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Pulmonary haemorrhage as a predominant cause of death in leptospirosis in Seychelles

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Abstract

We examined the cause of death during a 12-month period (1995/96) in all consecutive patients admitted to hospital with leptospiral infection in Seychelles (Indian Ocean), where the disease is endemic. Leptospirosis was diagnosed by use of the microscopic agglutination test and a specific polymerase chain reaction assay on serum samples. Seventy-five cases were diagnosed and 6 patients died, a case fatality of 8%. All 6 patients died within 9 days of onset of symptoms and within 2 days of admission for 5 of them (5 days for the 6th). On autopsy, diffuse bilateral pulmonary haemorrhage (PH) was found in all fatalities. Renal, cardiac, digestive and cerebral haemorrhages were also found in 5, 3, 3 and 1 case(s), respectively. Incidentally, haemoptysis and lung infiltrate on chest radiographs, which suggest PH, were found in 8 of the 69 non-fatal cases. Dengue and hantavirus infections were ruled out. In conclusion, PH appeared to be a main cause of death in leptospirosis in this population, although haemorrhage in other organs may also have contributed to fatal outcomes. This cause of death contrasts with the findings generally reported in endemic settings.

Keywords: leptospirosis, case fatality, pulmonary haemorrhage, Seychelles

Introduction

Human leptospirosis, which is caused by *Leptospira* pathogenic species, occurs mostly after contact, often through skin wound, with soil or water contaminated by urine of infected animals. It is an acute febrile illness with protean manifestations so that clinical diagnosis is difficult. Its severity varies from mild to rapidly fatal. Severe forms are characterized by hepatic involvement, acute renal failure (ARF), carditis, and haemorrhagic syndrome; case fatality varies from a few percent to 25% (GAUTHIER *et al.*, 1969; FAINE, 1994). Haemorrhagic presentation of leptospirosis can easily be mistaken for dengue haemorrhagic fever (DHF; dengue epidemics occurred in 1979 in Seychelles and in 1993 in the neighbouring Grand Comoro Island) or haemorrhagic fever with renal syndrome (HFRS) caused by Old World hantavirus infections (KIM *et al.*, 1985). Definite diagnosis of leptospirosis relies essentially on biological tests. The microscopic agglutination test (MAT) currently represents the reference test of serological diagnosis, but rarely helps diagnosis during the first week of the disease when titres have not yet largely increased (FAINE, 1994). In contrast, techniques that detect leptospiral DNA by polymerase chain reaction (PCR) are more informative during the first few days of the disease (BROWN *et al.*, 1995; MÉRIEN *et al.*, 1995).

Leptospirosis is endemic in Seychelles. Previous reports indicated a high annual incidence ranging from 45 to 101 per 100 000, and frequent hepatic, renal, and haemorrhagic complications (PINN, 1992; YERSIN *et al.*, 1998).

In this report we examine the cause of death in a series of consecutive acute cases identified from a prospective surveillance study conducted over 1 year, at a period during which haemodialysis was available and may have reduced mortality due to renal failure.

Materials and Methods

Study area and population

The study area covered the whole territory of Seychelles (445 km^2), a tropical country which consists of 115 islands situated just south of the equator in the Indian Ocean. The population of 74 331 concentrates on 3 islands with about 90% on the main island, Mahé.

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Study design

The study was a population-based surveillance conducted between 1 April 1995 and 31 March 1996. The detailed study design was reported previously (YERSIN *et al.*, 1998). In brief, all doctors of the country were asked to refer during this period any patient who had fever or any of the following signs or symptoms: myalgia, tender liver, jaundice, ARF, bleeding tendency, radiological lung infiltrates, and meningism which could not be accounted for by a definite diagnosis other than leptospirosis. All patients were submitted to a questionnaire investigating demographic, socio-economic, occupational and environmental variables [methods and results are detailed elsewhere (BOVET *et al.*, 1999)]. Definite leptospirosis diagnosis was based on serology and PCR assay with blood being collected as soon as the patient was enrolled in the study (on admission for most patients) and 2–4 weeks later, except for the patients who died before collection of the second sample. All patients who died were autopsied. Patients included in the study received a course of benzylpenicillin. Informed consent was obtained from the patients or from relatives in the case of early death of the patient. The study was approved by the Ministry of Health of Seychelles.

Laboratory methods

Biochemical tests were done at Victoria Hospital laboratory and maximum values observed during the hospital stay were considered in this study. For serology and PCR assay, sera were immediately stored at −20° Celsius and submitted to diagnostic tests within 3–40 months at Institut Pasteur, Noumea, New Caledonia (leptospirosis and dengue) and at University Hospitals Leuven, Belgium (hantavirus).

MAT was performed by standard methods (FAINE, 1994) using a battery of 24 live antigens (provided by the WHO Collaborating Centre for Leptospirosis, Institut Pasteur, Paris, France) representing all major serogroups including the recently described new serogroup Hurstbridge (PEROLAT *et al.*, 1998). The PCR assay was performed as previously described (MÉRIEN *et al.*, 1995), with the amplification of a 331-bp fragment designed from the 16S rRNA gene of *Leptospira* spp. followed by a dot blot hybridization. Definite acute leptospirosis was defined if the MAT demonstrated a seroconversion, i.e., a negative first serum sample and a titre $\geq$1:100 in the second sample; or if the MAT showed a $\geq$ 4-fold increase in antibody titre in subsequent samples; or if the PCR was positive in 1 serum sample.

The serogroup giving the highest titre by the MAT in serial samples was considered to be the presumptive infecting serogroup (FAINE, 1994).

The sera from fatalities (except 1 because of shortage of serum) and from other patients with pulmonary haemorrhage (PH) were examined for dengue and hantavirus infections. To detect dengue virus infection, sera were tested for total antibodies by haemagglutination inhibition assay (HIA) (CLARKE & CASALS, 1958), for IgM antibodies by immunocapture Mac-ELISA (BUNDO & IGARASHI, 1985), and 4 sera from fatalities (shortage of serum for 2 cases) by reverse transcriptase-PCR and semi-nested PCR (LANCIOTTI *et al.*, 1992). To detect hantavirus infection, sera were tested for IgG and IgM antibodies by recombinant nucleocapsid protein-ELISA (Hantaan strain 76-118 and Puumala strain Cg 18-20) (IVANOV *et al.*, 1988; ZÖLLER *et al.*, 1991). Sera were also tested by strip immuno-blot assay using an IgG-format as a variant of Western blot, using as antigen the recombinant-expressed truncated immunodominant peptide of the hantavirus nucleocapsid protein amino-terminus of SEO 80/39, resp. of PUU P360 virus, applied to a nitrocellulose membrane (courtesy of Professor B. Hjelle, University of New Mexico, USA).

Definition of pulmonary haemorrhage

PH was defined in fatalities on autopsy findings based on visual inspection (haemorrhagic congestion and/or consolidation) and conventional microscopy (intra-alveolar and interstitial haemorrhage). PH in non-fatal cases was defined as concomitant haemoptysis and radiological lung infiltrates reported by a radiologist and 2 physicians.

Statistical analysis

Student's t-test and χ^2 test were used to compare continuous and categorical variables, respectively. Two-tailed P values below 0·05 were considered to indicate statistical significance.

Case report and Results

Case report

The history of this patient is reported in detail because of its characteristic clinical presentation and its remarkable dramatic evolution.

In August 1995, a 23-year-old man was brought dead to the emergency room of Victoria Hospital. He had never been sick until 6 days before, when fever, headache, myalgia and low back pain started. The initial symptoms were treated, after medical advice, using paracetamol (acetaminophen). On day 4, diarrhoea and abdominal pain occurred, followed the day after by cough and several episodes of haemoptysis. Relatives noticed a yellow discoloration of his eyes. On the 6th day, he was referred in emergency to hospital and died during transportation. This young man was working as a barman and used to garden around the home. He used untreated water for drinking and for washing. Relatives reported that rats were present around his house and sometimes inside the house. Ten days before his death, he received a laceration on his hand. Autopsy revealed severe diffuse bilateral PH. Multiple haemorrhages were also seen in the heart, the kidneys and the stomach. The liver showed degenerative and necrotic changes. The MAT gave titres of 1:1600, 1:400, 1:400 and 1:200 for the serogroups Hurstbridge, Hebdomadis, Semaranga and Icterohaemorrhagiae, respectively, and the PCR assay was positive (Case 2, Table 1).

Results

Because only 1 serum was suitable for seroanalysis and PCR was negative, 5 eligible patients (none of them died) were excluded so the study included 124 out of 129 eligible patients. Leptospirosis was diagnosed in 75 patients among whom 6 died, a case-fatality rate of 8·0%. Death occurred 2–9 days after the onset of symptoms and within 48 h of admission for 5 of them and 5 days for the 6th.

The 6 fatalities had only 1 serum available for diagnostic tests as they died before a second sample could be taken and PCR assay was positive in all of them. MAT antibody titres for serogroups not listed on Table 1 were negative. The tested sera were negative for acute dengue and hantavirus infections.

Three fatalities had high-risk occupational activities for leptospiral infection during the 15 days before the onset of symptoms (2 were farmers and 1 worked in forest and field), 2 had medium-risk activities (cleaning and gardening around the habitat) and 1 had low-risk activities (staying at home because of handicap). All but 1 case had foot or hand wounds during the 15 days before the onset of symptoms. Rats were present around the habitat and/or at work areas in all 6 cases, and were seen even during daytime by 5 of them.

Selected clinical, pathological and laboratory characteristics in the 6 fatalities and in the 69 non-fatal leptospirosis cases are presented in Table 2. Compared to the non-fatal cases, the 6 fatalities had significantly higher values for urea nitrogen, total bilirubin, and tended to have higher values for white blood cells, creatinine, and creatine kinase and lower values for platelets and gamma-glutamyl transferase. Chest radiograph was normal in 2 cases, showed mixed nodular lesions and confluent air-space consolidation in middle and lower fields of both lungs in 1 (Figure), and multiple, predominantly small nodular densities in the lower field of the left lung in 1. Electrocardiogram demonstrated atrial fibrillation in 1 case, ST segment depression in 1 and was normal in 2. Chest radiograph and electrocardiogram were not obtained in 2 cases

Table 1. Microscopic agglutination test (MAT) titres and polymerase chain reaction (PCR) assay results in fatal leptospirosis cases in Seychelles

	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6
	Day of illness since onset of symptoms					
Serogroup	7	7	9	2	5	4
Semaranga	0	1:400	1:100	0	0	1:200
Hurstbridge	1:200	1:1600	1:100	0	1:100	1:200
Icterohaemorrhagiae	0	1:200	1:3200	0	1:100	0
Pyrogenes	0	0	0	0	1:100	0
Hebdomadis	0	1:400	0	0	0	0
Australis	0	0	1:1600	0	0	0
Autumnalis	0	0	1:1600	0	0	0
Ballum	0	0	1:1600	0	0	0
Cynopteri	0	0	1:800	0	0	0
Louisiana	0	0	1:6400	0	0	0
PCR	+	+	+	+	+	+

Table 2. Selected clinical, laboratory, and pathological characteristics in 6 fatal and 69 non-fatal leptospirosis cases in Seychelles

Variable	Fatalities ($n = 6$)[a]	Non-fatal cases ($n = 69$)[a]	P
Age (years)	53 ± 17·0	34 ± 14·8	0·004
Sex (M/F)	6/0	4·8/1·0	NS
Fever	100%	96%	NS
Headaches	67%	81%	NS
Myalgia	67%	62%	NS
Cough	50%	38%	NS
Haemoptysis	50%	12%	NS
Meningism	0%	12%	NS
Disease duration before admission (days)	4·5 ± 2·4	3·9 ± 2·1	NS
Duration of admission (days)	1·8 ± 1·7	7·5 ± 3·2	0·000
Infiltrates on chest radiograph	50% (2/4)	19% (13/68)	NS
ECG abnormalities	50% (2/4)	31% (21/68)	NS
Erythrocyte sedimentation rate (mm/h)	72 ± 47·5	68 ± 38·1	NS
Haemoglobin (g/dL)	13·4 ± 2·9	12·8 ± 2·2	NS
White blood cells (×1000/mm^3)	17·6 ± 10·4	12·9 ± 6·6	NS
Neutrophils (%)	87 ± 4·3	78 ± 4·3	NS
Platelet count (×1000/mm^3)	62 ± 42·5	112 ± 82·3	NS
Prothrombin time (INR)	1·4 ± 0·4	1·1 ± 0·2	NS
Sodium (mmol/L)	139 ± 13	135 ± 5	NS
Potassium (mmol/L)	3·5 ± 0·8	3·7 ± 0·6	NS
Urea nitrogen (mmol/L)	24·8 ± 17·8	10·1 ± 10·3	0·005
Creatinine (μmol/L)	343 ± 130	191 ± 277	NS
Total bilirubin (μmol/L)	264 ± 69	125 ± 75	0·018
Aspartate aminotransferase (U/L)	83 ± 63	89 ± 73	NS
Alanine aminotransferase (U/L)	52 ± 33	62 ± 54	NS
Alkaline phosphatase (U/L)	213 ± 69	171 ± 74	NS
γ-Glutamyl transferase (U/L)	42 ± 13	126 ± 177	NS
Creatine kinase (U/L)	1889 ± 2011	940 ± 1982	NS
Autopsy			
Pulmonary haemorrhage	100%	12%[b]	
Renal haemorrhage	83%	NA	
Cardiac haemorrhage	50%	NA	
Digestive haemorrhage	50%	NA	
Cerebral haemorrhage	17%	NA	

[a]Continuous variables are expressed as mean ± SD; categorical variables are expressed as proportion in percent.
[b]Defined as concurrent haemoptysis and pulmonary infiltrates on the chest radiograph.
NS, not significant; NA, not available.

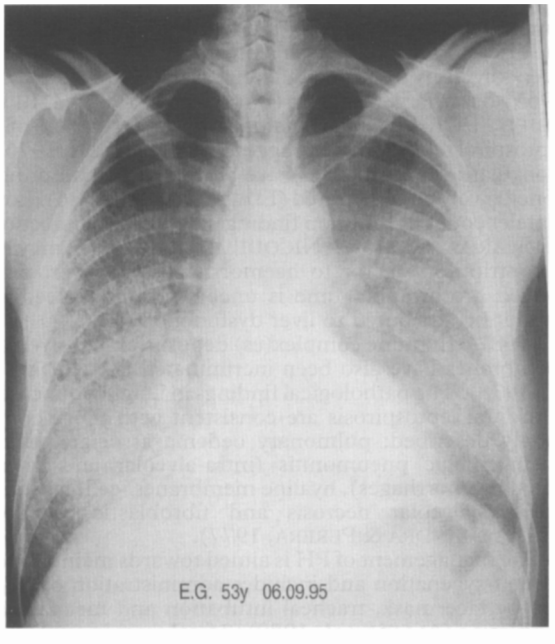

Figure. Radiograph of the chest in Case 3 showing bilateral multiple lungs infiltrates.

because of early death (death occurring in these cases before admission and within 2 h of admission, respectively).

In the 6 fatalities autopsy showed diffuse bilateral PH, which was considered by the pathologists to be the cause of death. In addition, Case 1 had pericardial petechiae and acute cortical necrosis of the kidneys. Case 2 presented degenerative and necrotic changes of the liver, multiple haemorrhages in the heart, the kidneys and the stomach. Degeneration of distal and proximal convoluted tubules and multiple foci of haemorrhage in both kidneys were seen in Case 3. Case 4 had cardiac interstitial oedema, oedema and petechial haemorrhages in the kidneys and the pancreas, and soft congested liver. Case 5 presented foci of haemorrhage in the myocardium, the kidneys, the liver, the adrenal glands, the cerebral ventricular walls, and the digestive mucous membrane. Case 6 had cardiac interstitial oedema, patchy ulcerative oesophagitis, bleeding ulcers in the small intestine, and oedematous kidneys with petechial haemorrhages.

Eight of the 69 non-fatal leptospirosis cases had PH defined as concurrent haemoptysis and pulmonary infiltrates on the chest radiograph (no statistical test provided, as PH definition differs in fatal and non-fatal cases). The presumptive infecting serogroup was Icterohaemorrhagiae in 5 and undetermined in 3 of them. All 8 sera were negative for acute dengue and hantavirus infections.

Discussion

In the 1-year study period, 75 cases of acute leptospirosis were diagnosed (an incidence of 101/100 000), among whom 6 died (a case-fatality of 8%). Noticeably, the 6 fatalities had positive PCR assay, demonstrating the value of this diagnostic tool during the early stage of the disease when the serodiagnosis is not decisive (BROWN *et al.*, 1995; MÉRIEN *et al.*, 1995). Cases and fatalities occurred throughout the year, confirming the endemic nature of the disease in Seychelles. A separate analysis showed that risk factors associated with the disease in Seychelles were mostly related to the environment (BOVET *et al.*, 1999) and typically those found in other tropical settings (FAINE, 1994).

PH seemed to be the main cause of death in all 6 fatalities. All 6 fatalities had PH ascertained by autopsy, with 5 developing clinical symptoms and signs of acute respiratory failure and the 6th, who died before admission, was reported to have had haemoptysis. Respiratory distress among the 5 fatalities occurring in hospital was associated with haemoptysis in 2, with 1 presenting a fulminant course resulting quickly in death, and the other presenting cough, breathlessness and chest pain. Respiratory distress in the 3 other fatalities occurring in hospital was characterized by restlessness, breathlessness, tachypnoea, and cyanosis. Arterial blood gas determination to quantify severity of respiratory failure was not available. Autopsy based on visual inspection and conventional microscopy (more specific investigations were not available) demonstrated haemorrhagic congestion and/or consolidation of the lungs in all 6 patients. Haemorrhages were also present in the kidneys of 5, in the myocardium and the digestive mucosa of 3, and in the brain of 1 patient. However, even if the haemorrhagic lung involvement was visually predominant, haemorrhages in other organs (e.g., heart, brain) cannot be excluded as contributory causes of death.

PH developed on a rapid to fulminant mode, with 5 cases dying within 48 h of admission and 1 on the 5th day of admission. In addition, the duration of symptoms before admission was short (1–8 days, average of 4·5 days) and was not different from that for non-fatal cases. It is unlikely that any patient delayed hospital admission as there are no financial barriers (free health care in Seychelles) and transportation to the main hospital does not exceed 2 h from the remotest locations of the main islands. Chest radiograph done 5 and 40 h before death was normal in 2 of 4 cases. It has already been reported that lesions on chest radiograph do not always coincide with respiratory symptoms (PARK *et al.*, 1989). Interestingly, platelet count and prothrombin time did not substantially differ between fatal and non-fatal cases. Other tests to investigate coagulation disorders were not available. No sign, symptom or biological test (except urea nitrogen and total bilirubin) appeared to be associated with fatal PH, although the small number of fatalities conveys only limited statistical power to identify such relationships.

As only 1 serum sample was available in the 6 fatalities, the infecting serogroup(s) could not be determined by the MAT because of common cross-agglutination between leptospiral serogroups. But Icterohaemorrhagiae was the presumptive infecting serogroup in 5 patients with non-fatal PH.

The increased occurrence of jaundice and renal failure in fatal compared to non-fatal cases can be interpreted as a reflection of the severity of the disease. PH concomitant with jaundice or renal failure has been reported (BOURDAIS *et al.*, 1988; NICODEMO *et al.*, 1997). However, TREVEJO *et al.* (1998) reported recently that death in epidemic leptospirosis with PH in Nicaragua occurred without any jaundice and without renal manifestations; no laboratory data or necropsy findings were given to assess the degree of hepatic and/or renal involvement in this patient series.

Eight non-fatal cases were likely to have some PH based on haemoptysis and pulmonary infiltrates on the chest radiograph. Our minimal criteria to define PH in non-fatal cases probably underestimate the number of non-fatal cases with PH as COURTIN *et al.* (1998) reported that broncho-alveolar lavage may demonstrate PH in patients without haemoptysis or with normal chest radiograph.

Dengue and Old World hantavirus infections can mimic perfectly the classical clinical presentation of leptospirosis and their complicated forms, respectively known as DHF and HFRS, can mimic leptospirosis with PH. Aetiological viral agents of these 2 conditions are widely distributed in tropical countries but dengue and hanta viruses could be ruled out in both the fatal and non-fatal leptospirosis cases with PH.

The cause of death in leptospirosis in Seychelles has largely changed since haemodialysis has been available in 1992. ARF and septicaemia (as a complication of peritoneal dialysis), which were frequent causes of death previously, did not cause death in our present series (8 of our 75 patients had to undergo haemodialysis including 1 fatal case, and 4 non-fatal cases with PH). In 1994, 2 fatal leptospirosis cases were reported and death was attributed to PH as ascertained by autopsy, with jaundice and ARF in one case and ARF alone in the other.

Classically, death in leptospirosis has been related to ARF and haemorrhagic syndrome (FAINE, 1994). Cardiac complications have been less frequently reported (PERTUISET *et al.*, 1988; EVERARD *et al.*, 1995) but cardiac involvement has not been always actively looked after. However, pulmonary manifestations in leptospirosis have been well recognized (POH & SOH, 1970) but massive haemoptysis and acute respiratory distress syndrome have been rarely reported (HEATH *et al.*, 1965; TURNER & WILLCOX, 1989) except in severe cases of leptospirosis (RAGNAUD *et al.*, 1987; PARK *et al.*, 1989) or during epidemic settings in Korea (SHIM *et al.*, 1980), Brazil (RIOS-GONÇALVES *et al.*, 1990) or Nicaragua (TREVEJO *et al.*, 1998). It may be difficult to relate leptospirosis to PH in cases where fever and respiratory symptoms are not found together with typical signs of liver or renal impairment (TREVEJO *et al.*, 1998; O'NEIL *et al.*, 1991). Our findings indicate that the diagnosis of leptospirosis should be considered for all patients with fever and haemoptysis or respiratory distress syndrome in regions where leptospirosis is endemic.

Physiopathology of the pulmonary complications in leptospirosis is not well understood. Respiratory symptoms occur usually during the leptospiraemic phase of the disease. The presence of leptospires in lung tissue has been diversely reported, either rarely (HEATH *et al.*, 1965) or in large number (PARK *et al.*, 1989; ZAKI & SHIEH, 1996). Capillary damage by direct action of a leptospiral toxin has been suggested (POH & SOH, 1970). Coagulation disturbances have been postulated but not conclusively demonstrated (EDWARDS *et al.*, 1986). Low platelet count, a common finding in leptospiral infection (EDWARDS *et al.*, 1982; NICODEMO *et al.*, 1997), may be a contributory factor to haemorrhage. However, prolonged prothrombin time is uncommon and bleeding cannot be attributed to liver dysfunction (RAGNAUD *et al.*, 1987). Immune complex(es) deposits on the alveolar membranes have also been incriminated (DAOUDAL *et al.*, 1978). The pathological findings in lungs of our cases with fatal leptospirosis are consistent with those commonly described: pulmonary oedema associated with haemorrhagic pneumonitis (intra-alveolar and interstitial haemorrhages), hyaline membranes, cellular infiltration, alveolar necrosis and fibroblastic reaction (RAMACHANDRA & PERERA, 1977).

The management of PH is aimed towards maintaining tissue oxygenation and includes administration of oxygen by face mask, tracheal intubation and mechanical ventilation (ALLEN *et al.*, 1989) (2 fatal cases in our series had been mechanically ventilated). Transfusions of platelets or fresh whole blood are likely to help control

of the associated coagulopathy. Successful administration of steroids in severe bleeding has been anecdotally reported (KAHN, 1982) but not confirmed experimentally (YUKAWA *et al.*, 1994). Penicillin is the antibiotic of first choice in leptospirosis (WATT *et al.*, 1988) and resistance to this drug has not been reported so far. Further studies are needed to clarify the mechanisms resulting in pulmonary inflammation and haemorrhage and the appropriate measures to prevent and control PH in leptospirosis.

Conclusion

PH appeared to be the major contributory cause of death in leptospirosis in Seychelles. This cause of death contrasts with findings generally reported. PH appeared to run on a fulminant mode and death occurred within a few days of symptom onset.

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