ACTA MICROBIOLOGICA BULGARICA Volume 34 / 4 (2018)

Chlamydia abortus and *Coxiella burnetii*- Related Abortions in Small Ruminants in Bulgaria during a Five-Year Period (2013-2018)

Konstantin Simeonov*, Mariela Chilingirova

Laboratory for Chlamydial and Rickettsial Diseases, National Diagnostic and Research Veterinary Medical Institute "Prof. Dr. George Pavlov", 1606 Sofia, Bulgaria

Abstract

The results of the polymerase chain reaction (PCR) and serological testing for *Chlamydia abortus* and *Coxiella burnetii* of abortion submissions (placentae or foetal organ samples) and sera from 56 sheep and 54 goats are presented. The samples were submitted for routine diagnostic procedures at the Laboratory for Chlamydial and Rickettsial Diseases, National Diagnostic and Research Veterinary Medical Institute (NDRVMI), during a five-year period (2013-2018). Samples from 48 sheep and 28 goats were tested by conventional PCR for the presence of one or both infections, while other 34 small ruminants were investigated only serologically by ELISA. In some cases one and the same animal was tested both serologically and by PCR. Of the 43 ovine and 24 caprine samples tested for *C. abortus* by PCR, positive results were obtained in 24 (35.8%), 18 (41.9%) ovine and 6 (25%) caprine samples, respectively. Five of the 12 PCR-tested goat samples were positive for *C. burnetii*, whereas no sample of the 17 sheep tested showed presence of this agent. Antibodies against these two intracellular bacteria were detected in 20 (23%) out of 87 sera from aborted animals, suggesting, or confirming their probable etiological role. The results show that *C. abortus* plays a leading role as a cause of abortions in small ruminants as a whole, but *C. burnetii*, too, should not be ignored, especially presuming its zoonotic potential.

Keywords: *Chlamydia abortus, Coxiella burnetii*, small ruminants, abortions

Резюме

Представени са резултатите от молекулно-биологични (PCR) и серологични изследвания на проби от фетуси и плаценти, както и серуми от 56 абортирали овце и 54 кози по отношение на *Chlamydia abortus и Coxiella burnetii*. Материалите са постъпили за рутинно диагностично изследване в лаборатория „Хламидии и рикетсии" на НДНИВМИ в периода 2013-2018 г. Проби от 48 овце и 28 кози бяха тестирани чрез конвенционална PCR за наличие на едната или на двете инфекции, докато други 34 дребни преживни бяха изследвани само серологично чрез ELISA. Някои от животните бяха изследвани както чрез PCR, така и серологично.От изследваните чрез PCR за *C. abortus* 43 проби от овце и 24 от кози, положителен резултат бе получен при 24 (35,8%) от тях, съответно 18 (41.9%) овце и 6 (25%) кози. Пет от 12 PCR тестирани кози бяха положителни за наличие на *C. burnetii*, докато този агент не бе доказан в нито една от 17 изследвани овце. Антитела срещу тези две вътреклетъчни бактерии бяха доказани в 20 от 87 серума от абортирали животни, което предполага или потвърждава етиологичното им участие в наблюдаваната патология. Резултатите показват, че *C. abortus* играе водеща роля като абортоген при дребните преживни животни като цяло, но значението на *C. burnetii* също не трябва да бъде подценявано, особено от гледна точка на зоонозния и потенциал.

Introduction

Keeping stock ruminants in a good reproductive condition is a problem of great importance, since, apart from obtaining offspring, it is related to lactation after birth. Therefore, any failures in the reproductive process, including abortions, could lead to significant economic losses (Longbottom *et al*., 2013).

The spectrum of pathogenic agents causing reproductive disorders in small ruminants is too

* Corresponding author: kbsimeonov@yahoo.com

wide, including infectious agents of viral origin (caprine herpesvirus-1 (CHV-1), Border disease virus, Bluetongue virus), bacteriae (*Brucella spp., Salmonella spp., Listeria monocytogenes, Campylobacter spp, Chlamydia abortus, Coxiella burnetii*) and parasites (*Neospora caninum, Toxoplasma gondii*) (Masala *et al*., 2007; Givens and Marley, 2008; Moreno *et al*., 2012). The identification of abortion causes is essential not only for the development of rational therapeutic and prophylactic measures, but also because it is also relevant to human health as some of these agents have zoonotic potential (EFSA Panel on Animal Health and Welfare, 2010; Moreno, 2014).

In this study we present data on abortions in small ruminants in the Republic of Bulgaria, diagnosed for a five-year period in the National Diagnostic and Research Veterinary Medical Institute, Sofia, with emphasis on those caused by *C. abortus* and *C. burnetii*.

Materials and methods

Samples from placental cotyledons and fetal organs, or blood samples from aborted animals were submitted for routine diagnostic procedures to the National Diagnostic and Research Veterinary Medical Institute. All samples (up to three) from animals from one and the same farm were processed and reported as one. The placentae and fetal organs were tested for *C. abortus* and *C. burnetii* by conventional PCR. Total tissue DNA was extracted using commercial kits (Tissue & Cell Genomic DNA Mini Kit, Guangzhou Geneshun Biotech, Ltd. and Animal and Fungi DNA Preparation Kit, Jena Bioscience) according to the manufacturer's instructions. PCR for the detection of *C. abortus* was performed with primers CpsiA (5'-ATG AAA CAT CCA GTC TAC TGG-3') and CpsiB (5'-TTG TGT AGTATAT ATC AAA-3') and cycling parameters as described by Laurucao *et al*., 2001. Two variants of PCR using differed primer sets were used to detect *C. burnetii*. In the first one, primer pair C.B.1/ C.B.2 with sequences 5'-TTG TGT AGT AAT ATT ATC AAA-3'and 5' -TAG CTG AAG CCA ATT CGC C-3', respectively, was used to amplify a region of 257 base pairs (bp), encompassing the gene encoding superoxide dismutase (Stein and Raoult, 1992). Primers Trans1/2, targeting the insertion sequence IS111 gene, were used in the second PCR reaction and generated a product of 678 bp (Berri *et al*., 2000).

All PCR reactions were performed on a QB-96 (LKB) thermocycler, and the amplification products obtained were analyzed by 1.5% agarose gel electrophoresis.

To test for the presence of antibodies against *C. burnetii* and *C. abortus*, commercial ELISA kits (IDEXX Laboratories) were used according to the manufacturer's instructions.

Results

Aborted placenta and fetal organ samples from 48 sheep and 28 goats were received at the Laboratory for PCR testing for *C. abortus* and/or *C. burnetii* between 2013 and 2018. In addition, only blood samples from 8 aborted sheep were sent for detection of antibodies against *C. abortus* and another 22 sheep blood samples were sent simultaneously with tissue samples from the same animals in order to refine, or confirm the diagnosis. Thirty-one blood samples from goats were sent for serological diagnosis of these two infections.

The results of PCR detection of *C. abortus* and *C. burnetii* from placental cotyledons and aborted fetuses, and serology are presented in Table 1.

Of a total of 43 sheep and 24 goat samples, PCR testing identified presence of *C. abortus* in 24 (35.8%) cases, 18 (41.9%) in sheep and 6 (25%) in goats, respectively. In 15 out of 49 (30.6%) sheep and goat sera, the probable etiological role of *C. abortus* in abortions was suggested, or confirmed based on a positive result for the presence of antibodies. Five of the 12 *C. burnetii* PCR-tested goat samples were positive. However, none of the 17 sheep tested showed presence of this agent.

Antibodies against *C. burnetii* were found in 5 (13.2%) of a total of 38 sheep and goat sera tested. In one flock of goats, this infectious agent was demonstrated in the placentas of two animals, however, the sera from the same animals showed a negative result for antibodies against *C. burnetii* when assayed by ELISA. Two sera from one goat flock were positive for antibodies against both *C. abortus* and *C. burnetii*. However, co-infection with both agents was not observed in any of the samples tested by PCR.

Discussion

Although many infectious agents have been implicated as causes of abortion in small ruminants, some of them have a major role in the context of their zoonotic potential or economic losses they cause. Thus, it is strongly recommended that they should be included in the diagnostic protocol, especially if there is a relationship with the available clinical and epizootiological data. In our laboratory,

Table 1. Number of tested animals and positive for antibodies and DNA of *C. abortus* and *C. burnetii* by years

Year	sheep				goat			
	PCR		serology		PCR		serology	
	C. abortus	*C. burnetii*	*C. abortus*	*C. burnetii*	*C. abortus*	*C. burnetii*	*C. abortus*	*C. burnetii*
2013	2/ 2	3/0	7/1	-	3/ 1	1/1	4/3	3/2
2014	12/ 2	1/0	9/1	-	6/ 3	2/1	1/0	-
2015	5/ 4	4/0	3/0	2/0	4/ 1	3/0	7/0	7/0
2016	6/ 0	3/0	5/2	4/0	4/ 0	1/0	4/2	4/0
2017	11/7,	2/0	3/3	2/0	4/1	3/1	-	9/1
2018	7/3,	4/0	3/3	2/2	3/0,	2/2	3/0	5/0
Total	43/18	17/0	30/10	10/2	24/6	12/5	19/5	28/3

Note: The number of tests performed for C. abortus, C. burnetii does not correspond to the total number of sample submissions since some of them were tested simultaneously for both infections and the samples from animals originated from one and the same farm were processed and reported as one.
The given results are only from serological testing for antibodies against C. burnetii of blood samples from aborted animals or animals from herds with increased abortion rates, sent for the purpose of diagnosis. The results from serological surveys within the frame of monitoring programs or official surveillance are not included.

all samples received from aborted sheep and goats were tested for presence of *C. abortus* and *C. burnetii* as the most probable *abortifaciens*.

The analysis of the results from the diagnostic investigations showed that *C. abortus* played a major role as an abortifacient agent in the small ruminant population in Bulgaria during the period 2013-2018, inducing 35.8% of the abortions. This is not surprising, taking into account the results from the available literature, indicating its major economic importance as a problem in reproductive pathology in a number of countries (Szeredi *et al*., 2006; Longbottom *et al*., 2013; Van Engelen *et al*., 2014). Previous serological and etiological investigations (isolation in CE, IF identification or EM observation) in Bulgaria have also noted the important role of *C. abortus* as a cause of abortion in small ruminants in this country (Martinov, 2009). This indicates a sustainability of the infection from epizootological point of view, regardless of the changes that have occurred in the sheep and goat farming practices in the last 20 years. Serological detection of antibodies in the sera obtained from the same animals that tested positive by PCR, confirmed the diagnosis and showed that it could be successfully used as a method for establishing the circulation of the infection at herd level, especially if no tissue samples were available.

Although *C. burnetii* infection is a well-known etiological factor in small ruminant abortions (EFSA Panel on Animal Health and Welfare, 2010; Agerholm, 2013), this agent was demonstrated by PCR in only five clinical cases during the whole study period, all of them in goats. Such a phenomenon was also noted by Van Engelen *et al*. (2014) in the Netherlands during the 2012-2013 lambing season, when no case of abortion caused by *Coxiella burnetii* was reported, in contrast to previous years (up to 2010) (Van den Brom *et al*., 2012), when these cases exceeded 10% and there were epidemic outbreaks of Q-fever in the country in 2007-2010, affecting more than 4000 people. In addition, of the 33 blood samples obtained from aborted animals or randomly selected from flocks with a high rate of abortions, antibodies were found in only 5 (15.2%). These results significantly differ from previous surveys of sheep and goats with abortions or similar pathology (premature birth or miscarriage), when seroprevalences of 22.6% in goats and 43.65% in the sheep were reported (Martinov, 2005; 2007). The reasons for these differences are not clear. Firstly, it is a well-known fact that some animals infected with *C. burnetii* do not seroconvert (Rodolakis *et al*., 2007; Serano-Perez *et al*., 2015). In this study, two goats which had aborted and tested positive for *C. burnetii* by PCR showed negative results when tested by ELISA two weeks after abortion. Secondly, the number of blood samples tested in this study is too small as compared to previous surveys, so the difference is not statistical-

ly trustworthy.

All five *C. burnetii* PCR-positive samples identified in our investigation were from goats. From the epidemiological point of view, this is important since infected small ruminants and especially goats are suspected as the main source of Q fever cases in humans (EFSA Panel on Animal Health and Welfare, 2010; Mori and Roest, 2018). This underlines the necessity to include *C. burnetii* in the diagnostic plan in all cases of abortion in order to quickly identify its presence and undertake appropriate therapeutic and prophylactic measures to prevent zoonotic risk.

References

Agerholm, J. (2013). *Coxiella burnetii* associated reproductive disorders in domestic animals-a critical review. *Acta Vet. Scand.* **55**: 13.

Berri, M., K. Laroucau, A. Rodolakis (2000). The detection of *Coxiella burnetii* from ovine genital swabs, milk and fecal samples by the use of a single touchdown polymerase chain reaction. *Vet. Microbiol*. **72**: 285-293.

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

Givens, D., M. Marley (2008). Infectious causes of embryonic and fetal mortality. *Theriogenology* **70:** 270-285.

Laroucau, K., A. Souriau, A. Rodolakis (2001). Improved sensitivity of PCR for *Chlamydophila* using pmp genes. *Vet. Microbiol*. **82**: 155-164.

Longbottom, D., G. Entrican, N. Wheelhouse, H. Brough, C. Milne (2013). Evaluation of the impact and control of enzootic abortion of ewes. *Vet. J.* **195**: 257-259.

Martinov, S. (2005). Serological investigations and isolation of *Coxiella burnetii* from sheep. *Vet. Med.* **IX**: 16-22.

Martinov, S. (2007). Studies on the Q-Rickettsial infection in goats. *Vet. Med.* **XI**: 18-25.

Martinov, S. (2009). Etiological and clinical studies on chlamydioses of ruminants. *Vet Med.* **XIII**: 26-34.

Masala, G., R. Porcu, C. Daga, S. Denti, G. Canu, C. Patta, S. Tola (2007). Detection of pathogens in ovine and caprine abortion samples from Sardinia, Italy, by PCR. *J. Vet. Diagn. Invest*. **19**: 96-98.

Moreno, B., E. Collantes-Fernández, A. Villac, A. Navarroc, J. Regidor-Cerrillo, L. M. Ortega-Mora (2012). Occurrence of *Neospora caninum* and *Toxoplasma gondii* infections in ovine and caprine abortions. *Vet. Parasitol*. **187**: 312-318.

Moreno, E. (2014). Retrospective and prospective perspectives on zoonotic brucellosis. *Front. Microbiol*. **5**: 213.

Mori, M., H. J. Roest (2018). Farming, Q fever and public health: agricultural practices and beyond. *Arch. Public Health* **76**: 2.

Rodolakis, A., M. Berri, C. Héchard, C. Caudron, A. Souriau, C. Bodier, B. Blanchard, P. Camuset, P. Devillechaise, J. Natorp, J. Vadet, N. Arricau-Bouvery (2007). Comparison of *Coxiella burnetii* shedding in milk of dairy bovine, caprine, and ovine herds. *J. Dairy Sci*. **90**: 5352-5360.

Serrano-Pérez, B., S. Almería, J. Tutusaus, I. Jado, P. Anda, E. Monleón, J. Badiola, I. Garcia-Ispierto, F. López-Gatius (2015). *Coxiella burnetii* total immunoglobulin G, phase I and phase II immunoglobulin G antibodies, and bacterial shedding in young dams in persistently infected dairy herds. *J. Vet. Diagn. Invest.* **27**: 167-176.

Stein A., D. Raoult (1992). Detection of Coxiella burnetii by DNA amplification using polymerase chain reaction. *J. Clin. Microbiol.* **30**: 2462-2466.

Szeredi L., S. Jánosi, M. Tenk, L. Tekes, M. Bozsó, Z. Deim, T. Molnár (2006). Epidemiological and pathological study on the causes of abortion in sheep and goats in Hungary (1998-2005). *Acta Vet. Hung.* **54**: 503-515.

Van den Brom R., K. Lievaart-Peterson, S. Luttikholt, K. Peperkamp, W. Wouda, P. Vellema (2012). Abortion in small ruminants in the Netherlands between 2006 and 2011. *Tijdschr Diergeneeskd*. **137**: 450-457.

Van Engelen, E., S. Luttikholt, K. Peperkamp, P. Vellema, R. Van den Brom (2014). Small ruminant abortions in the Netherlands during lambing season 2012–2013. *Vet. Rec*. **174**: 506.