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), Chlamydophila 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 [10]. 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 [10]. 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 [9]. Communal grazing is common in Southern Europe, Middle East and African nations [27], where Brucella spp. is a frequent cause of severe human illness, thus the first pathogen suspected when small ruminant abortions are investigated [2]. 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 [17].

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[3,4,11,25,26]. Testing is difficult because the Microscopic Agglutination Test (MAT) [6,13,29], which is the internationally recognized method for investigating leptosirosis, 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[8,24,26]. 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., Chlamydophila 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 Chlamydophila 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 immunofluerescence (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	*T. gondii*	C. *brunetii*	Brucella spp.	Chlamydophila spp.	Leptospira 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 chlamydophila 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[19]. 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[18,27]. Thus, low antibody titers to Brucella spp could also result from vaccinating adults with Rev-1[19]. The ELISA method is influenced by vaccination with Rev-1[5,27] 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. Chlamydophila spp. and C. burnetii have been concurrently involved in abortions of small ruminants[1]. Both microorganisms are not only of economic importance, but also of public health. They also show similarities in their pathogenesis[16], 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 others12. Some have reported a significant association between high numbers of strongly positive samples to C. burnetii and abortions[23]. 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[7,22]. 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[7,22], 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[21], 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[3] and to those among apparently healthy animals[4]. 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[14]. 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[15,26]. 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[14,15]. 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[15].

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 appears as one of the most important causes of naturally occurring abortion, its pathogenesis should be further investigated.

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