Rev. sci. tech. Off. int. Epiz., 2008, **27** (3), 885-892

Leptospira infection in animals and humans: a potential public health risk in India

N.L. Gangadhar (1), K. Prabhudas (1), Sashi Bhushan (2), Munasira Sulthana (3), S.B. Barbuddhe (4) & H. Rehaman (5)

(1) Indian Council of Agricultural Research Project Directorate on Animal Disease Monitoring and Surveillance, Campus of the Indian Veterinary Research Institute, Hebbal, Bangalore 560024, India
(2) Vijayanagar Institute of Medical Sciences, Bellary, India
(3) No. 21, 30th Cross, Bannerughatta Road Layout, 4th Block East, Jayanagar, Bangalore 560041, India
(4) Research Complex of the Indian Council of Agricultural Research, Goa, India
(5) Indian Council of Agricultural Research Complex for North Eastern Hill Region, Sikkim Centre, Tadong-Post, Gangtok, Sikkim-737 102, India

Submitted for publication: 2 June 2005
Accepted for publication: 3 October 2007

Summary

Leptospirosis is recognised as one of the commonest zoonotic infections in the world.

In India, where animals provide the draught power for agriculture, which is the main profession of the population, the incidence of leptospirosis among animals and humans is high.

In this paper, the isolation of pathogenic leptospires from human and animal hosts from several parts of India is reported. Because there are only limited facilities for serotyping within the country, most of the isolates were typed to the serogroup level only. In addition, the potential of leptospirosis to be a serious public health problem in India is discussed.

Keywords

Abortion – Carrier – Jaundice – Leptospira inadai – Rat – Zoonosis.

Introduction

Leptospirosis is an infectious disease of most warm-blooded animals, including humans, and is recognised as one of the most common zoonoses in the world. It is widely prevalent in India, as in most tropical countries. Given that farming is the main occupation of the general population in India, and that livestock provide the draught power for agriculture, there is abundant opportunity for cross-infection between animals and humans. Sporadic cases of leptospirosis occur throughout the year in all parts of the country. Cities such as Mumbai and Chennai, parts of Kerala state, and the Andaman and Nicobar Islands are known endemic areas in India. The occurrence of leptospirosis in outbreak form in humans and animals in India is rare.

Incidents in animals are abundant but very few are documented. Only infection with *Leptospira* spp. in dogs receives veterinary attention; little work has been done in other species (7, 15, 18, 20, 21, 22).

The family Leptospiraceae contains two genera, *Leptospira* and *Leptonema* (8). Based on antigenic determinants, the genus *Leptospira* is classified into two species, *Leptospira interrogans* and *L. biflexa*, the parasitic and saprophytic forms, respectively. A more recent molecular classification, based on DNA relatedness among leptospires, divides *Leptospira* into several species. Accordingly, the main pathogenic species in the genus *Leptospira* are: *L. interrogans*, *L. alexanderi*, *L. fainei*, *L. inadai*, *L. krischneri*, *L. meyeri*, *L. borgpetersenii*, *L. weillii*, *L. noguchii* and *L. santarosii*. The saprophytic species are *L. biflexa* and *L. wolbachii* (3, 17, 19).

Determination of the prevalence of leptospirosis among livestock and study of the zoonotic impact of this disease are among the priorities of the Project Directorate on Animal Disease Monitoring and Surveillance (PD-ADMAS)

of Bangalore, India. This is a project that was established by the Government of India under the Indian Council of Agricultural Research (ICAR). It is responsible for monitoring, surveillance and forecasting of livestock diseases in the country, and focuses particularly on diseases that have an economic impact on farming and those that have zoonotic significance. These activities are carried out through 15 substations of PD-ADMAS that are located in different parts of the country, using diagnostic tools that were developed at PD-ADMAS in Bangalore.

The objectives of this study were:

– to study the disease burden attributable to leptospirosis in livestock in India

– to develop a diagnostic protocol for monitoring leptospirosis in livestock under field conditions

– to study the zoonotic significance of leptospirosis in India.

To realise these objectives, a study that involved isolation of *Leptospira* spp. was launched with the collaboration of selected human health and veterinary institutions. This was intended to provide isolates of the aetiological agent to be used in the development of a suitable diagnostic kit.

Materials and methods

The leptospira laboratory protocol

The leptospira laboratory of PD-ADMAS follows a strict protocol of quality control in handling media and isolates. Ellinghausen-McCullough-Johnson-Harris (EMJH) semisolid medium is used for isolation of leptospires. The medium is freshly prepared every month with bovine serum albumin fraction V and locally available ingredients, and undergoes quality control checks with regard to nutritional standards and sterility. The medium is stored in 5 ml screw-cap tubes until use. A colour code is followed in the laboratory to identify cultures from different host species. The isolates are stored and maintained separately in independent incubators for each host species to prevent misidentification of isolates or cultures.

Samples

The clinical samples were collected from different host species by the collaborating centres and the main centre at Bangalore. Blood samples were collected aseptically in pre-sterilised 5 ml disposable syringes. Each blood-filled syringe, with needle attached and covered with the needle cap, was taped individually and packed in a self-sealing plastic pouch. The plastic pouches were packed in cardboard boxes and transported to the main laboratory at PD-ADMAS in Bangalore for further processing.

A total of 922 samples were inoculated into EMJH semisolid medium. They comprised:

a) blood and urine samples from cows with a history of abortions and repeat breeding, but showing no clinical manifestations of leptospirosis (sample size: 372, see Table I);

b) samples of goat sera that were referred to the PD-ADMAS laboratory from two goat farms for routine screening (sample size: 102);

c) serum samples from dogs with clinical signs compatible with leptospirosis (sample size: 22);

d) blood samples collected by ear vein puncture from piglets that were randomly selected at the ICAR Research complex, Goa (sample size: 20);

e) blood, serum, urine and cerebrospinal fluid (CSF) samples received from different human health centres and paediatric hospitals. These blood samples were collected from patients of different ages and sexes at the peak of fever. Blood samples from paediatric patients were collected from children of between 1 and 15 years of age.

Table I
***Leptospira* isolates from different host species**

Clinical sample	Cattle			Goats			Dogs			Pigs			Humans		
	Sample size	Isolates	Percentage	Sample size	Isolates	Percentage	Sample size	Isolates	Percentage	Sample size	Isolates	Percentage	Sample size	Isolates	Percentage
Blood/ serum	342	66	19.2	102	21	20.5	22	12	54.5	20	2	10.0	394	90	22.8
Urine	30	2	6.6										8	2	25.0
Cerebro-spinal fluid													4	2	50.0
Total	**372**	**68**	**18.3**	**102**	**21**	**20.5**	**22**	**12**	**54.5**	**20**	**2**	**10.0**	**406**	**94**	**23.1**

In four patients, CSF was aspirated aseptically by lumbar puncture between lumbar vertebrae four and five. The preliminary case selection criteria were based on the presence of clinical symptoms such as fever, jaundice, myalgia, conjunctival suffusion, vomiting and bleeding (sample size: 406).

Microbiological methods

The inoculated culture tubes were incubated at 30°C in a biological oxygen demand (BOD) incubator for 1 to 12 weeks. The cultures were examined under a dark field microscope every week. The presence of leptospires was confirmed by the ADMAS leptospira staining technique (9). This silver impregnation technique, developed at the PD-ADMAS leptospira laboratory, is an improvement of the earlier Fontana's staining technique (2). It has the advantage of staining the organisms in the clinical material while reducing staining of the background, allowing clear visualisation of the organism. Viewed under the oil immersion objective, the highly coiled morphology of the leptospires can be appreciated clearly. The stain aids differentiation of a leptospiral organism from debris, staining artefacts, fibrin strands and other organisms (Fig. 1). Some of the serious limitations of Fontana's staining technique, which have led leptospirologists to have serious reservations about the usefulness of the technique (2), have been overcome by this modification.

The cultures were subjected to routine tests to differentiate the organisms from saprophytic leptospires (11) and from *Leptonema* spp. (8). The isolates that were found to be pathogenic were referred to the World Health Organization/Food and Agriculture Organization/World Organisation for Animal Health (WHO/FAO/OIE) Collaborating Centre for Reference and Research on Leptospirosis in Brisbane, Australia, and to the Division of Microbiology, Defense Research Development Establishment (DRDE) in Gwalior, India, for typing.

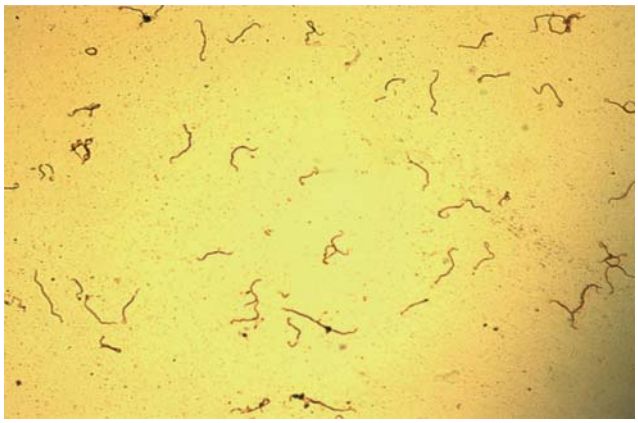

Fig. 1
***Leptospira inadai* isolated from a fatal case of human leptospirosis (ADMAS staining technique × 100)**

Results

Following 1 to 12 weeks of incubation, leptospires were demonstrated in 197 out of 922 samples using dark field microscopy, giving an isolation rate of 21.36%. Some of the isolates required filtration through 0.22 µm filter membrane, and then were successfully purified into pure cultures.

Studies to ascertain the pathogenic character of the isolates revealed that all isolates except one were pathogenic.

Out of 372 bovine samples, leptospires were isolated from 66 blood samples and two urine samples. There were 21 isolations from the 102 blood samples from goats. Two of the 20 porcine serum samples and 12 of the 22 canine serum samples revealed leptospira infection. Sixteen of the canine serum samples were from the collaborating unit at Umiam, Shillong. These dogs were of different age groups and sexes and had a history of fever, up to 103°F, lasting 4 days to 1 week. The dogs showed haematuria and oliguria. Leptospires were demonstrated using dark field microscopy, followed by isolation of organisms from eight of the clinical samples. None of the eight dogs with leptospirosis responded to treatment. Culturing of 406 human clinical samples yielded 94 leptospira isolations. Of these, 90 were obtained from blood or serum samples and two each from CSF and urine samples. Details of the leptospira isolations are presented in Table I.

Leptospira typing

There are limited serotyping facilities in India, therefore most of the isolates were typed to serogroup level only. Out of the 23 isolates that were typed, 15 isolates were from serogroup Autumnalis, and one each from serogroups Hebdomadis, Grippotyphosa, Canicola and Icterohaemorrhagiae (for details of host species see Table II). One human isolate was typed to serovar level and was identified as *Leptospira borgpetersenii* serovar Hardjo. Two other human isolates belonged to species *L. inadai*. To the knowledge of the authors this is the first record of the isolation of *L. inadai* from a fatal human case of leptospirosis. One cattle isolate was identified as being from genus *Leptonema*. The *L. inadai* and *Leptonema* isolates were typed by the WHO/FAO/OIE Centre for Reference and Research on Leptospirosis in Brisbane. The other isolates were typed by DRDE in Gwalior, India.

The classification by the typing centres was performed using initial serological investigations followed by DNA sequencing, polymerase chain reaction (PCR) and PCR–restriction fragment length polymorphism (PCR–RFLP) techniques. The detailed reports from the typing centres on the typing protocols used are not

Table II
Results of serotyping of leptospira isolates

Host	No. of isolates serotyped	Leptospira identified		
		Isolates typed to serogroup level	Isolates typed to serovar level	Isolates typed to species level
Cattle	6	Autumnalis (2)		
		Hebdomadis (1)		
		Grippotyphosa (1)		
		Canicola (1)		
		Genus *Leptonema* (1)		
Goats	1	Autumnalis (1)		
Dogs	1	Autumnalis (1)		
Pigs	1	Autumnalis (1)		
Humans	14	Autumnalis (10)	*L. borgpetersenii* serovar Hardjo (1)	*Leptospira inadai* (2)
		Icterohaemorrhagiae (1)		

Figures in parentheses indicate number of isolates typed

discussed in this paper. The results of serotyping of the isolates are presented in Table II.

Discussion

Incidents of leptospirosis in humans and animals have been increasingly reported in India in recent times. In an ecosystem characterised by interdependence of humans and animals, and where contact with the rodent population is unavoidable, each sharing the same source of food, water and shelter, a high incidence of leptospirosis is likely. Despite the modern technologies that have been introduced into agricultural operations, the storage of agricultural produce, particularly in rural areas, is primitive and provides opportunities for the rodent population to flourish. Rodents are the most prolific source of leptospirosis. For example, *Microtus oeconomus* (Tundra vole: a rat-like mammal) has been shown to carry 500 to 800 million leptospira organisms, and approximately 100 million leptospires are excreted in the urine daily (12).

Study of the natural rodent reservoir hosts of leptospires has resulted in 428 isolations of leptospires to date (10, 11). The rodent species *Rattus rattus wroughtoni hinton, Rattus rattus rufiscens*, *Bandicota indica* and *Bandicota bengalensis*, which are the most common rodent species in India, are distributed all over the country. *Rattus hinton* is the variety predominantly found in agricultural fields and *R. rufiscens* inhabits human and animal dwellings. The *bandicota* varieties are ubiquitous, and are found particularly in granaries, animal feed mixing units, storehouses, catering establishments and in underground drainage systems. Rural human dwellings are rarely weatherproof. Urban waste management is not of a good standard, and awareness of the disease in both urban and rural settings is inadequate. A further complicating factor is the practice of barefoot working, which is common among Indian farmers. This list of factors perhaps adequately explains the isolation of most of the known pathogenic strains of leptospires from a diverse range of hosts, including humans.

Our experience with studies of leptospira isolation has revealed that, in addition to whole blood, other clinical samples, such as clotted blood, serum, CSF, urine, aqueous and vitreous humours of the eye, kidney cortex, liver tissue and heart tissue, can also be good specimens from which to isolate leptospires. It was found to be possible to isolate leptospires from a 10-day-old clotted blood sample. This sample of blood was aseptically drawn in a sterile disposable syringe and was dispatched to the PD-ADMAS laboratory from a peripheral centre more than 1,000 km away. This practice of sending whole blood samples drawn in disposable syringes is followed at this laboratory with success. However, fresh blood, free of contamination, less than six hours old, and drawn during the febrile course of the disease, is most suitable for diagnosis by simple dark field microscopy.

Isolation of leptospires from cattle

Leptospira of serogroup Grippotyphosa have been isolated from the kidney cortex and maternal cotyledons of an aborted bovine foetus (Gangadhar and Prabhudas, unpublished data) (Fig. 2); nevertheless, isolation of organisms of serogroups Canicola and Grippotyphosa from cattle is unusual (Table II). Canicola is normally associated with canine species, particularly dogs, whereas Grippotyphosa is usually associated with human infections. These host ranges suggest a complex pattern of cross-infection between species. Only dogs are vaccinated against leptospirosis in India; other domestic animals and humans are not.

The association of leptospirosis with bovine abortions and repeat breeding has been long speculated, but there are very few reports from India or from other countries. The available literature is suggestive but not conclusive. An interesting incident involved the isolation of leptospires from five cows from a village in Karnataka state. All had a history of repeated abortions and are now confirmed repeat breeders. Blood samples from these animals tested negative for bovine brucellosis and infectious bovine rhinotracheitis (IBR). Veterinary health managers routinely suspect brucellosis and IBR to be the main causes of bovine abortions and infertility and rarely investigate beyond these two aetiologies. It is too premature to report leptospirosis as a primary cause of bovine abortion, but the cases discussed above are unlikely to have been mere coincidences and deserve further investigation. Cases of

Fig. 2
Bovine abortion caused by *Leptospira* of serogoup Grippotyphosa
Leptospires were isolated from the kidney cortex and maternal cotyledons of this aborted foetus (Gangadhar and Prabhudas, unpublished data)

abortions and repeat breeding should be critically analysed with leptospirosis in mind as a differential diagnosis.

Canine leptospirosis

The incidence of leptospirosis in dogs is relatively high in India, and canine leptospirosis has great public health significance. (A report from the United States of America described the isolation of leptospires from dogs that did not manifest any clinical signs: 41 out of 500 dogs had leptospira infection and only four had clinical signs [13]). These are the first recorded isolations of leptospira from the North Eastern Hill region of India. The lone isolate from a dog that was identified to serogroup level in the current study belonged to serogroup Autumnalis. Infection with leptospires of serogroup Autumnalis is rare in dogs. Serogroups Icterohaemorrhagiae, Canicola and Pomona more commonly infect dogs in India.

Isolation of leptospira from goats and pigs

In the course of conducting serological studies on *Brucella melitensis*, the PD-ADMAS laboratory attempted the isolation of leptospires from goats, because a few deaths had occurred on the goat farm a few months earlier. Among the 21 leptospires isolated from goats, the lone isolate that was identified was from serogroup Autumnalis. There was no report of clinically affected animals on the farm at the time of sample collection, suggesting that the goat was a healthy carrier of leptospires.

The ICAR Research Complex at Goa has a piggery unit that houses 70 pigs of different age groups. These animals are maintained for training, demonstration and research purposes. As part of a routine examination, 20 randomly collected blood samples from these animals were screened. Two samples revealed the presence of leptospires. The piglets from which these isolations were made were four weeks old and were apparently in good health. They were maintained on the farm up to 16 weeks of age and then slaughtered or sold to private piggeries. At no time did these animals exhibit any signs of ill health, and they demonstrated a normal rate of increase in body weight. It is possible that there is a natural carrier status in pigs for *Leptospira*. As healthy carriers of leptospires, pigs may contribute in passing on the infection to other healthy animals and possibly to humans. Given that pig rearing in rural India occurs in a traditional free-range system close to human habitation, there is a high risk of zoonotic transmission of leptospirosis from urine contamination.

Incidents of human leptospirosis in India

Despite the presence of a good public health system in India, the threat posed by leptospirosis has been overlooked (6). This is probably because:

– leptospirosis mimics the symptoms of other infections and escapes early differential diagnosis

– subclinical infection is common in endemic areas (1, 3, 4, 17)

– there are only a few laboratories in the country that work on leptospirosis

– the preliminary diagnostic tests are not straightforward, and are not easy to perform at the field level

– the organism is difficult to isolate and maintain

– the bacterium has biohazard potential and work with it requires trained personnel.

In this study leptospires were isolated from human patients who presented with diverse clinical manifestations of disease. For example, leptospires were isolated from:

– two patients infected with human immunodeficiency virus (HIV)

– a patient admitted to the National Institute of Mental Health and Neurosciences (NIMHANS) in Bangalore suspected of a neurological disorder, from whom leptospires were isolated from the CSF

– a case of fatal septic abortion

– paediatric patients of 1 to 14 years of age

– a veterinary surgeon with concurrent infection with leptospires and *Brucella abortus* isolated from a blood sample

– a child from the family of a dairy farmer from whom serovar *L.* Hardjo was isolated.

People associated with dairy farming run a considerable risk of contracting leptospira infection from cattle. An infected animal can remain asymptomatic but shed leptospires in its urine for its entire life, thus posing a potential health hazard (16).

Leptospira inadai was recovered from two fatal human cases. One patient, an agriculturist by profession, showed petechiae on the left arm, thigh, hard palate and buccal mucosa, together with conjunctival suffusion and hepatomegaly. Haematological examination showed normocytic, normochromic to microcytic, hypochromic erythrocytes and anisopoikilocytic white blood cells, with a reduced platelet count. As the case advanced, the patient developed altered sensorium, haemoptysis, haematemesis, dyspnoea and hypotension. The patient did not respond to treatment.

The second patient from whom *Leptospira inadai* was isolated was a goldsmith. The patient had a history of headache, fever and abdominal pain for 15 days, with recent onset of giddiness, drowsiness, generalised myalgia, weakness and haematemesis. He also had icterus, hypotension and neck stiffness. Initial haematological examination revealed normocytic normochromic anaemia with thrombocytopenia. Renal insufficiency was evident. A chest X-ray showed bilateral infiltrative lesions. Despite antibiotic and supportive therapy including blood transfusion and haemodialysis, the patient died as a result of adult respiratory distress syndrome (ARDS).

It is premature to attribute these two human deaths to *L. inadai* infection, but the clinical manifestations in the two cases, supported by isolation of *L. inadai*, justify the speculation of a possible aetiology.

On the basis of physiology, morphology, the pattern of flagellar insertion, fatty acid extracts of methyl esters, the G+C ratio and DNA analysis by PCR, *L. inadai* has been classified as pathogenic (5, 14, 24). There is an emerging opinion that *L. inadai* represents a group of leptospira in evolutionary transition from a free-living and non-pathogenic form to a parasitic and pathogenic form (23).

Conclusion

Leptospirosis is a widely prevalent zoonosis in India. The disease burden and the public health risk are enormous. There is a strong case for declaring leptospirosis as a notifiable disease in India. The differential diagnostic examination of all cases of fever and cases of abortion in human patients and livestock should include screening for leptospirosis. The protocol adapted for this study could form the basis for future studies. All food processing and eating establishments should undertake strict surveillance for leptospirosis and should be free of rodent pests. Monitoring of incidents of leptospirosis has received little attention, except at times of outbreaks, and awareness of the disease among the public, and among rural and urban health managers, needs to be upgraded.

Acknowledgements

The authors thank Dr K.M. Bajurbaruah (Deputy Director General) and Dr Lalkrishna (Assistant Director General) of the Indian Council of Agricultural Research, New Delhi; Dr. M. Rajasekhar, Director (retired), PD-ADMAS, Bangalore; Dr L.D. Smythe, Supervising Scientist, WHO/FAO/OIE Collaborating Centre for Reference and Research on Leptospirosis, Brisbane, Australia; Dr H.V. Batra, Joint Director, Division of Microbiology, Defense Research and Development Establishment, Gwalior; Ms Girija, Dr Vidya Gangadhar and Mr Vinay of the Indian Institute of Science, Bangalore, for their support for this research.

Infections à *Leptospira* chez l'homme et les animaux : une menace potentielle pour la santé publique en Inde

N.L. Gangadhar, K. Prabhudas, Sashi Bhushan, M. Rajasekhar, Munasira Sulthana, S.B. Barbuddhe & H. Rehaman

Résumé

La leptospirose est considérée comme l'une des infections zoonotiques les plus répandues dans le monde.

En Inde, pays où la majorité de la population vit de l'agriculture et où les animaux de trait fournissent la principale source d'énergie dans ce secteur, la leptospirose a un taux d'incidence élevé aussi bien chez l'homme que chez les animaux.

Les auteurs font état d'isolements de leptospires pathogènes réalisés en plusieurs endroits de l'Inde à partir de prélèvements humains et animaux. En raison du manque d'installations dans le pays permettant de caractériser les sérotypes, seul le sérogroupe a pu être identifié pour la plupart des isolats. Les auteurs envisagent la possibilité que la leptospirose puisse devenir un grave problème de santé publique en Inde.

Mots-clés

Avortement – Inde – Jaunisse – Leptospira inadai – Porteur – Rat – Zoonose.

La leptospirosis en animales y humanos, posible riesgo de salud pública en la India

N.L. Gangadhar, K. Prabhudas, Sashi Bhushan, M. Rajasekhar, Munasira Sulthana, S.B. Barbuddhe & H. Rehaman

Resumen

La leptospirosis es sin duda una de las zoonosis más comunes del mundo.

En la India, donde los animales proporcionan la fuerza de tracción utilizada en agricultura, que es la actividad principal de la población, hay una elevada incidencia de leptospirosis tanto en animales como en personas.

Los autores informan del aislamiento de leptospiras patógenas en personas y animales en varias regiones de la India. Dado que en el país hay pocas instalaciones adecuadas para la determinación de serotipos, la mayoría de especímenes sólo fueron caracterizados hasta el nivel de serogrupo. Por otro lado, los autores examinan también las probabilidades de que la infección acabe causando un grave problema de salud pública en la India.

Palabras clave

Aborto – Ictericia – Leptospira inadai – Portador – Rata – Zoonosis.

References

1. Ashford D.A., Kaiser R.M. & Spiegel R.A. (2000). – Asymptomatic infection and risk factors for leptospirosis in Nicaragua. *Am. J. trop. Med. Hyg.*, **63**, 249-254.

2. Baskerville A. (1986). – Histological aspects of diagnosis of leptospirosis. *In* The present state of leptospirosis diagnosis and control (W.A. Ellis & T.W.A Little, eds). Martinus Nijhoff publishers, Dordrecht, the Netherlands, 33-43.

3. Bharti A.R., Nally J.E., Ricaldi J.N., Mathias M.A., Diaz M.M., Lovett M.A., Levett P.N., Gilman R.H., Willig M.R., Gotuzzo E., Vinetz J.M. & Peru-United States Leptospirosis Consortium (2003). – Leptospirosis: a zoonotic disease of global importance. *Lancet infect. Dis.*, **3** (12), 757-771.

4. Bovet P., Yersin C., Merien F., Davis C.E. & Perolat P. (1999). – Factors associated with clinical leptospirosis: a population based case control study in the Seychelles (Indian Ocean). *Int. J. Epidemiol.*, **28**, 583-590.

5. Cacciapuoti B., Ciceroni L. & Attard Barbani D. (1991). – Fatty acid profiles, a key for the classification of strains of the family Leptospiraceae. *Int. J. syst. Bacteriol.*, **41**, 295-300.

6. Chaudhry R., Premalata M.M., Mohanthy S., Dhavan B., Singh K.K. & Day A.B. (2002). – Emerging leptospirosis, North India. *Emerg. infect. Dis.*, **8**, 1526-1527.

7. Clerke A.M., Leuva A.C., Joshi C. & Trevedi S.V. (2002). – Clinical profile of leptospirosis in South Gujarat. *J. postgrad. Med.*, **48** (2), 117-118.

8. Gangadhar N.L., Prabhudas K. & Rajasekhar M. (2005). – Isolation of *Leptonema* from rodents and cattle in India. *Indian J. anim. Sci.*, **75** (5), 508-510.

9. Gangadhar N.L. & Rajasekhar M. (1998). – A modified silver impregnation staining technique for leptospires. *Indian vet. J.*, **75**, 349-351.

10. Gangadhar N.L. & Rajasekhar M. (1998). – Record of rodent natural reservoir host of *Leptospira javanica* in Karnataka. *Indian vet. J.*, **75**, 563-564.

11. Gangadhar N.L., Rajasekhar M., Smythe L.D., Norris M.A., Symonds M.L. & Dhont M.F. (2000). – Reservoir hosts of *Leptospira inadai* in India. *Rev. sci. tech. Off. int. Epiz.*, **19** (3), 793-799.

12. Gulubev M.V. & Litvin Vlu (1983). – Population ecology of Leptospires – an experience with evaluation of leptospira count in a carrier and intensity of elimination in urine. *Zhurnal Mikrobiol. Epidemiol. Immunobiol. (Moskva)*, **6**, 60-63.

13. Harkin K.R., Rohto Y.M., Sullivan J.T., Purvis T.J. & Chengappa M.M. (2003). – Comparison of polymerase chain reaction assay, bacteriological culture, and serologic testing in assessment of prevalence of urinary shedding of leptospires in dogs. *J. Am. vet. med. Assoc.*, **222**, 230-233.

14. Hookey J.V., Bryden J. & Gatehouse L.G. (1993). – The use of 16S rDNA sequence analysis to investigate the phylogeny of leptospiraceae and related spirochetes. *J. gen. Microbiol.*, **139**, 2585-2590.

15. Karande S., Gandhi D., Kulkarni M., Baradwaj R., Pol S., Thakare J. & De A. (2005). – Concurrent outbreak of leptospirosis and dengue in Mumbai, India 2002. *J. trop. Pediatr.*, **51** (3), 174-181.

16. Leonard F.C., Quinn P.J., Ellis W.A. & O'Ferrel K. (1992). – Duration of urinary excretion of leptospires by cattle naturally or experimentally infected with *Leptospira interrogans* serovar *hardjo*. *Vet. Rec.*, **131**, 435-439.

17. Levett P.N. (2003). – *Leptospira* and *Leptonema*. *In* Manual of clinical microbiology (P.R. Murray, E.J. Baron & M.A. Pfaller, eds), 8th Ed. ASM Press, Washington, DC, 929-936.

18. Pappachan M.J., Mathew S., Aravindan K.P., Kadar A., Bhargavan P.V., Kareem M.M., Tuteja U., Shukla J. & Batra H.V. (2004). – Risk factors for mortality in patients with leptospirosis during an epidemic in Northern Kerala. *Natl med. J. India*, **17** (5), 240-242.

19. Paster B.J., Devhirst F.E. & Weisburg W.G. (1991). – Phylogenic analysis of the spirochetes. *J. Bacteriol.*, **173**, 6101-6109.

20. Ramakrishnan R., Patel M.S., Gupte M.D., Manickam P. & Venkataraghavan S. (2003). – An institutional outbreak of leptospirosis in Chennai, South India. *J. communic. Dis.*, **35** (1), 1-8.

21. Ratnam S., Sundaraj T. & Subramanian S. (1983). – Serological evidence of leptospirosis in the human population following an outbreak of the disease in cattle. *Trans. roy. Soc. trop. Med. Hyg.*, **77** (1), 94-98.

22. Sethi S., Sood A., PoojaSharma S., Sengupta C. & Sharma M. (2003). – Leptospirosis in Northern India: a clinical and serological study. *Southeast Asian J. trop. Med. public Hlth*, **34** (4), 822-825.

23. Woo T.H.S., Patel B.K.C., Smythe L.D., Symonds M.L., Norris M.A., Wyant R.S. & Dhont M.F. (1998). – Identification of *Leptospira inadai* by continuous monitoring of fluorescence during rapid cycle PCR. *Syst. appl. Microbiol.*, **21**, 89-96.

24. Yasuda P.H., Steigerwalt A.G., Sulzer K.R., Kaufmann A.F., Rogers F. & Brenner D.J. (1987). – Deoxyribonucleic acid relatedness between serogroups and serovars in the family Leptospiraceae with proposal for seven new leptospira species. *Int. J. syst. Bacteriol.*, **37**, 407-415.