

# A Serological Investigation Of Some Abortion Causes Among Small Ruminant Flocks In Greece

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## Abstract

Four hundred sixty three (463) serum samples collected from aborted ewes (289) and does (174) were tested for antibodies to five selected pathogens having public health importance. The investigation aimed in serologically ranking the importance of *Leptospira* spp infection, as compared to other causes of small ruminant abortion in Southern Greece. It involved 37 sheep flocks and 18 goat herds. All investigated farms were positive for antibodies to one or more of the selected microorganisms. Three hundred sixty eight (79.48%) of the examined serum samples were antibody positive. From them 219 ewe samples were positive to *Brucella* spp. (44 samples), *Chlamydia* spp. (43 samples), *Leptospira* spp. (72 samples), *Coxiella burnetii* (141 samples) and *Toxoplasma gondii* (144 samples). From the 149 positive doe samples 39, 37, 32, 110 and 52 were positive to the above causes respectively. None of the positive ewes and does was positive to only *Leptospira* spp., of which the highest antibody titers were observed for serovar Australis (ewes) and Copenhageni (does). Serovar Tarassovi had a significant presence in both animal species, but it showed lower titers.

## INTRODUCTION

Abortions of food producing animals are the cause of considerable economic losses for the farmer<sup>10</sup>. In addition, they may have a public health importance, if they are the result of microorganisms causing disease to man. Some microorganisms of public health importance causing also abortion are *Brucella*, *Listeria*, *Coxiella*, *Chlamydia* and *Toxoplasma*<sup>10</sup>. These infectious agents are easily spreading among animals and man in all farming systems, but they are of increased importance where communal grazing is common and farms are family holdings<sup>9</sup>. Communal grazing is common in Southern Europe, Middle East and African nations<sup>27</sup>, where *Brucella* spp. is a frequent cause of severe human illness, thus the first pathogen suspected when small ruminant abortions are investigated<sup>2</sup>. This approach to the investigation of abortions is often overlooking other causes, such as *Coxiella*, *Toxoplasma* or *Chlamydia*, vigorously investigated in nations where production is mostly intensive or they are free of brucellosis<sup>17</sup>.

However, regardless of production system, *Leptospira* spp. infection is rarely investigated as a possible cause of abortion among small ruminants, because the systematic study of animal leptospirosis is rather difficult. Leptospirosis, a disease of animals and man, is a "re-emerging" infection of economic and public health

importance. Its prevalence varies according to animal species and area where the disease is investigated. The reported prevalences of infection among the various animal species across the world are between 2 and 46%, the variation depending not only on the animal species, but also the method of testing, the geographic area and time of the year testing was performed<sup>3,4,11,25,26</sup>. Testing is difficult because the Microscopic Agglutination Test (MAT)<sup>6,13,29</sup>, which is the internationally recognized method for investigating leptospirosis, is neither easy to perform nor cheap. The MAT uses as antigen live leptospira serovars, thus requires a well equipped laboratory and trained personnel to maintain the large number of serovars needed. These difficulties affect the systematic and in depth investigation of the impact of the infection on animal production, including this of small ruminants<sup>8,24,26</sup>. However, to safely implicate *Leptospira* spp in abortion other infectious causes should be excluded. The present investigation attempted to serologically document the proportional importance of the most frequently investigated small ruminant abortion causes in Southern Greece, in relation to leptospirosis, which is systematically excluded. The aim was to serologically rank and further study the effects of *Leptospira* spp. infection on small ruminant production.

**MATERIAL AND METHODS**

**SAMPLES SELECTED**

The investigation was performed during 2005-2006 in collaboration with the Veterinary Diagnostic Laboratory of Tripolis, Ministry of Agriculture, Southern Greece. The laboratory was receiving samples from a variety of cases among which were also serum samples from small ruminant abortion. Within a period of two years, 463 serum samples, 289 from ewes and 174 from does, were collected originating from 37 sheep flocks (totaling 2751 ewes), and 18 goat herds (totaling 3605 does).

**LABORATORY EXAMINATION**

Serum samples were tested for *Brucella* spp., *Chlamydomphila* spp., *Leptospira* spp., *Coxiella burnetii* and *Toxoplasma gondii*.

The cELISA kit COMPELISA 400 (Veterinary Laboratories Agency (VLA), New Haw, Addlestone, Surry, UK) was used to test for brucella antibodies in serum. The kit is detecting antibodies to smooth *Brucella* spp. A sample with optical density equal to or higher than 60% of the mean optical density of the control was considered positive.

The CHEKIT Chlamydia ELISA Test Kit (Idexx Laboratories, USA) was used for the detection of antibodies against *Chlamydomphila abortus* (formerly known as *Chlamydia psittaci*). A sample having an OD equal or over 40% to that of the control was considered positive.

The CHEKIT Q-Fever ELISA Test Kit (Idexx Laboratories, USA) was used for the detection of antibodies against *C. burnetii* in serum samples of ruminants. A sample having an OD equal or over 80% to that of the control was considered positive.

Antibodies to *T. gondii* were detected with immunofluorescence (IFAT) using the Toxo-IF slides (DIACHEL, USA). Samples with titers of 1/160 and above were considered positive.

The MAT was used to test for leptospirosis. The MAT was performed according to the Standard Operating Procedures of the Veterinary Laboratories Agency (VLA), UK, which uses as antigen 19 live serovars of *Leptospira* spp. belonging to six serogroups. A positive sample showed 50% or more of antigen agglutination in a titer of 1/100.

**RESULTS**

From the ewe samples 219 (75.8%) were positive to one or

more of the investigated causes. From the doe samples 149 (85.6%) were positive to one or more causes.

The proportion of positive samples from each ewe flock ranged from 33.4% to 75%, while the same for doe herds was from 48.32% to 85.7%.

The numbers of positive samples to each of the investigated causes are given in Table 1.

**Figure 1**

Animal species	<i>T. gondii</i>	<i>C. brunetii</i>	<i>Brucella</i> spp.	<i>Chlamydomphila</i> spp.	<i>Leptospira</i> spp.
Sheep N°289	144 (49.8%)	141 (48.8%)	44 (15.2%)	43 (14.9%)	72 (24.9%)
Goats N°174	52 (29.9%)	110 (63.2%)	39 (22.4%)	37 (21.2%)	32 (18.4%)
Total	196	251	83	80	104

The highest number of positive ewe samples was positive to *T. gondii* and for doe samples to *C. brunetii*. *Leptospira* spp was third among ewes and fifth among does. *Leptospira* spp serovars most frequently identified were Tarassovi, Australis and Bratislava for sheep, and Australis, Tarassovi and Copenhageni for goat serum samples. However serovar Tarassovi showed low titers (1/100-1/440) compared to the other serovars (1/100-1/3200).

A higher number of ewe samples 53 (36.6%) had high antibody titers to *T. gondii*, while a higher number 21(56.7%) of doe samples had high titers to chlamydomphila infection compared to the other causes of abortion. All brucella positive samples had low titers (very close to 60% of OD). They originated from four sheep and six goat farms. All farms were serologically positive to two or more of the investigated causes, while 11 sheep and 10 goat farms had between one and five animals positive to four of the investigated abortion causes. Positive samples to *C. brunetii* were found in all sheep and goat farms. This microorganism was the one identified as the sole infectious agent in 19 ewes and 27 does.

Eight sheep flocks and 13 goat herds were positive to leptospirosis, but only four sheep and two goat farms had samples with rather high antibody titers (1/800 and above). In respect to high titers, the predominant serovars were Australis for ewes and Copenhageni for does.

Table 1: Positive serum samples to five abortion infectious

agents

### DISCUSSION

Common grazing in Greece contributes to the spreading, between farms, of pathogens such as *Brucella* spp<sup>19</sup>. Thus, control measures are necessary for limiting brucellosis infection. The Rev-1 vaccine administered by instillation in the conjunctiva of young and adult animals is used since 1998 for the control of brucellosis<sup>18,27</sup>. Thus, low antibody titers to *Brucella* spp could also result from vaccinating adults with Rev-1<sup>19</sup>. The ELISA method is influenced by vaccination with Rev-1<sup>5,27</sup> when testing of animals is within a few months from administration. Unfortunately, due to the control measures when a farm is brucellosis positive, farmers do not volunteer information on farm procedures, animal history or origin of individual animals. *Chlamydomphila* spp. and *C. burnetii* have been concurrently involved in abortions of small ruminants<sup>1</sup>. Both microorganisms are not only of economic importance, but also of public health. They also show similarities in their pathogenesis<sup>16</sup>, thus, they should be considered together when the causes of abortion are investigated. The investigation of both microorganisms in cases of abortion could lead to a better understanding of the role of the two in the epidemiology of abortion, thus contribute toward a more accurate estimation of the role other pathogens may have. In the present investigation the highest proportion of serum samples considered positive to *C. burnetii* were from goats. Perhaps, this species is of a greater risk to public health than sheep, as also is suggested by others<sup>12</sup>. Some have reported a significant association between high numbers of strongly positive samples to *C. burnetii* and abortions<sup>23</sup>. This was not appearing to be the case in the present investigation as only 36(14.3%) were strong positive.

The overall proportion of seropositive animals to *T. gondii* observed here was lower than that reported from other parts of the world using the same testing method<sup>7,22</sup>. Toxoplasmosis causes high rates of abortion among small ruminants. Thus, to contribute the abortions to *T. gondii* or any of the investigated infectious agents, paired serum samples should have been tested and this was not possible. The lower proportion of seropositive animals observed here compared to those reported by others<sup>7,22</sup>, could have resulted from the differences in the production system. Samples examined were from a semi-extensive system for sheep and extensive for goats. In these kinds of production systems, animals have fewer chances to come in contact with young cats<sup>21</sup>, the source of the parasite.

The information in the above discussed causes of abortion was considered necessary for better evaluating the role of *Leptospira* spp in the epidemiology of abortion, thus further study its impact on the production of small ruminants on farm level. The proportions of positive sheep and goat samples to leptospirosis were similar to those found in an earlier investigation of abortion among small ruminants in Greece<sup>3</sup> and to those among apparently healthy animals<sup>4</sup>. However, a difference in the prevalence of the reported serovars was recorded compared to these previous investigations. The variation was, perhaps, resulting from limiting the investigation to a smaller geographic area. Of interest was the predominance of serovar Tarassovi among sheep samples (second in goats), but not showing high antibody titers. High leptospira antibody titers in small ruminants have been associated to the presence of *Leptospira* spp in vaginal fluids and semen<sup>14</sup>. Perhaps, there is in such cases a higher risk for venereal transmission of *Leptospira* and abortion, although these species are considered more resistant to leptospirosis than others. Higher titers could, thus help in increasing the probability to isolate important *Leptospira* serovars and successfully study the pathogenesis of naturally occurring leptospirosis. The microorganism is difficult to isolate, thus the selection of method, animals and tissue is critical for accurate identification of leptospira as cause of abortion<sup>15,26</sup>. Therefore, a combination of methods, such as serologic examination of target flocks, isolation and PCR, are necessary to reliably and economically investigate leptospirosis under field conditions<sup>14,15</sup>. The reliable identification of serovar carriers could help in deciding upon the importance of leptospirosis in the pathogenesis of abortion, thus effective control programs. By determining, which serovars are found in vaginal fluids or colonize kidney tissue or cause clinical disease mainly expressed as abortion, the best commercial or locally produced vaccine for leptospirosis could be selected for maximum protection. PCR has been found as one such method significantly related to observed high antibody titers, as estimated by the MAT<sup>15</sup>.

### CONCLUSION

The main conclusion of the present investigation is that when other pathogens are also serologically identified as possible causes of abortion, the role of *Leptospira* spp in the pathogenesis of naturally occurring abortions could not be easily defined. None of the animals or flocks/herds investigated were positive to only *Leptospira* spp.. Fortunately, none of the flocks/herds positive to leptospirosis had animals positive to brucellosis, but the

same was not true with the serologic evidence from the other microorganisms. Thus, abortions among small ruminants in Greece should be attributed to mixed infections, explaining partially their high prevalence. Furthermore, although *Leptospira* spp infection does not appear as one of the most important causes of naturally occurring abortion, its pathogenesis should be further investigated.

## References

- Berri M, Rekiki A, Boumedine K, Rodolakis A: 2009, Simultaneous differential detection of *Chlamydochloa abortus*, *Chlamydochloa pecorum* and *Coxiella burnetii* from aborted ruminants clinical samples using multiplex PCR. *BMC Microbiol* 9:1-8.
- Blancou J and Lefèvre, PC: 2006, Diseases that Threaten Livestock. In *Encyclopedia of Infectious Diseases: Modern Methodologies*, editor M. Tibayrenc, publ John Wiley & Sons Inc., Hoboken, NJ, USA.
- Burriel AR, Magana-Vougiouka O, Butsini S, Nomikou K et al: 2002, A serologic investigation of some causes of reproductive failure among small ruminants in Greece. *OJVR* 1:57-63
- Burriel AR., Dalley C, Woodward MJ: 2003, Prevalence of *Leptospira* species among farmed and domestic animals in Greece. *Vet Rec* 153:146-148
- European Commission: 2001, Health and Consumer Protection Directorate-General. *Brucellosis in Sheep and goats (Brucella melitensis)*, SANCO.C.2/AH/R23?2001, 20-5-2010
- Faine S, Adler B, Bolin C, Perolat P: 2000, *Leptospira* and *Leptospirosis*. MediSci Press, Melbourne, Australia.
- Filho MFS, Erzinger E, da Cunha IAL, Bungi FM, et al.: 2008, *Toxoplasma gondii*: abortion outbreak in a goatherd from Southern Brazil. *Ciências Agrárias Londrina* 29: 887-894
- Guitian FJ, Garcia-Pena FJ, Oliveira J, Sanjuan ML et al.: 2001, Serological study of the frequency of leptospiral infections among dairy cows in farms with suboptimal reproductive efficiency in Galicia, Spain. *Vet Microbiol* 80:275-284
- Gwaze Rumsa F., Chimonyo M. and Dzama K 2009. Communal goat production in Southern Africa: a review. *Trop Anim Health Prod* 41: 1157-1168
- Jonker HF: 2004, Fetal death: comparative aspects in large domestic animals. *Anim Rep Sci* 82-83:415-430
- Kawaguchi L, Sengkeopraseuth B, Tsuyuoka R, Koizumi N, et al.: 2008, Seroprevalence of leptospirosis and risk factor analysis in food-prone rural areas in Lao PDR. *An J Med Hyg* 78:957-961
- Khalili M, Sakhaee E: 2009, An update on a serologic survey of Q fever in domestic animals in Iran. *Am J Trop Med Hyg* 80:1031-1032
- Levett PN: 2004 *Leptospirosis: A forgotten zoonosis?* *Clin Appl Immunol Rev* 4:435-448
- Lilenbaum W, Varges R, Brandao FZ, Cortez A, et al.: 2008, Detection of *Leptospira* spp in semen and vaginal fluids of goats and sheep by polymerase chain reaction. *Theriogenol* 69:837-842
- Lilenbaum W, Varges R, Ristow P, Cortez A, et al.: 2009, Identification of *Leptospira* spp carriers among seroreactive goats and sheep by polymerase chain reaction. *Res Vet Sci* 87:16-19
- Lukacova M: 1996, Are *Coxiella burnetii* and *Chlamydia* related? Antigenic properties of *Coxiella burnetii* and *Chlamydiae*. *Alpe Adria Microbiol J* 5:3-13
- Menzies PI: 2006, The Ontario Health Program: A structured health management program for intensively reared flocks. *Small Rum Res*, 62: 95-99
- Minas A, Minas M, Stournara A, Tselepidis S: 2004, The "effects" of Rev-1 vaccination of sheep and goats on human brucellosis in Greece. *Pev Vet Med* 64:41-47.
- Minas A: 2006, Control and eradication of brucellosis in small ruminants. *Small Rum Res* 62:101-107
- Natarajaseenivasan K, Prabhu N, Selvanayagi K, Savalaikarankulam S, et al.: 2004, Human leptospirosis in Erode, South India: Serology, isolation, and characterization of the isolates by randomly amplified polymorphic DNA (RAPD) fingerprinting *Jpn J Infect Dis* 57:193-197
- Pinheiro Jr JW, Mota RA, da Fonseca Oliveira AA, Faria EB. Et al.: 2009. Prevalence and risk factors associated to infection by *Toxoplasma gondii* in ovine in the State of Alagoas. *Brazil Parasitol Res* 105:709-715
- Reis CR, Lopes FMR, Freire RL, Goncalves DD. Et al.: 2007, Occurrence of anti-*Toxoplasma gondii* antibodies in caprines from Pitanga city, Parana State Brazil. *Brazil. J Vet Res An Sci* 44:358-363.
- Rouset E, Durand B, Berri M, Dufour P. et al.: 2007, Comparative diagnostic potential of three serological tests for abortive Q fever in goat herds. *Vet Microbiol* 124:286-297.
- Saglam YS, Yener Z, Tenur A, Yalcin E: 2008, Immunohistochemical detection of leptospiral antigens in cases of naturally occurring abortions in sheep. *Small Rum Res* 74:119-122
- Sambasiva RR, Naveen C, Bhalla P, Agarwal SK: 2003, *Leptospirosis in India and the rest of the world*. *Braz J Inf Dis* 7:178-193
- Szeredi L, Haake DA: 2006, Immunohistochemical identification and pathologic findings in natural cases of equine abortion caused by leptospira infection. *Vet Pathol* 43:755-761
- Spickler RA and Roth AJ: 2008, *Brucellosis*. In: *Emerging and Exotic diseases of animals*. 3rd edition, Iowa State University, Ames Iowa, USA, pp 141-143
- Stournara A, Minas A, Chatzopoulou-Bourtzi E, Stack J, et al.: 2007, Assessment of serological response of young and adult sheep to conjunctival vaccination with Rev-1 vaccine by fluorescence polarization assay (EPA) and other serological tests for *B. melitensis*. *Vet Microbiol* 119:53-64.
- World Health Organization: 2006, *Guidelines for the prevention and control of leptospirosis*. Zoonosis Division, National Institute of Communicable Diseases, 22-Sham Nath Marg, Dehli-110 054

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