Leptospirosis: an important zoonotic diseasesis

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1. Introduction

Leptospirosis, the infection with **Leptospira spp**., is an apparent or unapparent disease of animals and man. It is caused by a continuously changing number of pathogenic leptospira species and serogroups. The accumulation of information in recent years on leptospirosis has shown that serovar distribution around the world is neither uniform nor stable. In other words, the most prevalent serovars in one country or area are not necessarily the same with those of another. Thus, before deciding the appropriate measures for prophylaxis of domestic animals and man in one area or country serovar distribution in nature must be determined. In the study of serovar distribution the role of wild life in the spread of **Leptospira spp** must also be accounted. Wide epidemiological investigations among domestic and wild animals will also define the role of these animals in human infections. Toward this aim, various national and international organizations have been created, one of which is the "**International Leptospirosis Society**" formed in 1994, disseminating current knowledge between all those studying an important and scientifically interesting microorganism. The disease is also included in the list of diseases surveyed by the wildlife Conservation Society through its **Field Veterinary Program** formed in 1989. With the above in mind, an attempt is made here to review a zoonosis increasingly emerging as a public health hazard having also an economic importance among food producing animals.

2. The causative agent

The genus **Leptospira** belongs to the family of **Leptospiraceae**. The genus has currently, thirteen pathogenic species and six non-pathogenic (Table 1). More species are expected to be included in the genus [1,2,3] with the help of the newly created LepBank aiming in the accumulation of current information concerning the genome of **Leptospira spp** [4]. The species are divided in more than 250 serovars [3] distributed according to some in 23 serogroups [2,5,6] and according to others in 24 or 25 [1,7]. During the many decades of knowledge accumulation on the role of the microorganism in animal and human infections [8,9], a rather large number of serovars has emerged as pathogenic [3,7,10]. However, the great wealth of information accumulated is difficult to objectively evaluate for the purpose of epidemiologically defining the role of each serovar in disease observed among man and the various animal species [2,7,11]. This difficulty increases due to the adaptation of serovars to host animal species, becoming non-pathogenic for them, but causing disease in others [12,13].

Many animal species, including rodents, are considered natural hosts of the microorganism [3,11,14,15,16,17]. Natural hosts are disseminating the agent in nature through their urine [18], because leptospiras remain and multiply in the kidney tissue for long time and in some instances for the life of the host [3,11,19].

The microorganism survives in the environment if mean temperature remains at about 22^0 C year around and the fluctuations are not more than 5^0C [11]. Thus, leptospirosis is an extremely important disease in tropical and subtropical climates [6,20,21,22]. In all other parts of the world the disease is of seasonal importance, observed during spring and autumn, usually after heavy rainfall [10,23,24]. Regardless of where and when leptospirosis is observed, the epidemiological investigation of it reveals a plethora of serovars infecting animals and man in a variety of serovar prevalence within the various animal species [25,26,27,28,29,30,31,32,33,34]. This very wide proportional distribution of infecting serovars raises a multitude of questions needing scientific explanation. Areas needing further scientific investigation are those concerning the mechanisms of serovar adaptation to specific animal species, the mechanisms of animal species resistance to these serovars, the role of climatic factors in the survival of certain serovars and most importantly the mechanisms or microbial characteristics, which determine serovar pathogenicity [3,30,33,35,36].

Table 1: Current species of Leptospira spp [3]

Pathogenic Leptospira spp	**Non-pathogenic Leptospira spp**
Leptospira alexanderi	*Leptospira biflexa*
Leptospira alstonii	*Leptospira kmetyi*
Leptospira borgpetersenii	*Leptospira meyeri*
Leptospira fainei	*Leptospira yanagawae*
Leptospira interrogans	*Leptospira wolbachii*
Leptospira inadai	*Leptospira vanthielii*
Leptospira kirschneri	
Leptospira licerasiae	
Leptospira noguchii	
Leptospira santarosai	
Leptospira terpstrae	
Leptospira weilii	
Leptospira wolffii	

3. The infection and its clinical appearance

The multitude of factors contributing to the infection and its clinical expression are such that keep scientific interest around the world. These factors have become scientific challenges for many energetic researchers investigating the microorganism because,

1. it is a serious threat to human health
2. it is a serious economic problem among food producing animals needing control and prevention
3. the agent is difficult to study, since it is not easily isolated. Thus, methods for studying it need be developed
4. the agent is difficult to maintain under laboratory conditions. Thus, scientists working with it become dedicated experts.
5. the laboratory means currently available for accurately investigating the infection and specifically serovar distribution are using live leptospiras causing, in addition to public health concerns, an economic burden. Thus, there is a continuous need for developing economic, safe and reliable diagnostic methods.
6. the maintenance of live leptospiras for investigating human and animal infections restricts many scientists and laboratories around the world for contributing to the understanding of the microorganism [30,33,38,39,40]. Thus, a better understanding of this very interesting microorganism needs the improvement of molecular methods. The research for easier and cheaper molecular methods keeps scientists dedicated to study further the microorganism.

Currently, the bulk of the knowledge concerning human and animal leptospirosis is coming from countries having the economic and technologic means to systematically study Leptospira, its serovar distribution in nature and the pathogenicity of certain serovars for various animal species and man. According to this knowledge, man is infected through direct or indirect contact with the agent. As for the infecting serovars, they are these prevailing among domestic and wild animals.

The infection has traditionally been considered a serious zoonosis of the poor farmers from tropical and subtropical countries. They, working in wet fields contaminated by the urine of carrier wild animals, are at a high risk. Thus, the disease is common among rice field workers or others working in such an environment [8,22]. Additionally, the infection is often observed among the poor of rodent infested slums [22]. Furthermore, at risk are animal keepers working in environments contaminated with leptospiras from either urine of infected animals or the products of abortions caused by the microorganism [41]. In more recent times, an increase of cases is associated to people of a higher standard of living engaging in water sports and mountaineering [6].

Notably, there is a significant increase on the information concerning all aspects of leptospirosis in man and animals. Some translate this accumulation as evidence of a worldwide increase in reported cases referring to the infection as a "re-emerging" disease [3,19]. Others contribute the increases of the reported cases to the increase in the number of scientists working with the microorganism as new methods continuously improve safety, thus decreasing public health concerns among researchers and laboratory workers [21,23,24,35,42,43].

Regardless of the reasons the number of reported cases has increased, the microorganism could cause two forms of clinical disease; the acute or icteric and the chronic.

Acute or icteric disease could lead to death. In man it causes death to 25-50% of hospitalized cases, depending on the virulence of the infecting serovar [3,6]. The acute disease shows, in addition to jaundice and haemoglobinuria, signs of meningitis, pulmonary hemorrhage and signs from complete systemic collapse due to kidney failure. If the acute disease

is milder in its clinical appearance, it shows flue-like signs and remains mainly undiagnosed. Man is seldom chronically infected [3,6,11,30,33,35].

Chronic disease is mostly found among animals. Abortions and neonatal deaths are the common outcome of animal chronic infection. They are also of economic importance when food producing animals are infected [30,37,38,39,40]. However, among man, dogs and horses chronic infection could also be expressed by recurrent uveitis [44,45,46].

4. Economic importance of Leptospirosis among animals

The reported prevalence values of animal infection across the world are between 2% and 46% depending on the animal species [15,24,26,27,28,29,32,36,47,48]. Given this wide variation in reported prevalence values and the contributions to it of factors such as climatic, animal species, time of the year, method of investigation (serovar inclusion in testing), there is not a safe way to calculate the economic impact of the infection among animals.

However, it appears that the disease is of major economic concern when it is involved in the reproductive failure of food producing animals [10,38,40]. Infection of the reproductive system could result in a "storm of abortions" causing considerable economic losses from meat and milk reductions [49,50]. Furthermore, these losses appear as more significant among cattle and pigs, because these animal species are considered less resistant than small ruminants [51,52].

As research derived information accumulates and the disease is better understood, its economic impact could better be estimated. This needed evaluation, depends greatly on the available means to reliably investigate suspect cases, but also the importance of unapparent infection among farm animals.

5. Laboratory investigation of leptospirosis

Leptospirosis of man and animals is investigated by direct and indirect laboratory methods.

1. Direct methods of investigating leptospirosis are the isolation of the causative agent and the identification of **Leptospira spp** antigens in tissue and body fluids using such methods as immunofluorescence, immunochemistry and various methods of Polymerase Chain Reaction (PCR) [7,10,11].

2. Indirect methods of investigating leptospirosis are based on the detection of specific serum antibodies. These methods are either methods detecting serum antibodies without discriminating on serovars, such as various ELISA tests, indirect immunofluorescence, the spot agglutination test or methods reliably identifying the infecting serovars, such as the Microscopic Agglutination Test (MAT).

However, with the exception of isolation, none of the currently available diagnostic methods are suitable for studying microbial pathogenicity (structure, products, biochemical characteristics). Isolation is also required for accurately placing the agent into serogroups [2,53,54,55].

5.1 Direct methods

1. Isolation of the microorganism

Leptospira organisms could be isolated from body fluids, mainly urine, [3,19,53]. Nevertheless, tissue from dead animals is giving a greater opportunity of a successful isolation, if target tissue is not autolysed. Such target tissue is kidney, liver, lungs, brain [3,19,25,31,56]. If the agent is suspect for abortions, isolation could be attempted from non-autolysed abortion materials or tissue samples from a freshly aborted fetus. Isolation of the microorganism from fetal tissue (kidney, liver, lungs) confirms maternal infection [37,40].

Isolation requires expensive and properly prepared and kept culture media [57,58]. Inoculated media are incubated at $29+/-1^0$ C for several weeks or months. Cultures are incubated in dark and quite environment. Time of incubation depends on the serovar. Serovars such as Pomona and Grippotyphosa require the least time incubation (10 days) [58,59]. Regardless of time required for isolation, the inoculated culture media must be protected from contamination, thus require the addition of antimicrobial agents selected to inhibit growth of contaminants, but allow the multiplication of **Leptospira spp** serovars [58,60]. Evident is from the above that the isolation and systematic study of **Leptospira spp** is not an easy job for most scientists and laboratories around the world. Unfortunately, the majority of them are from parts of the world that the disease is most significant [19].

2. Other direct methods

Immunofluorescence and immunochemistry methods for identifying the agent in suspect clinical material have been used during the years, but they are not easy or successful [17,40,58,60]. Their sensitivity is influenced by the number of microorganisms present in the sample. In addition, they require reagents that are not commercially available, thus must be produced in situ or after a special order resulting in additional expenses and labor. Laboratories having money and

time have attempted improving these methods, but they have not overcome the problems of serovar recognition [3,19]. Therefore, these methods are used more as research tools and are study subjects of researchers and not work tools for diagnosticians.

The difficulties of isolating the microorganism, identifying its serovars or producing specific reagents continue to exist despised the efforts to overcome them. Thus, there is this past decade an effort to improve various PCR methods in an attempt to simplify systematic investigations. To date, there is not a PCR method for identifying serovars, although some attempts are promising [2,16,54,55] and could in the future successfully combine sensitivity with specificity. Until then, indirect methods are the preferred tool for investigating man and animal leptospirosis.

5.2. Indirect Methods

The detection of specific serum antibodies is the preferred way for investigating most of the important animal infections, and leptospirosis is not excluded. Among the available serologic methods are various IgM and IgG ELISA methods, the spot agglutination test, indirect immunofluorescence and the MAT [3,10,11,19,40,43,62]. Between them, the easiest to perform are the various ELISA tests, although they are not successful for the epidemiological investigation of leptospirosis in animals.The various ELISA methods used are good and safe screening tools, easy, quick and relatively cheap for investigating human leptospirosis. ELISA screening of human sera is a quick and useful method for determining the status of a suspect case, but not for determining the infecting serovar of **Leptospira spp**. Among the most useful ELISA tests are those detecting IgM antibodies, thus acute leptosirosis. The indirect immunofluorescence and spot agglutination test are not used as extensively as the various ELISA methods, because they are not sensitive and serovar specific [3,40,43,63]. Furthermore, the indirect immunofluorescence is faced with the same problems as the direct method due to difficulties of purchasing reagents.

Although these methods are successfully used for human acute disease and the purpose of screening animals, they are not useful for determining the infecting serovar. In animals recognition of prevalent serovars is required for selecting the most appropriate measures for prevention or control of the disease. In addition, the above mentioned methods do not compare well with the “gold standard” for the diagnosis of leptospirosis, which is the MAT. The MAT is currently the official and widely accepted method as accurately investigating the serovars possibly involved in an infection [3,10,11]. Therefore, when one investigates animals, the MAT is the method of choice, although it has important disadvantages.

5.2.1. Advantages and disadvantages of the MAT

The MAT is a reliable method for investigating leptospirosis in man and animals because it is not influenced by the investigated animal species. This method is based on the agglutination of live leptospira organisms. Thus, the method requires live leptospiras of various serovars, therefore having advantages and disadvantages needing a brief mention [3,19,61,64].

- The method has a good specificity because the presence of heterologous antibodies is not interfering in the results.
- Although there is a possibility of cross reactions from antigenically related serovars - serovars belonging to the same serogroup - the MAT remains up to now the only reliable method for investigating the spread of serovars among animals. This type of investigation is needed for determining the most important serovars infecting the various animal species, thus decide on the best vaccination scheme for preventing either the infection or the development of clinical disease.
- In addition, if paired serum samples are examined, the method reveals reliably not only acute disease, but also the serovar causing the disease, thus considered pathogenic for the examined animal species [3,19].

As is evidenced from the above, the method is advantageous only if a large number of serovars is used to test a serum sample.

- This requires the continuous maintenance of live serovars adding to the costs of the method due to material and labor. Costs and labor improve when a nation dedicates a laboratory where all national testing (routine and for research) could be performed.
- Understandably, such a dedication to this difficult, but important microorganism is a luxury for nations that are at most risk. They are not also in a position to allocate personnel to keep continuously the microorganism live and in conditions for reliable testing.
- Only if the investigation of leptospirosis is systematic and involves a large number of available worldwide serovars, the sensitivity and specificity of the MAT increase [3,19]. The two are also improving if a representative number of locally isolated serovars is included among those participating in the MAT.
- The last requires successful isolation of local serovars something that is very hard to accomplish, if it is not impossible.
- Furthermore, the MAT is influenced by vaccinal antibodies [3], thus a good history of the tested animal should be taken before the testing of sera.
- The method is also of poor value when investigating either or both, chronic infections or carrier animals.

However, regardless of its disadvantages, the MAT is currently the only available method for deciding on the appropriate vaccination scheme for prevention and control of this important economically and of public health importance microorganism. Prevention and control require serovar recognition and the MAT is the only method defining serovar distribution in nature.

6. Prevention and control of leptospirosis

The prevalence of leptosirosis among animals (2-46%) indicates that for minimizing its economic impact, the infection must be controlled mainly among food producing animals [15,24,26,27,28,29,32,34,36,47,48]. Its control in animals will decrease the chances for animal keeper infection and environmental contamination, possibly decreasing the spreading of pathogenic serovars in nature [41]. Although the infection cannot be eliminated either in the farm environment or the wild, because it is very widely spread, efforts to decrease the prevalence of pathogenic serovars could benefit farmers and the public.

Thus, an increasing interest about the disease across the world has increased recognition of the infection among man and animals making many to consider it as a "re-merging" disease [3,19,22]. Under the circumstances, the current interest of scientists is in creating effective vaccines for man and the various animal species covering them for as many pathogenic serovars as possible. This requires overcoming the absence of cross-immunity between serovars [3]. Toward this end, an international information bank, freely shared by workers across the world, has been created aiming in the accumulation of the great wealth of information concerning **Leptospira spp.** [4]. This knowledge will improve vaccine technology.

To date various types of vaccines have been experimentally considered as good candidates for effectively preventing infection, or at least clinical disease [65]. Recombinant, lipopolysaccharide, DNA and inactivated - attenuated vaccines have been experimentally tested with various results on effectiveness and safety among animals. However, there is yet a fully safe and effective vaccine to be produced for man. Those widely investigated for use in animals are attenuated and inactivated vaccines, but the protection conferred by them is partial, due to lack of cross immunity among serovars. Thus, current work aims in overcoming the problems of partial protection by applying new knowledge on the genomics of **Leptospira spp** to vaccine making technology [4,65]. Until an effective vaccine for the various animal species and safe for man is produced, prevention of losses is possible through antimicrobial treatment.

The current choices of treatment for leptospirosis include penicillin, doxycycline, cefotaxine, ceftriaxone and azithromycin, tetracycline, ampicilin, moxiflocacin, levofloxacine and others [66,67]. Although they are found effective, resistance to them could develop regionally, thus needing extensive susceptibility testing and this is not easy to accomplish, if there is not a national policy on leptospirosis. The reasons for the difficulties are those mentioned earlier concerning the isolation and maintenance of the microorganism.

7. References

[1] World Health Organization. Human leptospirosis: Guidance for diagnosis, surveillance and control. *WHO Library Cataloguing in – Publication Data, ISBN 92 4 154589 5*. 2003

[2] Morey RE, Galloway RL, Bragg SL, Steigerwalt AG, Mayer LW, Levett PN. Species-specific identification of Leptospiraceae by 16S rRNA gene sequencing. *Journal of Clinical Microbiology*. 2006;44:3510-3516

[3] Adler B, Moctezuma PA. Leptospira and Leptospirosis. *Veterinary Microbiology*. 2010;140:287-296

[4] Eslabao MR, Dellagostin OA, Cerqueira GM. LepBank: A Leptospira sequence repository and a portal for phylogenetic studies. *Infection, Genetics and Evolution*. 2010;10:586-590

[5] Vijayachari P, Hartskeerl RA, Sharma S, Natarajaseenivasan K, Roy S, Terpstra WJ, Sehgal SC. A unique strain of *Leptospira* isolated from a patient with pulmonary haemorrhages in Andaman Islands: a proposal of serovar Portblairi of serogroup Sehgali. *Epidemiology and Infection*. 2004;132:663–673.

[6] Ricaldi NJ, Vinetz MJ. Leptospirosis in the tropics and in travelers. *Current Infectious Disease Reports*. 2006;8:51-58

[7] Cerqueira MG, Picardeau M. A century of Leptospira strain typing. *Infection, Genetics and Evolution*. 2009;9:760-768

[8] Levett PN. Leptospirosis**.** *Clinical Microbiology Reviews*. 2001 April; 14(2): 296–326.

[9] Sambasiva RR, Naveen C, Bhalla P, Agarwal SK. Leptospirosis in India and the rest of the world. *Brazilian Journal of Infectious Diseases*. 2003;7:178-193

[10] Levett PN. Leptospirosis: A forgotten zoonosis? *Clinical Applied Immunology Reviews*. 2004;4:435-448

[11] World Health Organization. Guidelines for the prevention and control of leptospirosis. *Zoonosis Division, National Institute of Communicable Diseases, 22-Sham Nath Marg, Dehli-110 054*; 2006

[12] Mahajan S, Chabra D. Leptospirosis: A re-emerging Disease. *Veterinary World*. 2008;1:182-185

[13] Vijayachari P, Sugunan AP, Shriram AN. Leptospirosis: an emerging global public health problem. *Journal of Biosciences*. 2008;33:557-569

[14] Bunnel JE, Hice CL, Watts DM, Montrueil V, Tesh RB, Vinetz JM. Detection of pathogenic Leptospira spp. Infections among mammals captured in the Peruvian Amazon basin region. *The American Journal of Tropical Medicine and Hygiene*. 2000;63:255-258.

[15] Michel V, Branger C, Andre-Fontaine G. Epidemiology of leptospirosis. *Revista Cubana de Medicina Tropical*. 2002;54:7-10

[16] Turk N, Milas Z, Margaletic J, Staresina V, Slavica A, Riquelme-Sertour N, Bellenger E, Baranton G, Postic D. Molecular characterization of Leptospira spp strains isolated from small rodents in Croatia. *Epidemiology and Infection*. 2003;130:159-166
[17] Cox TE, Smythe LD and Leung LKP. Flying foxes as carriers of pathogenic Leptospira spp. *Journal of Wildlife Diseases*. 2005;41:753-757
[18] Monahan AM, Callanan JJ, and Nally JE. Review paper: Host – pathogen interactions in the kidney during chronic leptospirosis. *Veterinary Pathology*. 2009;46:792-799
[19] Ko AI, Goarant C and Picardeau M. Leptospira: the dawn of the molecular genetics era for an emerging zoonotic pathogen. *Nature Reviews Microbiology*. 2009;7:736-747
[20] Bharti AR, Nally JE, Ricaldi JN, Matthias MA, Diaz MM, Lovett MA, Levett PN, Gilman RH, Willig MR, Gotuzzo E, Vinetz JM. Leptospirosis: a zoonotic disease of global importance. *The Lancet Infectious Diseases*. 2003;3:757-771
[21] Pappas G, Papadimitriou P, Siozopoulou V, Christou L, Akritidis N. The globalisation of leptospirosis : worldwide incidence trends. *International Journal of Infectious Diseases*. 2008;12:351-357
[22] Reis RB, Ribeiro GS, Felzemburgh RD, Santana FS, Mohr S, Melendez AX, Queiroz A, Santos AC, Ravines RR, Tassinari WS, Carvalho MS, Reis MG, Ko AI, Gurtler RE. Impact of Environment and Social Gradient on Leptospira Infection in Urban Slums. *PLoS Neglected Tropical Diseases,* 2008;*2*: e228.
[23] Sanders EJ, Rigau-Perez JG, Smits HL, Deseda CC, Vorndam VA, Aye T, Spiegel RA, Weyant RS, Bragg SL. Increase of leptospirosis in Dengue-negative patients after a hurricane in Puerto Rico. *The American Journal of Tropical Medicine and Hygiene*. 1999;61:399-404
[24] Leal-Castellanos CB, Carcia-Suarez R, Gonzalez-Figueroa E, Fuentes-Allen JL, Escobedo-De La Rena J. Risk factors and the prevalence of leptospirosis infection in a rural comunity of Chiapas, Mexico. *Epidemiology and Infection*. 2003;131:1149-1156
[25] Baker TF, McEwen SA, Prescott JF, Meek AH. The prevalence of leptospirosis and its association with multifocal interstitial nephritis in swine at slaughter. *Canadian Journal of Veterinary Research*. 1989;53:290-294
[26] Ciceroni L, Lombardo D, Pinto A, Ciarrocchi S, Simeoni J. Prevalence of antibodies to Leptospira serovars in sheep and goats in Alto Adige-South Tyrol. *Journal of veterinary science*. 2000;47B:217-223
[27] Epsi A, Prieto JM, Fernandez M, Alvarez M. Seroloxical prevalence of six leptospira serovars in cattle in Asturias (North Spain). *Epidemiology and Infection*. 2000;124:599-602
[28] Burriel AR, Magana-Vougiouka O, Butsini S, Nomikou K, Patakakis M. A serologic investigation of some causes of reproductive failure among small ruminats in Greece. *Online Journal of Veterinary Research*. 2002;1:57-63
[29] Burriel AR, Dalley C, Woodward MJ. Prevalence of Leptospira species among farmed and domestic animals in Greece. *Veterinary Record*. 2003;153:146-148
[30] Szeredi L, Haake DA. Immunohistochemical identification and pathologic findings in natural cases of equine abortion caused by leptospira infection. *Veterinary Pathology*. 2006;43:755-761
[31] Hamir AN, Hanlon CA, Niezgoda M, Rupprecht CE. The prevalence of interstitial nephritis and leptospirosis in 283 raccons (Procyon lotor) from 5 different sites in the United States. *Canadian Veterinary Journal*. 2001;42:869-871
[32] Richardson DJ, Gauthier JL. A serosurvey of leptospirosis in Connecticut Peridomestic Wildlife. *Vector-born and Zoonotic Diseases*. 2003;3:187-192.
[33] Ortega-Pacheco A, Colin-Flores RF, Gutierrez-Blanco E, Jimenez-Coelloo M. Frequency and type of renal lesions in dogs naturally infected with Leptospira species. *Annals of the N. York Academy of Science*. 2008;1149:270-274
[34] Kawaguchi L, Sengkeopraseuth B, Tsuyuoka R, Koizumi N, Akasi H, Vongphracham P, Watanabe H, Aoyama A. Seroprevalence of elptospirosis and risk factor analysis in food-prone rural areas in Lao PDR. *The American Journal of Tropical Medicine and Hygiene*. 2008;78:957-961
[35] Plank R, Dean D. Overview of the epidemiology, microbiology and pathogenesis of Leptospira spp in humans. *Microbes and Infection*. 2000;2:1265-1276
[36] Faria MT, Athanazio DA, Ramos EAG, Silva EF, Reis MG, Ko AI.
Morphological alterations in the kidney of rats with natural and experimental Leptospira infection. *Journal of Comparative Pathology*. 2007;137 :231-238
[37] Vemulapalli R, Langohr IM, Sanchez A, Kiupel M, Bolin CA, Wu CC, Lin TL. Molecular detection of Leptospira kirschneri in tissue of a prematurely born foal. *Journal of Veterinary Diagnostic Investigation*. 2005;17:67-71
[38] Bomfim MRQ, Koury MC. Evaluation of LSSP-PCR for the identification of Leptospira spp in urine samples of cattle with clinical suspicion of leptospirosis. *Veterinary Microbiology*. 2006;118:278-288
[39] Leon A, Pronost S, Tapprest J, Foucher N, Blanchard B, Andre-Fontaine G, Laugier C,
Fortier G, Leclercq R. Identification of pathogenic leptospira strains in tissues of a premature foal by use of polymerase chain reaction analysis. *Journal of Veterinary Diagnostic Investigation*. 2006;18:218-221
[40] Saglam YS, Yener Z, Tenur A, Yalcin E. Immunohistochemical detection of leptospiral antigens in cases of naturally occurring abortions in sheep. *Small Ruminant Research*. 2008;74:119-122
[41] Natarajaseenivasan K, Boopalan M, Selvanayaki K, Suresh SR, Ratman S. Leptospirosis among rice mill workers of Salem, South India. *Japanese Journal of Infectious Diseases*. 2002;55:170-173
[42] Natarajaseenivasan K, Prabhu N, Selvanayaki K, Savalaikarankulam S, Raja S, Ratnam S.
Human leptospirosis in Erode, South India: Serology, isolation, and characterization of the isolates by randomly amplified polymorphic DNA (RAPD) fingerprinting. *Japanese Journal of Infectious Diseases*. 2004;57:193-197
[43] Doungchawee G, Kositanont U, Niwetpathomwat A, Inswisai T, Sarasaeranee P, Haake
DA. Early diagnosis of leptospirosis by immunoglobulin M immunoblot testing. *Clinical and Vaccine Immunology*. 2008;15:492-498
[44] Faber NA, Crawford M, LeFebvre RB, Buyukmihci NC, Madigan JE, Willits NH. *Journal of Clinical Microbiology*. 2000;38:2731-2733
[45] Townsend WM, Stiles J and Krohne SG. Leptospirosis and pauveitis in a dog. *Veterinary Ophthalogy*. 2006;9:169-173

[46] Gupta A, Gulmar PD, Srinivasan R, Kaliaperumar S. Bilateral acute keratouveitis in leptospirosis: A new entity. *Indian Journal of Ophthalmology*. 2007;55:399

[47] Talbada MD, Garvey N, Sprowls R, Eugster AK, Vinetz JM. Prevalence of leptospirosis infection in Texas cattle: Implications for transmission to humans. *Vector-born and Zoonotic Diseases*. 2003;3:141-147

[48] Salina-Melendez JA, Narvaez-Arce C, Riojas-Valdes V, Cantu-Covarrubias A, Avalos-Ramirez R, Segura-Correa LC. Seroprevalence of leptospirosis in beef cattle of Nuevo Leon, Mexico. *Journal of Animal and Veterinary Advances*. 2007;6:1265-1268

[49] Guitian FJ, Garcia-Pena FJ, Oliveira J, Sanjuan ML, Yus E. Serological study of the frequency of leptospiral infections among dairy cows in farms with suboptimal reproductive efficiency in Galicia, Spain. *Veterinary Microbiology*. 2001;80:275-284

[50] Tooloei M, Abdollapour G, Karimi H, Hasanpor A. Prevalence of serum antibodies against six leptospira serovars in sheep in Tabriz, North-western Iran. *Journal of Animal and Veterinary Advances*. 2008;7:450-455

[51] OIE Terrestrial Manual. Leptospirosis. Chapter 2.1.9, pp 251-264;2008

[52] Lilenbaum W, Varges R, Ristow P, Cortez A, Souza SO, Richtzenhain LJ, Vaconcellos SA. Identification of Leptospira spp carriers among seroactive goats and sheep by polymerase chain reaction. *Research in Veterinary Science*. 2009;87:16-19

[53] Freitas JC, da Silva FG, de Oliveira RC, Delbem ACB, Muller EE, Alves LA and Teles PS. Isolation of Leptospira spp from dogs, bovine and swine naturally infected. *Ciência Rural* 2004;34:853-856

[54] Langoni H, Souza CL, Da Silva AV, Cunha ELP, Silva RC. Epidemiological aspects of leptospirosis. Research of anti-Leptospira spp antibodies, isolation and biomolecular research in bovines, rodents and workers in rular properties from Botucatu, SP, Prazil. *Brazilian Journal of Veterinary Research in Animal Sciences*. 2008;45:190-199

[55] Slack AT, Galloway RL, Symonds ML, Dohnt MF, Smythe LD. Reclassification of Leptospira meyeri serovar Perameles to Leptospira interrogans serovar Perameles through serological and molecular analysis: evidence of a need for changes to current procedures in Leptospira taxonomy. *International Journal Of Systematic And Evolutionary Microbiology*. 2009;59:1199-1203

[56] Sitprija V, Pipatanagul V, Mertowidjojo K, Boonpuchnavig V, Boonpucknavig S. Pathogenesis of renal disease in leptospirosis: Clinical and experimental studies. *Kidney International*. 1980;17:827-836

[57] Hookey JV. Detection of Leptospiraceae by amplification of 16S ribosomal DNA. *FEMS Microbiology Letters*. 1992;90:267–274.

[58] Ellis WA. The diagnosis of leptospirosis in farm animals. *In: The Present State of Leptospirosis Diagnosis and Control*, Ellis W.A. & Little T.W.A., eds. Dordrecht, The Netherlands, Martinus Nijhoff,; 1986;13–31

[59] Letocart M, Baranton G, Perolat P. Rapid identification of pathogenic *Leptospira* species (*Leptospira interrogans*, *L. borgpetersenii*, and *L. kirschneri*) with species-specific DNA probes produced by arbitrarily primed PCR. *Journal Of Clinical Microbiology*. 1997;35:248–253.

[60] Adler B, Faine S, Christopher WL, Chappel RJ. Development of an improved selective medium for isolation of leptospires from clinical material. *Veterinary Microbiology*. 1986;12:377–381.

[61] Faine S, Adler B, Bolin C, Perolat P. *Leptospira* and Leptospirosis, 2nd Ed., Melbourne, Australia, Medisci Press; 2000

[62] Cullen PA, Xu X, Matsunaga J, Sanchez Y, Ko AI, Haake DA, Adler B. Surfaceome of *Leptospira* spp. *Infection And Immunity.* 2005;73:4853–4863.

[63] Lilenbaum W, Ristow P, Fraguas S,A, da Silva ED. Evaluation of a rapid slide agglutination test fort he diagnosis of acute canine leptospirosis. *Revista Latinoamericana De Microbiología*. 2002;44:124-128.

[64] National Veterinary Services Laboratories. Microtitre technique for detection of *Leptospira* antibodies. *Proceedings, Annual Meeting Of The United States Animal Health Association.* 1987;91:65–73.

[65] Wang Z and Wegrzyn A. Leptospirosis vaccines. *Microbial Cell Factories*. 2007;6:39-51

[66] Griffith ME, Hospenthal DR, Myrray CK. Antimicrobial therapy of leptospirosis. *Current Opinion on Infectious Diseases*. 2006;19:533-537

[67] Ressner RA, Griffith ME, Beckius ML, Pimentel G, Miller RS, Mende K, Fraser SL, Galloway RL, Hospenthal DR, Murray CK. Antimicrobial Susceptibilities of Geographically Diverse Clinical -Human Isolates of *Leptospira. Antimicrobial Agents and Chemotherapy*. 2008; 2750–2754