

ORIGINAL ARTICLE

Leptospirosis in Cattle: A Challenging Scenario for the Understanding of the Epidemiology

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Summary

All over the world, leptospirosis has been reported as one of the major causes of reproductive failure in cattle and other ruminants, determining abortions, still-birth, weak newborns and decrease in their growth rate and milk production. Nevertheless, despite its importance, it is still a challenging disease, from what scarce information about epidemiology, prophylaxis and control is available nowadays. During the last decades of the last century, many epidemiological studies have been conducted in several countries, mainly based on serology. According to those studies, a seroepidemiological scenario has been stated for different regions, where different serovars were reported for cattle. Nevertheless, a huge problem is that, when efforts are made in order to increase the collection of local strains (isolates), it has been demonstrated that the scenario that emerges from those studies contrasts with those previously determined by serology. Despite the large number of serological studies worldwide, the number of isolates is scarce. Isolation technique is a very delicate procedure that needs no contamination, fast processing and long delay to produce a positive result, what may corroborate to the lack of information for the comparison between serology versus bacteriological data, mainly in developing countries. It is noteworthy that the epidemiological scenario now acknowledged may not represent what really occurs in many parts of the world, particularly on those tropical regions where the disease is endemic. Consequently, the current knowledge about epidemiology and control, as well as the available diagnostic tools and the commercial vaccines, may not be adequate for those regions, what leads to a frustrating scenario of endemicity and difficulties on the control of the disease. Without a huge effort in the culturing of local strains, besides the advances on molecular typing, leptospirosis will not be defeated and will probably remain endemic in the developing countries, leading to important economic hazards in animal production and risks to public health in those regions.

Introduction

The world needs more protein of animal origin (FAO, 2013). According to FAO, a high global economic growth, together with continuing population gains, is expected to significantly (27%) increase the demand for high-quality food. It will happen mainly in China, India and Brazil, driving growth in their regions not only in the near term, but throughout the next 10 years (OECD-FAO, 2010).

Nevertheless, that need leads us to a dilemma: How to increase the production of food of animal origin without compromising the environment? Simply multiplying the number of heads (swine, poultry or cattle) cannot be the answer, as we are close to the sustainable limits and already occupying almost the entire cultivable surface of the Earth (FAO, 2013). Therefore, the response to that problem should be increasing productivity per animal, that is, producing more with the same number of heads in the same

area. It can be achieved by the development of new technologies or, in a simpler and faster way, improving the transference of the available knowledge to the farmers.

In the particular case of cattle, it has been estimated in Brazil that about 30% of the cows present reproductive failure every year (Baruselli et al., 2012); so, an increase of only 10% in the reproduction rate could represent 7 million more calves every year, without increasing the used area, environmental or human resources. Although the exact role of the infectious diseases in that scenario has not been clearly quantified, it is obvious that they represent an important cause of reproductive failure, mainly in developing countries (Tschopp et al., 2013). All over the world, leptospirosis has been reported as one of the major causes of reproductive failure in cattle and other ruminants, determining abortions, stillbirth, weak newborns and decrease in their growth rate and milk production (Grooms, 2006; Martins et al., 2012a). Nevertheless, despite its importance, it is still a challenging and frequently neglected disease, from what scarce information about epidemiology, prophylaxis and control is available nowadays.

Leptospirosis in Cattle

Leptospirosis is a worldwide zoonosis determined by pathogenic spirochaetes that belong to the genus *Leptospira*. Leptospirae colonize the proximal renal tubules of various mammals and are intermittently 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 non-pathogenic species, defined according to DNA relatedness. Approximately 250 serovars were recognized among the pathogenic *Leptospira* spp. Antigenically, related serovars are grouped into serogroups, 26 of which have been described for pathogenic strains (Murray et al., 2013).

The infection is classically divided into two major groups. The first is determined by strains adapted to and carried by an animal host (e.g. serovar Hardjo in cattle) and usually leads to subclinical infection, becoming an important source of infection for humans or other animals (Suepaul et al., 2011). Although reported to be less dependent of the region or environmental conditions, as topography or rainfall in countries with temperate climate, in the tropics it has been described that even for those infections the environment plays an important role facilitating the spreading of the infection (Martins et al., 2010). Another group consists of incidental infections caused by strains carried by other animals (domestic or wild) and are more dependent of environmental factors and management practices, what facilitates the contact of the animal with the

urine of the reservoirs of the bacterium (Ellis, 1994). This last group frequently presents as outbreaks, as has been recently described for serogroup Icterohaemorrhagiae in small ruminants (Martins et al., 2012b; Giangaspero et al., 2013). Although maintenance hosts and the serovars they carry vary throughout the world, a basic knowledge of serovars and their maintenance hosts is important to a better understanding of the epidemiology of leptospirosis in a determined region (Chappel and Smythe, 2012; Desvars et al., 2012; Martins et al., 2012a; Miraglia et al., 2012).

Leptospirosis in cattle is mainly characterized by reproductive problems, such as infertility, increasing in the number of services per conception and prolonged calving intervals, abortion, occurrence of stillbirths and weak offspring, leading to important, but not quantified, economic hazards. Persistent infection of the reproductive tract may be the most important manifestation of leptospirosis in ruminants, mainly when serovar Hardjo is involved (Ellis, 1994; Langoni et al., 1999; Grooms, 2006; Pereira et al., 2013).

As acute cases of leptospirosis are very rare, control of leptospirosis in cattle evolves the identification and treatment of the urinary carriers (apparently healthy), quarantine for acquired animals (and treatment with antibiotics of those infected) and systematic immunization with commercial vaccines containing the circulating serovars in the herd (Pereira et al., 2013; Mughini-Gras et al., 2013). The association of serological tests as microscopic agglutination test (MAT) as a screening test (detection of seroreactive herds) and further urine analysis by PCR (individual approach) was considered suitable for detection of renal carriers of leptospirae in cattle (Otaka et al., 2012). Noteworthy that, in the case of infection determined by host-adapted strains, for example Hardjo, carriers are considered to be the source of spreading of the disease in herds, and their detection and treatment represent a fundamental key to the adequate control of the infection in the herd (Mughini-Gras et al., 2013).

Vaccination plays an important role in the control of leptospirosis and may significantly reduce the occurrence of clinical symptoms (e.g. abortions) in the herd (Grooms, 2006; Pereira et al., 2013). Nevertheless, commercially available vaccines (bacterins) are inefficient to avoid the development of renal carriers (Adler and de la Peña Moctezuma, 2010). Thus, vaccinated animals can remain as a source of infection for other animals and the environment. As adaptive immunity in leptospirosis is serotype specific, the protection conferred by vaccination is directed towards the homologous serovars of the vaccine, with no cross-immunity (Murray et al., 2013). Thus, the identification of the infective serovar affecting each herd and the adequate choice of the vaccine is crucial to the control of the disease.

Challenges and New Approaches

During the last decades of the last century, many epidemiological studies have been conducted in several countries, based on serology. According to those studies, a seroepidemiological scenario has been stated for different regions, where different serovars were reported for cattle. An enormous variability has been reported regarding the seroreactions in bovine leptospirosis worldwide, with a large predominance (endemicity) of incidental serovars in tropical countries, such as serovar Javanica (India), Mini (Thailand) and Icterohaemorrhagiae (Trinidad) (Natarajaseenivasan et al., 2011; Suepaul et al., 2011; Suwancharoen et al., 2013). Nevertheless, serology presents many limitations, and information generated in those studies may not represent the real epidemiological scenario. Serology (MAT) is not a reliable tool for detecting individual carriers or chronically infected animals, nor for determining the infective serovar, as it is at maximum serogroup specific (Otaka et al., 2012). Additionally, despite the official recommendation of including local serovars in the antigen battery (OIE, 2008), few studies were conducted with such local strains, as in regions of Australia (Murray et al., 2011), New Caledonia (Desvars et al., 2012) and Brazil (Araújo et al., 2005; Chiareli et al., 2012), as far as the authors know for other tropical or developing countries. Moreover, it is well known that MAT must be interpreted carefully, especially in case of acute infection and multiple positive reactions towards different strains. Although useful for detecting seroreactive animals, using just local strains as antigens can lead to lose the standardization among different laboratories and therefore their comparability in terms of reproducibility. Furthermore, it is recommended to periodically check the antigenic panel, by means of hyperimmune positive control sera, in order to verify the identity of the strains used as antigens, and avoid cross-contaminations among strains.

Nevertheless, a huge problem is that, when efforts are made in order to increase the collection of local strains (isolates), it has been demonstrated that the scenario that emerges from those studies contrasts with those previously determined by serology. Despite the large number of serological studies worldwide, the number of isolates is scarce. Isolation technique is a very delicate procedure that needs no contamination, fast processing and long delay to produce a positive result, what may corroborate to the lack of information for the comparison between serology versus bacteriological data, mainly in developing countries. Thus, there is a lack of information for the comparison between serology versus bacteriological data, mainly in developing countries. Considering bovine leptospirosis, it has been demonstrated that in regions of Brazil, serogroups Australis, Sejroe, Pomona and Icterohaemorrhagiae are referred to

be predominant in serology, while obtained isolates are from serogroups Autumnalis, Canicola, Grippotyphosa, Sejroe and Pomona (Santa Rosa et al., 1980; Langoni et al., 1999; Lilenbaum and Souza, 2003; Miraglia et al., 2012). A similar scenario may be observed in other tropical countries, such as in regions of India, where serogroups Autumnalis, Javanica, Icterohaemorrhagiae and Pomona appear as frequent in serological studies, while Javanica, Grippotyphosa, Autumnalis, Hebdomadis and Canicola serogroups were recovered (Gangadhar et al., 2008; Natarajaseenivasan et al., 2011). Therefore, despite the paucity of bacteriological data, it is possible to verify that in many regions, particularly in tropical regions and developing countries, as parts of Africa, Thailand and Malaysia, the available information about local strains does not corroborate the previously determined seroepidemiological scenario (Table 1).

There are many possibilities that may participate on that lack of correspondence between serological and bacteriological results. If the serovars that were isolated have not been detected by means of serology, it is important to verify if MAT includes an homologous antigen, and if the panel of antigen strains was appropriate to the geographical area; In case of serology without correspondent isolates, it is important to consider the possible production of specific antibodies due to a exposure to the antigen without any shedding or clinical disease as well as the difficulties to growth of some strains of *Leptospira* in culture (Faine et al., 2000).

A promising and encouraging approach employs molecular tools, VNTR and sequencing of specific genes, particularly *secY* and *rpoB* for the classification of leptospiral DNA without the need of isolating the bacterium (Adler and de la Peña Moctezuma, 2010; Desvars et al., 2012; Miraglia et al., 2012). Nowadays, although molecular procedures cannot reliable type the *Leptospira*, they permit an easier direct diagnosis, leading to a more targeted effort when working with isolation, and can be helpful to confirm the circulation and the shedding of *Leptospira* among animals/herds in an easier and faster way with respect to isolation in culture.

Therefore, it is noteworthy that the epidemiological scenario now acknowledged may not represent what really occurs in many parts of the world, particularly on those tropical regions where the disease is endemic. The current knowledge about epidemiology and control, as well as the available diagnostic tools and the commercial vaccines, may not be adequate for those regions, what leads to a frustrating scenario of endemicity and difficulties on the control of the disease.

What could be done to overpass that frustrating scenario? First, veterinarians, epidemiologists, physicians, bacteriologists and molecular biologists should collaborate in a more integrated basis, conducting multispecies studies and

Table 1. Correlation between serological and isolates data for leptospirosis in cattle in tropical countries

Country	Serology	Isolates	References
Brazil	Bratislava, Grippotyphosa , Hardjoprajitno , Pomona , Icterohaemorrhagiae	Autumnalis, Goiano Grippotyphosa , Guaicurus, Hardjo , Icterohaemorrhagiae , Panama, Pomona , Sarmin, Shermani, Tarassovi, Wolffi	(Santa Rosa et al., 1980; Langoni et al., 1999; Lilenbaum and Souza, 2003; Araújo et al., 2005; Miraglia et al., 2012)
India	Autumnalis , Javanica , Icterohaemorrhagiae, Pomona	Javanica , Grippotyphosa, Autumnalis , Hebdomadis, Canicola	(Natarajaseenivasan et al., 2011; Suwancharoen et al., 2013)
South-East Asia (Thailand, Malaysia)	Tarassovi, Mini, Sejroe , Hebdomadis , Pomona.	Bataviae, Autumanalis, Javanica Hebdomadis , Pyrogenes Hardjo , Canicola, Australis, Ballum, Pomona	(Heisey et al., 1988; Bahaman and Ibrahim, 1988; Bahaman et al., 1988, 1990; Suwancharoen et al., 2013)
Africa (Zimbabwe, Tanzania, Nigeria, South Africa)	Sejroe , Pyrogenes , Hebdomadis , Tarassovi , Bataviae , Pomona , Icterohaemorrhagiae , Canicola	Australis, Bataviae , Grippotyphosa, Icterohaemorrhagiae , Pomona , Hebdomadis , Pyrogenes , Tarassovi , Sejroe	(Diallo and Dennis, 1982; Te Brugge and Dreyer, 1985; Feresu, 1987; Ezech et al., 1990; Feresu, 1992; Feresu et al., 1995, 1996, 1999; Mgode et al., 2006; Hesterberg et al., 2009; Swai and Schoonman, 2012)
Australia	Hardjo , Tarassovi , Pomona, Szwajizak	Hardjo , Zaroni, Tarassovi	(McClintock et al., 1993; Black et al., 2001; Corney et al., 2008)

Bold indicates isolates corroborates serological data.

employing serological, bacteriological and molecular tools. The concept of 'one health' must be disseminated, and leptospirosis is certainly one infection that would clearly benefit of that approach, as it has implications on human and animal health, with intense participation of wildlife as carriers and a strong impact of environmental conditions, particularly in the tropics (Chappel and Smythe, 2012). The main goal of researchers in those countries should be the isolation of local strains. Nowadays, a large part of what we know about transmission, the role of the carriers, influence of environmental conditions, treatments and vaccines are based on the available reference strains, which have been obtained, mostly, in developed countries. Culturing of leptospires is laborious and difficult, but is imperative. Local isolates are important for the developing of new diagnostic tools, as well as new vaccines and control protocols, probably more adequate to the local conditions of the tropics.

It is essential for the developing countries to equip laboratories and train specialized personal for the culturing of local strains of leptospires. Cooperation with international reference centres is also important. Based on the knowledge of the circulating strains in a region, the adequacy of the current vaccines and control protocols must be reanalysed. Additionally, the decision to vaccinate must be based on perspective clinical studies, in which usually serology is used as a screening, molecular diagnosis as a confirmation and the isolation in culture represents the final step to decide which vaccine to use. Without a huge effort in that direction, leptospirosis will not be defeated and will probably remain endemic in the developing countries, leading to important economic hazards in animal production and risks to public health in those regions.

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