LEPTOSPIROSIS IN SOUTH AFRICA

THE OCCURRENCE OF CASES OF LEPTOSPIRAL MENINGO-ENCEPHALITIS ON THE WITWATERSRAND

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Leptospiral jaundice of human beings is a rare disease in South Africa. Buchanan,[1] who had had considerable experience in the study of this condition in Britain, systematically tested over 200 South African cases of jaundice for evidence of infection with *Leptospira icterohaemorrhagiae*, but found none. He also examined 231 rodents, including 212 black rats (*Rattus rattus*), 8 gerbils (*Tatera*) and 3 striped mice (*Rhabdomys pumilio*) captured in and around Johannesburg, and 8 brown rats (*Rattus norvegicus*) from Durban, but detected no sign of leptospiral infection. He observed leptospirae in samples of stagnant water, but these produced no ill-effects in inoculated guinea pigs and were therefore presumed to be non-pathogenic.

A systematic study of the other leptospiral infections in animals in South Africa has not yet been carried out, but Malherbe and Kashula[2] have reported on the occurrence of leptospirosis in dogs in South Africa. They noted that dogs were frequently seen presenting the syndrome of

Stuttgart disease, often associated with severe kidney damage, and this responded readily to treatment with penicillin. Leptospirae were isolated from the blood, urine and organ emulsions of sick dogs by inoculation into guinea pigs, and demonstrated by the dark-field examination technique. Six dogs which had shown clinical symptoms of leptospirosis were bled after recovery and their sera tested by complement fixation and agglutination lysis tests. One serum gave a positive reaction with *L. canicola* and 2 with *L. sejroe*. This paper[2] recorded for the first time that canine leptospirosis existed in the Union of South Africa. In the discussion which followed the presentation of this paper at the 48th Annual Conference of the South African Veterinary Medical Association on 19 August 1953, Dr. V. Cooper reported that Weil's disease had been diagnosed in 2 human beings in Cape Town and that Dr. J. F. Brownlie had encountered both *L. canicola* and *L. icterohaemorrhagiae* in dogs.

The prevalence of these infections and the serotypes of leptospirae occurring in this country, their animal hosts, and their importance in causing human disease, have not yet been clearly defined. It thus will be of some interest to report the findings in 5 cases of meningo-encephalitis which on serological findings were proved to be due to leptospiral infections.

These serological tests were carried out as part of a wider programme to elucidate the causes of meningo-encephalitis and the aseptic meningitis syndrome in this region.

SEROLOGICAL METHODS

The serological tests used in this investigation for the detection of leptospiral antibodies were the complement-fixation and agglutination tests.

Preparation of Antigen. The antigens for the complement-fixation tests were prepared from egg cultures after it was found that antigens prepared from cultures in Fletcher's and Korthof's media were anticomplementary. To establish the infection, embryonated eggs were inoculated with 0·3-0·5 c.c. amounts of infected culture fluid, directly into the allantoic and amniotic sacs by means of the window technique. In the early subcultures, the eggs showed variation in the growths of leptospira obtained, but once established the growths became consistent and profuse.

The procedure then followed in preparing antigen was as follows: After 7 days primary incubation, the eggs were candled and the non-fertile and dead eggs were discarded. The living eggs were inoculated through a hole punched in the blunt end directly into the allantoic cavity each with 0·01-0·1 c.c. of heavily infected allantoic fluid derived from the previous subculture. These eggs were incubated for 7 days at 37°C. They were then candled again. The dead eggs were discarded, the eggs with living embryos were opened by burning a ring round the shell at the blunt end just above the shell membrane and flicking off the top. The shell membrane was reflected and the allantoic fluid aspirated with a syringe. The profuseness of growth was checked by examining the fluid under dark-ground illumination. If this was sufficiently rich the fluid was centrifuged at about 1,000 r.p.m. for 10 minutes to sediment the red cells. The supernatant was drawn off and heated at 60°C for 5 minutes. Merthiolate was then added to give a final concentration of 1 : 10,000. This fluid now constituted the antigen, which was kept at 4°C. Occasionally precipitates of urates formed, but this could be avoided by diluting the fluid in an equal volume of veronal buffer saline of pH 7·2.

Preparation of Antisera. Control antisera were prepared by inoculating rabbits at 6-day intervals with 0·3, 0·5, 0·75, and 1·0 c.c. of live cultures of the leptospira. One week after the last inoculation the rabbits were bled aseptically. The serum was separated from the clot and stored in a deep freeze at about −18°C.

Titration of Antigens. The antigens were titrated by the 'box' method to determine their strength. One example of the results obtained with *L. canicola* antigen in such a titration is given, as follows:

Antigen dilution	*Antiserum dilution*						*Controls*	
	1 : 25	1 : 50	1 : 100	1 : 200	1 : 400	1 : 800	*Serum dilution* 1 : 10	*Saline*
1 : 2	+	+	+	+	+	+	−	−
1 : 4	+	+	+	+	+	±	−	−
1 : 8	+	+	+	+	±	−	−	−
1 : 16	±	±	±	−	−	−	−	−

One antigen dose was taken as being contained in a dilution of 1 : 8, the highest dilution which gave clear-cut positive reaction in high titre against 2 full doses of complement. As 2 full doses of antigen were used, this was diluted 1 : 4 for the test.

The Complement-fixation Test Proper. The diluent used throughout was veronal buffer saline of pH 7·2.

Before dilution the sera were heated to 60°C for 20 minutes. In the preliminary screening the sera were tested in a dilution of 1 : 5 against each antigen. Two full minimum haemolytic doses (m.h.d.) of complement determined in the presence of the antigens individually by the overnight fixation method were added to each mixture. The haemolytic system consisted of a 1·5% suspension of washed sheep cells sensitized with 2 m.h.d. of haemolysin. The volumes used in the test were respectively

0·1 c.c. diluted serum
0·1 c.c. complement diluted to contain 2 full m.h.d.
0·1 c.c. diluted antigen
0·2 c.c. 1·5% sensitized sheep cells.

The fixation period allowed was 18-20 hours at 4°C. The tubes were then warmed in a 37°C water bath for 10 minutes before the addition of the sensitized cells. The racks were then thoroughly shaken and incubated for a further 30 minutes. The results were then read. The titre of complement fixation of all sera giving a positive reaction in this screening test was then determined.

The results given in this series of cases are noted in the individual cases. These were confirmed in the leptospiral agglutination test, with antigens prepared from cultures in Fletcher's medium. The identity of the antigens used was checked in comparative tests with antigens prepared from cultures recently received from Dr. Broom of the Wellcome Medical Research Foundation.

CLINICAL AND LABORATORY FINDINGS

Case 1

This patient, S.G.G. aged 25, a post-office clerk, was admitted to the Johannesburg Fever Hospital on 12 February 1957 complaining of headache, nausea and vomiting, and backache. He had been ill for the previous 7 days. This illness began with pain in the neck, fever and moderate headache. The fever subsided after 2 days, but on the 4th day again rose to 102°F and he now complained of severe headache. The temperature returned to normal the following day, but the headache remained, becoming excruciating at times. Two days later he developed stiffness of the neck and he was admitted to hospital with a diagnosis of meningo-encephalitis.

On examination he was found to be afebrile but seemed to be acutely ill. His conjunctivae were red and suffused and appeared to be acutely inflamed. No abnormal enlargement of the cervical glands was detected. The parotid glands and Stensen's duct opening were normal. His throat was slightly reddened. His chest moved well, air entry was good, and no adventitious sounds were heard. The heart was not enlarged and the sounds were closed. The blood pressure was 130/80 mm. Hg. His abdomen was soft and not tender and the liver and spleen were not enlarged. His neck and back became painful on flexion. The Kernig's sign was weakly positive. The tendon reflexes were present and equal on both sides; those of the knee were slightly depressed. A flexor plantar response was obtained.

The urine was darker than normal and had a specific gravity of 1020; a trace of protein was detected, bilirubin was absent, urobilin and urobilinogen was present. Microscopic examination of a centrifuged specimen showed the presence of 2 polymorphonuclear leucocytes per high-power field, with a few epithelial

cells. Bacteriological culture resulted in no growth. Leptospiral culture was not attempted.

A blood count taken on admission showed a haemoglobin of 16·7 g.%, 5,610,000 red cells per c.mm., and 9,600 white cells per c.mm., of which 69% were neutrophil, 0·5% monocyte, 29·5% lymphocytes, 0·5% eosinophil and 0·5% basophil leucocytes. The red cells were normal in appearance.

Examination of the cerebrospinal fluid collected on 12 February showed 295 cells per c.mm., of which 140 were polymorphonuclear leucocytes and 155 were lymphocytes. The protein was 90 mg. per 100 ml., sugar 50 mg. and chlorides 718 mg. The Wassermann reaction was negative. No bacteria were detected on direct or cultural examination. These findings confirmed that this patient had meningo-encephalitis.

A throat swab yielded a culture of pneumococci, scanty haemolytic streptococci and *Micrococcus catarrhalis*. *C. diphtheriae* was not detected.

Liver function tests, the first taken on admission and the second a week later, gave the following results:

Tests	Date of collection of blood in test (1957)	
	13 *Feb.*	22 *Feb.*
Thymol turbidity	1·5	8·0
Thymol flocculation	negative	+ +
Colloidal red		+ +
Cephalin cholesterol		
Flocculation test	negative	negative
Takata-Ara test	negative	negative
Alkaline phosphatase	7·6	8·2
van den Bergh	negative	negative
Bilirubin direct	0·4	0·3
Total	0·8	0·8
Total protein	7·7	8·4
Albumin	4·0	4·3
Globulin	3·7	4·1
Gamma globulin	1·34	1·99
Cholinesterase		100% of normal

These tests reveal some positive reactions resulting from an increase in the gamma globulin, but do not give other evidence of severe liver damage. The plasma amylase was less than 160 units.

The Widal test on admission gave a positive agglutination of *S. typhi H* in a titre of 1 : 50. A week later the titre had risen to 1 : 100, but this finding was considered not significant and probably an anamnestic reaction. The Weil-Felix test in a titre of 1 : 50 and the brucella agglutination tests in a titre of 1 : 10 gave negative results. The modified Coombs test for brucellosis also gave a negative result as did the Paul-Bunnell test in a titre of 1 : 7.

The rickettsial and the toxoplasma complement-fixation test both yielded negative results on the specimens taken on admission and again 1 week and 2 weeks later.

The leptospiral complement fixation tests gave the following results:

Antigen	Date of collection of blood in test (1957)		
	13 *Feb.*	22 *Feb.*	27 *Feb.*
L. canicola	0	1 : 320	1 : 320
L. icterohaemorrhagiae	0	1	1 : 80
L. pomona	0	0	0

These results indicated clearly that this patient's illness was caused by a leptospiral infection, and suggested that *L. canicola* was the serotype responsible.

Case 2

This patient, E.v.S., an 8-year-old European girl, was admitted to the Johannesburg Fever Hospital on 12 March 1957 from Brenthurst, a suburb of Brakpan, as a suspected case of non-paralytic poliomyelitis. Seven days before admission she had complained of headache and had vomited. A doctor was called, who thought the child had scarlet fever. On the day of admission, when symptoms had become aggravated, the child complained of headache, pain in the legs and stiff neck and back. No weakness was detected. The appetite was poor. The child had not had poliomyelitis vaccine and had had her tonsils removed 5 months previously.

On examination in hospital it was noted that both conjunctivae were mildly injected. The throat was clear, the tonsils were absent and the cervical glands were not enlarged. Her chest moved well, air entry was good, and no adventitious sounds were heard. was no enlargement of the heart and the sounds were closed. Her abdomen was soft and not tender, and no masses were felt. The spleen and liver were not enlarged. The neck and back were mildly stiff. Kernig's sign was negative. The tendon reflexes were somewhat exaggerated. No motor weakness was detected.

A blood count taken on the day after admission showed 15·1g.% haemoglobin, 5,030,000 red cells per c.mm., and 9,000 white cells per c.mm., of which 32% were neutrophil leucocytes, 2% monocytes, 62% lymphocytes, 2% eosinophil leucocytes and 2% plasma cells. The red cells and platelets were normal in appearance, but it was noted that there was a reversal of the neutrophil: lymphocyte ratio and that many lymphocytes had an atypical appearance. However, the Paul-Bunnell test gave a negative result.

The routine biochemical tests on a catheter specimen of urine showed the presence of a trace of protein; sugar was absent. Microscopical examination showed the presence of occasional polymorphonuclear leucocytes. Bacteriological culture yielded no growth.

The liver function tests gave normal readings except that of the total protein of 8·0 g.%, 3·9 g. was albumin and 4·1 g. globulin, of which 1·15 g. was gamma globulin.

Examination of the cerebrospinal fluid taken on the day of admission showed 230 cells per c.mm., of which 20 were polymorphonuclear leucocytes and 210 were lymphocytes. The total protein was 19 mg. per 100 ml., sugar 56 mg. and chloride 758 mg. These findings thus confirmed the diagnosis of meningo-encephalitis.

The routine bacterial agglutination tests, including the Widal, Weil-Felix and brucella tests, and the routine toxoplasma, rickettsial and viral complement-fixation tests gave negative results.

The leptospiral complement-fixation tests gave the following results:

Antigen	Date of collection of blood in test (1957)	
	13 *Mar.*	21 *Mar.*
L. canicola	1 : 5	1 : 160
L. icterohaemorrhagiae	1 : 5	1 : 40
L. pomona	0	1 : 20

This significant increase in the titre of complement fixation with these leptospiral antigens indicated that this patient had had a leptospiral infection. The highest titre being obtained with *L. canicola* suggested that this, or a serologically related leptospira, was the cause of the patient's illness.

Case 3

J.D., a girl aged 6 years, was admitted to the Johannesburg Fever Hospital on 5 May 1957, having been sent from the Out-Patient Department of the Transvaal Memorial Hospital by Dr. V. North with a diagnosis of meningo-encephalitis. She had been ill for the past 4 days with headache, vomiting and stiffness of the neck. She had not noticed any weakness of the limbs, but had a slight cough.

On examination she did not appear to be very ill. Her complexion was sallow, but she had no conjunctivitis, and no rash was seen. Her throat was normal and no enlarged glands were found in the neck. Her chest moved well and the breath sounds were normal. A systolic murmur could be heard all over the precordium, but the heart was not enlarged. Her neck and back were slightly stiff, and the hamstring muscles were tight. The cranial nerves were intact. The reflexes were present and equal on both sides. A diagnosis of meningo-encephalitis was made.

In her blood count, it was noted that the haemoglobin was 13·6 g.%, the red-cell count 4,550,000 per c.mm., and the white-cell count 6,800 per c.mm., of which 58·5% were lymphocytes, 41·0% neutrophil and 0·5% eosinophil leucocytes. The red cells and platelets were normal in appearance. The Kolmer and Paul-Bunnell tests gave negative results.

The bacteriological agglutination tests gave negative results except for agglutination of *S. typhi H* antigen in a titre of 1 : 50, which on re-test had risen to a titre of 1 : 200. However, there was no other indication of enteric fever.

In the virus complement-fixation tests, the *Herpes simplex* virus antigen reacted in a titre of 1 : 10. When repeated 1 week later, there had been no increase in titre and this reaction was therefore considered to be the result of a previously acquired infection and not related to the patient's present illness. The

rickettsial and toxoplasma complement-fixation test gave negative results on both occasions.

The leptospiral complement-fixation test gave the following results:

Antigen	Date of collection of blood in test (1957) 6 May	11 May
L. canicola	0	1 : 160
L. icterohaemorrhagiae	1 : 5	1 : 10
L. pomona	0	1 : 10

On the result of these complement-fixation tests the diagnosis of leptospiral meningo-encephalitis was made.

The patient's temperature returned to normal on the evening of the day of admission and she made an uninterrupted recovery and was discharged well 19 days later.

This case is of interest in that marked conjunctivitis, a prominent feature of the preceding cases, was not noted.

Case 4

D.J., a girl aged 12 years, was admitted to the Johannesburg Fever Hospital on 8 May 1957. She complained of pain in the legs and back at the onset of illness, vomiting and nausea, headache, and fever of 1 week's duration. She came home from school on Thursday 1 May 1957, 1 week before admission, complaining of pain in her legs and back. This lasted 1 day. On Friday she was very flushed and bilious, and vomited and had severe frontal headache and a temperature of 103°F. Her doctor was called and prescribed sulphadiazine. On Saturday she felt much better. On Sunday her improvement was maintained. On Monday she developed a swollen left cheek, and on Monday and Tuesday she was given penicillin intramuscularly. On Wednesday she developed severe headache and cried all night. Her cheek was still swollen. She was feverish and had a stiff neck and her admission to hospital was arranged.

On examination she was noted to be a well-nourished girl sitting comfortably in bed. She had no pallor, jaundice or cyanosis. Her temperature was 100·2°F, respirations 24 per minute, pulse rate 108, and blood pressure 130/80 mm. Hg. Mild stiffness of the neck was present. Her eyes were clear, the tongue had a strawberry appearance, the tonsils were enlarged, and small glands were felt in the neck. Her chest movements were good and there was no intercostal tenderness. The heart sounds were distant, but no murmurs were heard. Her back was stiff but not painful. Her abdomen was flat and no enlargement of the organs was detected and no rash was seen. There was slight tightness of the hamstring muscles. The cranial nerves were normal. Reflexes were all present and equal, except for the abdominals, which were absent. A diagnosis of meningo-encephalitis was made.

The blood count showed 15·9 g.% of haemoglobin, 5,300,000 red cells per c.mm. and 7,300 white cells per c.mm., of which 66% were neutrophil leucocytes, 7% monocytes, 25% lymphocytes, and 1% eosinophil and 1% basophil leucocytes. The Paul-Bunnell test was negative.

The cerebrospinal fluid was found to contain 73 cells per c.mm., of which 65 were polymorphonuclear leucocytes and 8 were lymphocytes. The protein was 45 mg. per 100 ml., sugar 42 mg. and chloride 730 mg. The Wassermann reaction was negative and no bacteria were detected on direct or cultural examination.

A throat swab culture yielded a mixed growth of *Streptococcus viridans, Micrococcus catarrhalis* and pneumococci. No bacteria were isolated from a blood culture in nutrient broth. The serum was found to contain 400 units of streptococcal antihaemolysin 0 per ml. The Widal and the brucella agglutination tests gave negative results. In the Weil-Felix test *Proteus OXK* was agglutinated in a titre of 1 : 50.

A poliovirus tissue-culture protection test revealed the presence of antibody to each of the three types of poliovirus.

The routine rickettsial and toxoplasma complement-fixation tests gave negative results.

The leptospiral complement-fixation tests gave the following result:

Antigen	Date of collection of blood in test (1957) 9 May	21 May
L. canicola	1 : 5	1 : 80
L. icterohaemorrhagiae	1 : 5	0
L. pomona	1 : 5	0

A significant increase in the complement-fixation test with *L. canicola* was thus demonstrated. This result indicated clearly that the patient's illness was caused by *L. canicola*, or a serologically related organism.

Case 5

A youth aged 15 years, who lived in the same house as the preceding case, was admitted to the Johannesburg Fever Hospital on 21 May 1957. Two days before admission he had been suffering from severe headache, sore throat, and stiffness of the neck and back, but had not noticed any weakness of his limbs. On examination he appeared ill. His conjunctivae were injected. His throat was red but there were no follicles or membrane present on the tonsils. No abnormal signs were detected in his chest. The heart was not enlarged and the sounds were closed. His abdomen was soft and not tender. No masses were detected. His neck was slightly stiff. Kernig's sign was positive. No abnormality of the cranial nerves was elicited and no motor weakness was detected. The reflexes were present and equal. A diagnosis of meningo-encephalitis was made.

A blood count gave the following results: Haemoglobin 16·6 g. %, red cells 5,600,000 per c.mm., white cells 14,000 per c.mm., of which 60·5% were neutrophil leucocytes, 11·5% monocytes, and 28% lymphocytes. The red cells and platelets were normal in appearance and the sedimentation rate 10 mm. in an hour. The Paul-Bunnell tests gave negative results.

The cerebrospinal fluid showed 5 lymphocytes per c.mm., and protein 27 mg. per 100 ml., sugar 67 mg. and chloride 730 mg. No bacteria were detected on direct or cultural examination. The Widal, Weil-Felix and brucella agglutination tests gave negative results. The serum contained 500 units streptococcal antihaemolysin 0 per ml. Culture of a throat swab yielded a mixed growth of pneumococci and *Micrococcus catarrhalis* and non-haemolytic streptococci. Bacteria were not isolated from blood cultures taken on the day after admission.

The liver function tests yielded essentially normal results, except for a ++ reaction in the colloidal-red test.

No protein was found on examination of the urine, and bilirubin and urobilinogen were not detected; urobilin was present. Microscopic examination of the deposit from a centrifuged specimen showed the presence of amorphous urates. Cultivation yielded no growth. Porphyrin was not detected.

The routine rickettsial, virus, and toxoplasma complement-fixation tests gave negative results.

The leptospiral complement-fixation tests gave the following results:

Antigen	Date of collection of blood (1957) 23 May	3 June
L. canicola	—	1 : 320
L. icterohaemorrhagiae	—	1 : 40
L. pomona	—	1 : 40

The leptospiral agglutination test gave the following results:

Antigen	Date of test (1957) 23 May	3 June
L. canicola	0	1 : 10, 240
L. pomona	0	0

These results confirmed that the patient had an infection with *Leptospira canicola*.

All five patients made an uninterrupted recovery and were discharged from hospital feeling well 2-3 weeks after their admission.

Comment

These 5 cases presented an illness lasting a week or longer showing a diphasic fever, during the second phase of which signs and symptoms of meningo-encephalitis developed. Of particular interest were the sudden onset, the pains in the back and limbs, especially in the leg muscles, and, in 3 of the cases, a marked conjunctivitis. These features suggested the diagnosis of leptospirosis, which was made provisionally on clinical grounds in the 3 cases with conjunctivitis. This diagnosis was confirmed by the results

of the leptospiral complement-fixation and agglutination tests.

ORIGIN OF THE INFECTION

Enquiries directed to finding the origin of the infection were made, and in particular the contact of the patients with animals was investigated. In Case 1 (S.G.G.) it was ascertained that the post office in which he worked was infested with rats. He also had a relatively young dog as a pet, and occasionally helped his wife to prepare meals, in the course of which he handled raw meat, usually beef, but occasionally pork. He had not been swimming, picnicking or camping recently, nor had he been in any area where cattle and pigs roamed. About 10 days before the onset of his illness he had attended a motor-car race meeting in a rural area. An inspection of this area subsequently showed that there were no cattle or pigs or damp or swampy ground in its immediate neighbourhood. It seemed more likely, then, that his infection was acquired from contact with the animals in his home environment.

Case 2 (E.v.S.) often played with three dogs in her home, but gave no history of contact with other animals.

Blood was collected from the 4 dogs associated with cases 1 and 2, and submitted to the leptospiral fixation tests, which gave the following results:

Antigens	*Serum dilution*							
	10	20	40	80	160	320	640	1280
Case 1, Dog G								
L. canicola	+	+	−	+	+	+	±	−
L. icterohaemorrhagiae	±	±	−	−	−	−	−	−
L. pomona	±	±	−	−	−	−	−	−
Case 2, Dog Sn								
L. canicola	+	+	−	+	−	−	−	−
L. icterohaemorrhagiae	±	−	−	−	−	−	−	−
L. pomona	−	±	±	−	−	−	−	−
Case 2, Dog Sp								
L. canicola	±	−						
L. icterohaemorrhagiae	−							
L. pomona	−							
Case 2, Dog B								
L. canicola	+	+	±	±	−	−	−	−
L. icterohaemorrhagiae	−	−	−	−	−	−	−	−
L. pomona	−	−	−	−	−	−	−	−

These relatively high titres of complement fixation given by the sera from 3 of these 4 dogs clearly indicate that these dogs had or recently had had an infection with *L. canicola*, or a serologically related organism. The history given by the two human patients (cases 1 and 2) of close contact with these dogs, taken in conjunction with these serological findings, clearly suggest that the source of the patients' infection was their dogs.

The relevant history was not obtained from J.D. (case 3), whose parents were unhelpful. However, it was found that the patients D.J. (case 4) and R.W. (case 5) lived in the same house and that D.J. had recently been given a young dog, with which both of them frequently played. This dog was not examined, but in view of the findings in the first two patients it seems probable that it was the source of infection of both these patients.

Several rats caught in the post office where S.G. (case 1) worked were tested for leptospiral antibodies, but these tests gave negative results.

For many years rats and other rodents have been trapped at strategic points in the municipal area of Johannesburg to check the incidence of murine typhus and tick-bite fever infections amongst them. A number of these rats, all *Rattus rattus*, were bled and their sera tested in the complement-fixation tests for leptospiral antibodies. Of 60 rats tested, 58 gave negative results with each of the three leptospiral antigens. One gave a weakly positive reaction with *Leptospira canicola* in a serum dilution of 1 : 10, but not in the higher dilutions, and negative reactions with the other two antigens, and one serum proved to be anticomplementary in the tests. The titre of the reaction in the serum giving a weakly positive reaction was so low that it was considered of doubtful significance. However, further study of the rats and other rodents for evidence of leptospiral infection is warranted. At present, the serological findings clearly incriminate the patients' dogs as being the source of the patients' infection.

REVIEW OF RECENT HISTORY OF LEPTOSPIROSIS

Leptospiral infections are now known to be one of the commonest causes of benign meningo-encephalitis. Outbreaks have been reported from Europe, Asia, Australasia, America and North and Central Africa. The findings reported in this paper reveal that they are also a common cause of the condition in South Africa. It will therefore be of some interest to give a brief general account of leptospirosis. Fuller accounts of these infections have recently been given by Broom[3] and by Kalz.[4]

Since the discovery of the first pathogenic leptospira, *L. icterohaemorrhagiae*, by the Japanese workers Inada and Ido[5] in 1915, about 40 antigenic types have been differentiated by serological methods. Many of these serotypes are closely related antigenically and may be assembled into groups. Some of the serotypes have a wide distribution, others are more restricted, possibly because of more limited distribution of its host of election. Leptospira are primarily parasites of animals and each serotype appears to have a host for which it has specific affinity, though under experimental conditions they may have a wide range of susceptible hosts. Rodents and other small animals are the main reservoirs of infection. However, these organisms are responsible for widespread and often serious disease of a number of domestic animals, including dogs, pigs and cattle, and have been shown to cause infection in a number of wild animals, including mice, voles, bats, mongooses, bandicoots, foxes, jackals and opossum.

Although contact, direct or indirect, with rats and dogs remains amongst the most frequent sources of infection of Man, infection may be acquired from similar contact with other animals or their environment. In their host animals the leptospirae often form colonies in the tubules of the kidneys and are shed in the urine and thus contaminate soil and water and, provided conditions of pH, moisture, and temperature are favourable, may survive for prolonged periods.

Man may acquire his infection from contact with water, mud or damp soil in such a contaminated environment or directly from contact with the urine or tissues of infected animals. The leptospira gain entrance through cuts or abrasions of the skin, or through the mucous membranes of the eye and nose. They then give rise to a generalized infection, which may penetrate the blood-brain barrier causing a meningo-encephalitis. The brunt of this infection is borne by the kidneys and liver, which in fatal cases show characteristic lesions. The liver may be swollen and on microscopical examination show dissociation of the liver cords. Sometimes necrosis of the cells round the central

vein is apparent and there is an infiltration of cells in the portal tracts. The kidneys are often enlarged and show changes varying from cloudy swelling to necrosis of the convoluted tubules and loops of Henle. The medullary tubules contain cellular casts. The glomeruli are little affected. There may also be interstitial oedema and peritubular infiltration of inflammatory cells.

The spleen may be enlarged and diffluent and show focal haemorrhages. The fibres of voluntary muscles, especially of the gastrocnemius may show loss of striation and hyaline degeneration.

Amongst the commonly recognized diseases of Man caused by leptospiral infections are Weil's disease, Canicola fever, and swineherd's disease. There are a number of others less well known, such as 'mud' fever, 'cane cutters' fever and rice-field fever. Fort Bragg fever, a condition affecting soldiers in the United States Army in several of the training camps in America during World War 2, has also been shown to be caused by a leptospiral infection.[6] The salient features of the more important of these diseases will be noted:

Weil's Disease

Weil's disease is caused by *Leptospira icterohaemorrhagiae*, of which the sewer rat, *Rattus norvegicus*, is the most important reservoir host. A number of other rodents have also been shown to harbour the infection. Man usually acquires the disease in a rat-infested environment, where the water, slime, or soil may be heavily contaminated by infected urine. Outbreaks of this origin have occurred amongst the workers in coal mines, fisheries, and sewers. Cases are also frequently reported after deliberate or accidental immersion in water of rivers, canals or ponds.

The incubation period has an average of 7—14 days. The onset is sudden, with high fever, headache, chilly feelings and muscle pain, particularly of the calf muscles, followed by anorexia, vomiting and abdominal pain. Conjunctivitis and nose-bleeding are features of most cases. Leptospirae are present in the blood and may be demonstrated by culture, animal inoculation or, more rarely, by dark-field microscopic examination of the serum sediment. This first septicaemic phase of the disease lasts 3—7 days, when the fever falls by lysis, but is followed in many cases by a second wave of fever, during which signs of involvement of the liver become apparent. Jaundice may be seen in some cases, but tenderness and enlargement of the liver are also found in many cases without icterus. A tendency to haemorrhage results in petechial haemorrhages, haematuria and melaena. The liver function tests show impairment at this stage.

Clinical signs and symptoms of renal involvement also become manifest. The urinary output is decreased and albumin, red cells, white cells and hyaline and granular casts are found in the urine. Cases which end fatally usually do so between the 10th and 17th day and death is most often due to renal failure.

Some cases develop signs and symptoms of meningeal involvement, but involvement of the central nervous system appears to be less prominent a feature of Weil's disease than of other leptospiral infections. Convalescence is often protracted and may be interrupted by further febrile relapses and by the development of complications such as iritis, iridocyclitis and optic neuritis.

Canicola Fever

Canicola fever is caused by *Leptospira canicola*, of which the dog is an important but not the only reservoir. Man most often acquires the infection from contact with dogs. These animals may have overt signs of disease, including bloodshot eyes, anorexia and vomiting, fever, and signs of renal damage, followed sometimes by death from kidney failure. Often they have relatively silent infections. Pigs, cattle, horses, donkeys and jackals may also be sources of infection.

The infection in Man may cause an illness resembling Weil's disease. However, it is as a cause of aseptic meningitis that *L. canicola* has attracted most attention. The clinical features of this illness have been illustrated in the descriptions of the cases in the first part of this paper.

Swineherd's Disease

Leptospira pomona was first identified as a distinct serotype by Clayton *et al.*[7] in Australia, where it has been incriminated as the cause of red water in calves. Pigs infected with this leptospira may show no clinical signs of illness, but may suffer impoverishment and lowered resistance to other infections. The infection may cause serious losses in herds of cattle.

In 1944 it was shown by Gsell[8] that this leptospira was the cause of swineherd's disease in Europe. The clinical features of this illness are an acute onset, with photophobia, myalgia, transient skin rashes, and high fever, often showing a biphasic course. During the second bout of fever the patient often develops a severe headache, stiff neck and back, and other signs of meningitis. The cerebrospinal fluid usually shows a pleocytosis mainly of lymphocytes, and an increase in protein, with relatively normal values of sugar and chlorides.

The condition in Man is usually benign and the patient usually makes an uninterrupted and complete recovery without any sequelae, although convalescence may be protracted.

DIFFERENTIAL DIAGNOSIS

In the differential diagnosis of Weil's disease the other causes of illnesses with an acute onset, high fever, and enlargement and tenderness of the liver, associated sometimes with jaundice and other signs of hepatic and renal dysfunction, have to be considered. These include infective hepatitis, yellow fever and Rift Valley fever, glandular fever, Q fever, the enteric fevers, relapsing fever, and the bilious remittent form of malaria.

In the differential diagnosis of Canicola and Pomona fever with involvement of the central nervous system, the other causes of benign aseptic meningitis have to be considered. Chief amongst these are the viral causes of meningoencephalitis, including infections due to poliovirus, Coxsackie and ECHO viruses, and mumps virus and herpes virus. There are a number of other viral and non-viral conditions which may also cause difficulty.

The differentiation of Weil's disease from clinically similar illnesses, and of Canicola and Pomona meningitis from other causes of the aseptic meningitis syndrome, is usually only possible by a comprehensive series of laboratory tests specifically designed for this purpose. In most advanced countries these laboratory facilities are now available. In South

Africa they are provided by the South African Institute for Medical Research and the Poliomyelitis Research Foundation.

TREATMENT

A number of antibiotics have been shown to have a lethal or inhibitory effect on leptospiral infections under experimental conditions. Favourable results have also been reported in cases treated with penicillin in large doses, streptomycin, chloramphenicol, aureomycin and tetracycline, or these antibiotics in various combinations. Other reports are less favourable. It is clear that the evaluation of these antibiotics in a disease so variable in its severity and course as leptospirosis is difficult. However, although it has not yet been proved that these antibiotics are of specific value, they may be beneficial and should be given a trial, especially in patients who are severely ill.

Prevention

In the prevention of leptospiral infections, it is necessary first to define the extent of the problem and to detect the important reservoirs of infection and the conditions under which it is spread. In the cases in which rodents are the chief vectors of infection, anti-rodent measures may be successful in controlling it. Widespread infection of dogs would in practice be more difficult to control. Advice may be given to lessen intimate contact between potentially infected dogs and Man, but in practice it is doubtful whether it would be followed. It may prove possible to treat dogs prophylactically with drugs and antibiotics, which would eliminate their infection and so the danger of passing it on to their human masters. However, again it is doubtful whether such measures would be widely applied. Fortunately the infection acquired from dogs is usually relatively benign.

The public should be warned of the danger of paddling, bathing or swimming in rivers, canals and ponds, where these are known to be infected. In South Africa cases have not yet been traced to this source, but investigations should be carried out to determine the importance of contaminated water and soil in spreading leptospiral diseases.

SUMMARY

The clinical findings in 5 cases of meningo-encephalitis admitted to hospital with a provisional diagnosis of non-paralytic poliomyelitis are described. These patients had an illness lasting about 1 week, characterized by headache, conjunctivitis, muscle pains, and fever often showing a biphasic course, during the second wave of which they developed signs of meningitis, severe headache, stiff neck and back and a pleocytosis in the cerebrospinal fluid mostly of lymphocytes.

The diagnosis was established in each case by serological tests, the complement-fixation and agglutination tests, which showed the development of antibodies against leptospira in the convalescent-phase blood as compared with negative results given by the acute-phase blood. The titre of antibody was significantly higher against *L. canicola* than against *L. icterohaemorrhagiae* or *L. pomona* and it was concluded that *L. canicola*, or a closely related organism, was responsible for the illness. It was found that the blood sera of 3 of the 4 dogs belonging to 2 of the patients also gave high-titre complement fixation against *L. canicola* and that the other 3 patients had had close contact with dogs, but not with other animals. It was concluded that the source of the patients' infection was their dogs.

The epidemiological features of leptospiral infections, of which about 40 antigenic serotypes have been differentiated, are briefly reviewed, noting that Man acquires his infection either directly or indirectly through contact with animals. Rodents and dogs are the most important reservoirs of infection, though cattle and pigs and a number of other domestic and wild animals have also been shown to harbour and excrete leptospirae pathogenic to Man. The epidemiological and clinical features of leptospiral jaundice or Weil's disease, Canicola fever and Pomona fever, or swineherd's disease, are briefly noted.

The value of antibiotic treatment has not yet been clearly assessed. These diseases may be prevented by avoiding contact with infected animals and their contaminated environment, and by eliminating infected rodents and possibly by the appropriate treatment of infected domestic animals, but in practice these measures may be difficult to enforce.

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