Le Infezioni in Medicina, n. 4, 235-240, 2005

Lavori originali

Original articles

Human leptospirosis in the Vicenza area (Italy) from 1990 to 2003: an epidemiological and clinical study

La leptospirosi umana nell'area di Vicenza (Italia) dal 1990 al 2003: studio epidemiologico e clinico

[1]Emma Conti, [1]Luca Lazzarini, [2]Patrizia Reatto, [1]Giulia Tositti, [1]Fausto de Lalla

[1]Department of Infectious Diseases and Tropical Medicine;
[2]Department of Microbiology, Ospedale San Bortolo, Vicenza, Italy

INTRODUCTION

Leptospirosis is the most widespread zoonosis in the world, affecting many species of wild and domestic animals, which are the natural reservoirs [1]. It is caused by spirochaetes of the *Leptospira interrogans* complex that includes more than 200 serovars (serotypes). Transmission to humans occurs through contact with water contaminated with rat urine on open wounds or mucous membranes. Transmission can also occur through close contact with infected animals or from their bites. Following infection, the spirochaetes spread to multiple organs and cause direct damage to parenchymal and endothelial cells. Further damage in the form of immune complex glomerulonephritis and vasculitis then results from the host's immune response (2). Although human infection may occur sporadically throughout the year, it is more common during the summer because of seasonal nature of agricultural and recreational activities and because of water conditions [3]. The true incidence of leptospirosis is largely unknown because infections are frequently unrecognised and misdiagnosed; clinical manifestations can be protean and non-specific, ranging from influenza-like syndrome to severe hepatorenal failure and sometimes to death. In Italy, a decrease of leptospirosis associated with occupational exposures (typically rice-field workers and farmers) has been observed; however, there has been a progressive increase of cases linked to recreational activities, such as fishing, bathing, and canoeing [4].

Until the 1980s, the Veneto region (North-East Italy) and the Vicenza area, in particular, were characterised by high endemism for leptospiral infection [3, 5].

PATIENTS AND METHODS

All cases of leptospirosis admitted to S. Bortolo Hospital in Vicenza, a small town in North-East Italy, during the 14-year period between 1990 and 2003 were retrospectively reviewed. For each case, data including epidemiological, clinical, diagnostic, and therapeutic information were collected. The epidemiological data included sex and age of the patients, risk factors (occupational and recreational exposures, accidental events, or activities around the home), and date of symptoms onset.

Concerning clinical presentation, the following signs and symptoms were considered: fever, headache, arthromyalgia, jaundice, sub-conjunctival haemorrhage, rash, neurological abnormalities, neck stiffness, oliguria, anuria, nausea, asthenia, vomiting, diarrhoea, abdominal pain, hepatomegaly and/or splenomegaly. Laboratory results focused on the blood cell count (leukocytes and platelets), renal and hepatic dysfunction (creatinine and alanine aminotransferase levels), and serum triglycerides levels.

The H.S. (heat stable) Leptospira antigen (Bio-Rad, France) for slide macroscopic agglutination was carried out on admission as a screening test for every patient with possible exposure or clinical data suggesting leptospirosis. The diagnosis was definitively confirmed using the microscopic agglutination test (MAT): suspensions in serial dilutions of 19 different leptospira strains, representing the circulating serovars in Italy, were used as live antigens to detect agglutinating antibodies. A laboratory-confirmed case was defined as a seroconversion between paired serum samples or as the presence of antibodies in a single serum sample with titre ≥1:1000. Clinical cases were defined as those not confirmed by microbiological investigations. Culture was not performed.
Data recorded about treatment included antibiotic therapy (dosage, duration and side effects) and eventual use of corticosteroids. Complementary examinations (electrocardiogram, abdominal ultrasound scans), days of recovery, eventual use of haemodialysis, and outcome were also recorded.
In order to assess if any changes have occurred over the time, the authors compared the current data to a previous study investigating similar parameters between August 1979 and August 1990 [5]. Attention was focused on prevalence, monthly incidence, sex and age of patients, risk factors, serologic findings, and antibiotic therapy.

RESULTS

Overall, 38 cases of leptospirosis were diagnosed and treated at S. Bortolo Hospital between 1990 and 2003. Thirty-three patients were males (87%) and five were female (13%); the mean age was 50 years (range 21-68 years). Thirty-two cases (84%) occurred from June to November, with a peak of incidence in August and with a mean of 2.7 cases per year (Figure 1). The likely source of infection and the mode of transmission were identified in 23 cases (60%). In particular, source of infection was potentially contaminated freshwater in 17 cases (45%) and direct contact with animals or animal urine in six cases (16%). The method of exposure was ascribed to recreational activities (mostly fishing) in 12 cases (32%) and to occupational exposures in seven cases (18%) (six patients were farmers). Three cases (8%) were related to home-contracted infections and one case was due to accidental events. In 14 patients, information on occupation or risk factors was unknown while for one patient information was not available. The most frequently recorded signs and symptoms were fever (95%), muscle pains (74%) (expecially localized to the calf muscles), and jaundice (60%) (Table 1). Two patients with signs of meningeal irritation and one with ataxia underwent lumbar puncture, but cerebrospinal fluid examination was normal. Thrombocytopenia and leukocytosis were reported in 29 (76%) and in 22 patients (58%), respectively; elevations of creatinine levels in 28 patients (74%) and of hepatic enzymes in 27 patients (71%). Hypertriglyceridemia and hepatic steatosis were present in a high percentage of patients (68% and 43% respectively). All laboratory findings are shown in Table 1.
Nineteen cases (50%) were confirmed serologically. Eleven cases were confirmed on the basis of a single specimen and eight cases were confirmed on the basis of two or three specimens.

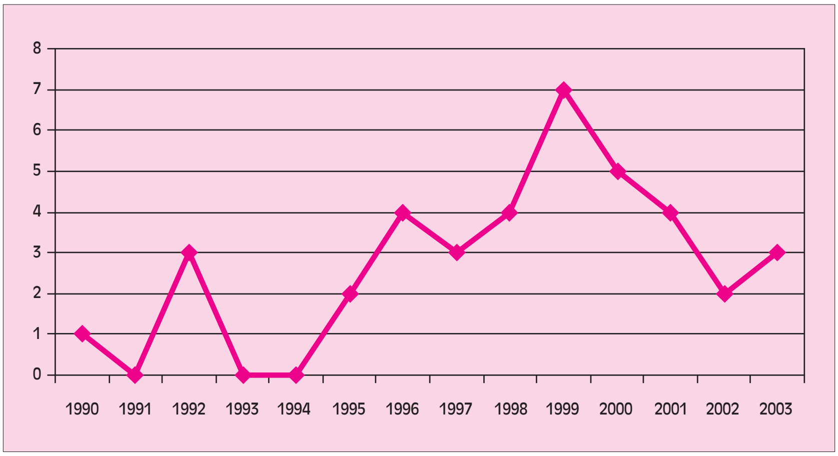

Figure 1 - Number of cases of leptospirosis annually, 1990-2003 (n = 38).

In 16 patients (42%), macroscopic agglutination was positive (four cases on a single serum sample, 12 cases on two subsequent serum samples) and MAT was not performed. One patient had a positive screening test (1:256), but it was not serologically confirmed. Another patient had a negative screening test and MAT was not performed. Finally, one patient had history, exams, and a clinical manifestation greatly suggestive for leptospirosis, but both macroscopic agglutination and serology persistently remained negative. Among the confirmed cases, the etiological serotype was identified in 13 patients (68%): *L. poi* was the most frequent serovar (six cases); *L. copenhageni* and *L. icterohaemorrhagiae* were responsible for two cases, and there was one case each of *L. canicola*, *L. hardjio* and *L. bratislava*. In six samples (31%), the presence of antibodies against more serovars (co-agglutinins) prevented the identification of the presumptively infecting serogroup.

Table 1 - Clinical, laboratory and instrumental results.

Signs and symptom	*No patients (%)*
Fever	36 (95%)
Arthromyalgia	28 (74%)
Hepato- and/or splenomegaly	26 (68%)
Jaundice	23 (60%)
Headhache	14 (37%)
Oliguria/anuria	13 (34%)
Asthenia	12 (32%)
Conjunctival suffusion	9 (24%)
Diarrhea	6 (16%)
Vomiting	5 (13%)
Rash	4 (10%)
Nausea	4 (10%)
Abdominal pain	4 (10%)
Neurological abnormalities	4 (10%)
Neck stiffness	2 (5%)
Laboratory results	
PLTS <150.000x10^9/L	29 (76%)
Creatinine >1.5mg/dL	28 (74%)
AST >37U/L	27 (71%)
TGL >170	26 (68%)
WBC >10.500x10^9/L	22 (58%)
USS	
Hepatic steatosis	10 (43%)
ECG	
Atrial fibrillation	7 (18%)
Alterations ventricular repol.	5 (13%)
I° atrio-ventricular block	3 (8%)

Electrocardiogram (ECG) was routinely performed, while abdominal ultrasound (US) scans were performed in 23 patients. On admission, ECG alterations (atrial fibrillation, alterations in ventricular repolarization, first-degree atrioventricular block) were documented in 15 patients (39%) and hepatic steatosis was observed in ten patients (43%) (Table 1).
In 36 patients, a single intravenous antimicrobial agent was administered: penicillin was the most common antibiotic (28 cases), followed by ampicillin (five cases), ceftriaxone (two cases) and piperacillin-tazobactam (one case). Combination treatment, including a penicillin-class antibiotic, was used in two patients. All patients received lower doses of antimicrobial agents in the fist 24 hours and corticosteroids were not used. Side effects, including Jarisch-Herxheimer reaction (JHR), were not observed. The mean treatment duration was 11 days (range 6-24 days) and hospitalization ranged from 3 to 40 days, with a mean of 14 days. Three patients were transferred to the intensive care unit because of severe Weil's syndrome (hepatorenal failure). A 48-year-old farmer died of renal insufficiency and respiratory failure, but the responsible serotype was not identified.
Thirteen patients (34%) required haemodialysis because of acute renal failure.

DISCUSSION

The incidence of leptospirosis in Italy, as well as that of other industrialized countries, is low, but a significant number of isolated cases are observed in all the infectious diseases centres [6].
In our study, the typical seasonal pattern of leptospirosis was evident. A peak in 1999 (four cases occurred in May and three in November) was observed, but an explanation for such increased incidence was not found (e.g. waterborne outbreak or other epidemiological factors).

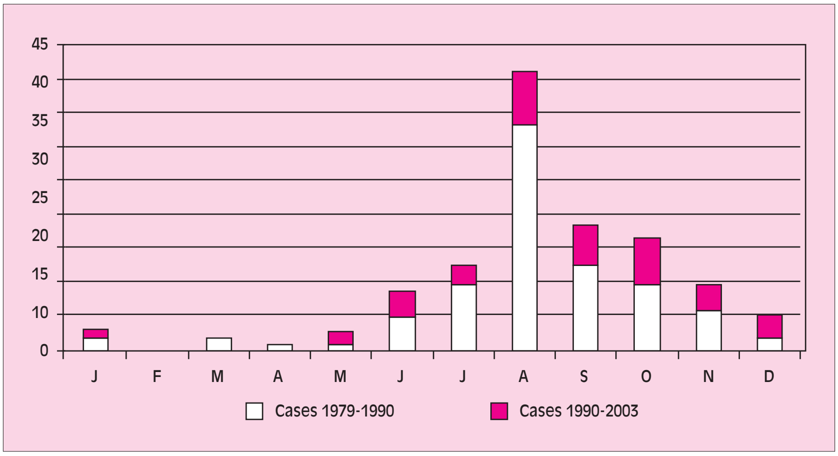

Figure 2 - Comparison of monthly incidence from 1979 to 2003 (n = 124).

The primary symptoms (fever, headhache, arthromyalgia) are very non-specific; for this reason, diagnosis of leptospirosis can be delayed or misdiagnosed, especially when there are no apparent contacts with any source of infection. Subconjunctival haemorrhage is considered by some authors as "pathognomonic" of leptospirosis [7, 8]. Since this sign is present in a minority of patients [9], its usefulness for clinical diagnosis is limited. Our data confirm the low prevalence of such a clinical sign.

Abnormal laboratory findings, such as thrombocytopenia and polymorphonuclear leukocytosis, were present in a high percentage of patients and are also described in the literature. Elevations of both creatinine levels and hepatic enzymes are also frequently found, even in mild cases of disease. The data from this study confirm that leptospiral infection can lead to myocardial involvement with ECG abnormalities in the acute phase, usually atrial fibrillation [10, 11]. As for the presence of hypertriglyceridemia and hepatic steatosis, there are no reports in the literature of such alterations in patients with leptospirosis. A possible explanation is the stimulation of Gram-negative bacteria on cytokines (TNF, IL1) that inhibit lipoprotein-lipase; this enzymatic inhibition would have as effect a reduced clearance of triglycerides and cholesterol from the blood, together with a reduction of lipids storage in the adipocytes [12].

Comparing the current results with the previous local study, some observations seem to be noteworthy. In both periods, males belonging to the working-age population were the most affected (86% in the earlier study and 92% in our study). This is a common feature of leptospirosis. Monthly incidence was similar during the two times periods (Figure 2), whereas total prevalence was significantly reduced: 38 cases (1990-2003) in comparison with 86 cases of the previous years (1979-1990). A probable explanation for this could be improved sanitation, education, and chemoprophylaxis [13].

As described in other reports, an increase of leptospirosis linked to recreational exposure was observed, together with a decline of acquired occupational infections [3, 14, 15]. By contrast, in the earlier analysis, occupational exposure was the predominant risk factor (48% of patients were farmers and construction workers).

In both time periods, macroscopic agglutination was used as a screening test and microscopic agglutination test (MAT) was used as a confirming test. Despite the widespread use of the MAT and other rapid serological tests, in our hospital about 50% of diagnoses were made on the basis of the MAT [16]. Lower sensibility and specificity of macroscopic agglutination are, indeed, counterbalanced because it is easy to perform, inexpensive, and quick. Moreover, the lack of serogroup identification does not influence the treatment strategy.

In the present case series, the most common serovar found was *L. poi* (46%), belonging to *Javanica* serogroup, while during the precedent decades *L. icterohaemorrhagiae* and *L. copenhageni* were the predominant serotypes, accounting for 32 cases (55.2%). Because cross-reactions between serogroups are common with the MAT and because of the small cluster of patients, this pattern of predominance is not a definitive indication of changing local epidemiology [17]. In recent studies, *L. poi* was the prevailing infecting serovar in Italy [18, 19].

No differences were found in the antimicrobial therapy during the two time periods. Intravenous penicillin was the treatment of choice, with a mean duration of 11 and 10 days respectively (p = ns). The mortality rate decreased compared to the previous period, in which five patients died within the first two days of hospitalization (shock, two cases, and pulmonary oedema, one case) or later (bronchopneumonia or intracerebral haemorrhage, one patient each). The Jarisch-Herxheimer reaction (JHR) has a variable occurrence in leptospirosis (20). Our data confirm the results of the earlier local report in which seven of 34 patients (40%) who received full antibiotic dosage showed a Jarisch-Herxheimer-like reaction, whereas the remaining patients (52, 60%), who received lower doses of antimicrobial agents in the first 24 hours combined with short course of corticosteroids, did not [5]. Reduced initial doses of penicillin were sufficient to avoid JHR, without corticosteroid administration.

In conclusion, the epidemiological trend of human leptospirosis in the Vicenza area shows a decreasing incidence, although it is probably underestimated because sub-clinical infections are common and because some cases probably have not been laboratory confirmed. Intravenous penicillin is still the treatment of choice and it should be started as soon as possible in order to avoid lethal complications.

Key Words: leptospirosis, infection, antibiotics, epidemiology.

SUMMARY

Sono stati analizzati retrospettivamente tutti i casi di leptospirosi umana osservati nell'Ospedale S. Bortolo di Vicenza, Italia, nel periodo settembre 1990 - dicembre 2003. Lo scopo dello studio è stato quello di valutare le caratteristiche epidemiologiche, cliniche, diagnostiche e terapeutiche di questa infezione e di confrontarle con un precedente studio locale (1979-1990) per analizzare le eventuali variazioni. Come test di screening è stata utilizzata l'agglutinazione macroscopica, come test diagnostico di conferma la reazione di agglutinazione microscopica (MAT). Il sierotipo eziologico è stato identificato in 13 pazienti (68%) e *Leptospira poi* è risultata la sierovarietà più frequente. Si è osservato interessamento epatico e renale in un'alta percentuale di pazienti (rispettivamente 71% e 74%), interessamento cardiaco nel 39%, ipertrigliceridemia e steatosi epatica rispettivamente nel 68% e 43% dei casi. Un paziente è deceduto per insufficienza renale acuta e insufficienza respiratoria. La penicillina per via endovenosa è stato il trattamento di scelta. Da questo studio è emersa una notevole riduzione della prevalenza (38 casi) rispetto al periodo precedente (86 casi); in entrambi i periodi il sesso maschile è risultato più colpito rispetto al sesso femminile. Nei paesi industrializzati la prevalenza della leptospirosi è in diminuzione, ciononostante questa infezione non è più limitata a specifiche categorie professionali e continua ad essere una malattia potenzialmente fatale, che dovrebbe entrare nella diagnosi differenziale per tutti i pazienti con febbre di origine sconosciuta.

RIASSUNTO

All cases of human leptospirosis observed at the S. Bortolo Hospital, Vicenza, Italy, in the period from September 1990 to December 2003 were retrospectively reviewed. The aim of the study was to define the epidemiological, clinical, diagnostic, and therapeutic aspects of this infection and to compare these with an earlier local study (1979-1990) in order to assess if any changes have occurred over time. The screening test was made using macroscopic agglutination and the diagnosis was definitively confirmed using the microscopic agglutination test (MAT). The etiological serotype was identified in 13 patients (68%) and Leptospira poi *was the most frequent serovar. Hepatic and renal involvements were present in a high percentage of patients (71% and 74%, respectively), cardiac involvement in 39%, and hypertriglyceridemia and hepatic steatosis were observed in 68% and 43% of cases, respectively. One patient died because of acute renal and respiratory failure. Intravenous penicillin was the treatment of choice. A consistent reduction in the prevalence was observed during the time period of this study (n = 38) compared with the previous period (n = 86); males were more affected than females in both time periods. In industrialized countries the prevalence of leptospirosis is decreasing; nevertheless, this infection is no longer limited to specific occupational groups and remains a potential fatal disease that should be included in the differential diagnosis of all the patients with unexplained fever.*

REFERENCES

[1] Kuriakose M., Eapen C.K., Paul R. Leptospirosis in Kolenchery, Kerala, India: epidemiology, prevalent local serogroups and serovars and a new serovar. *Eur. J. Epidemiol.* 13, 6, 691-697, 1997.

[2] Levett P.N. Leptospirosis, In *Principles and Practice of Infectious Diseases* (Mandell G.L., Bennett J.E., Dolin R., Eds), 2005, pp 2789-2794. Elsevier Churchill Livingstone, New York.

[3] Ciceroni L., Stepan E., Pinto A. et al. Epidemiological trend of human leptospirosis in Italy between 1994 and 1996. *Eur. J. Epidemiol.* 16, 79-86, 2000.

[4] Ciceroni L., Pinto A., Benedetti E. et al. Human leptospirosis in Italy, 1986-1993. *Eur. J. Epidemiol.* 11, 707-710, 1995.

[5] Caruso G., Rigoli R., Conz P., Cinco M., Banfi E., de Lalla F. Human Leptospirosis in the Vicenza Area, Italy. *Eur. J. Clin. Microbiol. Infect. Dis.* 11, 77-79, 1992.

[6] Istituto Superiore di Sanità. Epidemiologia delle Malattie Infettive: www.simi.iss.it.

[7] Faine S. Clinical leptospirosis in humans. *Leptospira and Leptospirosis* 1994, Chapter 12. CRC Press, Boca Raton, Florida USA.

[8] Watt G. Leptospirosis. In *Hunter's tropical medicine and emerging infectious diseases* (Strickland G.T., Ed), 2000, 452-458, W.B. Saunders, Philadelphia.

[9] Katz A.R., Ansdell V.E., Effler P.V., Middleton C.R., Sasaki D.M. Assessment of the Clinical Presentation and Treatment of 353 Cases of Laboratory-Confirmed Leptospirosis in Hawaii, 1974-1998. *Clin. Infect. Dis.* 33, 1834-1841, 2001.

[10] Sacramento E., Lopes A.A., Costa E. et al. Electrocardiographic alterations in patients hospitalized with leptospirosis in the Brazilian city of Salvador. *Arq. Bras. Cardiol.* 78, 3, 267-270, 2002.

[11] Rajiv C., Manjuran R.J., Sudhayakumar N., Haneef M. Cardiovascular involvement in leptospirosis. *Indian Heart J.* 48, 6, 691-694, 1996.

[12] Feingold K., Grunfeld C. Role of cytokines in inducing hyperlipidemia. *Diabetes* 41(Supp.2), 97-101, 1992.

[13] Edwards C.N., Levett P.N. Prevention and treatment of leptospirosis *Expert Rev. Anti Infect. Ther*. 2, 2, 293-298, 2004.

[14] Olszyna D.P., Jaspars R., Speelman P. et al. Leptospirosis in the Netherlands. *Ned. Tijdschr. Geneeskd.* 142, 1270-1273, 1998.

[15] Kariv R., Klempfner R., Barnea A., Sidi Y., Schwartz E. The changing epidemiology of leptospirosis in Israel. *Emerg. Infect. Dis*. 7, 6, 990-992, 2001.

[16] Bajani M.D., Ashford D.A., Bragg S.L. et al. Evaluation of four commercially available rapid serologic tests for diagnosis of leptospirosis. *J. Clin. Microbiol.* 41, 2, 803-809, 2003.

[17] Levett P.N. Usefulness of serologic analysis as a predictor of the infecting serovar in patients with severe leptospirosis. *Clin. Infect. Dis.* 36, 4, 447-452, 2003.

[18] Cacciapuoti B., Ciceroni L., Pinto A. et al. Survey on the prevalence of leptospira infections in the Italian population. *Eur. J. Epidemiol*. 10, 2, 173-180, 1994.

[19] Ciceroni L., Pinto A., Cacciapuoti B. Recent trends in human leptospirosis in Italy. *Eur J Epidemiol.* 4, 1, 49-54, 1988.

[20] Friedland J.S., Warrell D.A. The Jarisch-Herxheimer Reaction in Leptospirosis: Possible Pathogenesis and Review. *Rev. Infect. Dis.* 13, 2, 207-210, 1991.