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RECENT TRENDS IN HUMAN LEPTOSPIROSIS IN ITALY

L. CICERONI, A. PINTO, B. CACCIAPUOTI [1]

National Center for Leptospirosis - Department of Medical Bacteriology and Mycology Istituto Superiore di Sanità - 00161 Roma (Italy).

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The recent epidemiological trends of human leptospirosis in Italy were investigated using data collected for the years 1981-1985. A total of 626 hospitalized patients with clinical diagnoses of suspected leptospirosis were reported by hospital centers from several Italian regions. Epidemiological, clinical and seroimmunological data were collected in 517 of these cases and examined by the National Center for Leptospirosis.

Serological findings in 33.5% of these subjects met the criteria for confirmation of the disease. In 21.8% of the subjects, low titer antibodies were detected, which possibly reflected previous leptospiral infections. An early antibiotic treatment of the current infection may also have lowered the seroimmunological response in some of these patients.

In 59.3% of the confirmed cases, modes of transmission were allotted equally between accidental events and recreational or occupational activities. Drinking water from an open air fountain emerged as an uncommon mode of transmission; it was responsible for an outbreak of 33 cases of leptospirosis. In another 37.07% of the subjects, it was impossible to establish the mode of transmission.

Respiratory or influenza-like symptoms were the only clinical signs of illness in 21.2% of the patients with confirmed leptospirosis.

In comparison to the sixties and seventies, the prevalence of infecting serovars showed increasing incidence of infections due to serovars of the Javanica (11.0%) and Australis (11.0%) serogroups and an important decrease in the Bataviae serogroup infections (from 58.8% in rice-field workers in the forties to 0.6% in the years 1981-1985). Sejroe serogroup infections accounted for 4.5 per cent of confirmed cases of leptospirosis.

In 49.7% of subjects with confirmed leptospirosis, cross-agglutination at the same titre with two or more serowars of different sero-groups occurred, thus preventing the identification of the serogroup of the infecting strain.

INTRODUCTION

During the early 1960's in Italy, the leptospirosis morbidity rate in the rice-producing regions, and especially in rice-field workers, from 3,000 to 12,000 cases per annum (2,3).

At the end of the sixties, the mechanization of rice cultivation, the vaccine-prophylaxis measures for the individuals at risk (2), the use of herbicides instead of the manual weeding and the abolition of the transplanation of the young plants that required human labor had almost eliminated the occupational exposure of rice-field workers to *Leptospira* infections.

In the seventies, as a results of these radical changes, a different risk-group pattern began to emerge and there were frequent reports of leptospirosis contracted during recreational activities (5).

[1] Corresponding author.

In the eighties, a sero-epidemiological investigation carried out in the province of Viterbo — an area which seemed to be free of clinical cases of leptospirosis — demonstrated an unexpectedly high prevalence of this infection. About 3% of the urban population and about 12% of the rural population had serovar-specific leptospiral antibodies. On the basis of the observed frequency of seropositive subjects, an incidence of about 200 new cases of infection per year in the adult (14-45 years) population of this province was predicted. This prediction was not, however, confirmed by reports of clinical cases from the hospitals of the area.

The above phenomenon has been ascribed to concomitant clinical, epidemiological and diagnostic factors:

i) the prevalence of asymptomatic and subclinical forms of leptospirosis, and of vaguely defined clinical forms with influenza-like symptomatology;

ii) the failure to perform seroimmunological tests, or the performing of immunological tests that are inadequate to diagnose leptospirosis;

iii) the failure to include leptospirosis in the differential diagnosis of infectious diseases with benign courses.

The present work considers the clinical cases reported to the National Center for Leptospirosis for the years 1981-1985. The purpose of this investigation was to obtain information concerning the epidemiological trend of human leptospirosis in Italy during the 1980's and to compare it to that of the previous 2 decades.

MATERIALS AND METHODS

Clinical and epidemiological data. - Clinical and epidemiological data, collected by means of a questionnaire on hospitalized patients with clinical diagnoses of suspected leptospirosis, were sent to the National Center for Leptospirosis by hospital centers from several Italian regions.

Serological investigation. - Two serum samples from each patient, collected at intervals of 15-40 days, were examined serologically.

The microagglutination test (MA), using suspensions of living leptospires as antigen was used (11).

Leptospiral strains circulating in Italy, belonging to 16 serovars of 12 serogroups of the *Leptospira interrogans* pathogenic species, were chosen as antigens (5, 6, 7): Serovar *icterohaemorrhagiae,* Bianchi 1 strain; *copenhageni,* Wijnberg; *canicola,* Alarik; *pomona,* Mezzano I; *bataviae,* Pavia 1; *grippotyphosa,* Moskva V; *pyrogenes,* Zanoni; *poi,* Poi; *sejroe,* Topo 1; *saxkoebing,* Mus 24; *mini,* Sari; *castellonis,* Castellon 3; *tarassovi,* Mitis Johnson; *australis,* Ballico; *lora,* Riccio 37; *bratislava,* Riccio 2.

Each sample of serum was tested with the single strains at a 1:100 dilution. The positive sera were eventually titrated using interlocking dilutions on basis three: 1:300, 1:1000, 1:3000, 1:10000.

Case classification. - The clinical cases were classified according to the presence and titre of leptospiral antibodies. Three serological classes were chosen: negative sera (titre < 1:100); low-titre positive sera (titre 1:100-1:300); high-titre positive sera (titre ≥ 1:1000).

We defined as *serologically confirmed clinical cases* those with: i) seroconversion (titre < 1:100 in the first serum sample; titre ≥ 1:300 in the second sample); ii. antibody titre ≥ 1:100 in the first and/or in the second serum sample.

We defined as serologically non-confirmed clinical cases: iii) seronegative cases (titre < 1:100); iv. initially low-titre (1:100-1:300), seropositive cases which did not increase in subsequent samples.

RESULTS

During the period 1981-1985 a total of 626 hospitalized patients with clinical diagnoses of suspected leptospirosis were reported to the National Center for Leptospirosis by hospitals from several Italian regions. Epidemiological, clinical and sero-immunological data were collected and examined in 517 subjects.

The serological criteria for confirmation of leptospirosis were fulfilled in 33.5% of patients. In another 21.8% of these subjects low-titre leptospiral antibodies were detected, which could be ascribed either to past infection with leptospira or to an early antibiotic treatment of a current infection which had depressed the seroimmunological response of the host (Table 1).

TABLE 1.
Annual incidence of clinical cases of Leptospirosis.

Year	No. of cases			
	Reported	Confirmed titre ≥ 1:1000 or seroconversion	Non-confirmed titre 1:100-1:300	titre <1:100
1981	111	28	17	66
1982	95	30	10	55
1983	77	29	11	37
1984	147	54	54	39
1985	87	32	21	34
1981-1985	517	173	113	231

The regional distribution of the cases (Table 2) shows a higher incidence in some of the northern and central regions: Veneto, Latium and Emilia-Romagna. Nevertheless, leptospirosis cases have also been reported from southern Italy. In the Marche region, an uncommon outbreak connected with the drinking of fountain water was identified, which had a strong affect on the leptospirosis frequence in that region (6).

The case distribution of confirmed leptospirosis according to sex and age (Table 3) revealed a marked prevalence of male subjects (93.1%). The highest frequency was observed in subjects in the fifth decade of life, and no clinical cases were reported in children ≤ 10 years of age.

Residence in rural areas and open-air occupational and recreational activities were significantly associated with clinical cases of leptospirosis. One-hundred-nineteen of the 173 confirmed cases, lived and/or had occupations and recreational activities in rural environments.

Water contaminated with animal urine, direct contact with animals or with animal urine was established as the probable source of infection in 109/173 cases of confirmed leptospirosis (Table 4). In the other cases the source of infection remained unidentified.

TABLE 2.
Regional distribution of clinical cases of leptospirosis.

Region or country	No. of cases			
	Examined	Confirmed titre≥1:1000 or seroconversion	Non-confirmed titre 1:100-1:300	titre <1:100
Veneto	279	98	43	138
Friuli and Julian Veneto	4	1	1	2
Lombardy	3	2	1	—
Piedmont	1	—	1	—
Liguria	1	—	—	1
Emilia Rom.	74	24	23	27
Tuscany	1	—	—	1
Marche	55	25	17	13
San Marino	3	3	—	—
Umbria	4	2	1	1
Latium	61	7	19	35
Abruzzo	4	1	—	3
Campania	10	2	3	5
Calabria	3	—	3	—
Sicily	1	—	—	1
Sardinia	10	6	1	3
Germany	1	1	—	—
Algeria	1	1	—	—
Peru	1	—	—	1
Total	517	173	113	231

In the subjects with an identified source of infection, the probable mechanisms of transmission were ascribed to accidental events, recreational activities, occupational activities, or infections contracted at home (Table 4).

Among the accidental events, drinking of water that did not come from acqueducts was identified as the uncommon mode of transmission of the above recorded outbreak in the Marche region. Falls into ponds or streams were also associated with accidental contraction of leptospirosis. Among the causes connected with recreational activities, fishing is clearly predominant.

TABLE 3.
Sex and age distribution of 173 clinical cases of Leptospirosis.

Age	Males	Females	Total
0 - 10	—	—	—
11 - 20	12	1	13
21 - 30	22	1	23
31 - 40	32	—	32
41 - 50	35	3	38
51 - 60	31	3	34
61 - 70	15	2	17
≥ 71	8	2	10
not reported	6	—	6
Total	161	12	173

TABLE 4.
Probable source of infection and mode of transmission in 173 clinical cases of Leptospirosis.

Source of infection			Mode of transmission		
Water			*Accidental cause*		
running water	37		drinking water	34	
stagnant water	18		immersion in water	6	
waste water	7		contact with water	3	
drinking water	34		contact with animal	2	
Total		96	Total		45
Animals			*Recreational activity*		
mouse/rat	7		fishing	22	
swine	4		bathing	10	
cattle	1		other	3	
dog	1		Total		35
Total		13	*Occupational activity*		
			Agriculture/animal breeding	18	
			other	5	
			Total		23
			Home-contracted infection		6
Unknown		64	*Unknown*		64

Occupation-linked infections were most frequently seen in persons engaged in agricultural or animal breeding activities (Table 4).

The seasonal variation among the 173 confirmed cases demonstrated the typical leptospiral seasonal course, with a peak during the summer months. Nevertheless, a background of constant morbidity throughout the year was clearly demonstrated (Table 5).

Associated renal, hepatic and meningeal syndromes were observed in a total of 78.8 per cent of confirmed leptospira infections. The remaining 21.2 per cent of subjects had influenza-like or respiratory syndromes (Table 6).

The symptoms which were most frequently observed in the confirmed cases, besides fever, were disorders of the liver, of the urinary tract and of the meninges (Table 6). In a few patients, symptoms affecting the skin, the respiratory system and the alimentary tract, together with fever, were observed.

In the confirmed clinical cases, besides sera with serovar-specific antibodies, sera with antibodies at the same titre against several serovars (co-agglutinins) were observed in 49.7 per cent of cases (Table 7).

The most frequently identified serovars were *icterohaemorrhagiae, copenhageni, poi, bratislava* and *canicola.*

The *bratislava* serovar which had not previously been identified as a cause of human leptospirosis in Italy, was responsible for an outbreak of 33 cases in the town of San Leo. The *poi* serovar appears to be the causatory agent in an increasing number of subjects. Strains from the Sejroe serogroup were responsible for 4.5% of the confirmed cases.

TABLE 5.
Distribution according to the month of onset of 173 clinical cases of Leptospirosis.

Month	Frequency	Percentage
January	4	2.3
February	5	2.9
March	2	1.2
April	2	1.2
May	1	0.6
June	6	3.4
July	58	33.5
August	32	18.5
September	24	13.9
October	24	13.9
November	11	6.3
December	4	2.3
Total	173	100.0

TABLE 6.
Symptoms and clinical forms in 173 cases of human Leptospirosis.

Symptoms	Percentage	Clinical forms	Percentage
Fever	98.6	Icteric	8.2
Hepato and/or splenomegaly	67.1		
Jaundice	61.8	Hepato-renal	40.6
Acute renal failure	61.8		
Arthromyalgias	30.9	Hepato-meningo-renal	10.6
Meningitis	22.9		
Vomiting	17.4	Hepato-meningeal	2.3
Cephalalgy	16.8		
Conjunctivitis	16.8	Meningeal	6.5
Exanthema	6.5		
Cough	6.0	Meningo-renal	3.5
Diarrhoea	4.7		
Asthenia	4.0	Renal	7.1
Gastralgia	3.3		
Bronchopneumonia	2.7	Pulmonary	2.3
Pharyngeal symptoms	2.7		
Dyspnea	2.7	Influenza-like	18.9
Epistaxis	2.7		
Enanthema	2.0		

TABLE 7.
Agglutinins and Co-agglutinins in 173 clinical cases of Leptospirosis.

Serogroup	Serovar	Agglutinins 87 cases frequency (%)	Co-agglutinins 86 cases frequency (%)
Ictero-haemorrhagiae	*ictero-haemorrhagiae*	17 (19.5)	44 (51.2)
»	*copenhageni*	11 (12.6)	33 (38.4)
Canicola	*canicola*	6 (6.9)	26 (30.2)
Pomona	*pomona*	3 (3.4)	2 (2.3)
Bataviae	*bataviae*	1 (1.1)	2 (2.3)
Grippotyphosa	*grippotyphosa*	2 (2.3)	1 (1.2)
Pyrogenes	*zanoni*	1 (1.1)	4 (4.6)
Javanica	*poi*	19 (21.8)	30 (34.9)
Sejoroe	*saxkoebing*	1 (1.1)	2 (2.3)
»	*sejroe*	3 (3.4)	5 (5.8)
Mini	*mini*	3 (3.4)	4 (4.6)
Australis	*ballico*	0 (0.0)	18 (20.9)
»	*bratislava*	17 (19.5)	16 (18.6)
»	*lora*	2 (2.3)	21 (24.4)
Tarassovi	*tarassovi*	0 (0.0)	0 (0.0)
Ballum	*castellonis*	1 (1.1)	0 (0.0)

DISCUSSION

Several problems in both clinical and serological diagnosis of leptospirosis have emerged from this study.

Because of the variable clinical presentation of human leptospirosis, diagnosis must always be supported by laboratory and epidemiological investigations of the source of infection and the mode of transmission of the organism. The observation of acute febrile clinical forms associated with epidemiological data consistent with a leptospiral infection must be considered a sufficient motive to establish a differential diagnosis of leptospirosis and to test for leptospiral antibodies by serological tests.

The MA test, performed with live strains of leptospires circulating in the region, has been chosen for two reasons: i) the test is the reference test for leptospirosis; ii) serovar-specific antigens, one or more of which are common to all the serovars within a serogroup (9), are located on the outer-membrane of live leptospires. These antigens are reduced or completely absent in leptospires inactivated by physical or chemical methods.

Accordingly, titration of surface agglutinins by the MA test is reliable for the identification of the serogroup, and even the serovar, of the infecting strain, if more than one serovar in a serogroup is used as the diagnostic antigen.

The clinical diagnosis was serologically confirmed in only 33.5% of cases. A further 21.9% of the cases were found to have low antibody titres (1:100-1:300) which were not considered sufficient to confirm the clinical diagnosis. The other cases (44.6%) were seronegative (titre < 1:100).

The cases of leptospirosis recognized during the years 1981-1985 show that the epidemiological course of the disease in Italy has changed. Presently, the incidence is not confined to groups of subjects who work in rural areas.

The risk of infection does not seem to be limited to the regions of northern and central Italy: original cases of leptospirosis have been reported from different areas of southern Italy.

The frequency of the infecting serovars has significantly changed when compared to the years when rice-field leptospirosis was predominant. The emergence of new serovars of the Javanica and Australis serogroups, a strong reduction of infections due to the Bataviae group serovars, and the persistence of infectious caused by serovars belonging to the Icterohaemorrhagiae serogroup have been noted.

The infections caused by the Australis serogroup are second in frequency in our present case-report, but they are almost exclusively related to the clinical cases of the outbreak due to ingestion of contaminated water (6). Data about clinical cases of leptospirosis reported during 1986, show the endemic presence of infections caused by Australis serogroup leptospires: such pathogens must therefore be considered as a new emerging serogroup in the cause of human leptospirosis in Italy. The frequency of the cases of leptospirosis caused by the Javanica serogroup must also be monitored, since previously such cases were only 0.4%, while presently they account for 21.8% of total cases. A total of 4.5 per cent of confirmed cases of leptospirosis were due to strains of the Sejroe serogroup. The serovars *saxkoebing* and *sejroe* in the Sejroe serogroup were used as antigens in MA test. The serovar *hardjo* is presently added to the serovars used in the test.

Correlations between clinical forms (Table 6) and infecting serovars (Table 7) were not statistically significant because of the low frequencies of subjects infected by some of the circulating serovars in the region.

The absence of clinical cases in children below 10 years of age cannot be explained by the known lack of reactivity of young patients under two years of age to the lipo-polysaccharidic antigens, since the age range involves subjects up to 10 years of age.

We believe that the identified clinical cases represent only a small portion of leptospiral infections. This may be due to either the lack of serological diagnosis of the disease or to the unsatisfactory sensitivity of the methods. On the other hand — especially in patients who live in areas at risk — the clinical symptomatology of the leptospiral infection may be mild and non-specific, similar to those observed in the rice-pickers, during the 1960's.

The presence of co-agglutinins — at the same titre — against serovars belonging to different serogroups, observed in 49.7 per cent of subjects with confirmed leptospirosis, seems to be quite typical and prevents the identification of the infecting strain even at the serogroup level. The formation of co-agglutinins observed in human clinical cases is not reproducible in animal models after experimental infections. The appearance, in man, of antibodies against intergroup-specific leptospiral antigens confirms previous results, reported by several authors (1, 4, 8, 10).

On the whole, the present study indicates that leptospirosis is an endemic infection in many Italian areas which may, in certain particularly favourable circumstances, manifest itself in severe clinical cases.

For the verification of clinical cases of leptospirosis, it seems essential that laboratories capable of performing serological diagnosis with the mi-

croagglutination reference method be set up, at least on a regional level (9, 11). The sera of patients with suspected leptospirosis can thus be sent to these laboratories for serological confirmation. To this end, the National Center for Leptospirosis of the Istituto Superiore di Sanità has trained personnel from hospital and university laboratories to make Regional Reference Centers for Leptospirosis.

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