fever 1985-1998. Clinical and epidemiologic features of 1,383 infections. Medecine (Baltimore). 2000; 79 :109-23.

[5]. Van der Hoek W, Versteeg B, Meekelenkamp JC, Renders NH, Leenders AC, Weers-pothoff I, et al. Follow-up of 686 patients with acute Q Fever and detection of chronic infection. Clin Infect Dis. 2011. 52;12:1431-6.

[6]. Boone I, Henning K, Hilbert A, Neubauer H, Kalckreuth V et al. Are brucellosis, Q fever and melioidosis potentiel causes of febrile illness in Madagascar. Acta Tropica 2017: 255-262.

[7]. Cikman A, Aydin M, Gulhan B, Karakecili F, Ozciceck A, et al. The seroprevalence of *Coxiella burnetii* in Erzincan, Turkey: Identification of the risk factors and their relationship with geographical featues. J Vector Borne Dis. 2017;54: 157-163.

[8]. Raoult D. Re-emergence of Q fever after 11 September 2001. Clin Infect Dis. 2009;48: 558-559.

[9]. FOURNIER PE,Marrie T.J, and RAOULT D. Diagnosis of Q Fever. Journal Of Clinical Microbiology. 1998 July: 1823–1834.

[10]. Lachehab A, Raoult D. Seroprevalence of Q fever in Algeria. European Society of Clinical Microbiology and Infectious Diseases. 2009 ;15 : 167-168.

[11]. Dumas N. Rickettsioses et Chlamydioses au Hoggar (République Algérienne) : sondage épidémiologique. Bull Soc Pathol Exot 1984 ;77 :278-283.

[12]. Benslimani A, Fenollar F,Lepidi H, and Raoult D. Bacterial zoonoses and infective Endocarditis, Algeria. Emerging Infectious Diseases. 2005;11: No. 2.

[13]. Bessas A, Leulmi H, Bitam I, Zaidi S, Ait-Oudhia K, Raoult D, Parola P. Molecular evidence of vector-borne pathogens in dog and cat and their ectoparasites in Algiers, Algeria. Comparative Immunology, Microbiology and Infectious Diseases.2016;45: 23-28.

[14]. Khaled H, Sidi-Boumedine K, Merdja,P , Dahmani A, Rousset RTE ,.Bouyoucef.A. Serological and Molecular evidence of Q fever among small ruminant flocks in Algeria. Comparative Immunology, Microbiology and Infectious Diseases.2016;47: 19-25.

[15]. Aouadi A, Leulmi H, Boucheikhchoukh M, Benakhla A, Raoult D, Parola P. Molecular evidence of tick-borne hemoprotozoan-parasites (*Theileria ovis and Babesiaovis*) and bacteria in ticks and blood from small ruminants in Northern Algeria. Comparative Immunology, Microbiology and Infectious Diseases.2017;50: 34-39.

[16]. Kilic S,Yilmaz GR, Komiya T, Kurtogu Y, Karakoc EA. Prevalence of *Coxiella burnetii* antibodies in blood donors in Ankara, Central Anatolia, Turkey. New Microbiol. 2008;31:527-34.

[17]. Schelling E, Diguimbaye C, Daoud S, Nicolet I, Boerlin P, Tanner M, et al. Brucellosis and Q fever seroprevalences of nomadic pastoralists and their livestock in Chad. Prev Vet Med 2003;61: 279-93.

[18]. Bellazreg F, kaabia N, Hachfi W, Khalifa M, Ben jazia E, Ghanouchi N, Brahem A, Bahri F and Letaief A. Acute Q fever in hospitalised patients in Central Tunisia: report of 21 cases. European Society of Clinical Microbiology and Infectious Diseases .2009;15: 138-139.

[19]. Letaief A, Yacoub S, Dupon HT, LE Cam C, Ghachem L, Jemni L, Raoult D. Seroepidemiological survey of rickettsial infections among blood donors in central Tunisia. Trans R Soc Trop Med Hyg. 1995;83: 266-8.

[20]. kaabia, Rolain JM, Khalifa M,Ben Jazia E, BahriD F. Raoult D, and Letaief A. Serologic study of Rickettsioses among Acute Febrile Patient in Central Tunisia. N. Y Acad.Sci 1078. 2006; 176-179.

[21]. Meskini M, Beati L, Benslimani A, Raoult D. Seroepidemiology of Rickettsial infections in Morocco. Eur J Epidemiol, 1995;11: 655-60.

[22]. Mediannikov O, Fenollar F, Socolovschi C, Diatta G, Raoult D, et al. *Coxiella burnetii* in Humans and Ticks in Rural Senegal. Neglected Tropical Diseases. 2010;4: e654.

[23]. Angelakis E, Mediannikov O, Socolovschi C, Mouffok N, Bassene H, Tall A, Niangaly H, Raoult D, and al. *Coxiella burnetii*-positive PCR in febrile patients in rural and urban Africa. International Journal of Infectious Diseases .2014;28: 107-110.

[24]. Dabaja MF, Greco G, Villari S, Bayan A, Mortada M, and all. The First Serological Study of Q fever in Humans in Lebanon. Vector-Borne and Zoonotic Diseases. 2018.

[25]. Hamilton LTC LR, Dena M, George L, Scoville SL, and all. PCR for Rapid Diagnosis of Acute Q Fever at a combat Support Hospital in Iraq. Military Medecine. 2011;176, 1:103.

[26]. Mobarez AM, Bagheri F, Esmaeili S. Seroprevalenec of Q Fever among human and animal in Iran: A Systematic review and meta-analysis. Neglected Tropical Diseases. 201711 e0005521.

[27]. Czerwinski MS, Galinska EM, Niemczuk K, and Piotr-Knap J. Prevalence of *Coxiella burnetii* Infection in Humans Occupationally Exposed to Animals in Poland. Vector-borne and Zoonotic Diseases. 2015;15.

[28]. Maurin M, Raoult D. Q Fever. Clin Microbiol Rev. 1999 ; 12 :518-53.

[29]. Devine P. Diagnosis of Q fever. J Clin Microbial 1998;36:3446.

Partie III :

Contribution à l'étude de Coxiella burnetii l'agent causal de la fièvre Q chez différentes espèces animales en Algérie.

Avant-propos.

Fièvre Q chez l'Homme, ou Coxiellose chez le bétail, c'est une zoonose causée par *Coxiella burnetii*. Chez l'animal, la fièvre Q est principalement subclinique, mais elle a surtout été associée à des troubles de la reproduction tels que des avortements tardifs, des mortinatalités, une faiblesse au printemps, métrite et une infertilité chez les ruminants [9]. Le diagnostic sérologique de la fièvre Q au stade précoce de l'infection peut être infructueux en raison de la période de séroconversion qui s'étend sur **3-4** semaines après l'infection [9]. La littérature a montré qu'au moins **24 %** des chèvres séronégatives excrétaient la bactérie [1]. Par conséquent, la **PCR** est la méthode de choix pour retracer les excréteurs lorsque les résultats sérologiques positifs sont trouvés dans un troupeau [7]. En principe, le diagnostic de la fièvre Q en laboratoire doit être basé sur l'interprétation des résultats obtenus par différents types de méthodes de détection de la réponse sérologique et de la présence d'un agent pathogène. Les études de séroprévalence animale ont révélé une infection par *Coxiella burnetii* **(≤13%)** chez les bovins, à l'exception des études menées en Afrique occidentale et centrale **(18-55%)** [22]**.** En général, la séroprévalence des petits ruminants variait de **11 à 33 %**. Le génotypage par **MST**, a été introduit par Glazunova et al en **2005**, qui ont identifié **10 Spacers** très variables. Cette méthode est très discriminante et elle a été utilisée le plus fréquemment dans différentes études à travers le monde. Le génotypage par **MST** aide à retracer la dissémination de *Coxiella burnetii* d'une région à l'autre et des réservoirs animaux aux humains. Certains **MST** sont présents sur les cinq continents, tandis que d'autres sont très spécifiques aux situations épidémiques [9].

Visant à identifier les réservoirs et les sources de contamination, et les caractéristiques génétiques de *Coxiella burnetii* dans le nord de l'Algérie, nous avons démontré la présence moléculaire de *Coxiella burnetii* dans des échantillons de nature différente chez les bovins, les

ovins, les chiens et les chats, provenant des élevages bovins et ovins situés dans le nord-est de l'Algérie, et des ruminants abattus dans l'abattoir d'Alger, ainsi que des chiens et chats errants de la fourrière canine d'**El HARRACH**-Alger. À cet égard, un total de **599** échantillons ont été prélevés, dans divers échantillons de sang, de placenta, de foie, de rate et d'utérus. Nos résultats q PCR ont montré que sur **344** échantillons de sang total, seulement **15 (4,36 %)** étaient positifs pour *Coxiella burnetii*, alors que seulement **06 (2,35 %)** échantillons positifs sur un total de **255** échantillons d'organes collectés. Chez les bovins, ni les échantillons de rate ni ceux d'utérus n'ont donné des résultats positifs en q PCR, cependant, **03 (4%)** échantillons positifs ont été trouvés dans des échantillons de sang et de foie dans chacun d'eux. De plus, tous les échantillons de sang de mouton à l'abattoir étaient négatifs, seulement au niveau des fermes au nord est algérien, **01 (1,19%)** échantillon de sang de mouton a donné un résultat positif q PCR, et **03 (8,57%)** échantillons de placenta étaient positifs. A la fourrière canine d'Alger, **08 (10%)** et **03 (5%)** échantillons de sang ont montré des q PCR positives pour *Coxiella burnetii* chez les chiens et les chats respectivement. En outre, le génotypage **MST** a montré que le **MST 33** a été génotypé dans des échantillons de sang des bovins et des ovins, ainsi que dans des échantillons de placenta des ovins. Alors que, le sang total des chiens et de chats, a donné **MST 21**. De plus, le **MST 20** a été détectée dans des échantillons de foie de bovins.

A l'issue de cette étude, face à la réalité que *Coxiella burnetii* est hébergée dans des élevages de ruminants, des abattoirs, des chiens et des chats errants, nous devons insister pour sonner l'alarme devant les différents acteurs de santé publique en Algérie, et demander que les mesures et précautions nécessaires soient prises pour limiter la contamination et la propagation des *Coxiella burnetii*. En plus, nous devons sensibiliser le personnel à risque du fait que de bonnes pratiques d'hygiène pendant l'élevage et l'abattage du bétail sont un moyen important de réduire le risque de propagation des maladies infectieuses.

Article 3:

Molecular Detection and MST Genotyping of Coxiella burnetii in Ruminants and Stray Dogs and Cats in Northern Algeria.

GHAOUI H, ACHOUR N, SAAD-DJABALLAH A, Zaidi S, Zenia S, BITAM I, FOURNIER PE, RAOULT D

Molecular Detection and MST Genotyping of *Coxiella burnetii* in Ruminants and Stray Dogs and Cats in Northern Algeria

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Abstract.

Aiming to identify the reservoir and contamination sources of *Coxiella burnetii* in Northern Algeria, we demonstrated the molecular presence of *Coxiella burnetii* in samples of a different nature in cattle, sheep, dog and cat. In this respect, a total of **599** samples were collected, in various samples nature, blood, placenta, liver, spleen, and uterus. Our qPCR results showed that among **344** whole blood samples, only **15 (4.36%)** samples came back positives for *Coxiella burnetii*, while just **06 (2.35%)** positive samples from a total of **255** organ samples. In cattle, neither spleen samples nor uterus samples came back positive in qPCR. However, **03 (4%)** positive samples were found in blood and liver samples in each one. Moreover, all sheep's blood samples in the slaughterhouse were negative, only **01 (1.19%)** sheep blood sample gave positive q PCR, and **03 (8.57%)** placenta's samples were positive. At the Algiers dog pound, **08 (10%)** and **03 (5%)** blood samples showed qPCR positive for *Coxiella burnetii* in dogs and cats respectively. In addition, The **MST** genotyping showed that **MST 33** was genotyped in blood samples from cattle and also sheep, and also found in sheep placenta' samples. Whereas,

the total dog and cat blood, gave common **MST 21**. Furthermore, the **MST 20** was detected in bovine liver samples.

Key words: *Coxiella burnetii, MST genotyping, Q Fever Algeria, qPCR, Ruminants, Stray cats and dogs, Zoonosis.*

Introduction

Q fever in Humans, or Coxiellosis in Livestock, it is zoonosis caused by *Coxiella burnetii* **[1]**. This disease is regarded as endemic worldwide, with the exception of New Zeeland **[2]**. After the recent outbreaks occurred in Europe, *Coxiella burnetii* infection gained a renewed attention as an emerging disease impacting public health and livestock animal too **[1,3]**. In animals, Q fever is mainly subclinical but has especially been associated with reproductive disorders such as late abortions, stillbirths, weak off springs, metritis and infertility in ruminants **[2]**. The bacterium has a reservoir in a world-wide range of animal hosts; including mammals, arthropods, birds, fish, rodents, and reptiles **[4]**. Ruminants and ticks may play a major role in maintaining of the domestic cycle and the wild cycle respectively. The domestic cycle is considered as the main human source of infection, consequently the link between the wild and domestic cycle is poorly understood **[5]**. Domestic pets, as cats, dogs and wild-domestic birds are known to be an additional source of infection **[2]**. The main route of *Coxiella burnetii* infection, is the inhalation of contaminated aerosols, beside this route, the significance of ticks in transmitting the disease in ruminants and human has previously been documented. The bacteria are shed by mammals in urine, feces, milk, or birthing products and are very resistant to degradation **[4]**.

The dried spore-like material in the environment remain viable under a variety of environmental conditions, this high stability is due to the ability to the extracellular form. It has been reported that a very low infective dose (less than **10** organisms) can produce disease **[4,6]**. All these

characteristics made of this bacteria a potential bioterrorist agent. Goat outbreaks are generally associated with human exposures, and cattle are considered asymptomatic carries **[4]**.

Diagnosis of Q fever based on clinical symptoms or post-mortem examination is very difficult or almost impossible due to unspecific or missing symptoms or lesions caused by this disease **[7]**. The golden diagnosis tool remains serological, by the Immunofluerescence Assay. However, the detection of *Coxiella burnetii* shedding animals is not always possible by this serological method. Moreover, serological diagnosis of Q fever in the early stage of infection can be unsuccessful due to the time-frame of seroconversion spanning 3-4 weeks post infection **[7]**. The literature showed that at least **24%** of seronegative goats shed the bacteria. Consequently, the PCR is the method of choice to trace shedders when the positive serological results are found at the herd. Primarily, laboratory diagnosis of Q fever should be based on the interpretation of results obtained by different kind of methods both detecting the serological response as well as the presence of pathogen **[7]**.

Animal seroprevalence studies revealed infection by *Coxiella burnetii* **(≤13%)** among cattle except for studies in western and middle Africa (18-55%). Generally small ruminants seroprevalence ranged from 11-33% **[3]**.

MST genotyping, was introduced by Glazunova et al., who identified **10** highly variable spacers located between ORFs. This method is very discriminant and has been used most frequently in different studies around the world **[8]**. MST genotyping helps to trace the spread of *Coxiella burnetii* from one region to another and from animal reservoirs to humans. Some MSTs are present across the five continents, whereas others are very specific to epidemic situations. For example, MST 20 has been described in ruminants in Europe and in humans and ruminants in the United States, suggesting a spread of the disease by infected animals historically brought to the New World. In contrast, MST 17 has been isolated only from French Guiana to date, causing severe forms of the disease **[9].**

In Algeria, and other African countries, the animal Q fever seroprevalence is poorly known due to the lack of the diagnostic tools and also to the poor management of livestock farms. In addition, there is a glaring lack of relative research work to *Coxiella burnetii* animal nor human infection. However, research efforts have been carried out in Algeria on animal Q fever, on sheep, camels as well as ticks; where they have identified the presence of *Coxiella burnetii* serologically in animal sera, and molecularly and on ticks **[10,11,12]**. In light of these reasons, our study aims to detect the presence of *Coxiella burnetii* in different animal species in the Northern Algeria, as ruminants and stray dogs and cats, in order to identify the reservoir & contamination sources, and also to determine the genetic background of these sources using the **MST genotyping** method.

Material & Methods.

In order to study the animal cases for *Coxiella burnetii* & identify the reservoir & contamination sources, we carried out our samples on sheep and cattle farms, and on ruminants in the slaughterhouse, and also on cats and dogs at the Algiers dog pound. Between Marsh and October **2017**, we collected a whole blood, placenta, liver, spleen, uterus samples from the different animal species as described in **Table 1**.

Animals' selection and inclusion criteria.

First, we established a plan for selecting animals that best meet the pre-established selection criteria based on concerned species, and also the nature of the sample to be collected.

On Cattle farms, we selected females aged more **2** years (≥ 2 years), without special antecedents during the last year, clinically healthy, this selection is due to detect infectious sources of *Coxiella burnetii* among healthy livestock. We collected **60 whole blood samples** from jugular vein, using a safety BD Eclipse sampling needles, adapted to the vacutainer system, ***21 Gauge***, in **EDTA** tubes. The concerned farms situated in **Bouira** and **Bejaia** provinces, North-eastern Algeria.

On Sheep farms, in order to collect blood and placenta samples, we selected ewes with abortions' history and also those who had just given birth, thus we have more chance of identifying a *Coxiella* profile on these farms. A total of **34** whole blood and **35** placenta samples were collected, blood samples as described up, and the placenta in dry tube, from farms located in the same provinces listed up (**Bouira** and **Bejaia** provinces, situated in Northeastern Algeria).

On slaughterhouse, aiming to collect blood and organs samples from ruminants intended for slaughter, our study was conducted at **EL-HARACH, Algiers,** slaughterhouse, situated in Eastern Algiers (in the capital). On sheep and cattle females, whole blood was collected before slaughter, from jugular vein, while organs sampling was occurred after slaughter (liver, spleen uterus). The choice of organs was made on the basis of the target organs of *Coxiella burnetii*. A total of **60**, **75**, **75**, and **70** samples, from blood, liver, spleen, and uterus were collected from cattle respectively, while **50** blood samples were collected from sheep.

Stray dogs and cats. Aiming to screening for the presence of *Coxiella burnetii* organisms in stray cats and dogs in the region of Algiers, in the absence of any data available on that topic, we collected blood samples from dogs and cats that were captured in the **dog/cats pound** situated in eastern Algiers. These animals were captured in the **57** municipalities of the region of Algiers. Using an intracardial injection, blood samples were collected aseptically from the **3-4** intercostal space, using a **10 cc** syringe. A total of **80** and **60** blood samples were collected from dogs and cats respectively.

Sample's collection.

A total of **599** samples comprising whole blood/organs samples from the different animal species listed before. The samples were collected aseptically in suitable tubes; the organs in dry tube and the whole blood in **EDTA** tubes (EDTA: Ethylene-diaminetetraacetic acid). The samples were conserved at **-20°c** to handle them at the **URMITE** (Emerging Tropical Infectious

Diseases Research Unit at the Institut Hospitalo-Universitaire (**IHU**) Marseille; for q PCR for *Coxiella burnetii* targeting the IS1111 and IS30a genes.

Table 01. Number of samples collected according to animal species.

Animal species	**Samples' Number**				
	Whole Blood	**Placenta**	**Liver**	**Spleen**	**Uterus**
Cattle	120	-	75	75	70
Sheep	84	35	-	-	-
Dog	80	-	-	-	-
Cat	60	-	-	-	-

DNA extraction & qPCR.

A total of **200** µL of DNA was extracted using the QIAamp Tissue Kit by QIAGEN-BioRobot EZ1, according to the manufacturer's instructions (Qiagen, Hilden, Germany). Extracted DNA was stored at **-20**°C under sterile conditions until it was used in PCR assays. Extracted DNA was used in qPCR amplifications to detect *Coxiella burnetii* targeting ths specific genes IS1111 and IS30A. The final qPCR reaction mixture consisted of **5** µL DNA with **15** µL of mix from the Roche PCR Kit (Roche Diagnostics, Meylan, France). The PCR cycling parameters for the qPCR were 5 min at **95°C** followed by **39** cycles each consisting of **5** sec of denaturation at **95°C** and **30** sec of annealing at **60°C**.

Standard PCR, Multispacer sequence typing and sequence analysis.

Samples that tested positive by qPCR with Cycle threshold (**Ct)** values inferior to **32** were confirmed by standard PCR and sequencing in order to achieve a **100%** specificity. For the standard PCR, we used specific genes spacers with higher variation for differentiating the genotypes of *Coxiella burnetii*, we tested the following spacers: Cox2, Cox5, Cox18, Cox20, Cox22, Cox37, Cox56, Cox57, and Cox61, the Reverse/Forward probe sequence, amplified length, and hybridization of each spacer Cox are summarized in the table 2. The amplified PCR products were separated via gel electrophoresis using a 1.5% agarose gel stained with Sayber Safe (ThermoFisher, Paris, France). The DNA bands were visualized and photographed under ultraviolet light. PCR products were purified using the PCR filter plate Millipore Nucleo Fast 96 PCR kit in line with the manufacturer's recommendations (Macherey Nagel, Düren, Germany), and sequenced with *Cox spacers* as described Glazunova et al, 2005. All obtained sequences were assembled and edited using Chromas Pro (version 1.7.7). Then, Sequence types were determined using the MST database for MST *Coxiella burnetii*, of TIMONE, Marseille university-France: http://www.mediterranee-infection.com/article.php?larub=143&titre=base-de-donnees . The results were coupled with Excel file combination spacers to be able to identify the MST groups.

Table 2. Primers used for PCR amplification and sequencing of *Coxiella burnetii* genes spacers **[8].**

Spacers name	Nucleotide sequence	Hybridization	Amplified length (bp)
Cox 2F	Cox20766 CAACCCTGAATACCCAAGGA	59>>**56°C**-40 cycles	358 bp
Cox 2R	Cox21004 GAAGCTTCTGATAGGCGGGA	59>>**56°C**-40 cycles	
Cox 5F	Cox77554 CAGGAGCAAGCTTGAATGCG	59>>**56°C**-40 cycles	344 bp
Cox 5R	Cox77808 TGGTATGACAACCCGTCATG	59>>**56°C**-40 cycles	
Cox 18F	Cox283060 CGCAGACGAATTAGCCAATC	59>>**56°C**-40 cycles	556 bp
Cox 18R	Cox283460 TTCGATGATCCGATGGCCTT	59>>**56°C**-40 cycles	
Cox 20F	Cox365301 GATATTTATCAGCGTCAAAGCAA	59>>**56°C**-40 cycles	585 bp
Cox 20R	Cox365803 TCTATTATTGCAATGCAAGTGG	59>>**56°C**-40 cycles	
Cox 22F	Cox378718 GGGAATAAGAGAGTTAGCTCA	59>>**56°C**-40 cycles	340 bp
Cox 22R	Cox378965 CGCAAATTTCGGCACAGACC	59>>**56°C**-40 cycles	
Cox 37F	Cox647471 GGCTTGTCTGGTGTAACTGT	59>>**56°C**-40 cycles	422 bp
Cox 37R	Cox657794 ATTCCGGGACCTTCGTTAAC	59>>**56°C**-40 cycles	
Cox 56F	Cox886418 CCAAGCTCTCTGTGCCCAAT	59>>**56°C**-40 cycles	440 bp
Cox 56R	Cox886784 ATGCGCCAGAAACGCATAGG	59>>**56°C**-40 cycles	
Cox 57F	Cox892828 TGCAAATGGAAGGCGGATTC	59>>**56°C**-40 cycles	605 bp
Cox 57R	Cox893316 GGTGGAAGGCGTAAGCCTTT	59>>**56°C**-40 cycles	
Cox 61F	Cox956825 GAAGATAGAGCGGCAAGGAT	59>>**56°C**-40 cycles	569 bp
Cox 61R	Cox957249 GGGATTTCAACTTCCGATAGA	59>>**56°C**-40 cycles	

Statistical analyses:

In order to calculate the confidence interval and the significance level (p values) of the various results obtained, we used the web application (http://epitools.ausvet.com.au/content.php?page=CIProportion&SampleSize=127&Positive=7&Conf=0.95&method=1&Digits=4), as well as the application of the G Test (Log-Likelihood ratio). P-values less than 0.05 were considered statistically significant; and P-values between 0.05 and 0.1 were considered as Non-significant.

Ethics statement.

To allow us for the sampling at the slaughterhouse in Algiers, we received the authorization signed by the veterinary inspector responsible for the slaughterhouse, also for samples at the canine pound in Algiers we requested an access authorization from the HERBAL department

which manages this canine pound in order to be able to sample the animals. Concerning the sampling of farms, we have had the agreement of the farmers and also of the veterinarians who monitor these farms. Thereafter, to be able to transfer samples of animal origin from Algiers to IHU-Marseille, France, we requested a prior authorisation for the import into France of research and diagnostic samples of animal origin from countries outside the European Union. In **02/11/2017**, we had the agreement of Chief of the Animal Health and Production Department, Environment, of the Bouches-du-Rhone prefecture in Marseille. The samples were received in IHU-Marseille the **13/11/2017**.

Results.

From a total of **599** samples comprising whole blood/organs samples from the different animal species listed before, q PCR targeting the IS1111 and IS30A, and the MST genotyping, show promising results that deserve to be discussed.

Detection of *Coxiella burnetii* by quantitative q PCR.

q PCR was used for the detection of *Coxiella burnetii* in whole blood and organs samples, by employing *Coxiella burnetii* –specific primers and a probe designed to amplify the IS1111 gene and confirmed by the second gene IS30a which remains highly *Coxiella burnetii* -species specific. The table **3**, shows the q PCR positive samples according to each animal species.

Among **344** whole blood samples, only **15 (4.36%)** samples came back positives for IS1111 and IS30A too. Moreover, **06 (2.35%)** positive samples from a total of **255** organ samples for IS1111 and IS30a. Using the G Test (Log-Likelihood ratio), for blood samples the difference was significant with $\mathbf{p = 0.035} < 0.05$, furthermore, between ruminants' blood and the stray dogs and cats' blood, we note also high significant difference with **p = 0.0085**.

The **Ct** values of these positive q PCR are ranged from **19.77 to 31.35** corresponding to **8.8** log **10** DNA copies/ml and **5.4** log **10** DNA copies/ml, which are considered loaded positive. No swabs come back positive for the spleen and uterus, while *Coxiella burnetii* DNA was found in blood and liver bovine. Namely, the blood positive subjects are not the same for the liver, one (**01**) positive belong to the slaughterhouse in Algiers, and the two others belong to the bovine farms in North-eastern Algeria. The positivity of *Coxiella burnetii* in bovine liver, testifies the presence of the pathogen in the slaughterhouse, and it puts the slaughterhouse staff at major risk. However, all sheep in the slaughterhouse were negative, while the four positive ones belong to the farms described previously. The positive sheep placenta for *Coxiella burnetii*, it questions the way in which the bacteria are excreted via parturition products. One (**01**) sheep subject had both blood and placenta positive q PCR, which lead to suppose the bacteraemia around the calving process. Eight (**10%**) and Three (**5%**) blood samples belonging to stray dogs and cats respectively, which explains the major role that stray dogs and cats play in the transmission of *Coxiella burnetii* in urban areas. all these positive results, demonstrate the presence of *Coxiella burnetii* in small ruminant farms, in the slaughterhouse, and also in the dog pound in northeast Algeria.

Table 3. q PCR results for IS1111 and IS30A in blood and organs samples.

Species	Positive q PCR Samples									
	Blood (%), 95% CI		**Placenta** (%), 95% CI		**Liver** (%), 95% CI		**Spleen** (%), 95% CI		**Uterus**	
	+	**-**	**+**	**-**	**+**	**-**	**+**	**-**	**+**	**-**
Cattle	3 [2.5%] 2.49 to 2.55	117 [97.5%] 96.57 to 98.49	--	--	3 [4.00%] 3.99 to 4.08	72[96.0%] 95.08 to 97	0	75	0	75
Sheep	1 [1.19%] 1.17 to 1.22	83 [98.80%] 97.83 to 99.81 ***p = 0.0085** **Blood Rts Vs Blood Dog & Cat**	3 [8.57%] 8.56 to 8.75	32 [91.42%] 90.59 to 92.43	--	--	--	--	--	--
Dog	8 [10.0%] 9.96 to 10.16	72 [90.0%] 89.16 to 90.97	--	--	--	--	--	--	--	--
Cat	3 [5.0%] 4.99 to 5.10	57 [95.0%] 94.10 to 96.0	-- ***p = 0.035** **(Blood Vs Organs)**	--	--	--	--	--	--	--

CI, confidence interval; qPCR, real-time quantitative PCR. Rts Ruminants *Level of significance when p _ 0.05

MST genotyping results.

Aiming for genotyping the positive q PCR samples, we used **9** genes spacers specific of *Coxiella burnetii* by MST method, as described previously. The confirmation of positivity by standard PCR Cox spacers, makes a great challenge in laboratory, aiming to optimize our results, we had to balance on the dilutions of the samples to adjust the DNA concentration, in a tenth, a hundredth, even on the hybridization temperature in thermocyclors which is ranged between **56** and **59°C**, thus the results are well improved. All results after sequencing and MST genotyping are summarized in table 4.

Our results show a discrepancy between the **MST genotypes** found according to the animal species in question, and also to the nature of the sampling concerned. The **MST 33 genotype** dominated cattle blood samples and also the sheep' ones, and also found in sheep placenta' samples. Whereas, the total dog and cat blood, gave common **MST 21 genotype**. Furthermore, the **MST 20 genotype** was detected in bovine liver samples. Regrettably, we were unable to sequence and genotype two blood samples belonging to two dogs, as well as one sheep placenta' sample, and this despite all the efforts made by our team, it could be due to a lack of conservation of the DNA in concerned.

Table 4. MST genotyping results according to the animal species and corresponding samples.

Samples ID	Species	Samples nature	Ct values	Cox blasted results	MST groups
Ab/BV/B 01	Cattle	Blood	5,4 log 10 DNA copies/ml	Cox2.7;Cox5.5;Cox18.1; Cox37.9 ; Cox57.3	**33**
F/BV/B 01	Cattle	Blood	8,6 log 10 DNA copies/ml	Cox2.7;Cox5.5;Cox18.1; Cox37.9 ; Cox57.3	**33**
F/BV/B 02	Cattle	Blood	8,4 log 10 DNA copies/ml	Cox2.7;Cox18.1;Cox37.9 ; Cox57.3	**33**
Ab/BV/L 01	Cattle	Liver	7,3 log 10 DNA copies/ml	Cox2.3;Cox5.2;Cox22.5; Cox37.4 Cox56.10	**20**
Ab/BV/L 02	Cattle	Liver	8,7 log 10 DNA copies/ml	Cox5.2;Cox18.6; Cox20.1 ; Cox22.5; Cox37.4	**20**
Ab/BV/L 03	Cattle	Liver	6,0 log 10 DNA copies/ml	Cox5.2;Cox18.6;Cox22.5; Cox57.6	**20**

F/OV/B 01	Sheep	Blood	7,8 log 10 DNA copies/ml	Cox2.7;Cox18.1;Cox37.9 ; Cox57.3	**33**
F/OV/PL 01	Sheep	Placenta	8,8 log 10 DNA copies/ml	Cox2.7;Cox18.1;Cox37.9 ; Cox57.3	**33**
F/OV/PL 02	Sheep	Placenta	8,3 log 10 DNA copies/ml	Cox2.7;Cox5.5;Cox18.1; Cox37.9 ; Cox57.3	**33**
DOG/B 01	Dog	Blood	6,4 log 10 DNA copies/ml	Cox2.2;Cox5.1;Cox18.4;Cox57.1	**21**
DOG/B 02	Dog	Blood	7,3 log 10 DNA copies/ml	Cox2.2;Cox5.1;Cox18.4; Cox20.6;Cox22.2 ;Cox57.1	**21**
DOG/B 03	Dog	Blood	6,1 log 10 DNA copies/ml	Cox2.2;Cox56.11;Cox57.1; Cox61.1	**21**
DOG/B 04	Dog	Blood	6,2 log 10 DNA copies/ml	Cox2.2;Cox5.1;Cox18.4; Cox57.1	**21**
DOG/B 05	Dog	Blood	8,8 log 10 DNA copies/ml	Cox2.2;Cox22.2 ;Cox56.11; Cox57.1;Cox61.1	**21**
DOG/B 06	Dog	Blood	7,7 log 10 DNA copies/ml	Cox2.2;Cox5.1;Cox18.4;Cox56.11; Cox61.1	**21**
CAT/B 01	Cat	Blood	6,9 log 10 DNA copies/ml	Cox18.4;Cox37.3;Cox57.1; Cox61.1	**21**
CAT/B 02	Cat	Blood	6,0 log 10 DNA copies/ml	Cox2.2;Cox5.1;Cox18.4; Cox22.2.	**21**
CAT/B 03	Cat	Blood	7,4 log 10 DNA copies/ml	Cox2.2;Cox22.2 ;Cox56.11; Cox57.1;Cox61.1	**21**

Ab: abattoir, Bv: Bovine, Ov: Ovine F: farms, B: Blood, L: Liver, Pl: Placenta.

Discussion.

Aiming to identify the reservoir and contamination sources of *Coxiella burnetii* in Northern Algeria, we demonstrated the molecular presence of *Coxiella burnetii* in samples of a different nature in various animal species, namely cattle, sheep, dog and cat. The sampling was carried out on ruminants from farms in North-eastern Algeria, and also on slaughtered ruminants in Algiers, in addition, samples from stray cats and dogs were obtained from the Algiers dog pound.

Detection of aetiological agent by PCR requires biological material such as placenta, genital swabs, liver, spleen, uterus, samples from aborted foetuses. PCR assays provide results within hours, which facilitate diagnosis, therapeutic intervention, and surveys too. In this respect, a total of **599** samples were collected, in various samples nature, blood, placenta, liver, spleen, and uterus, the species sample's variation is described previously. Our qPCR results showed that among **344** whole blood samples, only **15 (4.36%)** samples came back positives for *Coxiella burnetii*, while just **06 (2.35%)** positive samples from a total of **255** organ samples. In cattle, neither spleen samples nor uterus samples came back positive in qPCR, however, **03 (4%)** positive samples were found in blood and liver samples in each one, these blood positive subjects don't match the positive one in the liver, one (**01**) positive belong to the slaughterhouse in Algiers, and two others belong to the bovine farms in North-eastern Algeria. Moreover, all sheep's blood samples in the slaughterhouse were negative, only **01 (1.19%)** sheep blood sample gave positive qPCR, and **03 (8.57%)** placenta's samples were positive belonging to farms subjects. At the Algiers dog pound, **08 (10%)** and **03 (5%)** blood samples showed qPCR positive for *Coxiella burnetii* in dogs and cats respectively. These findings testify the presence of *Coxiella burnetii* in cattle, sheep, dogs and cats in Northern Algeria, on the limit of the sampling carried out.

In African countries, aware of the potential danger of Q fever to public health and of the large gaps in the existing knowledge of the disease, studies on the causative agent of Q fever have taken on a new dimension, where we note an increase in the results obtained following research work in the subject, as well as collaborations between the various actors in the health sector in order to detect reservoirs and sources of Q fever contamination, and know more about the pathogen. *Coxiella burnetii* infection is detected in humans and in a wide range of animal species across Africa, but seroprevalence varies widely by species and location.

In an agropastoral region of **Algeria**, seroprevalence rates of **15%** with peaks up **30%** in villages where the disease is hyperendemic, have been observed in **Eastern Algeria** in **2009 [13].** The causative aspect was the close contact with infected animal and their products, this seroprevalence may explain the existence of *Coxiella burnetii* in Eastern Algeria where ruminant farming is very widespread, which could constitute a major source of contamination and also their parturition products. Moreover, in most African countries, seroprevalence rates are elevated in domestic ruminants, surveys in cattle showed rates ranging from **4%** in **Senegal** to **33%** in **Nigeria** and **18%** in **Ghana [26].**

In **Algeria**, Khaled in **2016**, conducted a study in order to identify the positive sources of Q fever in Algeria; where he found a seroprevalence of **14.1%** among small ruminant's flocks, and he determined the shedder flocks of *Coxiella burnetii* via the vaginal swab with **21.3%** qPCR positive **[14]**, these results confirm the presence and excretion of *Coxiella burnetii* in small ruminant farms in Algeria, and it supports our results where we report **1.96%** qPCR in cattle and sheep from North-eastern Algerian farms, which are considered important reservoirs for *Coxiella burnetii* infection. In addition, Bessas et al., reported the presence of *Coxiella burnetii* by qPCR in spleen dog and cat **,0.80%, and 0.93%** respectively in Northern Algeria **[15]**, our results are clearly higher, where we found **10%** and **5%** qPCR positive for *Coxiella burnetii* in stray dogs and cats blood respectively. The presence of *Coxiella burnetii* in canine

and feline species in Algeria could sound the alarm bell of the public health authorities, to respond in a timely manner and limit the risks of human *Coxiella burnetii* infections. In the same breath, Aouadi et al in Eastern Algeria, reported **5.5%** of positive qPCR for *Coxiella burnetii* in ticks, **5.8%** and **1.7%** in sheep and goats blood respectively **[10]**. These results could lead to the various sources of *Coxiella burnetii* contamination in Algeria. All these literature data reinforce our findings in terms of the existence of *Coxiella burnetii* among the cattle and sheep populations in Algeria. More recently, in **2017**, Benaissa studied the causative agent of Q fever in the dromedary camel (*Camelus dromedarius*) population in Algeria, where he found that antibodies to *Coxiella burnetii* were found in **71.2%** of all camels investigated and the true prevalence was calculated as **71.1% [16].** This high prevalence proves that even in southern Algeria where the highest temperature are registered in Algeria (**up 58 °C**) *Coxiella burnetii* could survive and infect one of the highest immune system in animal world, thus the pathogen's high resistance to high temperatures in the outside environment could be demonstrated.

In the eastern border of Algeria, Tunisia, Barkallah et al, in **2012**, enrolled a survey of infectious aetiologies of bovine abortion in dairy herds, where a total of **214** of each blood, of vaginal swabs, and milk samples were collected, Consequently, they concluded that *Coxiella burnetii* was not part of the infectious aetiologies of abortions in the studied dairy herds **[11].** These results show that the range of abortive agents extends beyond *Coxiella burnetii*, and during abortion episodes in cattle, other pathogens other than *Coxiella burnetii* should be thought of. In Turkey, Kirkanet al, reported **4.3%** positive PCR among **138** cattle blood samples collected from animals with abortion history and repeat breeding signs in the past in the herds studied **[17],** these results are in concordance with ours, with **2.5%** positive qPCR of bovine blood samples. In India, aiming to detect *Coxiella burnetii* DNA in sera of slaughtered ruminants (**11** goats, **4** sheep, **1** cattle, and **02** buffaloes); Pradeep et al. tested a total of **15** positive sera and **03** negative ones for *Coxiella burnetii* by the commercial agar gel PCR kit and in-house Trans

PCR, they found only one buffalo serum was positive with a band at **243** bp in in-hose Trans PCR **[12]**, these results support ours, where the slaughtered ruminants may be a potential contamination sources for slaughterhouse workers, in our study we report **0.6%** and **4%** positive qPCR in bovine blood and liver slaughtered respectively, which incriminate the slaughterhouse a source of contamination.

Shedding *Coxiella burnetii* coincides with its replication in epithelial (trophoblast) cells of the placenta, and those of the entry site (lung epithelium), also in the epithelial cells of gut and udder. Sobotta et al, in **2017**, showed that these cell lines in bovine hosts, exhibited different permissiveness for *Coxiella burnetii*, thus, the udder cells allowed the highest replication rates, the intestinal cells showed an enhanced susceptibility to invasion, and lung and placenta cells also internalized the bacteria **[18].** Cantas et al, in **2011**, conducted a study in Northern Cyprus, where they used a total of **27** foetal abomasal contents and **25** placental cotyledons, collected of aborted ruminants (cattle, sheep, and goat), results showed that **22%** and **19%** came back positive for qPCR *Coxiella burnetii* respectively **[2]**. Otherwise, In Poland, Niemczuk reported **16.64%** positive qPCR among **668** placental samples, and **20.58%** positive qPCR among **306** organs of aborted foetus, collected from ruminants during **04** years' study **[7].** This high prevalence among placental samples, could be explain the high shedding of *Coxiella burnetii* in farms ruminants in Poland and Cyprus. In our case, **8.57%** of placental tissue came back positive for qPCR targeting both IS1111 and IS30a too, consequently, sheep abortion products could be a crucial contamination's source of *Coxiella burnetii* in small ruminant's farms in Algeria. Furthermore, Muskens et al, in **2011**, tested *Coxiella burnetii* DNA detection in **45** dairy cow with metritis in Netherlands, they found only one uterine sample tested PCR positive, that suggests the fact that excretion of the bacterium by vaginal fluid of metritis cows in not continuously **[19],** this result may explain our result obtained of negative qPCR bovine uterine samples for *Coxiella burnetii*.

Ticks are also believed to probably play another crucial role in the transmission of the agent from infected wild vertebrates to domestic animals **[2]**. Otherwise, *Coxiella burnetii* IS1111 DNA was detected in **4** out **194** blood samples (**2.1%**) collected from peri-domestic rodents captured in a peri-urban setting in Negeria **[20].** The Canary Islands (Spain) are considered an endemic territory, with a high prevalence in both humans and livestock. A study conducted by Bolanos-Rivero et al, in **2017**, aiming to Detect *Coxiella burnetii* DNA in peridomestic and wild animals and Ticks in Canary Island. They found eight rodents (**8%**) and two rabbits (**1.5%**) were found to be positive, with the spleen being the most affected organ, and also **6.1%** of the processed ticks distributed between those removed from livestock (**11.3%**), domestic dogs (**6.9%**), and from wild animals (**6%**) **[21]**. The dog' Q fever prevalence in the previous study, is closer than our in stray dog, with **10%** positive qPCR, and the peri-domestic rodent' prevalence, is closer than our in stray cat, with **5%** positive qPCR.

Our **MST genotyping** results show a discrepancy between animal species and nature's samples. the most repeatable **MST** is the **MST 21 genotype**, which was genotyped in **6** blood samples from dogs, and **2** blood samples from cats, these results suggest the specificity of this MST for canine and feline species in Algeria. Furthermore, the same **MST 21**, was found only in Canada in Human, cat and dog too, these findings support our results about the specificity of **MST 21** for canine and feline species **[22]**. Based on the geographical and climatic nuances between Algeria and Canada, the only common factor that could respond to the existence of this **MST 21 genotype** in these two countries is the dogs and cats brought back from Canada to Algeria via Algerian immigrants, because the dogs and cats straying in Algiers, are of different breeds, namely, German Shepherd, Poodle, French Bulldog, Rottweiller, which could be a potential source of contamination. Secondly, **MST 33 genotype** was identified in blood samples from cattle and sheep, and also in the placenta of sheep, likewise, the **MST33 genotype** was identified on ewe placenta in Germany, and on clinical samples of human, goats, sheep and cow

in Netherlands too **[23,24].** in Algeria, the cattle population is made up of **70%** of cows imported from Europe, of different races, namely Holstein, Charolaize, Montbéliarde, Prim'Holstein, Blonde d'Aquitaine, Red Holstein. The entry of the various cattle breeds imported into Algeria requires an epidemiological control before release, but does not prevent positive carriers of *Coxiella Burnetii* from escaping the rule, on the one hand because of the lack of serological control of the Q fever of these animals, and on the other hand the positive animals can be **60%** asymptomatic. All these reasons may be the cause of the **MST 33** circulation among ruminants in Algeria. **MST 20 genotype** was identified in liver bovine samples, this later was also predominately harboured in Netherlands and Hungry on cattle, and on goats in England **[9,25]**.

At the outcome of this study, front of the reality that *Coxiella burnetii* is housed in ruminant farms, as well as in slaughterhouses and in stray dogs and cats, we must insist on sounding the alarm in front of the various public health actors in Algeria, and request that the necessary measures and precautions be taken to limit the contamination and spread of *Coxiella burnetii*. In addition, we have to raise awareness among at-risk personnel that good hygiene practices during livestock' farming and slaughtering are an important way of reducing the risk of spread of infectious diseases.

Conflict of interest.

None declared.

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Bibliographic references.

[1]. T.J. O'Neill, J.M. Sargeant and Z. Poljak. A systematic Review and Meta-Analysis of Phase I Inactivated Vaccines to Reduce Shedding of *Coxiella burnetii* from Sheep and Goats From Routes of Public Health Importance. Zoononse and Public Health,2013: doi: 10.1111/zph.12086.

[2]. Hasan Cantas, Adrian Muwonge, Baris Sareyyupoglu, Hakan Yardimici, Eystein Skjerve. Q fever abortions in ruminants and associated on-farm risk factors in northern Cyprus. BMC Vetrinary Research, 2011;7.13.

[3]. Sky Vanderburg, Matthew P. Rubach, Jo E.B. Halliday, Sarah Cleaveland, Elizabeth A. Reddy, John A. Crump. Epidemiology of *Coxiella burnetii* Infection In Africa: A OneHealth Systematic Review. Plos Neglected Tropical Diseases, April 2014; 8-4:e2787.

[4]. Michelle P. Emery, Eileen N. Ostlund, Mohamed Ait Ichu, Jeff D. Ballin, David McFarling, Luanne McGonigle. *Coxiella burnetii* Serology assays in goat abortion storm. Journal of Veterinary Diagnostic Investigation, 2014;26-1:141-145.

[5]. Javad Asadi, Mojtaba Kafi and Mohammad Khalili. Seroprevalenece of Q fever in sheep and goat flocks with a history abortion in Iran between 2011 and 2012. Veterinaria Italiana, 2013;49-2:163-168.

[6]. Maria Luisa Marenzoni, Valentina Stefanetti, Paola Papa, Patrizia Casagrande, et al. Is the horse a reservoir or an indicator of *Coxiella burnetii* infection? Systematic review and biomolecular investigation. Veterinary Microbiology,2013;167:662-669.

[7]. Krzysztof Niemczuk, Monika Szymanska-Czerwinska, Krzysztof Smietanka, Lukas Bocian. Comparison of diagnostic potential of serological, molecular and cell culture methods for detection of Q fever in ruminants. Veterinary Microbiology,2014;171:147-151.

[8]. O, Roux V, Freylikman O, Sekeyova Z, Fournous G, Tyczka J,Tokarevich N, Kovacava E, Marrie TJ, Raoult D. Coxiella burnetii genotyping. Emerg Infect Dis,2005;11:1211–1217.

[9]. Carole Eldin, Cléa Mélenotte, Oleg Mediannikov, Eric Ghigo, Matthieu Million,Sophie Edouard, Jean-Louis Mege, Max Maurin, Didier Raoult. From Q Fever to *Coxiella burnetii* Infection: A Paradigm Change. Clinical Microbiology Reviews, January 2017; 30-1:115-190.

[10]. Atef Aouadi, Hamza Leulmi, Mehdi Boucheikhchoukh, Ahmed Benakhla, Didier Raoult, Philippe Parola. Molecular evidence of tick-borne hemoprotozoan-parasites (*Theileria ovis and Babesiaovis*) and bacteria in ticks and blood from small ruminants in Northern Algeria. Comparative Immunology, Microbiology and Infectious Diseases,2017;50: 34-39.

[11]. Mohamed Barkallah, Yaakoub Gharbi, Amal Ben Hassena, Ahlem Ben Slima, Zouhir Mallek, Michel Goutier, Gilbert Greub, Radhouane Gdoura, Imen Fendri. Survey of Infectious Etiologies of Bovine Abortion during Mild-to Late Gestation in Dairy Herds. PlOS ONE,2014;9-3:e91549.

[12]. Jothimani Pradeep, Selvaraj Stephan, Pratheesh Pooja, Anbalagan Akshayavardhini, Balakrishnan Sangeetha and Prabkar Xavier Antony. Coxiellosis in domestic livestock of Puducherry and Tamil Nadu: Detection of *Coxiella burnetii* DNA by polymerase chain reaction in slaughtered ruminants. Veterinary Wolrd,2017;10:667-671.

[13]. A. Lacheheb and D. Raoult. Seroprevalence of Q-fever in Algeria. Clinical Microbiology and Infection, December 2009; 15-2.

[14]. H. Khaled, K.Sidi-Boumedine, S.Merdja,P. Dufour, A.Dahmani, R.Thiéry, E.Rousset,A.Bouyoucef. Serological and Molecular evidence of Q fever among small ruminant

flocks in Algeria. Comparative Immunology, Microbiology and Infectious Diseases ,2016;47: 19-25.

[15]. Amina Bessas, Hamza Leulmi, Idir Bitam, Sara Zaidi, Khatima Ait-Oudhia, Didier Raoult, Philippe Parola. Molecular evidence of vector-borne pathogens in dog and cat and their ectoparasites in Algiers, Algeria. Comparative Immunology, Microbiology and Infectious Diseases, 2016;45: 23-28.

[16]. Mohammed H. Benaissa, Samir Ansel, Abdallah Mohamed-Cherif, Karima Benfodil, Djamel Khelef, Curtis R. Youngs, Rachid Kaidi, Khatima Ait-Oudhia. Seroprevalence and risk factors for *Coxiella burnetii*, the causative agent of Q fever in the dromedary camel (*Camelus dromedarius*) population in Algeria. Onderstepoort Journal of Veterinary Research, 2017.

[17]. SuKru KIRKAN, Osman KAYA, Serten TEKBIYIK, Ugur PARIN. Detection of *Coxiella burnetii* in Cattle by PCR. Turk.j.Vet.Anim.Sci, 2008;32-3:215-220.

[18]. Katharina Sobotta, Katharina Bonkowski, Elisabeth Liebler-Tenorio, Pierre Germon, Pascal Rainard, Nina Hambruch, Christiane Pfarrer, Ilse D. Jacobsen and Christian Menge. Permissiveness of bovine epithelial cells from lung,intestine, placenta and udder for infection with *Coxiella burnetii.* Vet Res , 2017 ;48:23.

[19]. J. Muskens, C van Maanen, M.H Mars. Dairy cows with metritis: *Coxiella burnetii* test results in uterine, blood and bulk milk samples. Veterinary Microbiology, 2011;147:186-189.

[20]. Joshua Kamani, Gad Baneth, Ricardo Gutiérrez, Yaarit Nachum-Biala, Y. Mumcuoglu, Shimon Harrus. *Coxiella burnetii* and *Rickettsia conorii*: two zoonotic pathogens in peridomestic rodents and thir ectoparasites in Nigeria. Ticks and Ticks borne Diseases, 2017.

[21]. Margarita Bolanos-Rivero, Cristina Carranza-Rodrıguez, Noe F. Rodrıguez, Carlos Gutierrez, and Jose -Luis Perez-Arellano. Detection of *Coxiella burnetii* DNA in Peridomestic

and Wild Animals and Ticks in an Endemic Region (Canary Islands, Spain). VECTOR-BORNE AND ZOONOTIC DISEASES,2017.

[22]. F.D'amato, M.Million, S.Edourad, J.Delerce, C.Robert, T. Marrie and D Raoult. Draft genome sequence of *Coxiella burnetii* Dog Utad, a strain isolated from a dog-related outbreak of Q fever. NMNI, 2014;2:136-137.

[23]. Calvin K.Y. Cheng, Benjamin J. Cowling, Eric H.Y. Lau, Lai Ming Ho, Gabriel M. Leung, and Dennis K.M. Ip. Epidemic Genotype of *Coxiella burnetii* among Goats, Sheep, and Humans in the Netherlands. Emerging Infectious Diseases, 2012; 18-5:887.

[24]. D'Amato F, Rouli L, Edouard S, Tyczka J, Million M, Robert C, Nguyen TT, Raoult D. The genome of *Coxiella burnetii* Z3055, a clone linked to the Netherlands Q fever outbreaks, provides evidence for the role of drift in the emergence of epidemic clones. Comp Immunol Microbiol Infect Dis, 2014; 37:281–288.

[25]. Kinga M Sulyok, Zsuzsa Kreizinger, HeidieM Hornstra, Talima Pearson, Alexandra Szigeti, ÁdámDán, Eszter Balla, Paul S Keim and Miklós Gyuranecz. Genotyping of *Coxiella burnetii* from domestic ruminants and human in Hungary: indication of various genotypes. BMC Veterinary Research,2014 ; 10:107.

[26]. Kamga-Waladjo AR, Gbati OB, Kone P, Lapo RA, Chatagnon G, Bakou SN, Pangui LJ, Diop PEH, Akakpo JA, Tainturier D. Seroprevalence of Neospora caninum antibodies and its consequences for reproductive parameters in dairy cows from Dakar-Senegal, West Africa. Trop Anim Health Prod,2010; 42:953–959.

Conclusions et perspectives

A l'issue de notre travail portée sur l'étude de *Coxiella burnetii* l'agent causal de la fièvre Q chez l'Homme et différentes espèces animales en Algérie, en se basant sur nos résultats obtenus dans les différents secteurs relatifs à cette zoonose, allant des réservoirs et des sources de contaminations par *Coxiella burnetii* chez les ruminants et les chiens et chats en Algérie, arrivant à l'Homme qui est considéré un hôte accidentel de cette maladie. L'impact de la fièvre Q sur les différents secteurs de la santé public, professionnel de la santé animale et humaine, est une préoccupation majeure commune qu'on devrait tous s'y impliquer.

Aux services de gynécologie-Obstétrique, la sonnette d'alarme doit être tirée, vu la propagation de *Coxiella burnetii* au sein de ces services dans nos hôpitaux. L'aspect abortif de la bactérie est sous-estimé et passe à bas bruit, par conséquent une bombe à effet retardé menace le personnel des services obstétrique, surtout les sages-femmes et les médecins gynécologues praticiens. De ce fait, nous tenons à rappeler qu'il faut penser à la fièvre Q devant des épisodes abortifs répétés pour des patients présentant des facteurs de risque, à savoir le contact avec les animaux, le voyage dans des zones endémiques, et aussi la notion de morsures de tiques d'une part, et d'autre part de prendre toutes mesures préventives et hygiéniques afin d'éviter la contamination et la propagation du pathogène via les instruments et le matériel souillés dans les blocs opératoires et les salles de travail des services.

Tout de même, il faut tirer la sonnette d'alarme au niveau des services spécialisés en maladies infectieuses et aussi en médecine interne, où les moyens de diagnostic pour la fièvre Q ne sont pas disponibles, et surtout face à l'éventail des manifestations cliniques atypiques de la fièvre Q chez l'Homme. De ce fait, il faut penser à la fièvre Q devant tout épisode fébrile prolongé atypique, une arthromyalgie, asthénie chronique, des éruptions cutanées ou morsures de tiques, une hépatite granulomateuse et aussi une altération de l'état général. Les mesures préventives

et hygiéniques doivent être de règle pour limiter les conséquences et les séquelles de la maladie au stade aigu et surtout diagnostiquer et traiter à temps pour éviter le passage au stade chronique de la maladie.

Les ruminants et les espèces félines et canines peuvent être une source majeure de contamination et transmission de la fièvre Q. le personnel de la santé animale, des vétérinaires, personnel des abattoirs, des éleveurs, et aussi consomateurs, doivent avoir une attention particulière lors des épisodes suspects de la fièvre Q au sein des bétails, et il faut en penser devant chaque épisode abortif des ruminants au sein des élevages en tenant en compte la fréquence et l'effectif touché. Les mesures préventives et hygiéniques doivent être appliquées rigoureusement au niveau des abattoirs sur toutes les phases anté et post mortem, et à l'inspecteur vétérinaire de l'abattoir de faire méticuleusement l'examen général des ruminants avant l'abattage afin de déceler les animaux fébriles ou malades qui restent impropres à la consommation humaine. Par ailleurs, les chats et les chiens errants qui trainent dans nos villes et nos quartiers, font une source redoutable de contamination par *Coxiella burnetii*, d'où le risque majeur des épidémies de la fièvre Q dans les zones urbaines. Pour cela, les autorités habilitées doivent établir un plan de lutte ferme pour éliminer et éradiquer ses petits animaux menaçants de nos villes et nos quartiers.

Finalement, afin de mieux se placer devant cette situation critique où *Coxiella burnetii* est très répondue en Algérie, il faut mener un travail de langue halène multisectoriel, qui devrait réunir les différents acteurs de la santé public, la santé animale, et aussi de l'environnement, ayant pour but d'établir un plan de lutte contre la fièvre Q, et aussi d'élever la barre de l'épidémio-surveillance principalement sur les différentes espèces animales importées, en leurs exigeant l'indemnité de toute infection qui pourrait poser un risque sur la santé humaine et animale. D'autre part, il faut établir un plan de traçabilité de mouvement des différents cheptels à l'intérieur et à l'extérieur du territoire national afin d'avoir une traçabilité qui facilitera d'agir

à temps opportun. De plus, l'Algérie devrait profiter et suivre des expériences des pays où la fièvre Q est très endémique et où on a enregistré des épidémies très importantes. Par exemple, la Hollande, suite à la puissante épidémie de la fièvre Q qui a touché le pays en 2007, où ils ont rapporté 4000 cas humains de la fièvre Q. Par conséquent, les Hollandais ont établi un modèle type de lutte contre la fièvre Q que les pays européens le prennent comme exemple afin d'éviter et prévenir et aussi faire face aux épidémies de la fièvre Q.

ANNEXES

Article 4.

Urinary shedding of pathogenic Leptospira in stray dogs and cats, Algiers: A prospective study.

Zaidi S, Bouam A, Bessas A, Hezil D, Ghaoui H, Ait-Oudhia K, Drancourt M, Bitam I

PLOS ONE

RESEARCH ARTICLE

Urinary shedding of pathogenic *Leptospira* in stray dogs and cats, Algiers: A prospective study

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Abstract

Background

Leptospirosis is an important worldwide zoonosis. This disease is caused by pathogenic species of the genus *Leptospira* which are maintained in the environment via chronic renal infection of carrier animals which can be asymptomatic excretors of the organisms in their urines and become a source of infection for humans and other hosts. The prevalence of animal leptospirosis in Algiers, Algeria, is unknown.

Methodology/principal findings

Real-time PCR and standard PCR and sequencing were used to detect pathogenic *Leptospira* organisms in the urines of stray dogs and cats in Algiers. In the presence of appropriate controls, none of the 107 cat urine samples were positive while 5/104 (4.8%) canine urine samples (asymptomatic mixed-breed dogs, three females and two males) were positive in two real-time PCR assays targeting the *rrs* and *hsp* genes. The positivity of these samples was confirmed by partial PCR-sequencing of the *rpo*B gene which yielded 100% sequence similarity with *Leptospira interrogans* reference sequence. In this study, *L. interrogans* prevalence was significantly higher in dogs aged < one year (16.46% - 29.41%) than in adults (0%) (P value = 0.0001) and then in the overall dog population (2.68% - 4.8%) (P = 0.0007).

Conclusions/significance

These results suggest that dogs are maintenance hosts for zoonotic leptospirosis in Algiers, Algeria. To face this situation, effective canine vaccination strategies and raising public health awareness are mandatory. Further investigations incorporating a larger sample in more localities will be undertaken to document the epidemiology of urban animal leptospirosis in Algeria at large.

analysis, decision to publish, or preparation of the manuscript.

Competing interests: The authors have declared that no competing interests exist.

Introduction

Leptospirosis is a worldwide disease that affects wild and domestic animals and human populations. Affected persons are primarily farmers, fishermen, veterinarians and people working in sewers and slaughterhouses [1]. This zoonosis is caused by pathogenic spirochetes of the genus *Leptospira* which colonize the renal tubules where they reproduce before being excreted via the urines [2]. Infected urines or contaminated water are sources of leptospirosis infection and *Leptospira* can enter the body of mammalian hosts via lacerations in the skin, contacts with mucosa or conjunctiva and inhalation of aerosols [3–5]. Some host animals such as dogs may have an asymptomatic form or may suffer from a wide range of clinical manifestations, including hepatic and renal failure and severe pulmonary hemorrhage [6]. Asymptomatic and chronic carrier dogs can be maintenance hosts [7] acting as sources of infection and therefore cause a public health problem [8]. Formerly, it was thought that domestic cats were resistant to leptospirosis infection and many practitioners did not consider feline leptospirosis in the differential diagnosis of other diseases [9]. However, recently published reports on feline leptospirosis conclude that cats are exposed to *Leptospira* and may play a role in the epidemiology of this disease [10–12].

As a neglected tropical disease, leptospirosis has been increasingly observed in urban settlements, especially in slums in developing countries [6]. The prevalence of animal leptospirosis in Algiers, Algeria, is unknown. Only two studies were published about human leptospirosis in Algeria. These two serological investigations were conducted on patients of the Tizi-ouzou Hospital. The first one reported 48 cases of leptospirosis from 2006 to 2007 and the serogroup icterohaemorrhagiae was identified in 60% of cases [13]. In the second prospective study, 175 positive patients were diagnosed from 2005 to 2008, among the serovars identified, icterohaemorrhagiae and grippotyphosa were predominant [14].

The aim of the present work was to detect pathogenic *Leptospira* organisms in the urines of stray dogs and cats in Algiers.

Methods

Ethic statement

The study was submitted to and approved by the ethics committee and decision board (number 416/2017) of EPIC- H.U.P.E (EPIC: Entreprise publique à caractère industriel et commercial; H.U.P.E: Hygiène Urbaine et Protection de l'environnement) of Wilaya of Algiers (Ex: HURBAL). HURBAL was created in 1994 with a new status: EPIC-H.U.P.E under the register number: 16/00-0013132B00. EPIC- H.U.P.E is an institution affiliated with the Algerian Ministry of the Interior and the Local Government and the Algerian Ministry of Water Resources and Environment. In the context of the National Program for Rabies Control, EPIC- H.U.P.E captures stray dogs and cats in Algiers. Once captured, stray animals were housed in cages and euthanized after expiration of the seven day legal waiting time (in order to allow for owners to claim their pets), in compliance with the Algerian legislation for the protection of animals (Law 01/04/1994), which our protocol respected.

Study design and sampling

This study was designed to screen for the presence of *Leptospira* spp. organisms in stray cats and dogs in the region of Algiers, in the absence of any data available on that topic. Therefore, in this study, we aimed at collecting only the urines of the animals for the molecular detection of *Leptospira* spp. DNA. Urine specimens were aseptically collected between April 2017 and November 2017 were via cystocentesis from 211 stray animals (104 dogs and 107 cats). These

animals were captured in the 57 municipalities of the region of Algiers. The sampling was realized in animal shelters with an average of seven animals sampled per week. The age of each animal was estimated, based on dentition and physical aspect. Information concerning sex, breed and clinical status was noted. Samples were stored at -20˚C before being transported to the IHU Méditerranée Infection, Marseille, France, for PCR testing and culture was not performed. Up to 3 mL of each urine sample was centrifuged at 15,000 g for 20 minutes [15], the supernatant was discarded and the pellet was suspended in 200 μL of sterile phosphate-buffered saline solution (PBS, pH 7.2) [16].

DNA extraction

A total of 200 μL of DNA was extracted using the QIAamp Tissue Kit by QUIAGEN-BioRobot EZ1, according to the manufacturer's instructions (Qiagen, Hilden, Germany). Extracted DNA was stored at -20˚C under sterile conditions until used in PCR assays.

Real time PCR

Extracted DNA was used in qPCR amplifications to detect pathogenic *Leptospira* organisms. The final qPCR reaction mixture consisted of 5 μL DNA with 15 μL of mix from the Roche PCR Kit (Roche Diagnostics, Meylan, France). The components of the final reaction mixture of these PCR assays are given in Table 1. A homemade plasmid containing sequences specific to *Leptospira* spp. was used as a positive control. Three negative controls were incorporated into each PCR run. Results were recorded as positive when the cycle threshold (Ct) was lower than 33. We performed real-time PCR (qPCR) with two systems (Table 2) in order to confirm the positivity of the samples according to current standards in microbiology. The first system targets a 88-pb fragment of the *rrs* gene coding for 16S rRNA of pathogenic *Leptospira*: 16S rRNA Forward (5′-CCCGCGTCCGATTAG-3′), 16S rRNA Reverse (5′-TCCATTGTGGCCGRACAC-3′) and 16S rRNA Probe (5′-CTCACCAAGGCGACGTCGGTAGC-3′) were analyzed as previously described [17]. The second system targets a 103-pb fragment of the *hsp* gene of *L. interrogans*: Lint_hsp_MB Forward (5′-CCCGCGTCCGATTAG-3′), Lint_hsp_MB Reverse (5′-TCCATTGTGGCCGRACAC-3′) and Lint_hsp_MB Probe (5′-CTCACCAAGGCGACGTCGGTAGC-3′) were analyzed as previously described [18]. The PCR cycling parameters for the qPCR were 5 min at 95˚C followed by 39 cycles each consisting of 5 sec of denaturation at 95˚C and 30 sec of annealing at 60˚C.

Standard PCR and sequencing

Samples that tested positive by qPCR were confirmed by standard PCR and sequencing in order to achieve a 100% specificity. The final standard PCR reaction mixture consisted of 5 μL of DNA with 15 μL of mix from the Roche PCR Kit (Roche Diagnostics). The components of

Table 1. Concentration of components in the final reaction mixtures of the real-time polymerase chain reaction (qPCR) assays used in this study.

Reagent	16S rRNA qPCR (1X)	Hsp qPCR (1X)
Mix Roche (LightCycler® 480 Probes Master)	10 μL	10 μL
Water volume	3 μL	3 μL
Forward primer	0.5 μL	0.5 μL
Reverse primer	0.5 μL	0.5 μL
Probe	0.5 μL	0.5 μL
Uracyl DNA Glycosidase (UDG)	0.5 μL	0.5 μL

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Table 2. Primers and probes used in this study.

PCR assay	Primer and probe sequences	References
16S rRNA	Forward primer: (5'-CCCGCGTCCGATTAG-3') Reverse primer: (5'-TCCATTGTGGCCGRA/GACAC-3') Prob: (5'-CTCACCAAGGCGACGATCGGTAGC-3')	[17]
Lint_hsp_MB	Forward primer: (5'-CCCGCGTCCGATTAG-3') Reverse primer: (5'-TCCATTGTGGCCGRACAC-3') Prob: (5'-CTCACCAAGGCGACGTCGGTAGC-3')	[18]
rpoB	Forward primer: (5'-CCTCATGGGTTCCAACATGCA-3') Reverse primer: (5'-CGCATCCTCRAAGTTGTAWCCTT-3')	[19]

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the final reaction mixture of these PCR assays are given in Table 3. Samples were then confirmed by standard PCR using primers which amplified a 592-pb fragment of the *rpoB* gene: Lept 1900 Forward (5'-CCTCATGGGTTCCAACATGCA-3') and Lept 2500 Reverse (5'-CGCATCCTCRAAGTTGTAWCCTT-3'), as described by La Scola et al., 2006 [19] (Table 2). The PCR cycling parameters for the standard PCR were 15 min at 95˚C followed by 35 cycles of each consisting of 30 sec denaturation at 95˚C, 30 sec annealing at 51˚C and 6 min extension at 72˚C in an ABI Thermocycler (Applied Biosystems Gene Amp PCR System 2700, Villebon sur Yvette, France). Negative controls were incorporated into each PCR run.

The amplified PCR products were separated via gel electrophoresis using a 1.5% agarose gel stained with Sayber Safe (ThermoFisher, Paris, France). The DNA bands were visualized and photographed under ultraviolet light. PCR products were purified and sequenced with *rpoB* primers as described previously [19]. All obtained sequences were assembled and edited using ChromasPro (version 1.7.7). The sequences were then analyzed by Basic Local Alignment Search Tool (BLAST) and compared with sequences available in the GenBank database.

Statistical analyses

Statistical analyses were done by MEDCALC® online software https://www.medcalc.org/calc/comparison_of_proportions.php using the "N-1" Chi-squared test as recommended by Campbell., 2007 [20] and Richardson., 2011 [21]. The confidence interval was calculated according to the recommended method given by Altman et al., 2000 [22].

Results

Sample collection

From April 2017 to November 2017, a total of 104 stray dogs and 107 stray cats captured in Algiers, Algeria were sampled. These animals lived in urban areas, spending most of their time exclusively outdoors and did not receive any vaccine. Of the 104 dogs, 69/104 (66.34%) were males and 35/104 (33.65%) were females. The canine population consisted predominantly of mixed-breed dogs; other dogs belonged to the following races: German shepherd, American

Table 3. Concentration of components in the final reaction mixtures of the standard polymerase chain reaction assay used in this study.

Reagent	*rpoB* Standard PCR (1X)
Ampli Taq Master Mix	12.5 μL
Water volume	6 μL
Forward primer	0.75 μL
Reverse primer	0.75 μL

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Staffordshire, shepherd crosses and Pit-bull. The dogs' age ranged between 2 months and 11 years. Among the 107 cats, 66/107 (61.68%) were males and 41/107 (38.31%) were females. The cats were described as mostly belonging to mixed breeds, some belonging to European or Siamese crossbreeds. The 107 cats sampled were estimated to be under 5 years of age. All sampled animals were apparently healthy.

Real time PCR

qPCR targeting the 16S rRNA gene of pathogenic *Leptospira* and the hsp gene of *L. interrogans* revealed that none of the 107 urine samples of cats tested were positive while 5/104 (4.8%) dogs were positive. These five urine specimens were positive in the two qPCR systems (rrs and hsp). Using the Cts obtained from the 16S rRNA qPCR reactions and a calibration curve previously described for this system [23], we extrapolated the number of leptospira genomes per positive reaction (Table 4). Positive dogs were all very young, under one year of age. Three were females and two were males. All positive animals belonged to mixed-breeds (Table 4). In this study, *L. interrogans* prevalence was significantly higher in dogs aged < one year (5/17; 29.41%) than in adults (0/87; 0%) (P value = 0.0001, 95% CI: 12.73 to 53.13) and than in the overall dog population (5/104; 4.8%) (P = 0.0007, CI: 7.1929 to 48.2975). The sensitivity of our screening test based on the detection of 16S rRNA using qPCR was previously estimated to be of 56% [24], accordingly, the prevalence rate obtained here was estimated to be of 2.68%-4.8%. There was no significant difference regarding prevalence between males (3/69, 2.43% - 4.34%) and females (2/35, 3.19% - 5.71%) (p = 0.758).

Standard PCR and sequencing

All five urine samples detected positive by real-time PCR were confirmed with gel-based PCR assay targeting the *rpoB* and subjected to sequencing analysis. The BLAST (www.ncbi.nlm.nih.gov/blast) analysis of the *rpoB* gene sequence from all samples, once compared with sequences available in the GenBank database, confirmed *Leptospira* infection. The BLAST analysis yielded a 100% sequence homology with *L. interrogans* homologous gene fragment (GenBank accession no. CP020414.1).

Discussion

In Algeria, the exact morbidity and the mortality due to leptospirosis are unknown. In 1975, a study based on serology reported seven cases in a military group in Algiers' suburbs [25]. Two more recent studies reported cases of leptospirosis among hospitalized patients in the region of Tizi-Ouzou, located 100 km east of the capital Algiers. The investigation of 48 patients from 2006 to 2007 in the rural area of Tala-Athmane revealed that they were living in close contact

Table 4. Information relative to animals detected positive for *L. interrogans* DNA in urine samples: Age, sex, race, number of genomes per positive pPCR reaction. (The values were extrapolated from the calibration curve (21) using the Ct obtained from the 16S rRNA system).

Case (N°)	Age	Sex	Race	Ct rRNA (Log *Leptospira* genome/reaction)
1 (06)	4 months	M	Mixed-breed	26.6 (9.5×10^3)
2 (17)	7 months	M	Mixed-breed	23.71 (9.6×10^4)
3 (34)	4 months	F	Mixed-breed	23.47 (9.3×10^4)
4 (40)	5 months	F	Mixed-breed	25.26 (1.02×10^4)
5 (87)	4 months	F	Mixed-breed	17.54 (1.05×10^6)

M = male; F = female.

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to two garbage dumps invaded by rodents, the cases were confirmed serologically by the microagglutination test (MAT) and more than 60% (n = 29) were from the serogroup icterohaemorrhagiae [13]. A second prospective study conducted from 2005 to 2008 in the same region reported 173 cases among hospitalized patients, the cases were confirmed serologically with a predominance the of serovars icterohaemorrhagiae and grippotyphosa [14].

However, the prevalence of this zoonosis in reservoirs is totally unknown in Algeria as the only report of it is the observation of *Leptospira* organisms in histological sections of the liver in dogs presenting with severe jaundice, subcutaneous hemorrhages and acute nephritis [26]. For this pioneering study of animal leptospirosis in Algeria, we used urines in which leptospiral DNA can be found much longer than in blood [27–29]. The need for a rapid diagnosis of leptospirosis has led to the development of numerous PCR assays, which appeared to have more applicability in determining zoonotic risks [30]. This method is rapid, sensitive, specific and robust and many PCR assays were developed to detect universal *Leptospira* genes such as *gyr*B, *rrs* and *sec*Y genes or genes restricted to pathogenic species such as *lip*L32, *lfb*1, *lig*A and *lig*B2 [5]. In this study we aimed at retrospectively confirming the positive detection of *Leptospira* spp. DNA by targeting two different molecular targets. We chose the rrs and hsp as genus-level targets of identification and *rpo*B sequencing for the identification at the species level of pathogenic leptospira.

We confirmed the presence of *L. interrogans* in the urines of stray dogs in Algiers using qPCR and standard PCR-sequencing targeting universal and pathogen-related genes. We observed an overall prevalence of 2.68% - 4.8% and a high prevalence of 29.41% in the specific population of young dogs aged < one year. All animals were apparently healthy, indicating asymptomatic carriers. These dogs were always outside, in contact with garbage and small rodents which were likely sources of infection. Crowding animals in unsanitary quarters is associated with a high prevalence of infection since animals may acquire the disease through contact with urines from infected dogs or infected rodents [31]. Despite the fact that we did not attempt to isolate *Leptospira* spp. to confirm the role of stray dogs as reservoirs, present data indicate that stray dogs would indeed be good sentinels to know which serovars/groups are circulating in the rodent populations.

Subclinical, latent leptospirosis in dogs has regularly been reported and can also be observed in unsteady vaccinated animals [32]. In addition, there are data suggesting that clinically asymptomatic dogs can be chronic carriers, shedding *Leptospira* via urines into the environment [30, 33, 34]. Many epidemiological studies were conducted worldwide on the renal carriage of leptospirosis in dogs using molecular tools [8, 14, 34–43], showing a prevalence between 0.2% and 22% worldwide Fig 1). Some other studies have not found the presence of pathogenic *Leptospira* species in dogs in the USA (0/100) and in Egypt (0/25), but only the presence of antibodies as an evidence of the exposure to the disease [44] [45]. This may be due to the minute amount of *Leptospira* DNA in the blood and urine specimens of infected dogs. Therefore, a highly sensitive PCR platform is required to obtain an accurate diagnosis and an innovative approach must be adopted to maximize sample DNA input in the PCR or by increasing the volume of urine for DNA extraction. This could be achieved by high-speed sedimentation of a milliliter volume of urines and performing DNA extraction from the complete sediment, as performed in the present study [46]. Positive dogs in our study were very young dogs (under one year of age). However, it has been shown in the USA that dogs aged between 4 and 6.9 years and between 7 and 10 years faced a significantly higher risk of being infected than dogs under one year of age [47]. In a study conducted in Reunion Island, only adult dogs were positive for leptospirosis [48]. The difference in age range can be explained by the fact that the young stray dogs of our study were not vaccinated against leptospirosis and exposed at an early age to the bacterium in their

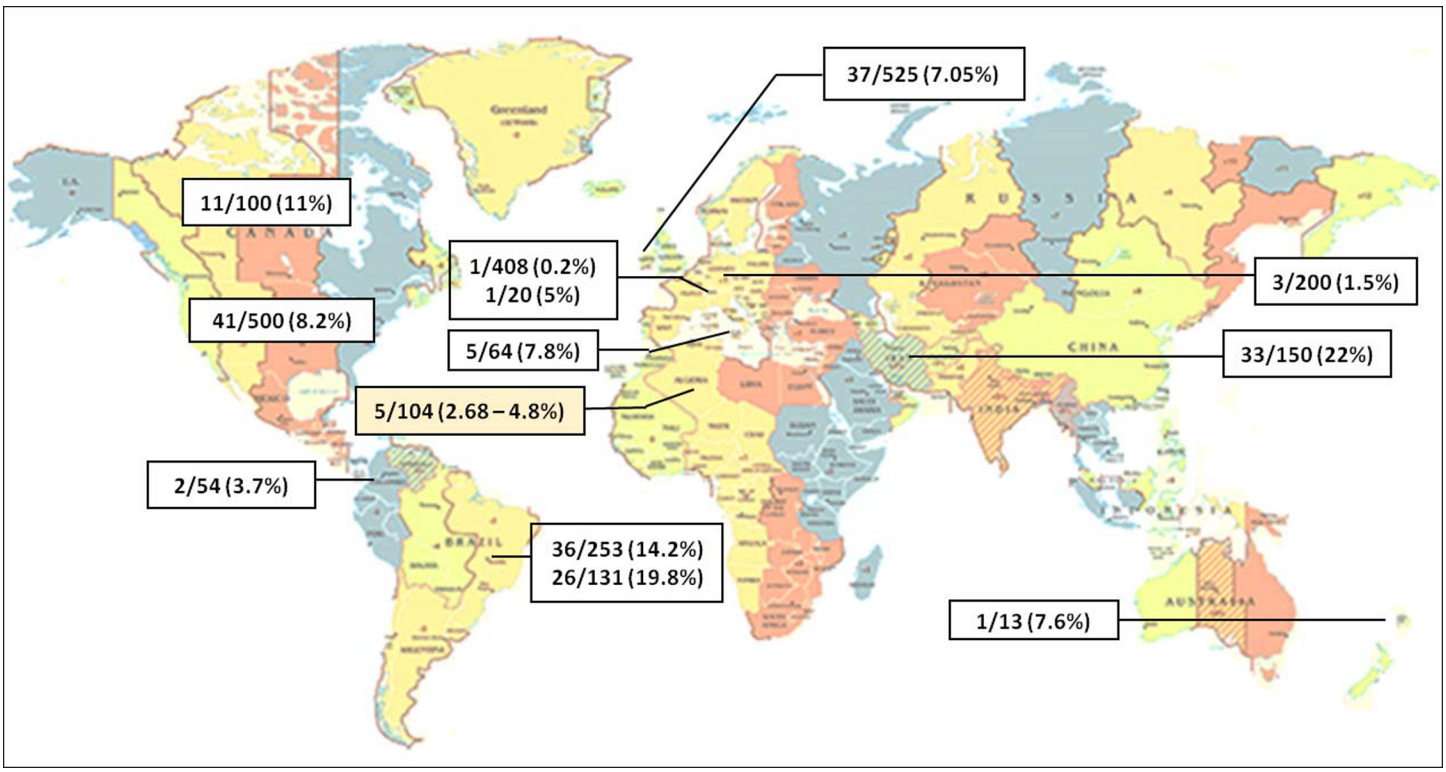

Fig 1. Molecular detection of *Leptospira* spp. DNA in the urines of dogs, worldwide. Germany, 3/200 (1.5%) [8], the USA, 41/500 (8.2%) [14], Teheran (Iran), 33/150 (22%) [34], Canada, 11% (11/100) [35], Sicily (Italy), 5/64 (7.8%) [36], Ireland, 37/525 (7.05%) [37], Porto Alegre City (Brazil), 36/253 (14.2%) [38], Switzerland, 1/20 (5%) [39], Colombia 2/54 (3.7%)[40], New Caledonia, 1/13 (7.6%) [41], Brazil, 26/131 (19.8%) [42], Switzerland, 1/408 (0.2%) [43], Algiers (Algeria), 5/104 (2.68% - 4.8%) [Present work].

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environment. Furthermore, the canine population of two previous studies [47, 48] was composed of domestic dogs with different risk factors and exposure to other maintenance host species.

In the present work, all sampled cats were qPCR-negative for *L. interrogans*. Some studies yielded a seroprevalence between 4% and 30% in different countries including Australia [49], Scotland [50], Greece [51], Iran [52], Spain [53], Canada [54], Taiwan [11], Chile [10], Colombia [55] and Brasil [56]. Some other studies found DNA sequences of *Leptospira* in urine samples indicating a renal carriage of leptospirosis in cats. In Taiwan, DNA of pathogenic *Leptospira* was detected in 67.8% (80/118) of the urine samples of cats including 71 stray cats and nine household cats [11]. In Canada, DNA of *Leptospira* was detected in the urines of sick cats (6/113) and healthy cats (2/125), corresponding to a prevalence of 8/238 (3.3%) [12]. In Germany, urine samples from 7/215 (3.2%) cats were PCR-positive [57]. In Quebec, PCRs on urines detected urinary excretion in 3.2% of the 250 cats sampled [58]. These results suggest that cats may have a role in the transmission of leptospirosis, as a reservoir or as an accidental host. The role of cats in the transmission of leptospirosis should be reevaluated, as it might in fact be underestimated.

In conclusion, this study demonstrates the high number of leptospiral carriers among asymptomatic young dogs. Improving the awareness of dog owners and the prevention of canine leptospirosis could be a valuable asset for human leptospirosis prevention [59]. The prevalence of leptospirosis in countries where dogs are correctly vaccinated remains high, this is mainly due to the difference in epidemiology between the different serovars and the absence of cross-protection in a vaccine, thus, a more effective vaccine needs to be developed [60]. The implementation of a

surveillance system for canine leptospirosis, using dogs as sentinels for human risk assessment, could also provide a valuable tool for estimating and in turn minimize the risk for humans [61]. Further studies on leptospirosis in other animals and other regions in Algeria should be considered to clarify the status of this disease in our country. Also, further studies in the region of Algiers and other regions of Algeria will have to determine the serotypes of circulating leptospira in order to refine the epidemiology of leptospirosis in Algeria.

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References

1. Brown PD, McKenzie M, Pinnock M, McGrowder D. Environmental risk factors associated with leptospirosis among butchers and their associates in Jamaica. Int J Occup Environ Med. 2011; 2(1):47–57. PMID: 23022818
2. Levett PN. Leptospirosis. Clin Microbiol. 2001; 14(2):296–326.
3. Baron S. Medical microbiology. University of Texas Medical Branch at Galveston; 1996. 1273 p.
4. Monahan AM, Miller IS, Nally JE. Leptospirosis: risks during recreational activities. J Appl Microbiol. 2009; 107(3):707–16. https://doi.org/10.1111/j.1365-2672.2009.04220.x PMID: 19302325
5. Musso D, La Scola B. Laboratory diagnosis of leptospirosis: A challenge. J Microbiol Immunol Infect. 2013; 46(4):245–52. https://doi.org/10.1016/j.jmii.2013.03.001 PMID: 23639380
6. Ko AI, Goarant C, Picardeau M. Leptospira: the dawn of the molecular genetics era for an emerging zoonotic pathogen. Nat Rev Microbiol. 2009; 7(10):736–47. https://doi.org/10.1038/nrmicro2208 PMID: 19756012
7. Muñoz-Zanzi C, Mason MR, Encina C, Astroza A, Romero A. Leptospira contamination in household and environmental water in rural communities in southern Chile. Int J Environ Res Public Health. 2014; 11(7):6666–80. https://doi.org/10.3390/ijerph110706666 PMID: 24972030
8. Llewellyn J-R, Krupka-Dyachenko I, Rettinger AL, Dyachenko V, Stamm I, Kopp PA, et al. Urinary shedding of leptospires and presence of Leptospira antibodies in healthy dogs from Upper Bavaria. Berl Munch Tierarztl Wochenschr. 2016; 129(5–6):251–7. PMID: 27344919

9. Arbour J, Blais M-C, Carioto L, Sylvestre D. Clinical leptospirosis in three cats (2001–2009). J Am Anim Hosp Assoc. 2012; 48(4):256–60. https://doi.org/10.5326/JAAHA-MS-5748 PMID: 22611217
10. Azócar-Aedo L, Monti G, Jara R. Leptospira spp. in Domestic Cats from Different Environments: Prevalence of Antibodies and Risk Factors Associated with the Seropositivity. Anim an open access J from MDPI. 2014; 4(4):612–26.
11. Chan K-W, Hsu Y-H, Hu W-L, Pan M-J, Lai J-M, Huang K-C, et al. Serological and PCR detection of feline leptospira in southern Taiwan. Vector Borne Zoonotic Dis. 2014; 14(2):118–23. https://doi.org/10.1089/vbz.2013.1324 PMID: 24359421
12. Rodriguez J, Blais M-C, Lapointe C, Arsenault J, Carioto L, Harel J. Serologic and urinary PCR survey of leptospirosis in healthy cats and in cats with kidney disease. J Vet Intern Med. 2014; 28(2):284–93. https://doi.org/10.1111/jvim.12287 PMID: 24417764
13. Afiri M, Amara Khorba A, Ait Kaid D. Renal manifestations of leptospirosis: 88 cases. Med Sante Trop. 2013; 23(2):234–5.
14. Afiri M, Toudeft F, Ait KD. Leptospirosis epidemic: 48 cases. Med Sante Trop. 2013; 23(2):234.
15. Branger C, Blanchard B, Fillonneau C, Suard I, Aviat F, Chevallier B, et al. Polymerase chain reaction assay specific for pathogenic Leptospira based on the gene hap1 encoding the hemolysis-associated protein-1. FEMS Microbiol Lett. 2005; 243(2):437–45. https://doi.org/10.1016/j.femsle.2005.01.007 PMID: 15686847
16. Harkin KR, Roshto YM, Sullivan JT. Clinical application of a polymerase chain reaction assay for diagnosis of leptospirosis in dogs. J Am Vet Med Assoc. 2003; 222(9):1224–9. PMID: 12725309
17. Smythe LD, Smith IL, Smith GA, Dohnt MF, Symonds ML, Barnett LJ, et al. A quantitative PCR (TaqMan) assay for pathogenic Leptospira spp. BMC Infect Dis. BioMed Central; 2002; 2:13.
18. Appelt S, Armougom F, Le Bailly M, Robert C, Drancourt M. Polyphasic analysis of a middle ages coprolite microbiota, Belgium. PLoS One. Public Library of Science; 2014; 9(2):e88376. https://doi.org/10.1371/journal.pone.0088376 PMID: 24586319
19. La Scola B La, Bui LTM, Baranton G, Khamis A, Raoult D. Partial rpoB gene sequencing for identification of Leptospira species. FEMS Microbiol Lett. 2006; 263(2):142–7. https://doi.org/10.1111/j.1574-6968.2006.00377.x PMID: 16978348
20. Campbell I. Chi-squared and Fisher–Irwin tests of two-by-two tables with small sample recommendations. Stat Med. 2007; 26(19):3661–75. https://doi.org/10.1002/sim.2832 PMID: 17315184
21. Richardson JTE. The analysis of 2 × 2 contingency tables-Yet again. Stat Med. John Wiley & Sons, Ltd.; 2011; 30(8):890–890. https://doi.org/10.1002/sim.4116 PMID: 21432882
22. Altman DG, Machin D, Bryant TN, Gardner MJ (Eds) (2000) Statistics with confidence, 2nd ed. BMJ Books. (p. 49).
23. Emily J. Viau, Alexandria B. Boehm. Quantitative PCR-based detection of pathogenic Leptospira in Hawai'ian coastal streams. Journal of Water and Health. 2011; 9(4):637–646. https://doi.org/10.2166/wh.2011.064
24. Thaipadunpanit J, Chierakul W, Wuthiekanun V, et al. Diagnostic Accuracy of Real-Time PCR Assays Targeting 16S rRNA and lipl32 Genes for Human Leptospirosis in Thailand: A Case-Control Study. PLoS ONE. 2011; 6(1):e16236. https://doi.org/10.1371/journal.pone.0016236 PMID: 21283633
25. Aubry P, Bordahandy R, Ferah T, Mailloux M, Thomas J. An anademy of ictero-hemorrhagic leptospirosis in a military group in Algeria. Bull Soc Pathol Exot Filiales. 1975; 68(4):370–6. PMID: 1243751
26. Donatien A, Gayot G, Poul J. Leptospirosis of the dog in Algeria. Arch l'Institut Pasteur d'Algerie Inst Pasteur d'Algerie. 1951; 29(1):20–4.
27. Bal AE, Gravekamp C, Hartskeerl RA, De Meza-Brewster J, Korver H, Terpstra WJ. Detection of leptospires in urine by PCR for early diagnosis of leptospirosis. J Clin Microbiol. 1994; 32(8):1894–8. PMID: 7989538
28. Brown PD, Gravekamp C, Carrington DG, van de Kemp H, Hartskeerl RA, Edwards CN, et al. Evaluation of the polymerase chain reaction for early diagnosis of leptospirosis. J Med Microbiol. 1995; 43(2):110–4. https://doi.org/10.1099/00222615-43-2-110 PMID: 7629850
29. Villumsen S, Pedersen R, Borre MB, Ahrens P, Jensen JS, Krogfelt KA. Novel TaqMan® PCR for detection of Leptospira species in urine and blood: pit-falls of in silico validation. J Microbiol Methods. 2012; 91(1):184–90 https://doi.org/10.1016/j.mimet.2012.06.009 PMID: 22750039
30. Harkin KR, Roshto YM, Sullivan JT, Purvis TJ, Chengappa MM. Comparison of polymerase chain reaction assay, bacteriologic culture, and serologic testing in assessment of prevalence of urinary shedding of leptospires in dogs. J Am Vet Med Assoc. 2003; 222(9):1230–3. PMID: 12725310
31. Scanziani E, Origgi F, Giusti AM, Iacchia G, Vasino A, Pirovano G, et al. Serological survey of leptospiral infection in kennelled dogs in Italy. J Small Anim Pract. 2002; 43(4):154–7. PMID: 11996391

32. Klopfleisch R, Kohn B, Plog S, Weingart C, Nöckler K, Mayer-Scholl A, et al. An emerging pulmonary haemorrhagic syndrome in dogs: similar to the human leptospiral pulmonary haemorrhagic syndrome Vet Med Int. Hindawi Limited;2010; 2010:928541. https://doi.org/10.4061/2010/928541 PMID: 21274452

33. Brown CA, Roberts AW, Miller MA, Davis DA, Brown SA, Bolin CA, et al. Leptospira interrogans serovar grippotyphosa infection in dogs. J Am Vet Med Assoc. 1996; 209(7):1265–7. PMID: 8837647

34. Zakeri S, Khorami N, Ganji ZF, Sepahian N, Malmasi A-A, Gouya MM, et al. Leptospira wolffii, a potential new pathogenic Leptospira species detected in human, sheep and dog. Infect Genet Evol. 2010; 10 (2):273–7. https://doi.org/10.1016/j.meegid.2010.01.001 PMID: 20074666

35. Cai HY, Hornby G, Key DW, Osuch MR, Maxie MG. Preliminary study on differentiation of Leptospira grippotyphosa and Leptospira sejroe from other common pathogenic leptospiral serovars in canine urine by polymerase chain reaction assay. J Vet Diagn Invest. 2002; 14(2):164–8. https://doi.org/10.1177/104063870201400214 PMID: 11939341

36. Vicari D, Percipalle M, Concetta LM, Li Vecchi L, Curro V, Vitale M, et al. Evidence of canine leptospirosis in kennels in Sicily, by PCR method. Rev Cubana Med Trop. 2007; 59(1):61–2. PMID: 23427421

37. Rojas P, Monahan AM, Schuller S, Miller IS, Markey BK, Nally JE. Detection and quantification of leptospires in urine of dogs: a maintenance host for the zoonotic disease leptospirosis. Eur J Clin Microbiol Infect Dis. 2010; 29(10):1305–9. https://doi.org/10.1007/s10096-010-0991-2 PMID: 20559675

38. Oliveira ST, Messick JB, Welker Biondo A, Pires A, Santos D, Stedile R, et al. Exposure to Leptospira spp. in Sick Dogs, Shelter Dogs and Dogs from an Endemic Area: Points to Consider. Acta Sci Vet. 2012; 40(403):1056–1056.

39. Fraune CK, Schweighauser A, Francey T. Evaluation of the diagnostic value of serologic microagglutination testing and a polymerase chain reaction assay for diagnosis of acute leptospirosis in dogs in a referral center. J Am Vet Med Assoc. 2013; 242(10):1373–80. https://doi.org/10.2460/javma.242.10.1373 PMID: 23634681

40. Calderón A, Rodríguez V, Máttar S, Arrieta G. Leptospirosis in pigs, dogs, rodents, humans, and water in an area of the Colombian tropics. Trop Anim Health Prod. 2014; 46(2):427–32. https://doi.org/10.1007/s11250-013-0508-y PMID: 24254419

41. Gay N, Soupé-Gilbert M-E, Goarant C. Though not Reservoirs, Dogs might Transmit Leptospira in New Caledonia. Int J Environ Res Public Health. 2014; 11(4):4316–25. https://doi.org/10.3390/ijerph110404316 PMID: 24747539

42. Sant'anna R, Vieira AS, Grapiglia J, Lilenbaum W. High number of asymptomatic dogs as leptospiral carriers in an endemic area indicates a serious public health concern. Epidemiol Infect. 2017; 145 (9):1852–4. https://doi.org/10.1017/S0950268817000632 PMID: 28367783

43. Delaude A, Rodriguez-Campos S, Dreyfus A, Counotte MJ, Francey T, Schweighauser A, et al. Canine leptospirosis in Switzerland-A prospective cross-sectional study examining seroprevalence, risk factors and urinary shedding of pathogenic leptospires. Prev Vet Med. 2017; 141:48–60. https://doi.org/10.1016/j.prevetmed.2017.04.008 PMID: 28532993

44. Fink JM, Moore GE, Landau R, Vemulapalli R. Evaluation of three 5' exonuclease-based real-time polymerase chain reaction assays for detection of pathogenic Leptospira species in canine urine. J Vet Diagn Invest. 2015; 27(2):159–66. https://doi.org/10.1177/1040638715571360 PMID: 25776541

45. Felt SA, Wasfy MO, El-Tras WF, Samir A, Rahaman BA, Boshra M, et al. Cross-species surveillance of Leptospira in domestic and peri-domestic animals in Mahalla City, Gharbeya Governorate, Egypt. Am J Trop Med Hyg. 2011; 84(3):420–5. https://doi.org/10.4269/ajtmh.2011.10-0393 PMID: 21363980

46. Xu C, Loftis A, Ahluwalia SK, Gao D, Verma A, Wang C, et al. Diagnosis of canine leptospirosis by a highly sensitive FRET-PCR targeting the lig genes. Dellagostin OA, editor. PLoS One. 2014; 9(2): e89507. https://doi.org/10.1371/journal.pone.0089507 PMID: 24586833

47. Ward MP, Glickman LT, Guptill LE. Prevalence of and risk factors for leptospirosis among dogs in the United States and Canada: 677 cases (1970–1998). J Am Vet Med Assoc. 2002:53–8.

48. Desvars A, Naze F, Benneveau A, Cardinale E, Michault A. Endemicity of leptospirosis in domestic and wild animal species from Reunion Island (Indian Ocean). Epidemiol Infect. 2013; 141(6):1154–65. https://doi.org/10.1017/S0950268812002075 PMID: 22998941

49. Dickeson D, Love DN. A serological survey of dogs, cats and horses in south-eastern Australia for leptospiral antibodies. Aust Vet J. 1993; 70(10):389–90. PMID: 8257320

50. Agunloye CA, Nash AS. Investigation of possible leptospiral infection in cats in Scotland. J Small Anim Pract. 1996; 37(3):126–9. PMID: 8683955

51. Mylonakis ME, Bourtzi-Hatzopoulou E, Koutinas AF, Petridou E, Saridomichelakis MN, Leontides L, et al. Leptospiral seroepidemiology in a feline hospital population in Greece. Vet Rec. 2005; 156 (19):615–6. PMID: 15879545

52. Jamshidi S, Akhavizadegan M, Maazi N, Ali AG, Bokaie S. Serologic study of feline leptospirosis in Tehran, Iran J Microbiol 2009; 1:32–36.

53. Millán J, Candela MG, López-Bao JV, Pereira M, Jiménez MA, León-Vizcaíno L. Leptospirosis in wild and domestic carnivores in natural areas in Andalusia, Spain. Vector Borne Zoonotic Dis. 2009; 9 (5):549–54. https://doi.org/10.1089/vbz.2008.0081 PMID: 18973450

54. Lapointe C, Plamondon I, Dunn M. Feline leptospirosis serosurvey from a Quebec referral hospital. La Rev Vet Can. 2013; 54(5):497–9.

55. Ramírez-Ramírez MM, León-Castañeda OM, Rodriguez-Morales AJ. Leptospirosis in an urban setting: cases diagnosed at a private medical center of Western Colombia, 2008–2012. Recent Pat Antiinfect Drug Discov. 2015; 10(1):59–63. PMID: 25873099

56. Dos Santos LF, Guimarães MF, de Souza GO, da Silva IWG, Santos JR, Azevedo SS, et al. Seroepidemiological survey on Leptospira spp. infection in wild and domestic mammals in two distinct areas of the semi-arid region of northeastern Brazil. Trop Anim Health Prod. 2017; 49(8):1715–22. https://doi.org/10.1007/s11250-017-1382-9 PMID: 28861677

57. Weis S, Rettinger A, Bergmann M, Llewellyn JR, Pantchev N, Straubinger RK, et al. Detection of Leptospira DNA in urine and presence of specific antibodies in outdoor cats in Germany. J Feline Med Surg. 2017; 19(4):470–6. https://doi.org/10.1177/1098612X16634389 PMID: 26927819

58. Davis MA, Evermann JF, Petersen CR, VancerSchalie J, Besser TE, Huckabee J, et al. Serological survey for antibodies to Leptospira in dogs and raccoons in Washington State. Zoonoses Public Health. 2008; 55(8–10):436–42. https://doi.org/10.1111/j.1863-2378.2008.01137.x PMID: 18631236

59. Renaud C, Andrews S, Djelouadji Z, Lecheval S, Corrao-Revol N, Buff S, et al. Prevalence of the Leptospira serovars bratislava, grippotyphosa, mozdok and pomona in French dogs. Vet J. 2013; 196 (1):126–7. https://doi.org/10.1016/j.tvjl.2012.10.002 PMID: 23141966

60. Hartmann K, Egberink H, Pennisi MG, Lloret A, Addie D, Belák S, et al. Leptospira species infection in cats: ABCD guidelines on prevention and management. J Feline Med Surg. 2013:576–81. https://doi.org/10.1177/1098612X13489217 PMID: 23813819

61. Langston CE, Heuter KJ. Leptospirosis. A re-emerging zoonotic disease. Vet Clin North Am Small Anim Pract. 2003; 33(4):791–807. PMID: 12910744

Communications internationales

République algérienne démocratique et populaire
Ministère de la sante et de la reforme hospitalière
Etablissement hospitalier spécialisé en maladies infectieuses
EL HADI FLICI EX EL KETTAR

ATTESTATION DE COMMUNICATION

Le président et le comité d'organisation des
2emes journées internationales d'infectiologie
De l'EHS EL HADI FLICI 13 et 14 décembre 2018.

Atteste par la présente que
Madame/Monsieur :

Dr Ghaoui

A présenté une communication Orale intitulée :

Fièvre Q

Co auteur Pr Achour

Présidente comité D'organisation Pr Achour .N	Président comité Scientifique Pr Mesbah	Président de séance

Centre Hospitalo Universitaire Tlemcen
Service de Maladies Infectieuses
Laboratoire de recherche VIH/SIDA

Université Abou Bekr Belkaid
Faculté de Médecine de Tlemcen

1éres Rencontres Internationales d'Infectiologie

Tlemcen 09 - 10 - 11 Mars 2018

Attestation de Communication

La présidente du comité d'organisation atteste que

Mr/Mme: **H.GHAOUI**

A participé à la 1éres Rencontres Internationales d'Infectiologie Tlemcen

Avec la communication affichée intitulée: **FIEVRE Q A ALGER.**

Co-Auteur(s): ***I.BITAM, N.ACHOUR,***

Pr S.Benchouk
Présidente du comité d'organisation

1er Rencontre internationale d'infectiologie Tlemcen 1er R.I.I.T 9-11 MARS 2018

Pr A.Benabdellah
Président du comité scientifique

Le président de Séance
Pr Brahimi

Networking & Refreshments 15:50-16:10 @ Foyer

16:10-16:35	Title: Prevalence and resistance of *Acinetobacter baumannii* **Said Hassan,** Huazhong University of Science and Technology, China
16:35-17:00	Title: *Coxiella burnetii* infection with women's febrile spontaneous abortion reported in Algiers **Hichem Ghaoui,** Aix-Marseille University, France
17:00-17:25	Title: Uropathogens among diabetic patients at Zagazig University Hospital's outpatient clinics: prevalence and their antibiotic susceptibility patterns **Walid Mohamed Attiah,** Zagazig University, Egypt

Panel Discussion

Awards & Closing Ceremony

Références Bibliographiques

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Article 1 :

[1]: Mediannikov O, Fenollar F, Socolovschi C, Diatta G, Bassene H, et al. *Coxiella burnetii* in Humans and Ticks in Rural Senegal. Negleted Tropical Diseases. April 2010; 4:654.

[2]: Quijada SG, Tera´n BM, Murias PS, Anitua AA, Cermen˜o JL and Frıas AB. Q fever and spontaneous abortion, Clin Microbiol Infect. 2012; 18: 533–538.

[3]: Munster JM, Leenders ACAP, Hamilton CJCM, Hak E, Aarnoudse JG, Timmer A. Placental histopathology after *Coxiella burnetii* infection during pregnancy. Elsevier Ltd. Placenta. 2011;11.012.

[4]: Lacheheb A and Raoult D. Seroprevalence of Q-fever in Algeria. Clinical Microbiology and Infection. December 2009; 15 (2).

[5]: Nielsen SY, Anderson NAM, Mølbak AK, Hjøllund NH, Kantsø B, Krogfelt KA and Henriksen TB. No Excess Risk of Adverse Pregnancy Outcomes Among Women with Serological Markers of Previous Infection with *Coxiella burnetii*: Evidence from the Danish National Birth Cohort. BMC Infectious Diseases. 2013:13:87.

[6]: Benslimani A, Fenollar F, Lepidi H, and Didier Raoult. Bacterial zoonoses and infective Endocarditis, Algeria. Emerging Infectious Diseases. 2005 ;11 (2).

[7]: Domonique R, Obadia Y, Tissot-Dupont H, Didier Raoult. Seroprevalence of Antibodies to *Coxiella burnetii* among Pregnant Women in South Eastern France. European Journal of Obstetrics & Gynecology and Reproductive Biology 93. 2000: 151-156.

[8]: Langley JM, Marrie TJ, LeBlanc JC, Almudevar A, Resch L, and Raoult D. *Coxiella burnetii* Seropositivity in Parturient women is Associated with Adverse Pregnancy Outcomes. Am J Obstet Gynecol. 2003;189: 228-32.

[9]: Roult D, Fenollar F, Stein A. Q Fever During Pregnancy. ARCHI INTER MED. 2002; 162:701-704.

[10]: McCaughey C, McKenna J, McKenna C, Coyle PV, O'Neill HJ, Wyatt DE, Smyth B, and Murray LJ. Human Seroprevalence to *Coxiella burnetii* [Q fever] in Northern Ireland. Zoonoses Public Health. 2008: 189–194.

[11]: Carcopino X, Raoult D, Boubli F, and Andreas Stein. Managing Q Fever during Pregnancy: The Benefits of Long-Term Cotrimoxazole Therapy. Clinical Infectious Diseases 2007; 45:548–55.

[12]: Vaidya MV, Malik SVS, Kaur S, Kumar S, and Barbuddhe SB. Comparison of PCR, Immunofluorescence Assay and Pathogen Isolation for Diagnosis of Q Fever in Humans with Spontaneous Abortions. Journal of Clinical Microbiology. June 2008; 2038–2044.

[13]: Fournier PE, Thomas JM, and RAOULT D. Diagnosis of Q Fever. Journal of Clinical Microbiology. July 1998: 1823–1834.

[14]: Nielson SY, Molbak K, Henriksen TB, Krogfelt KA, Larsen CS, and Villumsen S. Adverse Pregnancy Outcome and *Coxiella burnetii* Antibodies in ¨Pregnant Women, Denmark. Emerging Infectious Diseases. June 2014; 20 (6): 925-31.

[15]. Moodie CE, Thompson HA, Meltzer MI, and Swerdlow DL. Prophylaxis after Exposure to *Coxiella burnetii*. Emerging Infectious Diseases. Octobre 2008; 14(10): 1558-1566.

[16]. Coste Mazeau, Hantz S, Eyraud JL, Donadel L, Lacorre A, Rogez S, Aubard Y, Gauthier T. Q fever and Pregnancy: Experience from the Limoges Regional University Hospital. Arch Gynecol Obstet. Aug 2016; 294(2): 233-8.

[17]. Nielsen SY, Hjøllund NH, Anderson NAM, Henriksen TB, Kantso B, Krogfelt KA, Mølbak AK. Prsence of Antibodies Against *Coxiella burnetii* and Risk of Spontaneous Abortion: A Nested Case-Control Study. PLos ONE. February 2012; 7 (2): e31909.

[18]. Angelakis E, Million M, D'Amato F, Rouli L, Richet H, Stein A, Rolain JM, Raoult D. Q fever and Pregnancy: Disease, Prevention, and Strain Specificity. Eur J Clin Microbiol Infect Dis. 2013;32: 361-368.

[19]. Shpynov SN, Tarasevich IV, Skiba AA, Pozdnichenko NN and Gumenuk AS. Comparison of genomes of *Coxiella burnetii* strains using formal order analysis. New Microbes and New Infect;2018; 23:86-92.

[20]. Million M, Roblot F, Carles D, D'Amato F, Protopopescu C, Carrieri MP and Raoult D. Reevaluation of the Risk of Fetal Death and Malformation After Q fever. Clinical Infectious Diseases. 2014; 59 (2):256-60.

[21]. Edouard S, Mahamat A, Demar M, Djossou PAF and Raoult D. Comparison between Emerging Q Fever in French Guiana and Endemic Q fever in Marseille, France. The American Society of Tropical Medicine and Hygiene. 2013, pp. 000–000.

[22]. Fournier PE, Thuny F, Richet H, Lepidi H, Casalta JP, Arzouni JP, Maurin M, Célard M, Mainardi JL, Caus T, Collart F, Habib G, Raoult D. Comprehensive Diagnostic Strategy for Blood Culture–Negative Endocarditis: A Prospective Study of 819 New Cases. Clinical Infectious Diseases. 2010; 51(2):131–140.

Article 2 :

[1]. Oyston P.C.F and Davies C. Q fever: the neglected biothreat agent. Journal of Medical Microbiology. 2011; 60; 9-21.

[2]. Pavia C.S, McCalla C. Serologic detection of a rare case of Q fever in New York City having hepatic and unusual renal complications. Infection. 2010; 38; 325-329.

[3]. Raoult D, Marrie T. Q fever. Clin Infect Dis off Publ Infect Dis Soc Am. 1995; 20:489-95.

[4]. Raoult D, Tissot-Dupont H, Foucault C, Gouvernet J, Fournier PE, Bernit E, et al. Q fever 1985-1998. Clinical and epidemiologic features of 1,383 infections. Medecine (Baltimore). 2000; 79 :109-23.

[5]. Van der Hoek W, Versteeg B, Meekelenkamp JC, Renders NH, Leenders AC, Weers-pothoff I, et al. Follow-up of 686 patients with acute Q Fever and detection of chronic infection. Clin Infect Dis. 2011. 52;12:1431-6.

[6]. Boone I, Henning K, Hilbert A, Neubauer H, Kalckreuth V et al. Are brucellosis, Q fever and melioidosis potentiel causes of febrile illness in Madagascar. Acta Tropica 2017: 255-262.

[7]. Cikman A, Aydin M, Gulhan B, Karakecili F, Ozciceck A, et al. The seroprevalence of *Coxiella burnetii* in Erzincan, Turkey: Identification of the risk factors and their relationship with geographical featues. J Vector Borne Dis. 2017;54: 157-163.

[8]. Raoult D. Re-emergence of Q fever after 11 September 2001. Clin Infect Dis. 2009;48: 558-559.

[9]. FOURNIER PE,Marrie T.J, and RAOULT D. Diagnosis of Q Fever. Journal Of Clinical Microbiology. 1998 July: 1823–1834.

[10]. Lachehab A, Raoult D. Seroprevalence of Q fever in Algeria. European Society of Clinical Microbiology and Infectious Diseases. 2009 ;15 : 167-168.

[11]. Dumas N. Rickettsioses et Chlamydioses au Hoggar (République Algérienne) : sondage épidémiologique. Bull Soc Pathol Exot 1984 ;77 :278-283.

[12]. Benslimani A, Fenollar F,Lepidi H, and Raoult D. Bacterial zoonoses and infective Endocarditis, Algeria. Emerging Infectious Diseases. 2005;11: No. 2.

[13]. Bessas A, Leulmi H, Bitam I, Zaidi S, Ait-Oudhia K, Raoult D, Parola P. Molecular evidence of vector-borne pathogens in dog and cat and their ectoparasites in Algiers, Algeria. Comparative Immunology, Microbiology and Infectious Diseases.2016;45: 23-28.

[14]. Khaled H, Sidi-Boumedine K, Merdja,P , Dahmani A, Rousset RTE ,.Bouyoucef.A. Serological and Molecular evidence of Q fever among small ruminant flocks in Algeria. Comparative Immunology, Microbiology and Infectious Diseases.2016;47: 19-25.

[15]. Aouadi A, Leulmi H, Boucheikhchoukh M, Benakhla A, Raoult D, Parola P. Molecular evidence of tick-borne hemoprotozoan-parasites (*Theileria ovis and Babesiaovis*) and bacteria in ticks and blood from small

ruminants in Northern Algeria. Comparative Immunology, Microbiology and Infectious Diseases.2017;50: 34-39.

[16]. Kilic S,Yilmaz GR, Komiya T, Kurtogu Y, Karakoc EA. Prevalence of *Coxiella burnetii* antibodies in blood donors in Ankara, Central Anatolia, Turkey. New Microbiol. 2008;31:527-34.

[17]. Schelling E, Diguimbaye C, Daoud S, Nicolet I, Boerlin P, Tanner M, et al. Brucellosis and Q fever seroprevalences of nomadic pastoralists and their livestock in Chad. Prev Vet Med 2003;61: 279-93.

[18]. Bellazreg F, kaabia N, Hachfi W, Khalifa M, Ben jazia E, Ghanouchi N, Brahem A, Bahri F and Letaief A. Acute Q fever in hospitalised patients in Central Tunisia: report of 21 cases. European Society of Clinical Microbiology and Infectious Diseases .2009;15: 138-139.

[19]. Letaief A, Yacoub S, Dupon HT, LE Cam C, Ghachem L, Jemni L, Raoult D. Seroepidemiological survey of rickettsial infections among blood donors in central Tunisia. Trans R Soc Trop Med Hyg. 1995;83: 266-8.

[20]. kaabia, Rolain JM, Khalifa M,Ben Jazia E, BahriD F. Raoult D, and Letaief A. Serologic study of Rickettsioses among Acute Febrile Patient in Central Tunisia. N. Y Acad.Sci 1078. 2006; 176-179.

[21]. Meskini M, Beati L, Benslimani A, Raoult D. Seroepidemiology of Rickettsial infections in Morocco. Eur J Epidemiol, 1995;11: 655-60.

[22]. Mediannikov O, Fenollar F, Socolovschi C, Diatta G, Raoult D, et al. *Coxiella burnetii* in Humans and Ticks in Rural Senegal. Neglected Tropical Diseases. 2010;4: e654.

[23]. Angelakis E, Mediannikov O, Socolovschi C, Mouffok N, Bassene H, Tall A, Niangaly H, Raoult D, and al. *Coxiella burnetii*-positive PCR in febrile patients in rural and urban Africa. International Journal of Infectious Diseases .2014;28: 107-110.

[24]. Dabaja MF, Greco G, Villari S, Bayan A, Mortada M, and all. The First Serological Study of Q fever in Humans in Lebanon. Vector-Borne and Zoonotic Diseases. 2018.

[25]. Hamilton LTC LR, Dena M, George L, Scoville SL, and all. PCR for Rapid Diagnosis of Acute Q Fever at a combat Support Hospital in Iraq. Military Medecine. 2011;176, 1:103.

[26]. Mobarez AM, Bagheri F, Esmaeili S. Seroprevalenec of Q Fever among human and animal in Iran: A Systematic review and meta-analysis. Neglected Tropical Diseases. 201711 e0005521.

[27]. Czerwinski MS, Galinska EM, Niemczuk K, and Piotr-Knap J. Prevalence of *Coxiella burnetii* Infection in Humans Occupationally Exposed to Animals in Poland. Vector-borne and Zoonotic Diseases. 2015;15.

Article 3 :

[1]. T.J. O'Neill, J.M. Sargeant and Z. Poljak. A systematic Review and Meta-Analysis of Phase I Inactivated Vaccines to Reduce Shedding of *Coxiella burnetii* from Sheep and Goats From Routes of Public Health Importance. Zoononse and Public Health,2013: doi: 10.1111/zph.12086.

[2]. Hasan Cantas, Adrian Muwonge, Baris Sareyyupoglu, Hakan Yardimici, Eystein Skjerve. Q fever abortions in ruminants and associated on-farm risk factors in northern Cyprus. BMC Vetrinary Research, 2011;7.13.

[3]. Sky Vanderburg, Matthew P. Rubach, Jo E.B. Halliday, Sarah Cleaveland, Elizabeth A. Reddy, John A. Crump. Epidemiology of *Coxiella burnetii* Infection In Africa: A OneHealth Systematic Review. Plos Neglected Tropical Diseases, April 2014; 8-4:e2787.

[4]. Michelle P. Emery, Eileen N. Ostlund, Mohamed Ait Ichu, Jeff D. Ballin, David McFarling, Luanne McGonigle. *Coxiella burnetii* Serology assays in goat abortion storm. Journal of Veterinary Diagnostic Investigation, 2014;26-1:141-145.

[5]. Javad Asadi, Mojtaba Kafi and Mohammad Khalili. Seroprevalenece of Q fever in sheep and goat flocks with a history abortion in Iran between 2011 and 2012. Veterinaria Italiana, 2013;49-2:163-168.

[6]. Maria Luisa Marenzoni, Valentina Stefanetti, Paola Papa, Patrizia Casagrande, et al. Is the horse a reservoir or an indicator of *Coxiella burnetii* infection? Systematic review and biomolecular investigation. Veterinary Microbiology,2013;167:662-669.

[7]. Krzysztof Niemczuk, Monika Szymanska-Czerwinska, Krzysztof Smietanka, Lukas Bocian. Comparison of diagnostic potential of serological, molecular and cell culture methods for detection of Q fever in ruminants. Veterinary Microbiology,2014;171:147-151.

[8]. O, Roux V, Freylikman O, Sekeyova Z, Fournous G, Tyczka J,Tokarevich N, Kovacava E, Marrie TJ, Raoult D. Coxiella burnetii genotyping. Emerg Infect Dis,2005;11:1211–1217.

[9]. Carole Eldin, Cléa Mélenotte, Oleg Mediannikov, Eric Ghigo, Matthieu Million,Sophie Edouard, Jean-Louis Mege, Max Maurin, Didier Raoult. From Q Fever to *Coxiella burnetii* Infection: A Paradigm Change. Clinical Microbiology Reviews, January 2017; 30-1:115-190.

[10]. Atef Aouadi, Hamza Leulmi, Mehdi Boucheikhchoukh, Ahmed Benakhla, Didier Raoult, Philippe Parola. Molecular evidence of tick-borne hemoprotozoan-parasites (*Theileria ovis and Babesiaovis*) and bacteria in ticks and blood from small ruminants in Northern Algeria. Comparative Immunology, Microbiology and Infectious Diseases,2017;50: 34-39.

[11]. Mohamed Barkallah, Yaakoub Gharbi, Amal Ben Hassena, Ahlem Ben Slima, Zouhir Mallek, Michel Goutier, Gilbert Greub, Radhouane Gdoura, Imen Fendri. Survey of Infectious Etiologies of Bovine Abortion during Mild-to Late Gestation in Dairy Herds. PlOS ONE,2014;9-3:e91549.

[12]. Jothimani Pradeep, Selvaraj Stephan, Pratheesh Pooja, Anbalagan Akshayavardhini, Balakrishnan Sangeetha and Prabkar Xavier Antony. Coxiellosis in domestic livestock of Puducherry and Tamil Nadu: Detection of *Coxiella burnetii* DNA by polymerase chain reaction in slaughtered ruminants. Veterinary Wolrd,2017;10:667-671.

[13]. A. Lacheheb and D. Raoult. Seroprevalence of Q-fever in Algeria. Clinical Microbiology and Infection, December 2009; 15-2.

[14]. H. Khaled, K.Sidi-Boumedine, S.Merdja,P. Dufour, A.Dahmani, R.Thiéry, E.Rousset,A.Bouyoucef. Serological and Molecular evidence of Q fever among small ruminant flocks in Algeria. Comparative Immunology, Microbiology and Infectious Diseases ,2016;47: 19-25.

[15]. Amina Bessas, Hamza Leulmi, Idir Bitam, Sara Zaidi, Khatima Ait-Oudhia, Didier Raoult, Philippe Parola. Molecular evidence of vector-borne pathogens in dog and cat and their ectoparasites in Algiers, Algeria. Comparative Immunology, Microbiology and Infectious Diseases, 2016;45: 23-28.

[16]. Mohammed H. Benaissa, Samir Ansel, Abdallah Mohamed-Cherif, Karima Benfodil, Djamel Khelef, Curtis R. Youngs, Rachid Kaidi, Khatima Ait-Oudhia. Seroprevalence and risk factors for *Coxiella burnetii*, the causative agent of Q fever in the dromedary camel (*Camelus dromedarius*) population in Algeria. Onderstepoort Journal of Veterinary Research, 2017.

[17]. SuKru KIRKAN, Osman KAYA, Serten TEKBIYIK, Ugur PARIN. Detection of *Coxiella burnetii* in Cattle by PCR. Turk.j.Vet.Anim.Sci, 2008;32-3:215-220.

[18]. Katharina Sobotta, Katharina Bonkowski, Elisabeth Liebler-Tenorio, Pierre Germon, Pascal Rainard, Nina Hambruch, Christiane Pfarrer, Ilse D. Jacobsen and Christian Menge. Permissiveness of bovine epithelial cells from lung,intestine, placenta and udder for infection with *Coxiella burnetii.* Vet Res , 2017 ;48:23.

[19]. J. Muskens, C van Maanen, M.H Mars. Dairy cows with metritis: *Coxiella burnetii* test results in uterine, blood and bulk milk samples. Veterinary Microbiology, 2011;147:186-189.

[20]. Joshua Kamani, Gad Baneth, Ricardo Gutiérrez, Yaarit Nachum-Biala, Y. Mumcuoglu, Shimon Harrus. *Coxiella burnetii* and *Rickettsia conorii*: two zoonotic pathogens in peridomestic rodents and thir ectoparasites in Nigeria. Ticks and Ticks borne Diseases, 2017.

[21]. Margarita Bolanos-Rivero, Cristina Carranza-Rodrıguez, Noe F. Rodrıguez, Carlos Gutierrez, and Jose -Luis Perez-Arellano. Detection of *Coxiella burnetii* DNA in Peridomestic and Wild Animals and Ticks in an Endemic Region (Canary Islands, Spain). VECTOR-BORNE AND ZOONOTIC DISEASES,2017.

[22]. F.D'amato, M.Million, S.Edourad, J.Delerce, C.Robert, T. Marrie and D Raoult. Draft genome sequence of *Coxiella burnetii* Dog Utad, a strain isolated from a dog-related outbreak of Q fever. NMNI, 2014;2:136-137.

[23]. Calvin K.Y. Cheng, Benjamin J. Cowling, Eric H.Y. Lau, Lai Ming Ho, Gabriel M. Leung, and Dennis K.M. Ip. Epidemic Genotype of *Coxiella burnetii* among Goats, Sheep, and Humans in the Netherlands. Emerging Infectious Diseases, 2012; 18-5:887.

[24]. D'Amato F, Rouli L, Edouard S, Tyczka J, Million M, Robert C, Nguyen TT, Raoult D. The genome of *Coxiella burnetii* Z3055, a clone linked to the Netherlands Q fever outbreaks, provides evidence for the role of drift in the emergence of epidemic clones. Comp Immunol Microbiol Infect Dis, 2014; 37:281–288.

[25]. Kinga M Sulyok, Zsuzsa Kreizinger, HeidieM Hornstra, Talima Pearson, Alexandra Szigeti, ÁdámDán, Eszter Balla, Paul S Keim and Miklós Gyuranecz. Genotyping of *Coxiella burnetii* from domestic ruminants and human in Hungary: indication of various genotypes. BMC Veterinary Research,2014 ; 10:107.

[26]. Kamga-Waladjo AR, Gbati OB, Kone P, Lapo RA, Chatagnon G, Bakou SN, Pangui LJ, Diop PEH, Akakpo JA, Tainturier D. Seroprevalence of Neospora caninum antibodies and its consequences for reproductive parameters in dairy cows from Dakar-Senegal, West Africa. Trop Anim Health Prod,2010; 42:953–959.

Revue :

[1]. Kate D. Halsby, Hilary Kirkbride, Amanda L. Walsh, Ebere Okereke , Timothy Brooks , Matthew Donati and Dilys Morgan. The Epidemiology of Q Fever in England and Wales
2000–2015. Vet. Sci, 2017; 4: 28.

[2]. Sabatier F, Dignat-George F, Mege JL, Brunet C, Raoult D, Sampol J. CD4+ T-cell lymphopenia in Q fever endocarditis. *Clin Diagn Lab Immunol,* 1997; 4: 89–92.

[3]. Linda M. Kampschreur, Corine E. Delsing, et *al*. Chronic Q Fever in the Netherlands 5 Years after the Start of the Q Fever Epidemic: Results from the Dutch Chronic Q Fever Database. Journal of Clinical Microbiology, May 2014 ;52-5: 637–1643.

[4]. E. H. Derrick. Q Fever, a New Fever Entity: Clinical Features, Diagnosis and Laboratory Investigation. Reviews of Infectious Diseases,1983;5-4:790-800.

[5]. F.M. Burnet and Mavis Freeman. Experimental Studies of the Virus “Q” Fever. Reviews of Infectious Diseases,1983;5-4:800-808.

[6]. GORDON E. Davis and HERALD.R.Cox. A Filter-Passing Infectious Agent Isolated from Ticks. PuBLIC Health Reports, Dec 1938; 53-52:2259-2276.

[7]. R.E. Dyer. Human Infection. PuBLIC Health Reports, Dec 1938; 53-52:2277-2282.

[8]. Joseph E. McDade. Historical aspects of Q fever. Q fever: the disease,1990; I: 5-21.

[9]. Loïc Epelboin, Mathieu Nacher, Aba Mahamat, Vincent Pommier de Santi, et *al.* Q Fever in French Guiana: Tip of the Iceberg or Epidemiological Exception? Plos Neglected Tropical Diseases, May 2016.

[10]. Parker NR, Barralet JH, Bell AM. Q fever. Lancet,2006; 367:679–688.

[11]. Arricau-Bouvery N, Rodolakis A. Is Q fever an emerging or re-emerging zoonosis? Vet Res 2005; 36:327–349.

[12]. Armengaud A, Kessalis N, Desenclos JC, et al. Urban outbreak of Q fever, Briancon, France, March to June 1996. Euro Surveill, 1997; 2: 12–13.

[13]. Brouqui P, Badiaga S, Raoult D. Q fever outbreak in homeless shelter. Emerg Infect Dis, 2004; 10:1297–1299.

[14]. van Woerden HC, Mason BW, Nehaul LK, et al. Q fever outbreak in industrial setting. Emerg Infect Dis, 2004; 10:1282–1289.

[15]. Steiner HA, Raveh D, Rudensky B, et al. Outbreak of Q fever among kitchen employees in an urban hospital. Eur J Clin Microbiol Infect Dis, 2001; 20:898–900.

[16]. KA Bond, L Franklin, B Sutton, MA Stevenson and SM Firestone. Review of 20 years of human acute Q fever notifications in Victoria, 1994–2013. Australian Veterinary Journal, June 2018;96-6:223-230.

[17]. K. A. Bond, G. Vincent, C. R. Wilks, L. Franklin, B. Sutton, J. Stenos, et *al.* One Health approach to controlling a Q fever outbreak on an Australian goat farm. Epidemiol. Infect, 2016; 144 :1129–1141.

[18]. Brett N Archer, Cathie Hallahan, Priscilla Stanley, Kathy Seward, Margaret Lesjak, Kirsty Hope, Anthony Brown. Atypical outbreak of Q fever affecting low-risk residents of a remote rural town in New South Wales. CDI,2017; 42-2: E125-E132.

[19]. J.V IRONS, J.N MURPHY, DON M WOLFE. Q Fever in The United States. J.A.M.A. March 1947;133-12: 819-820.

[20]. J.H. MCQuiston and J.E. Childs. Q Fever in Humans and Animals in the United States. VECTOR BORNE AND ZOONOTIC DISEASES, 2002 ;2-3 :179-191.

[21]. Petros C. Karakousis, Michele Trucksis, and J. Stephen Dumler. Chronic Q Fever in the United States. JOURNAL OF CLINICAL MICROBIOLOGY, June 2006;2283–2287.

[22]. Adam Bjork, Nicola Marsden-Haug, Randall J. Nett, Gilbert J. Kersh, WilliamNicholson, et *al*. First Reported Multistate Human Q Fever Outbreak in the United States, 2011. VECTOR-BORNE AND ZOONOTIC DISEASES,2014 ;14.

[23]. Rare Q fever outbreak hits U.S medical tourists, 01/10/2015.
https://www.cbsnews.com/news/rare-q-fever-outbreak-hits-u-s-medical-tourists/ .

[24**].** Honarmand H. Q Fever: An old but still a poorly understood disease. Interdiscip Perspect Infect Dis, 2012.

[25]. S. N Shpynov, I.V Tarasevich, A.A. Skiba, N.N. Pozdnichenko and A.S. Gumenuk. Comparison of genomes of Coxeilla burnetii strains using formal order analysis. New Microbes and New Infections, 2018;23:86-92.

[26]. Roest HIJ, Tilburg JJ, Van der Hoek W, Vellema P, Van Zijderveld FG, Klaassen CH, Raoult D. The Q fever epidemic in The Netherlands: history, onset, response and reflexion. Epidemiol Infect, 2011;139:1-12.

[27]. P. Speelman. The largest Q fever outbreak ever reported. The Netherlans Journal of medicine, Dec 2010;68-12.

[28]. Van Steenbergen JE, Morroy G, Groot CAR, Ruikes FGH, Marcelis JH, Speelman P. Een uitbraak van Q-koorts in Nederland – mogelijk verband met geiten. Ned Tijdschr Geneeskd, 2007;8:151.

[29]. Diane Frankel, Hervé Richet, Aurélie Renvoisé, and Didier Raoult. Q fever in France, 1985-2009. Ermerging Infectious Diseases,2011; 17-3 :350-356.

[30]. Herve Tissot-Dupont, Sylvie Torres, Meyer Nezri, and Didier Raoult1. Hyperendemic Focus of Q Fever Related to Sheep and Wind. American Journal of Epidemiology,1999 ;150-1 :69-74.

[31]. Dellacasagrande J, Ghigo E, Capo C, Raoult D, Mege JL. *Coxiella burnetii* survives in monocytes from patients with Q fever endocarditis: involvement of tumour necrosis factor. *Infect Immun,*2000; 68: 160–64.

[32]. G Dupuis, J Petite , O Péter , M Vouilloz . An Important Outbreak of Human Q Fever in a Swiss Alpine Valley. *International Journal of Epidemiology,* June 1987 ;16-2: 282–287.

[33]. M. Maurin and D. Raoult. Q Fever. CLINICAL MICROBIOLOGY REVIEWS, Oct 1999;12-4:518–553.

[34]. Smith, D. L., J. G. Ayres, I. Blair, P. S. Burge, M. J. Carpenter, E. O. Caul, B. Coupland, U. Desselberger, M. Evans, I. D. Farrell, J. I. Hawker, E. G. Smith, and M. J. Wood. A large Q fever outbreak in the West Midlands: clinical aspects. Respir. Med, 1993; 87:509–516.

[35]. Hawker, J. L., J. G. Ayres, I. Blair, M. R. Evans, D. L. Smith, E. G. Smith, P. S. Burge, M. J. Carpenter, E. O. Caul, B. Coupland, U. Desselberger, I. D. Farrell, P. J. Saunders, and M. J. Wood. A large outbreak of Q fever in the West Midlands: windborne spread into a metropolitan area? Commun. Dis. Public Health,1998;1: 180–187.

[36]**.** Hugo C. van Woerden, Brendan W. Mason, Lika K. Nehaul, Robert Smith, Roland L. Salmon,Brendan Healy, *et al*. Q Fever Outbreak in Industrial Setting. Emerging Infectious Diseases, July 2004;10-7:1282-1289.

[37]. L. E. Wilson, S. Couper, H. Prempeh, D. Young, K. G. J. Pollock, W. C. Stewart, L. M. Browning and M. Donaghy. Investigation of a Q Fever Outbreak in a Scottish Co-LocatedSlaughterhouse and Cutting Plant. Zoonoses Public Health,2010; 57 :493–498.

[38]. A Wallensten, P Moore, H Webster, C Johnson, G van der Burgt, G Pritchard, J Ellis-Iversen, I Oliver. Q fever outbreak in Cheltenham, United Kingdom, in 2007 and the use of dispersion modelling to investigate the possibility of airborne spread. www.eurosurveillance.org. Surveillance and outbreak reports, March 2010.

[39]. C. Bellini, I. Magouras, C. Chapuis-Taillard, O. Clerc, E. Masserey, G. Peduto, O. Peter, S. Schaerrer, G. Schuepbach and G. Greub, Q fever outbreak in the terraced vineyards of Lavaux, Switzerland. New Microbes and New Infections, July 2014, 2-4 :93-99.

[40]. A. Hurtado, E. Alonso, I. Aspiritxaga, I. Lopez Etxaniz, B. Ocabo, J.F. Barandika, J.I. Fernandez-Ortiz De Murua, F. Urbaneja, R. Alvarez-Alonso, I. Jado, and A.L. Garcia-Perez. Enviromental sampling coupled with real-time PCR and genotyping to investigate the source of Q fever outbreak in a work setting. Edidemiol Infect, 2017.

[41]. Sabahattin Payzin , EPIDEMIOLOGICAL INVESTIGATIONS ON Q FEVER IN TURKEY. Bull. World Hlth Org, 1953 ;9 :553-559.

[42]. A. Gozalan, B. Esen, J.M. Rolain, L. Akin and D. Raoult. Is Q fever an emerging infection inTurkey? La Revue de Santé de la Méditerranée orientale,2005 ;11-3.

[43]. Hellenbrand W, Breuer T, Petersen L. Changing epidemiology of Q fever in Germany, 1947-1999. Emerging Infect Dis, 2001;7-5:789-796.

[44]. Berktas M, Ceylan E, Yaman G, Ciftci IH. Seroprevalence of *Coxiella burnetii* antibodies in high risk groups in eastern Turkey. Turkiye Klinikleri J Med Sci, 2011;3-1:45-50.

[45]. Celebi B, Babur C, Kilic C, Carhan A, Esen B, Ertek B. Investigation of Q fever, Listeriosis, Toxoplasmosis and Cytic Echinococcosis seroprevalence in risk group. Turk Hij Den Biyol Derg,2008;65-2:67-73.

[46]. Aytekin Cikman, Merve Aydin, Baris Gulhan, Faruk Karakecili, Adalet Ozciceck, et al. The seroprevalence of *Coxiella burnetii* in Erzincan, Turkey: Identification of the risk factors and their relationship with geographical featues. J Vector Borne Dis,2017;54:157-163.

[47]. Monika Szymanska-Czerwinska, Elzbieta Monika Galinska, Krzysztof Niemczuk, and Jozef Piotr Knap. Prevalence of Coxiella burnetii Infection in Humans Occupationally Exposed to Animals in Poland. Vector-Borne and Zoonotic Diseases, 2015;15-4: 261-267.

[48]. H. S. EL-Mahallawy, G. LU, P. Kelly, D. XU, Y. LI, W. Fan and C. Wang. Q fever in China: a systematic review, 1989–2013. Epidemiol. Infect, 2015 ;143 :673–681.

[49]. **The Europrean Surveillance Sysytem "TESSy", 2016:**

https://ecdc.europa.eu/sites/portal/files/documents/AER_for_2015-Q-fever.pdf

[50]. Blanc, G., Martin, L. A. & Maurice, A. Bull. Acad. Med,1947:131-138.

[51]. Halawani, E., El Dine, K. Z. & El Fiki, A. Y. J. Egypt. med. Ass,1952; 35:339.

[52]. MARTIN M. KAPLAN, P. BERTAGNA. THE GEOGRAPHEICAL DISTRIBUTION OF Q FEVER. Bull. Wld Hlth Org, 1955; 13 :829-860.

[53]. Giroud, P. et al. Bull. Acad. Mid,1953;137:29.

[54]. A. Lacheheb and D. Raoult. Seroprevalence of Q-fever in Algeria. Clinical Microbiology and Infection, December 2009; 15-2.

[55]. Raoult D, Marrie T. Q fever. Clin Infect Dis off Publ Infect Dis Soc Am,1995; 20:489-95.

[56]. Meskini M, Beati L, Benslimani A, Raoult D. Seroepidemiology of Rickettsial infections in Morocco. Eur J Epidemiol, 1995,11; 655-60.

[57]. Schelling E, Diguimbaye C, Daoud S, Nicolet J, Boerlin P, Tanner M, Zinsstag J. Brucellosis and Q-fever seroprevalences of nomadic pastoralists and their livestock in Chad. Prev Vet Med, 2003; 61:279 –293.

[58]. Potasman I, Rzotkiewicz S, Pick N, Keysary A. Outbreak of Q fever following a safari trip. Clin Infect Dis, 2000; 30:214 –215.

[59]. F. Bellazreg, N. kaabia, W. Hachfi, M. Khalifa, E. Ben jazia, N. Ghanouchi, A. Brahem, F. Bahri and A. Letaief. Acute Q fever in hospitalised patients in Central Tunisia: report of 21 cases. European Society of Clinical Microbiology and Infectious Diseases 15, 2009; 138-139.

[60]. Kobbe R, Kramme S, Kreuels B, Adjei S, Kreuzberg C, et al. Q fever in children, Ghana. Emerg Infect Dis,2008; 14: 344–346.

[61]. Sky Vanderburg, Matthew P. Rubach, Jo E. B. Halliday, Sarah Cleaveland, Elizabeth A. Reddy, John A. Crump. Epidemiology of *Coxiella burnetii* Infection in Africa: A OneHealth Systematic Review. PLOS Neglected Tropical Diseases,2014 ;8-4, e2787.

[62]. Amin WF, Ahmed SO. Detection of *Coxiella burnetii* in bovine milk samples using polymerase chain reaction. Assiut Vet Med J, 2009; 55: 23–31.

[63]. Kamga-Waladjo AR, Gbati OB, Kone P, Lapo RA, Chatagnon G, et al. Seroprevalence of Neospora caninum antibodies and its consequences for reproductive parameters in dairy cows from Dakar-Senegal, West Africa. Trop Anim Health Prod, 2010; 42: 953–959.

[64]. Scolamacchia F, Handel IG, Fevre EM, Morgan KL, Tanya VN, et al.
Serological patterns of brucellosis, leptospirosis and Q fever in Bos indicus cattle in Cameroon. PLoS One, 2010; 5: e8623.

[65]. Angelakis E, Mediannikov O, Socolovschi C, Mouffok N, Bassene H, Tall A, Niangaly H, Doumbo O, Znazen A, Sarih M, Sokhna C, Raoult D. *Coxiella burnetii*-positive PCR in febrile patients in rural and urban Africa. Int J Infect Dis,2014; 28:107–110.

[66]. Mediannikov O, Fenollar F, Socolovschi C, Diatta G, Bassene H, Molez J-F, Sokhna C, Trape J-F, Raoult D. Coxiella burnetii in humans and ticks in rural Senegal. PLoS Negl Trop Dis,2010 ; 4: e654.

[67]. Prabhu M, Nicholson WL, Roche AJ, Kersh GJ, Fitzpatrick KA, Oliver LD, Massung RF, Morrissey AB, Bartlett JA, Onyango JJ, Maro VP, Kinabo GD, Saganda W, Crump JA. Q fever, spotted fever group, and typhus group rickettsioses among hospitalized febrile patients in northern Tanzania. Clin Infect Dis,2011; 53: e8–e15.

[68]. Kamga-Waladjo AR, Gbati OB, Kone P, Lapo RA, Chatagnon G, Bakou SN, Pangui LJ, Diop PEH, Akakpo JA, Tainturier D. Seroprevalence of Neospora caninum antibodies and its consequences for reproductive parameters in dairy cows from Dakar-Senegal, West Africa. Trop Anim Health Prod,2010; 42:953–959.

[69]. Nahed HG, Khaled AAM. Seroprevalence of *Coxiella burnetii* antibodiesamong farm animals and human contacts in Egypt. J Am Sci,2012; 8: 619–621.

[70]. Hussien MO, ElFahal AM, Enan KA, Taha KM, Mohammed MS, et al. Seroprevalence of Q fever in goats in the Sudan. Vet World,2012; 5: 394–397.

[71]. Ghoneim N, Abdel-Moein K. Seroprevalence of *Coxiella burnetii* antibodies among farm animals and human contacts in Egypt. J Am Sci, 2012; 8:619–621.

[72]. Crump JA, Morrissey AB, Nicholson WL, Massung RF, Stoddard RA, Galloway RL, Ooi EE, Maro VP, Saganda W, Kinabo GD, Muiruri C, Bartlett JA. Etiology of severe non-malaria febrile illness in Northern Tanzania: a prospective cohort study. PLoS Negl Trop Dis,2013 7: e2324.

[73]. Knobel DL, Maina AN, Cutler SJ, Ogola E, Feikin DR, Junghae M, et al. *Coxiella burnetii* in humans, domestic ruminants, and ticks in rural western Kenya. Am J Trop Med Hyg, 2013;88-3:513–8.

[74]. Ratmanov P, Bassene H, Fenollar F, Tall A, Sokhna C, Raoult D, Mediannikov O. The correlation of Q fever and *Coxiella burnetii* DNA in household environments in rural Senegal. Vector Borne Zoonotic Dis,2013; 13:70 –72.

[75]. Amina Bessas, Hamza Leulmi, Idir Bitam, Sara Zaidi, Khatima Ait-Oudhia, Didier Raoult, Philippe Parola. Molecular evidence of vector-borne pathogens in dog and cat and their ectoparasites in Algiers, Algeria. Comparative Immunology, Microbiology and Infectious Diseases, 2016;45: 23-28.

[76]. H. Khaled, K.Sidi-Boumedine, S.Merdja,P. Dufour, A.Dahmani, R.Thiéry, E.Rousset,A.Bouyoucef. Serological and Molecular evidence of Q fever among small ruminant flocks in Algeria. Comparative Immunology, Microbiology and Infectious Diseases ,2016;47: 19-25.

[77]. Atef Aouadi, Hamza Leulmi, Mehdi Boucheikhchoukh, Ahmed Benakhla, Didier Raoult, Philippe Parola. Molecular evidence of tick-borne hemoprotozoan-parasites (*Theileria ovis and Babesiaovis*) and bacteria in ticks and blood from small ruminants in Northern Algeria. Comparative Immunology, Microbiology and Infectious Diseases,2017;50: 34-39.

[78]. Mohammed H. Benaissa, Samir Ansel, Abdallah Mohamed-Cherif, Karima Benfodil, Djamel Khelef, Curtis R. Youngs, Rachid Kaidi, Khatima Ait-Oudhia. Seroprevalence and risk factors for *Coxiella burnetii*, the

causative agent of Q fever in the dromedary camel (*Camelus dromedarius*) population in Algeria. Onderstepoort Journal of Veterinary Research, 2017.

[79]. Sulyok KM, Hornok S, Abichu G, Erdelyi K, Gyuranecz M. Identification

of novel Coxiella burnetii genotypes from Ethiopian ticks. PLoS One,2014; 9:e113213.

[80]. Bottieau E, Raeve DH, Colebunders R, Van den Ende J, et al. Q fever after a journey in Syria: A diagnosis suggested by bone marrow biopsy. Acta Clinica Belgica, 2000; 55:30–33.

[81]. Esmaeili S, Naddaf SR, Pourhossein B, Shahraki AH, et al. seroprevalence of brucellosis, leptospirosis, and Q fever among butchers and slaughterhouse workers in South-Eastern Iran. PLoS One 2016; 11:e0144953.

[82]. Faix DJ, Harrison DJ, Riddle MS, Vaughn AF, et al. Outbreak of Q fever among US military in western Iraq, June–July 2005. Clin Infect Dis, 2008; 46:e65–e68.

[83]. Esmaeili, Pourhossein B, Gouya MM, Bagheri Amiri F, Ehsan Mostafavi E. Seropepidemiological survey of Q fever and brucellosis in Kurdistan province western Iran. Vector Borne Diseases, 2014; 14:41-45.

[84]. Mohhamed Hassan Kayedi, Hamid Mokhayeri, Mehdi Birjandi, Ali Chegeni-Sharafi, Saber Esmaeili, Ehsan Mostafavi. Seroepidemiological study of Q fever in Lorestan province, western Iran, 2014. Iranian Journal of Microbiology, 2017;9-4:213-218.

[85]. Ashraf Mohabbati Mobarez, Fahimeh Bagheri Amiri, Saber Esmaeili. Seroprevalence of Q fever among human and animal in Iran; a Systematic review and meta-analysis. Plos Neglected Tropical Diseases, 2017;11-4:e0005521.

[86]. Garabedian GA, Djanian AY, Johsnton EA. Q fever in Lebanon (Middle East). Am J Hyg 1956; 63:313–318.

[87]. Mayssaa Fawaz Dabaja, Grazia Greco, Sara Villari, Ali Bayan, Gesualdo Vesco, Valeria Gargano, Maria Arnone, Mohamad Hneino, Rossella Lelli, Mohamad Ezzedine, Atika Berry, Hussein Mortada, Maria Tempesta, and Mohamad Mortada. The First Serological Study of Q Fever in Humans in Lebanon. VECTOR-BORNE AND ZOONOTIC DISEASES, 2018, DOI: 10.1089/vbz.2016.2102.

[88]. Ziva Amitai, Michal Bromberg, Michael Bernstein, David Raveh, Avi Keysary, Dan David, Silvio Pitlik, David Swerdlow, Robert Massung, Sabine Rzotkiewicz, Ora Halutz, and Tamy Shohat . A Large Q Fever Outbreak in an Urban School in Central Israel. Clinical Infectious Diseases, 2010; 50-11:1433–1438.

[89]. V. M. Vaidya, S. V. S. Malik, Simranpreet Kaur, Satish Kumar, and S. B. Barbuddhe. Comparison of PCR, Immunofluorescence Assay and Pathogen Isolation for Diagnosis of Q Fever in Humans with Spontaneous Abortions. JOURNAL OF CLINICAL MICROBIOLOGY, June, 2008; 2038–2044.

[90]. Loïc Epelboin, Mathieu Nacher, Elba Regina Sampaio de Lemos, Félix Djossou, et al. Q Fever in French Guiana: Tip of the Iceberg or Epidemiological Exception? PLOS Neglected Tropical Diseases, May 2016; DOI:10.1371/journal.pntd.0004598.

[91]. van der Hoek W, Meekelenkamp JC, Leenders AC, Wijers N, Notermans DW, Hukkelhoven CW. Antibodies against *Coxiella burnetii* and pregnancy outcome during the 2007–2008 Q fever outbreaks in The Netherlands. BMC Infect Dis, 2011; 11:44.

[92]. Edouard S, Mahamat A, Demar M, Abboud P, Djossou F, Raoult D. Comparison between emerging Q fever in French Guiana and endemic Q fever in Marseille, France. Am J Trop Med Hyg, 2014; 90-5:915–9.

[93]. Gardon J, Heraud JM, Laventure S, Ladam A, Capot P, Fouquet E, et al. Suburban transmission of Q fever in French Guiana: evidence of a wild reservoir. J Infect Dis, 2001;184-3:278–84.

[94]. Seshadri, R., Paulsen, I.T., Eisen, J.A., Read, T.D., Nelson, K.E., Nelson, W.C., et al. Complete genome sequence of the Q-fever pathogen *Coxiella burnetii*. Proc. Natl. Acad. Sci. U.S.A, 2003;100: 5455–5460.

[95]. Gimenez DF. Staining rickettsiae in yolk-sac cultures. Stain Technol, 1964; 39:135–140

[96]. Angelakis E, Raoult D. Q fever. Vet Microbiol,2010 ;140 :297–309.

[97]. Baca, O.G. et Paretsky, D. Q fever and *Coxiella burnetii*: a model for host-parasite interactions. Microbiol. Rev,1983;47: 127– 149.

[98]. Ben, Amara, A., Ghigo, E., Le Priol, Y., Lepolard, C., Salcedo, S.P., Lemichez, E., et al. *Coxiella burnetii*, the agent of Q fever, replicates within trophoblasts and induces a unique transcriptional response. PLoS One,2010; 5: e15315.

[99]. La Scola, B. et Raoult, D. Survival of *Coxiella burnetii* within free-living amoeba *Acanthamoeba castellanii*. Clin. Microbiol. Infect,2001 ;7: 75–79.

[100]. Bechah, Y., Verneau, J., Ben Amara, A., Barry, A.O., Lepolard, C., Achard, V., et al. Persistence of *Coxiella burnetii*, the agent of Q fever, in murine adipose tissue. PLoS One,2014;9 : e97503.

[101]. Voth DE, Heinzen RA. Lounging in a lysosome: the intracellular lifestyle of *Coxiella burnetii*. Cell Microbiol, 2007; 9:829–840.

[102]. Sandoz KM, Popham DL, Beare PA, Sturdevant DE, Hansen B, Nair V, Heinzen RA.Transcriptional profiling of *Coxiella burnetii* reveals extensive cell wall remodeling in the small cell variant developmental form. PLoS One? 2016;11: e0149957.

[103]. Sandoz KM, Sturdevant DE, Hansen B, Heinzen RA. Developmental transitions of *Coxiella burnetii* grown in axenic media. J Microbiol Methods, 2014; 96:104 –110.

[104]. Carole Eldin, Cléa Mélenotte, Oleg Mediannikov, Eric Ghigo, Matthieu Million,Sophie Edouard, Jean-Louis Mege, Max Maurin, Didier Raoult. From Q Fever to *Coxiella burnetii* Infection: A Paradigm Change. Clinical Microbiology Reviews, January 2017; 30-1:115-190.

[105]. Hendrik I.J Roest, Alex Bossers, Fred G. van Zijderveld and Johanna M.L rebel. Clinical microbiology of *Coxiella burnetii* and relevant aspects for the diagnosis abd control of the zoonotic disease Q fever. Veterinary Quarterly, 2013;33-3: 148-160.

[106]. Zerai Woldehiwet. Q fever (coxiellosis): epidemiology and pathogenesis. Research in Veterinary Science, 2004;93-100.

[107]. Vishwanath, S. et Hackstadt, T. Lipopolysaccharide phase variation determines the complement-mediated serum susceptibility of *Coxiella burnetii*. Infect Immun, 1988; **56** : 40–44.

[108]. Andoh M, Zhang G,Russell-Lodrigue Ke, Shive HR, Weeks BR, Samuel JE, T cells are essential for bacterial clearance, and gamma interferon, tumor necrosis factor alpha, and B cells are crucial for disease development in *Coxiella burnetii* infection in mice. Infect Immunolo, 2007; 75:3245-3255.

[109]. P.C.F Oyston and C. Davies. Q fever: the neglected biothreat agent. Journal of Medical Microbiology, 2011;60:9-21.

[110]. Coleman SA, Fischer ER, Cockrell DC, Voth DE, Howe D, Mead DJ, Samuel JE, Heinzen RA. Proteome and antigen profiling of Coxiella burnetii development forms. Infect Immunol, 2007;75:290-298.

[111]. Narasaki, C.T. et Toman, R. Lipopolysaccharide of *Coxiella burnetii*. Adv. Exp. Med. Biol, 2012;984: 65–90.

[112]. Hotta A, Kawamura M, To H, Andoh M, Yamagushi T, Fukushi H, Hirai K. Phase variation analysis of *Coxiella burnetii* during serial passage in cell culture by use of monoclonal antibodies. Infect Immunol, 2002;70:4747-4749.

[113]. Marrie TJ, Raoult D. Q fever areview and issues for the next century. Int J antimicrob Agents, 1997;8:145-161.

[114]. Million, M., Lepidi, H. et Raoult, D. [Q fever: current diagnosis and treatment options]. Med. Mal. Infect,2009; 39: 82–94.

[115]. Kampschreur, L.M., Wegdam-Blans, M.C., Wever, P.C., Renders, N.H., Delsing, C.E., Sprong, T., et al. Chronic Q fever diagnosis -consensus guideline versus expert opinion. Emerg. Infect. Dis,2015;21: 1183–1188.

[116]. Charles W. Heppell, Joseph R. Egan, Ian Hall. A human time dose response model for Q fever. Epidemics, 2017.

[117]. Russell-Lodrigue, K., Zhang, G., McMurray, D,. Sam,J,. Clinical and pathological changes in a guinea pig aerosol challenge model of acute Q fever. Infect Immun, 2006;74-11:6085-6091.

[118]. Baca, O.G., Klassen, D.A. et Aragon, A.S. Entry of *Coxiella burnetii* into host cells. Acta Virol, 1993;37: 143–155.

[119]. van Schaik, E.J., Chen, C., Mertens, K., Weber, M.M. et Samuel, J.E. Molecular pathogenesis of the obligate intracellular bacterium *Coxiella burnetii*. Nat. Rev. Microbiol, 2013: 11 : 561–573.

[120]. D Raoult, T J Marrie, J L Mege. Natural history and pathophysiology of Q fever. Lancet Infect DIS? 2005;52:19-226.

[121]. Capo C, Lindberg FP, Meconi S, et al. Subversion of monocyte functions by *Coxiella burnetii*: impairment of the cross-talk between vαβ3 integrin and CR3. *J Immunol*, 1999; 163**:** 6078–85.

[122]. Honstettre A, Ghigo E, Moynault A, et al. Lipopolysaccharide from *Coxiella burnetii* is involved in bacterial phagocytosis, filamentous actin reorganization, and inflammatory responses through Toll-like receptor 4. *J Immunol*, 2004; **172:** 3695–703.

[123]. McQuiston, JH., Childs, J.E? Thompson, H.A., Q fever. Journal of the American Veterinary Medical Association, 2002; 221:796-799.

[124]. Aboudharam G, Drancourt M, Raoult D. Culture of *C. burnetii*from the dental pulp of experimentally infected guinea pigs. *Microb Path*, 2004; 36: 349–50.

[125]. Shannon JG, Heinzen RA. Adaptive immunity to the obligate intracellular pathogen *Coxiella burnetii*. Immunol Res, 2009;43:138-148.

[126]. Russell-Lodrigue KE, Andoh M, Poels MW, Shive HR, Weeks BR, Zhang GQ, Tersteeg C, Masegi T, Hotta A, Yamagushi T, et al. *Coxiella burnetii* isolates cause genogroup specific virulence in mouse and guinea pigs moldels of acute Q fever. Infect Immunol, 2009;77:5640-5650.

[127]. Baumgartner W, Bachmann S. Histological and immune-cytochemical characterization of Coxiella burnetii associated lesions in the murine uterus and placenta. Infect Immunol, 19923;60:5232-5241.

[128]. Roest HIJ, van Gelderen B, Dinkla A,Frangoulidis D,van Zijderveld FG, Rebel J, van Keulen L. Q fever in pregnant goats: pathogenesis and excretion of *Coxiella burnetii*.Plos ONE, 2012;7:14.

[129]. Sanchez J, Souriau A, Buendia AJ, Arricau-Bouvery N, Martinez CM, Salinas J, Rodolakis A, Navarro JA. Experimental Coxiella burnetii infcetion in pregnant goats: a histhopathological and immunohistochemical study. J Comp Pathol, 2006;135:108-115.

[130]. J.L. Pellerin, A. Alsaleh, P. Mermillod, J.M.G. Souza-Fabjan, A. Rodolakis, E. Rousset, L. Dubreil, J.F. Bruyas, C. Roux, F. Fieni. Attachment of *Coxiella burnetii* to the *zona pellucida* of *in vitro* produced goat Embryos. Theriogenology, an iternational journal of animal production, 2017.

[131]. van Loenhout JAF, Wielders CCH, Morroy G, Cox MJM, van der Hoek W, Hautvast JLA, Paget WJ, van der Velden J. Severely impaired health status of non-notified Q fever patients leads to an underestimation of the true burden of disease. Epidemiol Infect,2015; 143:2580 –2587.

[132]. Maltezou HC, Raoult D. Q fever in children. Lancet Infect Dis,2002; 2:686–69.

[133]. Kampschreur LM, Wegdam-Blans Mc, Thijsen SF, Groot CA, Schneeberger PM, Hollander AA, et al. Acute Q fever related in hospital mortality in the Netherlands. Neth J Med, 2010; 68:408-413.

[134]. Marrie TJ. Q fever pneumonia. Infect Dis Clin North Am, 2010; 24:27– 41.

[135]. Pierre-Edourad Fournier, Thomas J, Marrie, and Didier Raoult. Diagnosis pf Q Fever. Journal of Clinical Microbiology, July 1998; 36-7:1823-1834.

[136]. Chang K, Yan J-J, Lee H-C, Liu KH, Lee NY, Ko W-C. Acute hepatitis with or without jaundice: a predominant presentation of acute Q fever in southern Taiwan. J Microbiol Immunol Infect, 2004; 37:103–108.

[137]. Skiba V, Barner KC. Central nervous system manifestations of Q fever responsive to steroids. Mil Med,2009; 174:857– 859.

[138]. Rustscheff S. Q fever as a cause of pure sensory polyneuropathy— the six-year itch: a follow-up of an indigenous Swedish case. Scand J Infect Dis,2005; 37:949–950.

[139]. Melenotte C, Million M, Audoly G, Gorse A, Dutronc H, Roland G, Dekel M, Moreno A, Cammilleri S, Carrieri MP, Protopopescu C, Ruminy P, Lepidi H, Nadel B, Mege J-L, Xerri L, Raoult D. B-cell non-Hodgkin lymphoma linked to *Coxiella burnetii*. Blood,2016; 127:113–121.

[140]. Figtree M, Miyakis S, Stenos J, Graves S, Botham S, Ferson M, Krilis S. Q fever cholecystitis in an unvaccinated butcher diagnosed by gallbladder polymerase chain reaction. Vector Borne Zoonotic Dis,2010;10: 421–423.

[141]. Wegdam-Blans MC, Kampschreur LM, Delsing CE, Bleeker-Rovers CP, Sprong T, van Kasteren ME, Notermans DW, Renders NH, Bijlmer HA, Lestrade PJ, et al. Chronic Q fever: review of the literature and a proposal of new diagnostic criteria. J Infect, 2012; 64:247-259.

[142]. Watt G, Pachirat O, Baggett HC, Maloney SA, Lulitanond V, Raoult D, Bhengsri S, Thamthitiwat S, Paupairoj A, Kosoy M, Ud-Ai N, Sukwicha W, Whistler T, Fournier P-E. Infective endocarditis in northeastern Thailand. Emerg Infect Dis,2014; 20:473– 476.

[143]. Correia MG, Barbosa GIF, Golebiovski WF, Weksler C, Lepidi H, Raoult D. Diagnosis of blood culture-negative endocarditis and clinical comparison between blood culture-negative and blood culturepositive cases. Infection,2016; 44:459–466.

[144]. Carcopino X, Raoult D, Bretelle F, Boubli L, Stein A. Q fever duringpregnancy: a cause of poor and maternal outcome. Ann N Y Acad Sci,2009;1166:79–89.

[145]. Tissot-Dupont H, Vaillant V, Rey S, Raoult D. Role of sex, age, previous valve lesion, and pregnancy in the clinical expression and outcome of Q fever after a large outbreak. Clin Infect Dis,2007;44:232–237.

[146]. Rey D, Obadia Y, Tissot-Dupont H, Raoult D. Seroprevalence of antibodies to *Coxiella burnetti* among pregnant women in South Eastern France. Eur J Obstet Gynecol Reprod Biol,2000; 93:151–156.

[147]. Langley JM, Marrie TJ, Leblanc JC, Almudevar A, Resch L, Raoult D. Coxiella burnetii seropositivity in parturient women is associated with adverse pregnancy outcomes. Am J Obstet Gynecol,2003; 189:228 – 232.

[148]. Baud D, Peter O, Langel C, Regan L, Greub G.Seroprevalence of Coxiella burnetii and Brucella abortus among pregnant women. Clin Microbiol Infect,2009;15:499 –501.

[149]. Nielsen SY, Molbak K, Nybo Andersen AM, Brink Henriksen T, Kantso B, Krogfelt KA, Hjollund NH. Prevalence of Coxiella burnetii in women exposed to livestock animals, Denmark, 1996 to 2002. Euro Surveill,2013 18-28: pii:20528.

[150]. Million M, Roblot F, Carles D, D'Amato F, Protopopescu C, Carrieri MP, Raoult D.Reevaluation of the risk of fetal death and malformation after Q fever. Clin Infect Dis,2014;59:256 –260.

[151]. Angelakis E, Million M, D'Amato F, Rouli L, Richet H, Stein A, Rolain J-M, Raoult D. Q fever and pregnancy: disease, prevention, and strain specificity. Eur J Clin Microbiol Infect Dis,2013;32:361–368.

[152]. CVI. 2007. *Coxiella burnetii* RSA 331. Craig Venter Institute. c. l., ed. Available from: http://gsc.jcvi.org/projects/msc/coxiella_burnetii/coxiella_burnetii_rsa_331/index.shtml.

[153]. Beare, P.A., Unsworth, N., Andoh, M., Voth, D.E., Omsland, A., Gilk, S.D., et al.Comparative genomics reveal extensive transposon-mediated genomic plasticity and diversity among potential effector proteins within the genus *Coxiella*. Infect Immun,2009;77: 642–656.

[154]. Runa Kuley, Eric Kuijt, Mari A. Smiths, Hendrik I.J. Roest,Hilde E.Smith and Alex Bossers. Genome Plasticity and Polymorphisms in Critical Genes Correlate with Increased Virulence of Dutch Outbreak-Related *Coxiella burnetii*. Frnotiers in Microbiology, August 2017;8:1526.

[155]. Olga Glazunova, Véronique Roux, Olga Freylikman, Zuzana Sekeyova, Ghislain Fournous, Didier Raoult et al. Coxiella burnetii Genotyping. Emerging Infect Diseases,2005 ;11-8 :1211-1217.

[156]. Roest, H.I.J, Ruuls, R.C, Tilburg, J.J.H.C, Nabuurs-Franssen, M.H., Klaassen.C.H.W, Vellema, O, et al. Molecular epidemiology of Coxiella burnetii from ruminants in Q fever outbreak, The Netherlands. Emerg Infect Dis, 2001;17:668-675.

[157]. Svraka S, Toman R, Skultety L, Slaba K, Homan WL.Establishment of a genotyping scheme for Coxiella burnetii. FEMS Microbiol Lett,2006;254:268 –274.

[158]. Arricau-Bouvery N, Hauck Y, Bejaoui A, Frangoulidis D, Bodier CC,Souriau A, Meyer H, Neubauer H, Rodolakis A, Vergnaud G.Molecular characterization of Coxiella burnetii isolates by infrequent restriction site-PCR and MLVA typing. BMC Microbiol,2006; 6:38.

[159]. Huijsmans CJJ, Schellekens JJA, Wever PC, Toman R, Savelkoul PHM,Janse I, Hermans MHA. Single-nucleotide-polymorphism genotyping of Coxiella burnetii during a Q fever outbreak in The Netherlands. Appl Environ Microbiol,2011 ;77 :2051–2057.

[160]. Anderson A, Bijlmer H, Fournier P-E, Graves S, Hartzell J, Kersh GJ, Limonard G, Marrie TJ, Massung RF, McQuiston JH, Nicholson WL, Paddock CD, Sexton DJ. Diagnosis and management of Q fever— United States, 2013: recommendations from CDC and the Q Fever Working group. MMWR Recomm Rep,2013; 62:1–30.

[161]. Dupont HT, Thirion X, Raoult D. Q fever serology: cutoff determination for microimmunofluorescence. Clin Diagn Lab Immunol,1994; 1:189 –196.

[162]. Imbert G, La Scola B. Diagnosis of Q fever using indirect microimmunofluorescence Methods Mol Biol,2006; 345:197–202.

[163]. OIE Terrestrial Manual 2010:

http://www.oie.int/fr/normes/manuel-terrestre/acces-en-ligne/

[164]. Stein A, Kruszewska D, Gouvernet J, Raoult D. Study of the 16S-23S ribosomal DNA internal spacer of Coxiella burnetii. Eur J Epidemiol,1997;13:471– 475.

[165]. Fenollar F, Fournier PE, Raoult D. Molecular detection of *Coxiella burnetii* in the sera of patients with Q fever endocarditis or vascular infection. J Clin Microbiol,2004;42:4919 – 4924.

[166]. Fenollar F, Raoult D. Molecular genetic methods for the diagnosis of fastidious microorganisms. APMIS,2004; 112:785– 807.

[167]. Eldin C, Angelakis E, Renvoise A, Raoult D. *Coxiella burnetii* DNA, but not viable bacteria, in dairy products in France. Am J Trop Med Hyg,2013;88:765–769.

[168]. Rolain J-M, Raoult D. Molecular detection of Coxiella burnetii in blood and sera during Q fever. QJM,2005;98:615–617–620.

[169]. Schneeberger PM, Hermans MHA, van Hannen EJ, Schellekens JJA, Leenders ACAP, Wever PC. Real-time PCR with serum samples is indispensable for early diagnosis of acute Q fever. Clin Vaccine Immunol,2010; 17:286 –290.

[170]. Edouard S, Raoult D. Lyophilization to improve the sensitivity of qPCR for bacterial DNA detection in serum: The Q fever paradigm. J Med Microbiol,2016;65:462– 467.

[171]. Kersh,G.J, Wolfe, T.M, Fitzpatrick, K.A, Candee, A.J, Oliver, L.D, Patterson, N.E, Self, J.S, Priestly, R.A, Loftis, A.D, Massung, R.F. Presence of *Coxiella burnetii* DNA in the environment of the United States, 2006 to 2008. Appl Environ Microbiol, 2010;76:4469-4475.

[172]. Raoult D, Host factors in the severity of Q Fever. Ann N Y Acad Sci, 1990;590:33-38.

[173]. M. Garner, at al. A Review of Q Fever in Australia 1991-1994. Aust. N.Z. J Public Health,1997;21:722-730.

[174]. EFSA Panel on Animal Health and Welfare (AHAW). 2010. Scientific Opinion on Q Fever. EFSA J. **8**(5): 1595. doi:10.2903/j.efsa. 2010.1595.

[175]. Roest HIJ, van Steenbergen J, Wijkmans C, van Duijnhoven Y, Stenvers O, Oomen T, Vellema P. Q fever in 2008 in The Netherlands and the expectations of 2009. Tijdschr Diergeneeskds, 2009;134:300-303.

[176]. Guatteo R, Beaudeau F, Berri M, Rodolakis A, Joly A, Seegers H. Shedding routes of *Coxiella burnetii* in dairy cows: implications for detection and control Vet Res, 2006;37:827-833.

[177]. Enserink, M. Questions abound in Q Fever explosion in the Netherlands. Science, 2010;327:266-267.

[178]. Tozer, et al. Potential animal and environmental sources of Q fever infection for humans in Queensland. Zoonoses Public Health, 2014;61:105-112.

[179]. H. Tissot-Dupont, et al. Wind in November, Q Fever in December. Emerg Infect Dis, 2004;10:1264-1269.

[180]. Carrieri, M.P., Tissot-Dupont, H., Rey, D., Brousse, P., Renard, H., Obadia, Y. et Raoult, D.Investigation of a slaughterhouserelated outbreak of Q fever in the French Alps. Eur. J. Clin. Microbiol. Infect Dis,2002;21: 17–21.

[181]. Gilsdorf, A., Kroh, C., Grimm, S., Jensen, E., Wagner-Wiening, C. et Alpers, K. Large Q fever outbreak due to sheep near residential areas, Germany, 2005. Epidemiol. Infec,2008;136: 1084–1087.

[182]. Schimmer, B., Ter Schegget, R., Wegdam, M., Zuchner, L., de Bruin, A., Schneeberger, P.M., et al. The use of a geographic information system to identify a dairy goat farm as the most likely source of an urban Q-fever outbreak. BMC Infect. Dis,2010;10: 69.

[183]. Hackert, V.H., van der Hoek, W., Dukers-Muijrers, N., de Bruin, A., Al Dahouk, S., Neubauer, H., et al.Q fever: single-point source outbreak with high attack rates and massive numbers of undetected infections across an entire region. Clin. Infect. Dis,2012;55: 1591–1599.

[184]. Berri, M., Rousset, E., Champion, J.L., Arricau-Bouvery, N., Russo, P., Pepin, M. et Rodolakis, A. Ovine manure used a garden fertiliser as a suspected source of human Q fever. Vet. Rec,2003;153: 269–270.

[185]. Hermans, T., Jeurissen, L., Hackert, V. et Hoebe, C. Landapplied goat manure as a source of human Q-fever in the Netherlands, 2006-2010. PLoS One,2014 ;9: e96607.

[186]. van der Hoek, W., Hunink, J., Vellema, P. et Droogers, P.Q fever in the Netherlands: the role of local environmental conditions. Int. J. Environ. Health Res,2011;21: 441–451.

[187]. P. Harris, et al. Acute Q fever in northern Queensland: variation in incidence related to rainfall and geographical location. Epidemiol. Infect, 2013;141-5:1034-1038.

[188]. N.R.Parker, J.H. Barralet, A.M. Bell. Q Fever. Lancet, 2006;367:679-688.

[189]. M. Benazis, et al. A survey of Western Australian sheep, cattle and Kangaroos to determine the prevalence of Coxiella burnetii. Vet. Micobial,2010;143:337-345.

[190]. Williams JC. Infectivity, virulence, and pathogenicity of *Coxiellaburnetii* for various hosts. In: Williams JC, Thompson HA, eds. Q fever: the biology of *Coxiella burnetii*. Boca Raton, Florida: CRC Press, 1991: 21–71.

[191]. Marrie TJ, Stein A, Janigan D, Raoult D. Route of infectiondetermines the clinical manifestations of acute Q fever. *J Infect Dis*,1996; **173:** 484–87.

[192]. La Scola B, Lepidi H, Raoult D. Pathologic changes during acute Qfever: influence of the route of infection and size in infected guinea pigs. *Infect Immun*, 1997; 65: 2443–47.

[193]. Leone M, Honstettre A, Lepidi H, et al. Effect of sex on *Coxiella burnetii* infection: protective role of 17_-estradiol. *J Infect Dis*, 2004; 189: 339–45.

[194]. Gikas A, Spyridaki I, Psaroulaki A, Kofterithis D, Tselentis Y.In vitro susceptibility of *Coxiella burnetii* to trovafloxacin in comparison with susceptibilities to pefloxacin, ciprofloxacin, ofloxacin, doxycycline, and clarithromycin. Antimicrob Agents Chemother,1998;42:2747–2748.

[195]. Gikas A, Spyridaki I, Scoulica E, Psaroulaki A, Tselentis Y.In vitro susceptibility of Coxiella burnetii to linezolid in comparison with its susceptibilities to quinolones, doxycycline, and clarithromycin. Antimicrob Agents Chemother,2001;45:3276 –3278.

[196]. Raoult D, Drancourt M, Vestris G. Bactericidal effect of doxycycline associated with lysosomotropic agents on *Coxiella burnetii* in P388D1 cells. Antimicrob Agents Chemother,1990;34:1512–1514.

[197]. Maurin M, Benoliel AM, Bongrand P, Raoult D. Phagolysosomal alkalinization and the bactericidal effect of antibiotics: The *Coxiella burnetii* paradigm. J Infect Dis,1992 ;166 :1097–1102.

[198]. Samira Boarbi, David Fretin et Marcella Mori. *Coxiella burnetii*, agent de la fièvre Q. Can. J. Microbiol ,2016 ;62 :102–122.

[199]. Angelakis, E. et Raoult, D . Q Fever. Vet. Microbiol,2010 ;140: 297–309.

[200]. Dorko E, Rimarova K, Pilipcinec E. of the environment and occupational exposure on the occurrence of Q fever. Cent. Eur. J. Public. Health,2012;20:208–214.

[201]. Kováčová E, Kazár J, Španělová D. Suitability of various Coxiella burnetii antigen preparations for detection of serum antibodies by various test. Acta Virol, 1998;42:365–368.

[202]. G. FLORES-RAMÍREZ, M. KMEŤOVÁ, M. DANCHENKO, E. ŠPITALSKÁ, V. HAVLÍČEK, Ľ. ŠKULTÉTY. Protein composition of the phase I *Coxiella burnetii* soluble antigen prepared by extraction with trichloroacetic acid. Acta virologica, 2017 ;61: 361 – 368.

[203]. Margarita Bolanos-Rivero, Cristina Carranza-Rodrıguez, Noe F. Rodrıguez, Carlos Gutierrez, and Jose -Luis Perez-Arellano. Detection of *Coxiella burnetii* DNA in Peridomestic and Wild Animals and Ticks in an Endemic Region (Canary Islands, Spain). VECTOR-BORNE AND ZOONOTIC DISEASES,2017.

[204] A. Joulié, E. Rousset, P. Gasqui, E. Lepetitcolin, A. Leblond, K. Sidi-Boumedine, E. Jourdain. *Coxiella burnetii* circulation in a naturally infected flock of sheep: individual follow-up of antibodies in serum and milk. American Society for Microbiology,2017.

[205]. Lindsay A. Petty, Helen S. Te, Kenneth Pursell. A case of Q fever after liver transplantation. *Transpl Infect Dis*, 2017; e12737.

[206]. Agerholm JS. *Coxiella burnetii* associated reproductive disorders in domestic animals—a critical review. Acta Vet Scand,2013; 55:13.

[207]. Markus Freick , Andrea Konrath , Haimo Enbergs , JörgWalraph , JimWeber, Karin Eulenberger. Detection of *Coxiella burnetii* DNA and anti-*Coxiella burnetii* IgG antibodies in precolostral blood samples of stillborn calves in an endemically infected Holstein dairy herd. Folia Microbiol,2017; DOI 10.1007/s12223-017-0553-4.

[208].Stephen S. Koh, Aldon Li, David S, Cassarino. Leukocytoclastic vasculitis presenting in association with *Coxiella burnetii* (Q fever); a case report. J Cutan Pathol., 2018 Jan;45-1:71-73.

[209]. Kampschreur LM, Oosterheert JJ, Koop AM, Wegdam-Blans MC, Delsing CE, Bleeker-Rovers CP, et al. Microbiological challenges in the diagnosis of chronic Q fever. Clin Vaccine Immunol, 2012;19:787–90.

[210]. Mostafa F. N. Abushahba, Abdelbaset E. Abdelbaset, Mohamed S. Rawy and Sylvia O. Ahmed. Cross-sectional study for determining the prevalence of Q fever in small ruminants and humans at El Minya Governorate, Egypt. BMC Res Notes, 20171 ;10:538.

[211]. Katharina Sobotta, Katharina Bonkowski, Elisabeth Liebler-Tenorio, Pierre Germon, Pascal Rainard, Nina Hambruch, Christiane Pfarrer, Ilse D. Jacobsen and Christian Menge. Permissiveness of bovine epithelial cells from lung,intestine, placenta and udder for infection with *Coxiella burnetii.* Vet Res , 2017 ;48:23.

[212]. G. FLORES-RAMÍREZ, M. KMEŤOVÁ, M. DANCHENKO, E. ŠPITALSKÁ, V. HAVLÍČEK, Ľ. ŠKULTÉTY. Protein composition of the phase I *Coxiella burnetii* soluble antigen prepared by extraction with trichloroacetic acid. Acta virologica,2017 ;61: 361 – 368.

[213]. Bonhan Koo, Choong Eun Jin, Se Yoon Park, Tae Yoon Lee, Jeonghun Nam, Young-Rock Jang, Sun Mi Kim, Ji Yeun Kim, Sung-Han Kim, Yong Shin. A rapid bio-optical sensor for diagnosing Q fever in clinical specimens. J Biophotonics., 2018 ;11-4:e201700167.

[214]. Centers for Disease Control and Prevention. Q fever. Atlanta: CDC, 2003: CDC fact sheet.

[215]. www.hpa.org.uk/deliberate_accidental_releases/biological.