ACIN
ASOCIACIÓN COLOMBIANA DE INFECTOLOGÍA

infectio

ARTÍCULO ORIGINAL

Coxiella burnetii infection in sheep and goats: a public risk health, Colombia

Verónica Contreras[1], Marco Gonzalez[1], Jaime Alvarez[1], Salim Mattar[1,*]

Abstract

Objective. The aim of this study was to provide molecular evidence of *C. burnetii* in sheep and goats from some herds of Valledupar, Cesar, Colombia.
Materials and methods. Fifteen herds of sheep and goats were chosen by convenience to investigate the infection by *C. burnetii,* during March and April of 2013. 328 female goats and 66 sheep from 15 herds were included in this study. Milk from ewes and vaginal mucus samples from goats were analyzed by Polymerase Chain Reaction for DNA detection of transposase gene (*IS*1111) of *C. burnetii.*
Results. DNA of *C. burnetii* in 6% (4/66) of sheep's milk and 0.6% (2/328) vaginal mucus from goats was found. 13% (2/15) of the herds had at least one infected animal.
Discussion. Our findings suggest the circulation of *C. burnetii* in sheep and goats from some herds of Valledupar, Colombia, and it highlights the possibility of occurrence of infections in humans and animals.
Conclusions. The detection of *C. burnetii* in sheep milk could represent a public health risk factor for people who consuming raw milk, cheeses or people associated to agriculture and livestock handling. Further studies are necessary to evaluate other routes such as tick's bite, feces, milk from goats and vaginal mucus from sheep of this region of Colombia.

Key words: Q fever, Zoonoses, Milk ejection, Disease Vectors, Health Services Research, Education, Communicable diseases.

Infección por *Coxiella burnetii* en ovinos y caprinos: un riesgo en salud publica en Colombia

Resumen

Objetivo. El objetivo de este estudio fue proporcionar evidencia molecular de infección por *C. burnetii* en ovinos y caprinos de algunos rebaños de Valledupar, Cesar, Colombia.
Materiales y métodos. Quince rebaños de ovinos y caprinos fueron seleccionados a conveniencia para investigar la infección por *C. burnetii,* durante marzo y abril de 2013. En este estudio se incluyeron 328 caprinos y 66 ovinos de 15 rebaños. La leche procedente de ovinos y muestras de moco vaginal de caprinos fueron analizados mediante PCR (Reacción en Cadena de Polimerasa) para la detección de ADN del gen transposasa (*IS*1111) de *C. burnetii*.
Resultados. Se encontró ADN de *C. burnetii* en 6% (4/66) de leche de oveja y 0,6% (2/328) de moco vaginal de cabras. El 13% (2/15) de los rebaños tenían al menos un animal infectado.
Discusión. Nuestros hallazgos sugieren la circulación de *C. burnetii* en ovinos y caprinos de algunos rebaños de Valledupar, Colombia, y destaca la posibilidad de ocurrencia de infecciones en humanos y animales.
Conclusiones. La detección de *C. burnetii* en la leche de oveja podría representar un factor de riesgo para la salud pública de las personas que consumen con frecuencia leche cruda, quesos o personas que trabajan en la agricultura y manipulación de ganado. Otros estudios son necesarios para evaluar otras rutas como la mordedura de la garrapata, las heces, la leche de las cabras y el moco vaginal de las ovejas de esta región de Colombia.

Palabras clave: Fiebre Q, Zoonosis, Secreción de leche, Vectores de enfermedades, Investigación de servicios de salud, Educación, Enfermedades contagiosas

Introduction

Coxiella burnetii is a Gram-negative bacterium causing of Q fever, a zoonosis that concerns public health throughout the world. Mammals, birds and arthropods, mainly ticks can be infected, but domestic ruminants (sheep, goats and cattle) are the main reservoirs[1], in which *C. burnetii* is shed through placenta, vaginal discharges, urine, feces and milk[2,3]. *C. burnetii* is a frequent cause of reproductive disorders mainly in minor ruminants[3-5]. In goats, it has been reported as a cause of abortions and stillbirths[6,7] and in cows, infection is asymptomatic, but metritis and subclinical mastitis have been described[8].

The risk of *C. burnetii* transmission between animals and from animals to humans depends of the prevalence of shedders and excretion's levels in ruminants[5]. *C. burnetii* is able to persist for long time adverse conditions[9] and it is easily transported by the wind[10]. Inhalation of contaminated dust with the

1 Instituto de Investigaciones Biológicas del Trópico, Universidad de Córdoba, Colombia.
* Autor para correspondencia.
Correo electrónico: mattarsalim@hotmail.com;
smattar@correo.unicordoba.ed.co

Recibido: 26/09/2017; Aceptado: 02/02/2018

Cómo citar este artículo: V. Contreras, *et al. Coxiella burnetii* infection in sheep and goats: a public risk health, Colombia. Infectio 2018; 22(4): 173-177

bacterium from products of infected animals is the main source of infection in humans. Nevertheless, infection by consuming raw milk has also been reported[11]. Clinical spectrum in humans is very broad; patients may experience asymptomatic seroconversion, nonspecific febrile syndrome, atypical pneumonia or hepatitis. Chronic Q fever is rare (<5%), but it occurs in patients with underlying conditions such as immunosuppression, vascular disease, aneurysm, etc., and is mainly expressed with endocarditis that could have a fatal course[12].

The epidemiology of Q fever is characterized by a complex interaction of factors like variety of hosts, low infectious dose, nonspecific symptoms, difficult access to diagnostic tools and the lack of epidemiological association, and they provide a masking with others febrile syndromes[1]. However, in the Q fever epidemic occurred in the Netherlands (2007-2012), more than 4,000 human cases related to farms with infected goats were reported, and it has highlighted its potential impact on human health. The confluence of concentration of infected goat farms near areas with high human density and a favorable meteorological context were among the facilitating factors of bacterial spreading[13].

In Colombia, seroprevalence studies of *C. burnetii* and some human cases of Q fever has been reported[14-16] Additionally, DNA of *C. burnetii* was detected in 45% of 11 bulk cow milk samples[17]. Furthermore, in Colombia there is report describing a case of a 56-year-old patient with an associated in agriculture and livestock handling, the diagnose was made using an indirect immunofluorescence assay showed high titers of IgG for *C. burnetii* anti-phase I (1: 256) and anti-phase II (1:1024)[18]. However, there is a lack of information of *C. burnetii* infection in minor ruminants, which are able to transmit the infection. The aim of this study was to provide molecular evidence of *C. burnetii* infection in sheep and goats from some herds of municipality of Valledupar, Cesar, Colombia.

Materials and Methods

Type of study, geographical area, ruminant's population, size sample and specimens.
A descriptive, prospective and transversal study was performed. Fifteen herds of small ruminants from six villages of municipality of Valledupar, department of Cesar (Colombia) (10º 27´N y 73º 15´O) were chosen at convenience (Figure 1), during March and April 2013. The number of herds and animals per farm were calculated by using of Free-Calc., software[19]. It was taking to account the population of 20,000 sheep and 8,000 goats and a regional register of 100 herds from Valledupar; a confidence level of 95%, maximum error of 5%, and an expected proportion of infected animals and herds according to previous reports were chosen[20]. The obtained results were analyzed through descriptive statistic.

328 female goats and 66 sheep with at least one birth were included in this study. Goats belonged to all 15 herds, whereas sheep came from 11 of them. 15 ml of milk's ewes and vaginal swabs were collected from goats using sterile cotton swabs. They were placed in sterile plastic tubes, transported to the laboratory at 4°C and subsequently preserved at -20°C. Milk samples showed normal physical characteristics (color, pH and density). The state of *C. burnetii* infection in herds was unknown at baseline. A herd was considered positive if at least one animal (sheep or goat) was found infected with *C. burnetii* (milk or vaginal mucus positive by PCR).

DNA extraction and molecular detection of *C. burnetii*.
Milk samples and vaginal swabs were subjected to DNA extraction using the DNA mini kit Purelink (Invitrogen, CA, USA). Milk's specimens directly from 300 µl of whole homogenized milk were analyzed. 300 µl of TE buffer (10 mM Tris Base, 1 mM EDTA and pH 8) was added to vaginal swabs and mixed by vortex. To ensure no contamination, negative controls (sterile water) were included. DNA was purified in a final volume of 100 µl; according to the manufacturer's conditions. Sample was stored at -20°C until use as template for Polymerase Chain Reaction (PCR).

A conventional PCR was performed using oligonucleotides CoxP4 (5'-GGCTGCGTGGTGATGG) (Gen bank accession number: M80806) and CoxM9 (GTCCCGGTTCAACAATTCG), previously described with some modifications (21), which amplify a fragment of (435 bp) transposase gene (*IS*1111) of *C. burnetii*. All amplified products were visualized in electrophoresis of agarose gel (1.5%); it was purified and sequenced by Macrogen, Korea services. The obtained sequences were edited with MEGA program (version 6.0) and analyzed in BLAST.

Results

Four of 66 (6%) sheep milk and two of 328 (0.6%) vaginal swabs of goats yielded *C. burnetii* DNA. The transposase gene sequences (*IS*1111) generated in this study had a percentage of identity of 100 and 99% with *C. burnetii* strain CbuK Q_154 (Genbank access number CP001020) and *C. burnetii* strain Guiana Cb175, respectively.

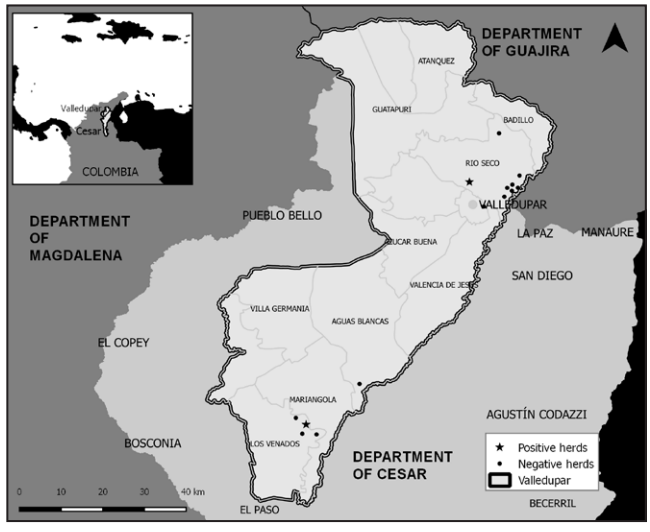

Figure 1. Geographical location of goats and sheep herds in the municipality of Valledupar, department of Cesar, Colombia.

Table 1. Description of studied herds.

Villages of Valledupar	Herds Code	Reproductive background´s	Herds size (n)		Positive PCR / Total obtained samples		Frequency of infection
			Sheep	Goats	Sheep's milk	Goat's vaginal swabs	
Los Venados	**A**	A*	102	30	4/11	1/20	16%
	B	A, M	26	29	0/1	0/25	0
	C	A, M	60	23	0/10	0/23	0
	D	M**	60	35	0/1	0/24	0
	E	A, M	35	27	0/1	0/23	0
Mariangola	**F**	A, M	56	29	0/2	0/23	0
El Jabo	**G**	A, M	130	28	0/9	0/26	0
Río Seco	**H**	A, M	22	27	0	1/16	6%
	I	M	28	47	0/1	0/16	0
Guacoche	**J**	M	25	28	0/1	0/16	0
	K	A	47	38	0/4	0/24	0
	L	A, M	38	25	0	0/24	0
	M	M	22	38	0/20	0/24	0
Guacochito	**N**	M	40	30	0/5	0/28	0
Valledupar	**O**	M	60	40	0	0/16	0
Total					4/66 (6%)	2/328 (0,6%)	13% (2/15)

* Abortions; **Mastitis

Thirteen percent (n=2) of 15 herds had at least one infected animal (Figure 1). Four milk's sheep (4/11; 36%) and one vaginal swab from goats (1/16; 5%) were found infected with *C. burnetii* from a same herd (Table 1). The average of animals in the herds was 77.2 (SD = 33.47, range 1-158). Sheep and goats were mixed in all herds and these latter were predominant. The type of production in sampled herds was 60% (9/15) of ruminants of double purpose (meat and milk production). Farms and its herds were not handled by technical means, and they did not have identification records and reproductive history from animals. However, the occurrence of mastitis, abortions or both were informed in all herds in previous deliveries. Positive herds had records of abortions (Table 1), but the causal agent of such conditions was never identified.

Discussion

The circulation of *C. burnetii* has been reported in some regions of Colombia. In 2006, a seroprevalence in rural workers from Cordoba and Sucre departments was informed[14]. A case of endocarditis and one of pneumonia by Q fever were reported in 2012[15,16], and a case of infection by *C. burnetii* was reported in a patient with a background in agriculture and livestock handling from a rural area of Monteria, Cordoba[18]. Furthermore, in a study performed in cattle farms from Monteria, *C. burnetii* DNA was found in 45% of 11-bulk milk, and 61% of farm workers and residents of the farms had antibodies against *C. burnetii*[17]. Herein, we report the infection by *C. burnetii* in sheep and goats from some herds of Valledupar, Colombia.

The shedding of *C. burnetii* in sheep and goats is a major issue for public health[22]. However, studies of frequency of infection in ruminants based on PCR analysis are uncommon[23] and this knowledge is important to determine the risk of transmission between animals and from animals to humans[5]. In the present study, 6% (n=4) of 66 sheep's milk and 0.6% (n=2) of 328 vaginal swabs from goats yielded *C. burnetii* DNA. In a study performed in Turkey, out of the 350 bovine milk samples and 250 ovine milk samples collected, 1,42% and 0,4% were found to be positive using the PCR technique, respectively[24]. The amplification of transposase gene (*IS*1111)[1] allowed for the sensitivity of the assay to be increased, because this a multi-copy gene (7-110 copies)[25]. DNA sequences generated in the present study confirm that *C. burnetii* is circulating in goats and sheep from some herds of Valledupar, Colombia.

As it was above described, shedding of *C. burnetii* can be intermittent, it could increase during postpartum periods, be different between species and vary according to the type of sample. *C. burnetii* in vaginal mucus of goats is less frequent, but more frequent in milk[2]. In the present work, this result is in according to the proportion of vaginal mucus samples from goats that yielded *C. burnetii* DNA (0.6%). Therefore, it is likely that the sample collection in the present study may have coincided with the shedding period for some individuals and not with others. Additionally, only one type of sample was obtained from each ruminant species (ovine milk and mucus vaginal from goats), and taken together these results, we suggest that a concomitant analysis of various

and different types of samples from the same animal might increase the probability of finding an infected animal with *C. burnetii*. Moreover, Guatteo *et al.,*[5] reported that infected animals shed *C. burnetii* mainly during parturition; in our study, samples were collected several months after delivery in herds. However, in a study carried out by Rodolakis *et al.,*[2], it was found that shedding of *C. burnetii* could not be related with the parturition. *C. burnetii* DNA was found in samples of milk, mucus vaginal and feces taken from 0 to 421 days after parturition in bovine herds, from 5 to 119 days in caprine and 11 to 238 days in ovine herds. The results of this study might suggest that the excretion of *C. burnetii* could be higher in the studied animals and could vary in different times. These results also suggest that the excretion of *C. burnetii* in infected animals could create a public health risk for people in the immediate surroundings as well as in surrounding areas. Additional studies are necessary, with a larger number of animals and with several samples in different periods. Likewise, it is important to carry out studies on other species, such as rodents and ticks, which have been described to be included in the epidemiological cycle of *C. burnetii*[26].

In this study, 13% (n=2) of 15 herds had at least one infected animal with *C. burnetii*. One herd had five infected animals, four sheep's shed *C. burnetii* in milk and one goat shed in vaginal mucus, and it showed that this herd had active circulation of *C. burnetii*. In a study performed in Germany, *C. burnetii* DNA was found in 5% of 39 flock's sheep[27]. In the Netherlands, *C. burnetii* DNA was detected in 33% of 292 goat farms[28]. In Italy, *C. burnetii* DNA was amplified in 18% of 199 goats and sheep farms with reproductive history[29]. In the present study, all herd owners reported the occurrence of reproductive problems (abortions and/or mastitis) in previous deliveries, but causal agent of such conditions were undetermined. One of positive herds to *C. burnetii* have a history of abortions and other one have history of abortions and mastitis, however, others studies are necessary to determine causal agent of this disorders.

On the other hand, Q fever is an important zoonosis. However, In Colombia and Latin American countries, Q fever is a neglected disease due to great multiplicity of symptoms, absence of knowledge of the disease and epidemiological data, which most expected lead to underdiagnoses and underreporting of the disease. This is the first report in the Caribbean area of Colombia, which the main reservoirs and sources of human infections were above described, however, ticks also are involved and the disease presents with a variety of clinical manifestations as atypical pneumonia, febrile hepatitis and endocarditis may also occur. The variability in the clinical manifestations of Q fever may lead to postponement of diagnosis. Therefore, anamnesis, epidemiological factors and serological tests are tremendously important in Colombia. Being exposed to livestock, living in rural area or living closely to farms are public health risk factors. Above that, the lack of direct contact with animals cannot disregard the diagnosis of Q fever, since airborne transmission of *C. burnetii* is also recurrent.

Conclusion

We report the *C. burnetii* infection in sheep and goats from some herds in Valledupar, Colombia. Due to environmental stability and potential aerosol dispersion of *C. burnetii*, our findings highlight the possibility of occurrence of infections in humans and animals in Valledupar, Colombia. The detection of *C. burnetii* in sheep milk could represent a public health risk factor for people who consuming frequently raw milk, cheeses or other products. Further studies are necessary to evaluate other routes such as tick's bite, feces, milk from goats and vaginal mucus from sheep of this region of Colombia. Clinical differential diagnoses including Q fever in high-risk people should be taken into account in Colombia.

Conflict of interest

None of the authors in this study declares having any conflict of interests.

Ethical statement

The research ethics committee of the Institute of Tropical Biological Research at Universidad de Córdoba approved the study through Act 026-2011. Ethical, technique, scientific and administrative standards contained in Resolution No. 008430 de 1993 and Law 84 of 27th of December 1989 were taken into account to their collection, manage and conservation of samples.

Protection of human and animal subjects. The authors declare that the procedures followed were in accordance with the regulations of the responsible Research Ethics Committee for animals manipulation.

Confidentiality of data. The authors declare that they have followed the protocols of their work center on the publication of owner´s of animal data.

Right to privacy and informed consent. The authors obtained the informed consent of the owners of the animals described in the article. The author for correspondence is in possession of theses document.

Acknowledgements

The authors gratefully acknowledge the owners of the farms who joined the project. We thank to Yuleida Martinez and Juan Carlos Rumbo for their help in collection samples and to Professor Oscar Vergara G. This research was funded by Universidad de Córdoba. Project CIUC, Code 1-2-08-110-26. FMV 04-11.

References

1. Contreras V, González M, Guzmán C, Máttar S. Fiebre Q: una zoonosis olvidada en Colombia. Rev Med Risaralda. 2013;19:137-46. DOI:doi.org/10.22517/25395203.8471
2. Rodolakis A, Hechard C, Caudron C, Souriau A, et al. Comparison of *Coxiella burnetii* shedding in Milk of Dairy Bovine, Caprine, and Ovine Herds. J Dairy Sci. 2007;90:5352-60. DOI:10.3168/jds.2006-815
3. Van den Brom R, Van Engelen E, Roest H, van der Hoek W, Vellema P. *Coxiella burnetii* infections in sheep or goats: an opinionated review. Vet Microbiology. 2015;181:119-29.

4. Berri M, Souriau A, Crosby M, Rodolakis A. Shedding of *Coxiella burnetii* in ewes in two pregnancies following an episode of *Coxiella* abortion in a sheep flock. Vet microbial. 2002;85:55-60. DOI:doi.org/10.1016/S0378-1135(01)00480-1
5. Guatteo R, Beaudeau F, Joly A, Seegers H. Assessing the Within-herd Prevalence of *Coxiella burnetii* Milk-shedder Cows using a Real-time PCR Applied to Bulk Tank Milk. Zoonoses Public Health. 2007;54:191-94. DOI:10.1111/j.1863-2378.2007.01043.x
6. Moeller RB. Causes of caprine abortion: diagnostic assessment of 211 cases (1991-1998). J Vet Diagn Invest. 2001;13:265-70. DOI:10.1177/104063870101300317
7. Masala G, Porcu R, Sanna G, Chessa G, et al. Occurrence, distribution, and role in abortion of *Coxiella burnetii* in sheep and goats in Sardinia, Italy. Vet Microbiol. 2004;99:301-5. DOI:doi.org/10.1016/j.vetmic.2004.01.006
8. Barlow J, Rauch B, Welcome F, Kim SG, et al. Association between *Coxiella burnetii* shedding in milk and subclinical mastitis in dairy cattle. Vet Res. 2008;39:23. DOI:10.1051/vetres:2007060
9. Astobiza I, Barandika JF, Ruiz-Fons F, Hurtado A, et al. Four-year evaluation of the effect of vaccination against Coxiella burnetii on reduction of animal infection and environmental contamination in a naturally infected dairy sheep flock. Appl Environ Microbiol. 2011;77:7405-07. DOI:10.1128/AEM.05530-11
10. Van der Hoek W, Hunink J, Vellema P, Droogers P. Q fever in The Netherlands: the role of local environmental conditions. Int J Environ Heal. 2011;21:441-51. DOI: dx.doi.org/10.1080/09603123.2011.574270
11. Signs KA, Stobierski MG, Gandhi TN. Q fever cluster among raw milk drinkers in Michigan, 2011. Clin Infect Dis. 2012;55:1387-89. DOI:10.1093/cid/cis690
12. Anderson A, Bijlmer H, Fournier P, Graves S. Diagnosis and Management of Q fever - Recommendations from CDC and the Q Fever Working Group. MMWR. 2013;62:1-23. [https://www.cdc.gov/mmwr/preview/mmwrhtml/rr6203a1.htm]
13. Dijkstra F, Hoek W, Wijers N, Schimmer B. The 2007–2010 Q fever epidemic in the Netherlands: characteristics of notified acute Q fever patients and the association with dairy goat farming. FEMS Microbiol Immunol. 2012;64:3-12. DOI: 10.1111/j.1574-695X.2011.00876.x
14. Máttar S, Parra M. Detection of antibodies to Anaplasma, Bartonella and Coxiella in rural inhabitants of the Caribbean area of Colombia. Rev. MVZ Córdoba. 2006;11:781-89.
15. Betancur CA, Múnera AG. Coxiella burnetii endocarditis: Q fever. Acta Med Colomb. 2012;37:31-3.
16. Meza-Cardona JC, Rosso-Suárez F. Neumonía por Coxiella burnetii: presentación de un caso y revisión de la literatura. CES Medicina. 2012;26: 201-8.
17. Contreras V, Máttar S, González M, Alvarez J, Oteo JA. *Coxiella burnetii* in bulk tank milk and antibodies in farm workers at Monteria, Colombia. Rev Colomb Cienc Pec. 2015;28:181-87. DOI: 10.17533/udea.rccp.v28n2a07
18. Máttar S, Contreras V, González M, Camargo F, Álvarez J, Oteo JA. Infection by *Coxiella burnetii* in a patient from a rural area of Monteria, Colombia. Rev Salud Publica. 2014;6:789-92.
19. Cameron AR, Baldock FC. Two-stage sampling in surveys to substantiate freedom from disease. Prev Vet Med. 1998;34:19-30.
20. Guatteo R, Seegers H, Taurel AF, Joly A, Beaudeau F. Prevalence of Coxiella burnetii infection in domestic ruminants: A critical review. Vet. Microbiology. 2011;149:1-16. DOI: 10.1016/j.vetmic.2010.10.007
21. Panning M, Kilwinski J, Greiner-Fischer S, et al. High throughput detection of *Coxiella burnetii* by real-time PCR with internal control system and automated DNA preparation. BMC Microbiology. 2008;8:77. DOI: https://doi.org/10.1186/1471-2180-8-77
22. Rousset E, Berri M, Durand B, et al. *Coxiella burnetii* shedding routes and antibody response after outbreaks of Q fever-induced abortion in dairy goat herds. App Environ Microbiol. 2009;75:428-33. doi: 10.1128/AEM.00690-08
23. Muskens J, Van Engelen E, Van Maanen C, Bartels C, Lam TJ. Prevalence of *Coxiella burnetii* infection in Dutch dairy herds based on testing bulk tank milk and individual samples by PCR and ELISA. Vet Rec. 2011;168:79. DOI: 10.1136/vr.c6106
24. Saglam AG, Sahin M. Coxiella burnetii in samples from cattle herds and sheep flocks in the Kars region of Turkey. Vet Med. 2016;1:17-22. DOI:10.17221/8678-VETMED
25. Klee SR, Tyczka J, Ellerbrok H, et al. Highly sensitive real-time PCR for specific detection and quantification of Coxiella burnetii. BMC Microbiology. 2006;6:2. DOI:10.1186/1471-2180-6-2
26. Rozental T, Ferreira MS, Guterres A, Mares-Guia et al. Zoonotic pathogens in Atlantic Forest wild rodents in Brazil: Bartonella and Coxiella infections. Acta tropica. 2017;168:64-73.
27. Hilbert A, Schmoock G, Lenzko H, et al. Prevalence of Coxiella burnetii in clinically healthy German sheep flocks. BMC research notes. 2012;5:152. DOI:10.1186/1756-0500-5-152
28. Van den Brom R, Van Engelen E, Luttikholt S, Moll L, Van Maanen K, Vellema P. Coxiella burnetii in bulk tank milk samples from dairy goat and dairy sheep farms in The Netherlands in 2008. Vet Rec. 2012;170:310. DOI:10.1136/vr.100304.
29. Parisi A, Fraccalvieri R, Cafiero M, et al. Diagnosis of Coxiella burnetii-related abortion in Italian domestic ruminants using single-tube nested PCR. Vet Microbiol. 2006;118:101-06. DOI: doi.org/10.1016/j.vetmic.2006.06.023