SHORT COMMUNICATION

Molecular detection of *Coxiella burnetii* in vaginal swabs from aborted cattle in Mexico

Detección molecular de Coxiella burnetii en hisopos vaginales de bovinos abortados en México

Detecção molecular de Coxiella burnetii em swabs vaginais de bovinos abortados no México

Carlos Flores-Pére[1]; Efrén Díaz-Aparicio[2]; Erika-Gabriela Palomares-Resendiz[2]; José-Luis Gutiérrez-Hernández[2*]; Enrique Herrera-López[2]; Rigoberto Hernández-Castro[3].

[1]*Facultad de Medicina Veterinaria y Zootecnia, Universidad Nacional Autónoma de México. Ciudad de México, México.*

[2]*Centro Nacional de Investigación Disciplinaria en Salud Animal e Inocuidad, Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias. Ciudad de México, México.*

[3]*Departamento de Ecología de Agentes Patógenos, Hospital General Manuel Gea González. Ciudad de México, México.*

To cite this article:

Flores-Pérez C, Díaz-Aparicio E, Palomares-Resendiz EG, Gutiérrez-Hernández JL, Herrera-López E, Hernández-Castro R. Molecular detection of *Coxiella burnetii* in vaginal swabs from aborted cattle in Mexico. Rev Colomb Cienc Pecu 2024; 37(1):52–59. https://doi.org/10.17533/udea.rccp.v37n1a3

Abstract

Background: *Coxiella burnetii* is an obligate intracellular bacterium that causes Q fever, a worldwide contagious and zoonotic disease. Q fever is primarily transmitted to humans by infected cattle, sheep, and goats. Ruminants do not always develop clinical signs, except for pregnant females, which can present reproductive failure such as abortions and stillbirths. Regardless of its worldwide distribution, in Mexico this is considered an exotic disease, although serological evidence of *C. burnetii* has been demonstrated and at least six confirmed human cases have been reported. **Objective:** To assess the presence of *C. burnetii* in cattle in Mexico. **Methods:** Genomic DNA was extracted from 153 vaginal swabs obtained from cattle dwelling in 12 Mexican states and analyzed by PCR. **Results:** The *C. burnetii* IS*1111* insertion sequence was identified by endpoint PCR in 33.33% (51/153) of the vaginal swabs. We obtained two nucleotide sequences that confirmed the genetic material of *C. burnetii*. **Conclusion:** Our results constitute a first step to elucidate the current epidemiology of Q fever in our country, and they indicate that cattle may be a reservoir of *C. burnetii*. To the best of our knowledge, this report provides the first molecular proof that this pathogen exists among cattle in Mexico.

Received: November 3, 2022. Accepted: April 19, 2023

***Corresponding author.** Centro Nacional de Investigación Disciplinaria en Salud Animal e Inocuidad, Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias. Carretera Federal México-Toluca Km 15.5 S/N. Col. Palo Alto. Cuajimalpa de morelos, Ciudad de México, México. C.P. 05110. E-mail: joseluisgh@comunidad.unam.mx

Keywords: *abortion; Coxiella burnetii; diagnosis; epidemiology; livestock; Mexico; Q fever; reproductive failure; ruminants; vaginal swabs.*

Resumen

Antecedentes: *Coxiella burnetii* es una bacteria intracelular obligada que causa la fiebre Q, una enfermedad contagiosa y zoonótica a nivel mundial. La fiebre Q se transmite principalmente a los humanos a través de bovinos, ovinos y caprinos infectados. Los rumiantes no siempre desarrollan signos clínicos; excepto las hembras preñadas, que pueden presentar fallas reproductivas como abortos y mortinatos. Independientemente de su distribución mundial, en México la enfermedad se considera exótica a pesar de que se ha demostrado evidencia serológica de *C. burnetii* y se han reportado al menos seis casos humanos confirmados. **Objetivo:** Evaluar la presencia de *C. burnetii* en bovinos en México. **Métodos:** Se extrajo ADN genómico de 153 hisopos vaginales obtenidos de ganado bovino en 12 estados de México y se analizaron mediante PCR. **Resultados:** La secuencia de inserción de *C. burnetii* IS*1111* se identificó mediante PCR de punto final en el 33,33% (51/153) de los hisopos vaginales. Se obtuvieron dos secuencias de nucleótidos que confirmaron el material genético de *C. burnetii*. **Conclusiones:** Nuestros resultados constituyen un primer paso para dilucidar la epidemiología actual de la fiebre Q en Mexico, e indican que el ganado bovino puede ser un reservorio de *C. burnetii*. Hasta donde sabemos, el presente informe proporciona la primera prueba molecular de que este patógeno existe entre el ganado bovino en México.

Palabras clave: *aborto; Coxiella burnetii; diagnóstico; epidemiología; falla reproductiva; Fiebre Q; ganado; hisopos vaginales; México; rumiantes.*

Resumo

Antecedentes: *Coxiella burnetii* é uma bactéria intracelular obrigatória, causadora da febre Q, uma doença contagiosa e zoonótica mundial. A febre Q é transmitida principalmente aos humanos por bovinos, ovinos e caprinos infectados. Nem sempre os ruminantes desenvolvem sinais clínicos, exceto as fêmeas grávidas, que podem apresentar falhas reprodutivas como abortos e natimortos. Independentemente de sua distribuição mundial, no México a doença é considerada exótica, embora evidências sorológicas de *C. burnetii* tenham sido demonstradas e pelo menos seis casos humanos confirmados tenham sido relatados. **Objetivo:** Avaliar a presença de *C. burnetii* em bovinos no México. **Métodos:** O DNA genômico foi extraído de 153 swabs vaginais obtidos de bovinos residentes em 12 estados mexicanos e analisados por PCR. **Resultados:** A sequência de inserção *C. burnetii* IS*1111* foi identificada por PCR de ponto final em 33,33% (51/153) dos esfregaços vaginais. Obtivemos dois sequências de nucleotídeos que confirmaram o material genético de *C. burnetii*. **Conclusões:** Nossos resultados constituem um primeiro passo para elucidar a epidemiologia atual da febre Q em nosso país e indicam que o gado pode ser um reservatório de *C. burnetii*. Até onde sabemos, o presente relatório fornece a primeira prova molecular de que esse patógeno existe entre o gado no México.

Palavras-chave: *aborto; Coxiella burnetii; diagnóstico; epidemiologia; esfregaços vaginais; falha reprodutiva; febre Q; gado; México; ruminantes.*

Introduction

Q fever is a worldwide zoonosis caused by *Coxiella burnetii*, a highly infectious intracellular and Gram-negative bacterium (España *et al.*, 2020; Škultéty, 2020) that can be isolated from an extended number of animals and arthropods (González-Barrio and Ruiz-Fons, 2019; Menadi *et al.*, 2020). Infection in animals and humans is frequently asymptomatic (Njeru *et al.*, 2016). Domestic ruminants are considered the primary reservoirs (Menadi *et al.*, 2020). Infection is associated with abortion at the end of gestation without previous clinical symptoms. It is also linked with stillbirths and pre-mature delivery. Particularly, cattle may present metritis, mastitis, and reproductive problems such as infertility (Roest *et al.*, 2013; Njeru *et al.*, 2016; Burette and Bonazzi, 2020).

The epidemiology of human infection reflects the circulation of the bacterium in animal reservoirs (Eldin *et al.*, 2016) because transmission to humans typically occurs by inhalation of contaminated aerosols from feces, urine, milk, or conception products, which contain large numbers of bacteria; but rarely by ingestion of raw milk from infected animals (Njeru *et al.*, 2016; Menadi *et al.*, 2020). The presentation of Q fever is especially variable: it is rarely a fatal disease in humans but is frequently debilitating. Infection can lead to asymptomatic seroconversion in up to 60% of infected people; several infected patients may develop acute disease ranging from a flu-like syndrome to severe pneumonia, or chronic disease mainly manifested as endocarditis. Around 0.9 to 2.4% of acute Q fever cases result in death (Hartzell *et al.*, 2008; González-Barrio and Ruiz-Fons, 2019).

Cattle production constitutes one of the main activities of the agricultural sector in Mexico This country ranks among the first ten meat and milk-producers worldwide (Rojo-Rubio *et al.*, 2009). Q fever is still considered an exotic disease by the Ministry of Agriculture and Livestock (Secretaría de Agricultura, by its name in Spanish) although at least three cases have been reported in humans (González-Canudas *et al.*, 1997; Sahagún-Sánchez *et al.*, 1998; Santamaría, 2009) and serological surveys in both animal and human population have detected *C. burnetii* (Silva, 1950; Salman *et al.*, 1990; González-Canudas *et al.*, 1997; Salinas-Meléndez *et al.*, 2002; Sifuentes-Osornio *et al.*, 2012). Nonetheless, the presence of Q fever in such an important species like bovine has been poorly studied. The aim of this study was to assess the presence of *C. burnetii* in cattle in Mexico.

Materials and Methods

Sample collection

A retrospective study was performed using samples submitted to the laboratory between 2019 and 2021. All of them were taken from cattle with a previous history of abortion within the first 30 days postpartum, or from cattle with recent abortion, no later than 30 days after the event. At the time of sampling, no herd had declared vaccinating against brucellosis and leptospirosis. These samples were originally analyzed to diagnose brucellosis by the Rose Bengal (RBT) and Rivanol (RT) tests under specifications of NOM-041-ZOO-1995 of the National campaign against brucellosis in animals. The samples were also analyzed through a microscopic agglutination test (MAT) for nine serovars of *Leptospira interrogans*. This diagnostic procedure was conducted because both diseases are endemic in Mexican cattle.

A total of 153 sera and vaginal swab samples were taken from herds dwelling in 12 Mexican states (i.e. Chiapas, Mexico City, State of Mexico, Guanajuato, Hidalgo, Jalisco, Oaxaca, Queretaro, Sinaloa, Sonora, Tamaulipas, Veracruz). To obtain blood sera, the coccygeal vein was punctured and blood was collected in 5-ml vacuum-sealed tubes kept at room temperature until coagulation. Subsequently, they were centrifugated at 3500 rpm to obtain the sera which were placed in 1.5 ml conical tubes and stored at -4 ºC for transport and subsequent use. Vaginal swabs were preserved in medium consisting of 2-ml sucrose phosphate glutamate (SPG) at refrigeration temperature (4 ºC), and

finally stored at -80 ºC until processing. All samples were analyzed at the INIFAP laboratory in Mexico City.

DNA extraction and molecular detection of C. burnetii

Genomic DNA from vaginal swabs was extracted using the DNeasy Blood & Tissue Kit (QIAGEN, Germany) following the manufacturer's instructions. An endpoint PCR was performed using a set of primers previously described by De Bruin *et al.*, 2011, IS1trg-f 5' AGAATTTCTATTTTCAAAAAAAGGAGAAG- and IS1trg-r 5'- CGGTTCAACAATTCGG TATACA AACAA-3', which amplifies a 605 bp fragment of the insertion sequence (IS*1111*) of *C. burnetii*. A positive control was previously donated by The University of Murcia, Spain.

The amplified products were purified using a QIAquick® gel extraction kit (QIAGEN, Germany) according to the manufacturer's instructions, and then sequenced. The nucleotide sequence was performed using a fluorescence-based Taq FS dye-terminator sequencing procedure and analyzed on an Applied Biosystems 3730 DNA sequencing system (Foster City, CA, USA). The obtained sequences were edited with the Molecular Evolutionary Genetics Analysis software (MEGA X; Pennsylvania State University, Pennsylvania, PA, USA) and sequence alignment was completed using the nucleotide BLAST tool at the NCBI website.

Determination of frequency

Frequencies of brucellosis, leptopirosis and Q fever were determined in the samples using the diagnostic tests results, as described by Thrusfield (2018).

Results

A total of 153 blood sera were analyzed and were negative for brucellosis and leptospirosis. The IS*1111* was identified in 51 out of 153 (33.33%) genomic DNA extracted from vaginal swabs from cattle with abortion history. Figure 1 shows the distribution and proportion of positive samples to PCR per state. It is necessary to make it clear that this identification is not a Q fever diagnosis and that our results might not be linked to abortion. The PCR products from two different areas were sequenced in both senses. A consensus sequence was compared with Gen Bank databases, and sequences showed homology of up to 99.23% with *C. burnetii* Schperling (CP014563.1) and AuQ31 (KT954146.1), among others. The complete sequences were deposited in the GenBank under accession numbers MT459149 and MT459148 (Table 1).

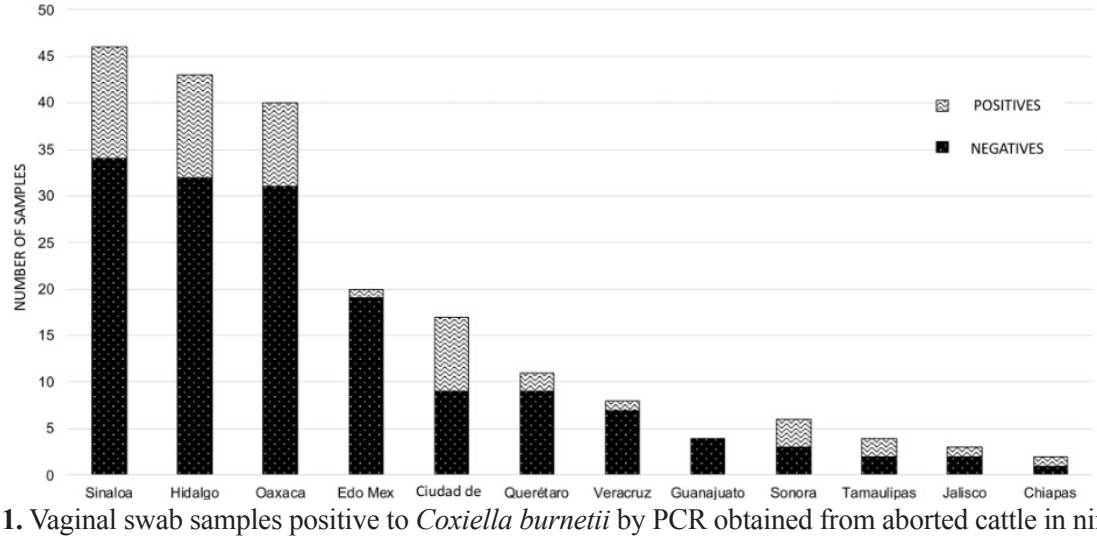

Figure 1. Vaginal swab samples positive to *Coxiella burnetii* by PCR obtained from aborted cattle in nine states of Mexico.

Table 1. Sequences of IS*1111* of *Coxiella burnetii* obtained by vaginal swabs from cattle in México and deposited in the GenBank.

Identification	Species	Identity	GenBank	Reference strain		
				Species	Country	GenBank
INIFAP BOV01	Bovine	99.65%	MT459148	Human	Australia	KT954146.1
INIFAP BOV02	Bovine	99.23%	MT459149	Human	Kirguistán	CP014563.1

Discussion

We demonstrated the presence of *C. burnetii* in vaginal swabs from aborted cattle in Mexico. As is true worldwide, Q fever is poorly studied in Mexico and is quite misunderstood. This disease is still considered exotic in México by Secretaría de Agricultura y Desarrollo Rural (AGRICULTURA) which complicates monitoring of the disease through diagnosis as well as establishing control strategies. Briefly, the study of Q fever in Mexico began in 1950. Since then, only serological evidence of *C. burnetii* has been reported in both human and animal populations. Animal seroprevalence ranges from 6 to 40% (Silva, 1950; Salman *et al.*, 1990; González-Canudas *et al.*, 1997; Salinas-Meléndez *et al.*, 2002; Sifuentes-Osornio *et al.*, 2012). At least six cases in humans have been confirmed since 1997 (González-Canudas *et al.*, 1997; Sahagún-Sánchez *et al.*, 1998; Santamaría 2009). Currently, Q Fever is not diagnosed in Mexican people, neither considered a report disease in the Epidemiological Bulletin of the National Epidemiological Surveillance System.

Diagnosis is an essential tool for the control of Q fever in livestock species; therefore, it is important to reduce the risk of zone since cattle, sheep and goats are the main reservoirs and transmitters of the disease to humans (Guatteo *et al.*, 2011; Borawski *et al.*, 2020; España *et al.*, 2020). Several methods are available for diagnosing Q fever. PCR is a common method for detecting the causal agent in clinical samples due to its high specificity and versatility. According to the World Organization for Animal Health (formerly known as OIE), this diagnostic test is highly recommended to demonstrate the absence of infection in the population, to establish an eradication program, or to confirm clinical cases (OIE, 2018). Unfortunately, there is a lack of studies worldwide aimed at elucidating the epidemiology of Q fever in cattle. In France, Guatteo *et al.* (2006) detected the *C. burnetii* IS*1111* insertion sequence by PCR in 15% (46/292) vaginal swabs from cattle. In the present study, most sampled animals did not present previous reproductive failure and only 46 of 153 animals presented abortion.

On the other hand, Biggs *et al.* (2016) identified the *C. burnetii* IS*1111* insertion sequence by PCR in 7% (2/28) of vaginal swabs from cattle dwelling in a region of the USA where five human cases of Q fever had been diagnosed within five months. This result can differ from ours because those animals did not present reproductive failure at the moment of sampling. Similarly, Bruin *et al.* (2011) detected *C. burnetii* IS*1111* by qPCR in 45% (136/300) vaginal swabs from cattle, sheep, and goats in The Netherlands. This finding varies from ours possibly due to the random sampling they conducted and also because some herds were considered to be *C. burnetii* free. More recently, in Latin America, Cornejo *et al.* (2020) identified *C. burnetii* IS*1111* by qPCR in 2.01% (2/105) in bulk-tank milk samples from dairy farms in Chile.

Cadmus *et al.* (2020) reported antibodies against *C. burnetii* in Cattle from Nigeria by iELISA in 23.5% (35/149) while seroprevalence for *Brucella* spp. was 11.4% (17/149) by RBT. In their study they also reported coinfection with *Brucella* spp. and *Coxiella burnetii* in 6% (9/149). In Mexico, the presence of various

diseases that cause abortions in cattle is recognized, such as leptospirosis, Bovine viral diarrhea (BVD), Infectious bovine rhinotracheitis (IBR), neosporosis, and chlamydia, among others (Solis-Calderón *et al.*, 2003; Escamilla *et al.*, 2007; Gutiérrez *et al.*, 2020, Gómez *et al.*, 2021); however, only brucellosis is officially monitored through the National Campaign against Brucellosis in Animals (NOM-041-ZOO-1995).

In the present report, all samples from aborted cattle were previously analyzed by serology with the official methods of the National Campaign and negative results were reported; therefore, the abortions could be related to other causes, including different ethological agents such as *C. burnetii*.

Amplification of bacterial DNA is a common method for detecting *C. burnetii*. Its efficiency has been demonstrated using different types of clinical samples such as blood, exudate and ectoparasites (Eldin *et al.*, 2017; Mori *et al.*, 2017). Given that Q fever is considered as an exotic disease in Mexico, it is impossible to conduct diagnostic tests or import serological tests for epidemiological studies. This situation restricts our knowledge of the impact this disease has on herd productivity. We have demonstrated the usefulness of a PCR technique for detecting the genetic material of the causal agent of Q Fever in Mexico, which allows to build greater knowledge about its presence and impact on the national livestock.

In conclusion, our study is the first of its kind in Mexico attempting to elucidate the epidemiology of Q fever in cattle. To the best of our knowledge, it is the first molecular proof of the presence of *C. burnetii* in cattle in Mexico and demonstrates that this microorganism is circulating among cattle in our country.

Declarations

Acknowledgements

The authors thank Julia Robert Farah for English revision of the manuscript.

Funding

This study was partially funded by the SAGARPA-CONACYT 2017-2-291311 project entitled "Desarrollo y transferencia de pruebas diagnósticas para-Lentivirus y microorganismos causantes de aborto: *Chlamydia* spp., *Brucella melitensis, Leptospira* spp. y *Coxiella burnetii*, en ovinos y caprinos" and for the fiscal resources project: "Detection of *Coxiella burnetii* in cattle raised under extensive grazing in the tropical and temperate regions of Mexico".

Conflict of interest statement

The authors declare they have no conflicts of interest with regard to the work presented in this report.

Author contributions

Flores-Pérez C: Diagnosis by PCR and wrote and translated the paper. Gutiérrez-Hernández JL: Administered the project and edited the paper. Palomartes-Resendiz EG: Diagnosis by RBT and RT. Herrera-López E: Provision of the samples, diagnosis by MAT. Díaz-Aparicio E: Responsible for the design and conception of the study. Hernández-Castro R: Bioinformatic analysis.

Use of artificial intelligence (AI)

No AI or AI-assisted technologies were used during the preparation of this work.

Data availability

The datasets generated during the current study are available in the GenBank, https://www.ncbi.nlm.nih.gov/nuccore/MT459149 and https://www.ncbi.nlm.nih.gov/nuccore/MT459148

References

Biggs HM, Turabelidze G, Pratt D, Todd SR, Jacobs-Slifka K, Drexler NA, McCurdy G, Lloyd J, Evavold CL, Fitzpatrick KA, Priestley RA, Singleton J, Sun D, Tang M, Kato C, Borawski K, Dunaj J, Pancewicz S, Król M, Czupryna P, Moniuszko-Malinowska A. *Coxiella burnetii* and Q fever - a review. Przegl Epidemiol 2020; 74(1):43–48. http://doi.org/10.32394/pe.74.05

Burette M, Bonazzi M. From neglected to dissected: How technological advances are leading the way to the study of *Coxiella burnetii* pathogenesis. Cell Microbiol 2020; 22(4): e13180. http://doi.org/10.1111/cmi.13180

Cadmus SI, Akporube KA, Ola-Daniel F, Adelakun OD, Akinseye VO. Seroprevalence and associated factors of brucellosis and Q-fever in cattle from Ibarapa area, Oyo State, South-western Nigeria. Pan Afr Med J 2020; 36:370. http://doi.org/10.11604/pamj.2020.36.370.24925

Cornejo J, Araya P, Ibáñez D, Hormazabal JC, Retamal P, Fresno M, Herve LP, Lapierre L. Identification of *Coxiella burnetii* in tank raw cow milk: first findings from Chile. Vector Borne Zoonotic Dis 2020; 20(3):228–230. http://doi.org/10.1089/vbz.2019.2535

De Bruin A, de Groot A, de Heer L, Bok J, Wielinga PR, Hamans M, van Rotterdam BJ, Janse I. Detection of *Coxiella burnetii* in complex matrices by using multiplex quantitative PCR during a major Q fever outbreak in The Netherlands. Appl Environ Microbiol 2011; 77(18):6516–6523. http://doi.org/10.1128/AEM.05097-11

Eldin C, Mélenotte C, Mediannikov O, Ghigo E, Million M, Edouard S, Mege JL, Maurin M, Raoult D. From Q Fever to *Coxiella burnetii* Infection: a paradigm change. Clin Microbiol Rev 2017; 30(1):115–190. http://doi.org/10.1128/CMR.00045-16

Escamilla HP, Martínez MJ, Medina CM, Morales SE. Frequency and causes of infectious abortion in a dairy herd in Queretaro, Mexico. Can J Vet Res 2007; 71(4):314–317. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1940280/pdf/cjvr71_pg314.pdf

España PP, Urania A, Cillóniz C, Torres A. Q Fever (*Coxiella burnetii*). Semin Respir Crit Care Med 2020; 41(04):509–521. http://doi.org/10.1055/s-0040-1710594

Gómez-Romero N, Ridpath JF, Basurto-Alcantara FJ, Verdugo-Rodriguez A. Bovine viral diarrhea virus in cattle from Mexico: current status. Front Vet Sci 2021; 8:673577. http://doi.org/10.3389/fvets.2021.673577

González-Barrio D, Ruiz-Fons F. *Coxiella burnetii* in wild mammals: a systematic review. Transbound Emerg Dis 2019; 66(2):662–671. http://doi.org/10.1111/tbed.13085

González-Canudas JA, Vega B, Nellen-Hummel H, Lisker-Halpert A, Laredo-Sánchez F. Hepatitis granulomatosa por fiebre Q [Granulomatous hepatitis caused by Q fever]. Gac Med Mex 1997; 133(5):475–477.

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(6):827–833. http://doi.org/10.1051/vetres:2006038

Guatteo R, Seegers H, Taurel AF, Joly A, Beaudeau F. Prevalence of *Coxiella burnetii* infection in domestic ruminants: a critical review. Vet Microbiol 2011; 149(1–2):1–16. http://doi.org/10.1016/j.vetmic.2010.10.007

Gutiérrez J, Palomares G, Hernández E, Leyva J, Díaz E, Herrera E. Frequency of reproductive impact diseases in dual-purpose cattle located in Oaxaca, Mexico. Abanico Vet 2020; 10:e114. https://doi.org/10.21929/abavet2020.22

Kersh GJ, Anderson A. *Coxiella burnetii* Infection in a community operating a large-scale cow and goat dairy, Missouri, 2013. Am J Trop Med Hyg 2016; 94(3):525–531. http://doi.org/10.4269/ajtmh.15-0726

Menadi SE, Mura A, Santucciu C, Ghalmi F, Hafsi F, Masala G. Seroprevalence and risk factors of *Coxiella burnetii* infection in cattle in northeast Algeria. Trop Anim Health Prod 2020; 52(3):935–942. http://doi.org/10.1007/s11250-019-02083-x

Mori M, Mertens K, Cutler SJ, Santos AS. Critical aspects for detection of *Coxiella burnetii*. Vector Borne Zoonotic Dis 2017; 17(1):33–41. http://doi.org/10.1089/vbz.2016.1958

Njeru J, Henning K, Pletz MW, Heller R, Neubauer H. Q fever is an old and neglected zoonotic disease in Kenya: a systematic review. BMC Public Health 2016;16:297. http://doi.org/10.1186/s12889-016-2929-9

Roest HI, Bossers A, van Zijderveld FG, Rebel JM. Clinical microbiology of *Coxiella burnetii* and relevant aspects for the diagnosis and control of the zoonotic disease Q fever. Vet Q 2013;33(3):148–160. http://doi.org/10.1080/01652176.2013.843809

Rojo-Rubio R, Vázquez-Armijo JF, Pérez-Hernández P, Mendoza-Martínez GD, Salem AZ, Albarrán-Portillo B, González-Reyna A, Hernández-Martínez J, Rebollar-Rebollar S, Cardoso-Jiménez D, Dorantes-Coronado EJ, Gutierrez-Cedillo JG. Dual purpose cattle production in Mexico. Trop Anim Health Prod 2009; 41:715–721. https://doi.org/10.1007/s11250-008-9249-8

Sahagún Sánchez G, Cotter Lemus L, Zamora González C, Reyes PA, Ramírez S, Buendía A. Endocarditis por *Coxiella burnetii*. Informe del primer caso diagnosticado en México [*Coxiella burnetii* endocarditis. A report of the first case diagnosed in Mexico]. Arch Inst Cardiol Mex 1998; 68(4):322–327.

Salinas-Melédez JA, Avalos-Ramírez R, Riojas-Valdez V, Kawas-Garza J, Fimbres-Durazo H, Hernández-Vidal G. Serologic survey in animals of 'Q' fever in Nuevo Leon [published correction appears in Rev Latinoam Microbiol] 2002; 44(3–4):111. https://www.medigraphic.com/pdfs/lamicro/mi-2002/mi022f.pdf

Salman MD, Hernández JA, Braun Y. A seroepidemiological study of five bovine diseases in dairy farms of the coastal region of Baja California, Mexico. Prevent Vet Med 1990; 9(2):143–153. http://doi.org/10.1016/0167-5877(90)90032-D

Santamaría, J.R. Fiebre Q en el Estado de Hidalgo, México. Reporte de caso. Perinatol Reprod Hum 2009; 23(1):34–37. https://www.medigraphic.com/pdfs/inper/ip-2009/ip091g.pdf

Sifuentes-Osornio J, Bobadilla-del-Valle JM, Aguilar-Cruz A, Torres-Ángeles O, Ramírez-González JL, Ponce-de-León A, Ruíz-Palacio GM, Guerrero-Almeida ML. What do we know about Q fever in Mexico? Rev Invest Clin 2012; 64(6 Pt 1):541–545.

Silva Goytia R. Fiebre Q en México. II. Estudios serológicos [Q fever in Mexico; serologic studies]. Medicina (Mex) 1950; 30(617):493–497.

Škultéty L. Q fever and prevention. Epidemiol Mikrobiol Imunol 2020 Spring;69(2):87–94. English. PMID: 32819108.

Solis-Calderon JJ, Segura-Correa VM, Segura-Correa JC, Alvarado-Islas A. Seroprevalence of and risk factors for infectious bovine rhinotracheitis in beef cattle herds of Yucatan, Mexico. Prev Vet Med 2003; 57(4):199–208. http://doi.org/10.1016/s0167-5877(02)00230-1

Thrusfield, M. *Veterinary epidemiology*. 4th ed. John Wiley-Blackwell. USA. 2018.