

Leptospirosis in sheep and goats under tropical conditions

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Abstract Inadequate management practices and poor reproductive performance have been reported as fundamental factors on reducing the levels of productivity in livestock. Different pathogens have been reported in small ruminants' herds/flocks with reproductive failures. The aim of the present study was to review aspects of leptospirosis in small ruminants, mainly its impact on reproduction and consequently on productivity of the herds/flocks under tropical conditions. Leptospiral infection in goats and sheep is common in several countries, and those species can also act as carriers of leptospires. Severe disease is often associated to young animals and is frequently associated to incidental serovars. In contrast, subclinical infection is mainly characterized by reproductive problems, such as infertility, abortion, occurrence of stillbirths, and weak lambs/goat kids. Moreover, laboratorial tests are essential to achieve an accurate diagnosis of the infection. Microscopic agglutination test is the most common indirect test of leptospirosis, being used worldwide. In small ruminants, PCR consists on a recommendable method for diagnosing animals that carry leptospires. Control of leptospirosis in small ruminants involves measures such as the identification and treatment of the carriers and other sources of infection, quarantine in acquired animals, and systematic immunization with commercial vaccines containing the circulating serovars in the herd/flock. Productivity of small ruminant breeding can dramatically increase with adequate sanitary conditions and control of leptospirosis. Immunization of all the animals combined to the treatment of carriers may successfully control the infection and importantly reduce the economic reproductive hazards that are observed under tropical conditions.

Keywords Leptospirosis · Small ruminants · Tropical scenario · Reproduction

Generality

Small ruminants (sheep and goats) are ubiquitous and important for subsistence and economic (income generation) and social sustenance (poverty alleviation) of the human population, mainly in developing countries (Pugh 2001). According to the Food and Agriculture Organization (FAO), in 2011, there were 875.5 million goats and one billion sheep in the world. Nevertheless, approximately 80 % of all small ruminants were located in developing countries, many of them located in tropical regions, representing more than 70 % of the production of these species (FAOSTAT 2011). These species are adaptable to a wide range of climatic and geographical conditions, even the roughest ones, presenting the largest distribution in livestock scenario worldwide. Moreover, sheep and goats present high prolificacy, small size, and are able to utilize a large variety of foods, including weeds (Kosgey et al. 2006).

Developing countries, due to the social, soil, and climate characteristics, present a suitable scenario for expansion and increase of goats and sheep breeding. In the last decades, despite the high number of small ruminant population in developing countries, its productivity remains lower than that in developed ones (Pollott and Wilson 2009). Despite a rising on the total production of small ruminants' products has been observed in the last few years, it was based on the increase of the total number of heads, and not on real productivity gains, exposing a severe situation regarding the low productivity of sheep and goats, mainly in tropical regions (FAOSTAT 2011).

Among other causes, as nutritional deficiencies and inadequate management practices, poor reproductive performance has been reported as a fundamental factor on reducing the

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levels of productivity in livestock. It may be characterized by disturbances in the reproductive physiology of male and female, with symptoms such as anestrus, repeating estrus, early embryonic death, abortion, retained placenta, and delayed sexual maturity (Ellis 1994). Infectious diseases, particularly those of the reproductive sphere, play a dramatic role in this scenario, determining important economic hazards in livestock (Grooms and Bolin 2005; Grooms 2006; Kosgey et al. 2006; Subharat et al. 2011).

Different pathogens have been reported in herds/flocks with reproductive failures (Grooms 2006; Lilenbaum et al. 2007a; Giangaspero et al. 2013). From those, lentiviruses (caprine arthritis encephalitis and maedi–visna), toxoplasmosis, neosporosis, brucellosis (*Brucella abortus*, *Brucella melitensis*, and *Brucella ovis*) have been often reported in tropical countries, with different prevalence according to the studied region (Lilenbaum et al. 2007a). Leptospirosis is an outstanding neglected disease, and since it is usually silent, its effects on livestock are often underestimated. As an example that may be considered for other tropical areas of the world, it was recently described as the most frequent and potentially the major infection impairing productivity in small ruminants in Rio de Janeiro, Brazil (Martins et al. 2012a).

The aim of the present study was to review aspects of leptospirosis in small ruminants, mainly its impact on reproduction and consequently on productivity of the herds/flocks under tropical conditions.

Leptospirosis

Introduction

Leptospirosis is a worldwide zoonosis determined by pathogenic spirochetes that belong to the genus *Leptospira*. Leptospire colonize the proximal renal tubules of various mammals and are excreted in the urine of carrier animals. Transmission of leptospirosis occurs mainly by exposure to water or soil contaminated by the urine of infected animals or by direct contact with infected animals (Adler and de la Peña Moctezuma 2010). The genus *Leptospira* consists of both pathogenic and nonpathogenic species, defined according to DNA relatedness. Approximately 250 serovars were recognized among the pathogenic *Leptospira* spp. Antigenetically related serovars are grouped into serogroups, 26 of which have been described for pathogenic strains (Adler et al. 2011).

The infection has been classically divided into two major groups. The first is determined by strains adapted to and carried by the affected host, which are less dependent of the region or environmental conditions, as topography or rainfall (e.g., serovar Hardjo in ruminants or pomona in pigs) and usually leads to subclinical infection, becoming an important source of infection for humans or other animals (Suepaul et al.

2011). Another group consists of incidental infections caused by strains carried by other domestic and free-living animals and are more dependent of environmental factors and management practices, which facilitate the direct or indirect contact of the animal with the urine of the reservoirs of the bacterium (Adler and de la Peña Moctezuma 2010). This last group constantly leads to an acute and severe leptospirosis syndrome and frequently presents as outbreaks, e.g., serovar Icterohaemorrhagiae (Martins et al. 2012b; Giangaspero et al. 2013). Though distinct variations in maintenance hosts and the serovars they carry can occur throughout the world, a basic knowledge on serovars and their maintenance hosts is required to understand the epidemiology of leptospirosis, either animal or human, in a determined region (Adler and de la Peña Moctezuma 2010; Suepaul et al. 2011; Giangaspero et al. 2013).

Epidemiology

There is a lack of studies regarding leptospirosis in small ruminants, and many of them represent simple reports on outbreaks or surveys with a poor study design and inadequate sampling. Those papers represent only a limited contribution to the understanding on the disease in those animals. Additionally, a significant part of what we nowadays recognize as correct on that disease has been studied in cattle and considered by analogy as adequate for small ruminants, which may or not be correct.

Serological studies have demonstrated that leptospiral infection in goats and sheep is common in several countries (Table 1). For many years, small ruminants had been considered as accidental hosts of leptospire, being affected only for incidental serovars, carried by other domestic and wild species (Leon-Vizcaino et al. 1987). Nevertheless, several studies have shown that leptospiral infection in goats and sheep are common and that those species can also act as carriers of leptospire and eliminate the agent on the environment for long periods (Cousins et al. 1989; Ellis 1994; Gerritsen et al. 1994; Silva et al. 2007; Lilenbaum et al. 2008a, 2009). It is noteworthy that the carrier status is important to the zoonotic potential, representing a major concern, particularly for people who present direct contact with those animals and their secretions and excretions, such as practitioners, farmers, and slaughterhouse workers (Dorjee et al. 2008).

Leptospirosis in small ruminants has often been associated to strains belonging to the serogroup Sejroe, particularly serovar Hardjo (Table 1). It is noteworthy that it is considered as the main agent of leptospirosis in cattle worldwide (Martins et al. 2010; Desvars et al. 2012), and its transmission occurs regardless of seasonal and environmental factors (Ellis 1994; Martins et al. 2010). After experimental infections with serovar Hardjo in sheep (Andreani et al. 1983; Farina et al. 1996) and goats (Tripathy et al. 1985), these species were also recognized

Table 1 Occurrence of anti-*Leptospira* agglutinins and predominant serovars detected by MAT in sheep and goats (numbers of animal surveys and percent positive) in various surveys worldwide

Country	Species	Survey A (sheep)	Survey B (goats)	Predominant serovar	Reference
Americas					
Grenada	Sheep/goats	51/146 (34.9)	11/44 (25.0)	At	Everard et al. 1985
Guyana	Sheep/goats	24/427 (5.6)	37/417 (8.9)	H, I, Py	Motie and Myers 1986
Jamaica	Sheep/goats	187/327 (57.2)	1,253/2,018 (62.1)	Pv, Ca, Ju, I, Pm, H	Grant et al. 1988
Lesser Antilles	Sheep/goats	8/442 (1.8)	42/579 (7.2)	Sj, At, I, G	Levett et al. 1996
Brazil	Goats	–	111/1,000 (11.1)	H	Lilenbaum et al. 2007a
Brazil	Sheep	5/157 (3.1)	–	H	Seixas et al. 2011
Trinidad and Tobago	Sheep/goats	11/222 (4.9)	6/180 (3.3)	At, I	Suepaul et al. 2011
Brazil	Goats	–	72/230 (31.3)	At, T, Py, I	dos Santos et al. 2012
Brazil	Sheep/goats	146/308 (47.4)	89/343 (25.9)	H, I, G, Pm	Martins et al. 2012a
Brazil	Goats	–	98/975 (10.1)	At	Higino et al. 2013
Asia					
India	Goats	–	79/805 (9.8)	Sj, G	Sawhney 1968
Thailand	Sheep/goats	52/1,110 (4.7)	41/516 (7.9)	M, Sh, R	Suwancharoen et al. 2013
Iraq	Sheep/goats	42/171 (24.6)	30/134 (22.4)	H, M	Al-Badrawi et al. 2010
Africa					
Congo and Ruanda	Sheep/goats	12/353 (3.4)	3/72 (4.1)	I, G, Bu	Van Riel and Van Riel 1956
Morocco	Sheep/goats	172/616 (27.9)	106/388 (27.3)	I, Ca, Ba, Py	Mailloux 1969
Egypt	Sheep/goats	14/330 (4.2)	83/195 (42.1)	At, Ja	Maronpot and Barsoum 1972
Sudan	Sheep/goats	4/200 (2.0)	37/120 (30.8)	G, Py	Shigidi 1974
Ethiopia	Sheep/goats	69/159 (43.4)	44/93 (47.3)	Bu, As, At	Moch et al. 1975
Tunisia	Sheep	46/182 (25.6)	–	Co	Khbou et al. 2010
Reunion Island	Goats	–	36/60 (60.0)	Pa	Desvars et al. 2012
Oceania					
Australia	Sheep	64/256 (25.0)	–	H	Gordon 1980
Australia	Sheep	907/2,160 (41.9)	–	H	Ellis et al. 1994

As Australis, *At* Autumnalis, *Ba* Ballum, *Bu* Butembo, *Ca* Canicola, *Co* Copenhageni, *G* Grippotyphosa, *H* Hardjo, *I* icterohaemorrhagiae, *Ja* Javanica, *Ju* Jules, *M* Mini, *Pa* Panama, *Pm* Pomona, *Pv* Portland-verre, *Py* Pyrogenes, *R* Ranarum, *Sj* Sejroe, *Sh* Shermani, *T* Tarassovi

as potential reservoirs for the serovar Hardjo. Additionally, a persistent leptospirosis caused by serovar Hardjo was found in sheep with no contact with cattle infected by the same serovar (Cousins et al. 1989), which reinforces the profile of adaptability of this strain to other ruminants than cattle.

Serological surveys, although useful for epidemiological studies, are not able to discriminate in a reliable level the infecting serovar (OIE 2008), which would require the bacterial culturing, which is difficult and fastidious (Limmathurotsakul et al. 2012). Thus, although Hardjo appears to be ubiquitous, there is a lack of reports regarding the bacterial culturing of this strain in small ruminants (Gordon 1980; Ellis et al. 1983; Kingscote 1985; Leon-Vizcaino et al. 1987; Cousins et al. 1989; Gerritsen et al. 1994; Cerri et al. 1996; Dorjee et al. 2008). Additionally, few reports on isolation of leptospires occurred in these species worldwide, but putative results demonstrate a great diversity of circulating serovars of *Leptospira* in sheep and goats (Table 2).

Many risk factors may influence the occurrence of leptospiral infection in animals. Surprisingly, although relatively common in cattle (Ellis 1994; Adler and de la Peña Moctezuma 2010; Otaka et al. 2012), a comprehensive study with adequate statistical analysis on those factors regarding the infection in sheep has never been conducted. In a recent study conducted in Brazil (dos Santos et al. 2012), adult goats had three times (relative risk [RR]) more risk of acquiring leptospiral infection than younger animals. These risks could be explained by the level of opportunities for contact with older animals as sources of infection. Additionally, in the same study, the researchers identified that animals with defined races (RR 1.59) presented more chances to present leptospiral infection than others. At the farm level, the intensive breeding system (RR 1.59) and association of other animals (RR 1.60) were all found to be related with the frequency of leptospirosis in goats. The results demonstrated that inadequate management might also favor the occurrence of infection in goats.

Table 2 List of *Leptospira* strains isolated from sheep and goats worldwide

Country	Animal species	Serogroups	Reference
Israel	Goats	Grippotyphosa	Van der Høden 1953
Argentina	Sheep	Ballum	Cacchione et al. 1963
Australia	Sheep	Sejroe	Gordon 1980
Australia	Sheep	Sejroe	Cousins et al. 1989
India	Sheep	Javanica	Natarajaseenivasan and Ratnam 1999
Brazil	Sheep	Unknown	Azevedo et al. 2004
Brazil	Sheep	Autumnalis	Silva et al. 2007
Brazil	Goats	Grippotyphosa	Lilenbaum et al. 2007b

It is noteworthy that human resources dedicated to the animals also influence the occurrence of leptospirosis. Veterinary supervision has been described as a protection factor against leptospirosis, where herds under frequent veterinary supervision or visited at least every 2 weeks showed a less rate of seroreactive animals than herds visited infrequently by a veterinarian and those without any veterinary assistance (RR 2.4) (Lilenbaum et al. 2008b). Additionally, the presence of family labors was demonstrated as a protection factor against leptospirosis (RR 1.5) (dos Santos et al. 2012).

Furthermore, environmental conditions are reported as fundamental to the maintenance of the leptospirosis, mainly incidental strains in tropical conditions (Martins et al. 2010; Desvars et al. 2012). Goats located under tropical climates have almost three times more chances to be seroreactive than those from temperate climates (Lilenbaum et al. 2008b). Tropics are recognized as ideal scenarios for the maintenance and spreading of leptospires, since the presence of a highest rainfall and a wide gamma of wild and domestic reservoirs contribute for the transmission of the agent. It is remarkable that, in this particular ecological scenario, the presence of rodents on goat herds has been shown to triplicate the chances of the occurrence of seroreactive animals (Higino et al. 2013).

Leptospirosis and reproduction

Leptospirosis in ruminants may present as an acute infection or, more often, subclinically. Acute infection is represented by loss of appetite, irritability, diarrhea, and opaque furs, epidemic abortions, and milk drop syndrome (Ellis 1994; Martins et al. 2012b). Severe disease is often associated to lambs and goat kids and is frequently associated to incidental serovars, mainly pomona, ballum, icterohaemorrhagiae, or grippotyphosa (Leon-Vizcaino et al. 1987; Vermunt et al. 1994). In contrast, subclinical infection is mainly characterized by reproductive problems, such as infertility, increase in the number of services per conception and prolonged calving intervals, abortion, and occurrence of stillbirths and weak lambs/goat kids. Persistent infection of the reproductive tract may be the most important manifestation of leptospirosis in ruminants, mainly when

serovar Hardjo is involved. Curiously, differences have been found in tissue tropisms exhibited by different strains of Hardjo. While some strains of Hardjovovis have been shown to persist primarily in the kidney, others have a predilection for the genital tract, and still others persist in both organ systems (Ellis 1994).

Small ruminants get infected through direct or indirect contact with urine or contaminated tissues. Following the period of leptospiremia (7–10 days after infection), the leptospires localize and persist in a number of organs, especially the proximal renal tubules (all ages) and, as stated in cows (Grooms and Bolin 2005), on the genital tracts of sexually mature small ruminants. The pathogenesis of these reproductive failures is not clearly understood; presumably the presence of *Leptospira* in the uterus and genital tract of infected ruminants interferes with the implantation of the embryo or other early pregnancy events (Ellis 1994; Grooms 2006).

The precise location of the persistent infection in the female genital tract is not known, but leptospiral DNA on this system has been detected in small ruminants by molecular tolls (Lilenbaum et al. 2008a), providing further indications of the association between leptospires in the female genital tract and impaired reproductive efficiency of small ruminants. It is noteworthy that venereal transmission of leptospirosis in these species has also been suggested (Ellis 1994; Cerri et al. 1996; Farina et al. 1996; Lilenbaum et al. 2008a).

Diagnosis

There is a lack of apparent clinical symptoms and characteristic lesions in leptospiral infection in small ruminants. Moreover, laboratorial tests are essential to achieve an accurate diagnosis of the infection (Limmathurotsakul et al. 2012). Laboratorial diagnosis of leptospirosis may be indirect, such as serology, or direct, such as dark-field microscopy, immunofluorescence, bacterial culturing, or DNA detection in tissues or fluids (Grooms and Bolin 2005).

Microscopic agglutination test (MAT) is the most common indirect test of leptospirosis, being used worldwide. Furthermore, this is the test recommended by the World Organization

for Animal Health for several animal species (OIE 2008). The major advantage of MAT is its high specificity. This technique requires specific equipment and/or laboratory and highly trained staff, making it restricted to a few reference laboratories (Bourhy et al. 2013). Although the use of other serological tests, such as ELISA, has been reported, it is serovar-specific and consequently limited to regions where the occurrence of the serovars is well defined. This technique presents a better sensitivity and specificity than MAT and permits the distinction between acute and chronic infections by the detection of specific immunoglobulin (IgM and IgG) (Bourhy et al. 2013). Furthermore, the use of ELISA as a single diagnostic method, replacing MAT, is not recommended (Adler and de la Peña Moctezuma 2010).

Although dark-field microscopy is described in textbooks as a useful method of demonstrating leptospires in fluids, it has sometimes proved to be of doubtful value even in the hands of very experienced staff. Furthermore, serum protein and fibrin strands and other cell debris in the blood resemble leptospires, while the concentration of organisms in the urine of humans and animals is frequently too low to be detectable by this method (Limmathurotsakul et al. 2012). Direct immunofluorescence has been used for detection of leptospires in tissues as liver, lung, kidney, brain, or placenta from sheep and goats (Ellis et al. 1983; Leon-Vizcaino et al. 1987). Nevertheless, the test is laborious and not serovar-specific. Therefore, bacterial culturing remains as the gold standard for the diagnosis of leptospirosis. Nevertheless, it presents important limitations such as low sensitivity (mainly due to the high level of contamination of samples) and fastidious growth of *Leptospira*, which may require more than 16 weeks to grow; moreover, this is an expensive technique (Grooms and Bolin 2005; Limmathurotsakul et al. 2012), which explains the reduced number of reports on leptospiral isolates worldwide (Table 2).

More recently, molecular methods, such as PCR, are becoming increasingly important in laboratorial routine for the diagnosis of leptospirosis in many animal species (Pinna et al. 2011; Otaka et al. 2012). PCR presents high sensitivity and specificity and may detect up to ten leptospires per milliliter of urine. Nevertheless, this technique requires technological apparatus and trained staff to be performed (Limmathurotsakul et al. 2012). In small ruminants, PCR consists on a recommendable method for diagnosing animals that carry leptospirosis by detecting the agent in the urine and other specimens. In this sense, different diagnostic techniques can act jointly in epidemiological studies and significantly contribute for the control of leptospirosis (Lilenbaum et al. 2009; Martins et al. 2012b).

Control and prevention

Control of leptospirosis in small ruminants involves measures such as the identification and treatment of the carriers and other sources of infection, quarantine in acquired animals, and

systematic immunization with commercial vaccines containing the circulating serovars in the herd/flock (Subharat et al. 2011). The association of MAT as a screening test (detection of seroreactive herds) and urine analysis by PCR (individual approach) was considered suitable for detection of renal carriers of leptospires in cattle (Otaka et al. 2012). It is noteworthy that, in the case of infection determined by host-adapted strains, e.g., Hardjo, carriers are considered to be the source of spreading of the disease in herds, and their detection and treatment represent a key to the adequate control of the infection among the herd/flock (Lilenbaum et al. 2009). Newly acquired animals must be tested and, if necessary, treated with antibiotics before the introduction in the herd/flock (Pinna et al. 2007).

Vaccination plays an important role in the control of leptospirosis and may reduce significantly the occurrence of clinical symptoms (e.g., abortions) in the herd (Subharat et al. 2011). Nevertheless, commercially available vaccines (bacterins) cannot avoid the infection of the animals or the development of renal carries (Adler and de la Peña Moctezuma 2010). Thus, vaccinated animals can remain as a source of infection for other animals and the environment. Since adaptive immunity in leptospirosis is serotype-specific, the protection of vaccination is only against homologous serovars of the vaccine solution, with no cross-immunity (Subharat et al. 2011). Thus, the identification of the infective serovar in each herd/flock is crucial to the control of the disease.

Antibiotic therapy represents a decisive tool to eliminate the carrier status and may lead to an effective control of leptospirosis in the herd (Martins et al. 2012b). Streptomycin (25 mg/kg, IM, single dose) has been reported as the recommended antimicrobial drug to the elimination of the carrier status of the animal, since it presents easy dispersion in renal tissue and high efficiency against this microorganism (Adler and de la Peña Moctezuma 2010).

A recent study reported on the control of a leptospirosis outbreak in goats in Brazil. In this herd, a broad-based management approach was conducted, including serological and molecular diagnostic methods, vaccination, antibiotic treatment, and correction of some environmental aspects. That approach was critical to the control of this outbreak, thereby minimizing subsequent reproductive failures and economic losses (Martins et al. 2012b).

Conclusion

Knowledge about leptospirosis in small ruminants is still scarce and disproportional to the hazards that the disease may lead to producers worldwide. Broader studies with adequate sampling as well as risk factors analysis must be carried on in several countries, as well as additional experiments regarding to the real role of goats and sheep as reservoirs of leptospires, particularly Hardjo. Controlling leptospirosis in

small ruminants is still empiric, and practically nothing is known about the particularities of those species regarding to adequate control programs, especially under tropical conditions. What we know at the moment is that the productivity of small ruminant breeding can dramatically increase with adequate sanitary conditions and control of leptospirosis. In order to obtain that benefit, the available diagnosis with indirect (serology) and direct (culturing, PCR) tools must be associated. Adequate detection of the sources of infection (as free-living animals and other environmental aspects), as well as the presence of animal carriers among the herds/flocks, is mandatory. Immunization of all the animals combined to the treatment of carriers may successfully control the infection and importantly reduce the economic reproductive hazards that are observed under tropical conditions.

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