Epidemiology of Leptospirosis*

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DURING the past 2 years the Epidemiological Intelligence Services of the West have repeatedly requested coöperation in the solution of the etiologic factors responsible for outbreaks or sporadic cases of jaundice. Epidemics involving small rural schools have occurred in May and June and affected as many as 42 children at one time. In another, the cases of catarrhal jaundice were seen in familial distribution shortly before or during the rainy season which was followed by floods.

Invariably, the serological examination, inoculations of guinea pigs, and the examination of rodents collected within the epidemic area yielded no evidence which would stigmatize the *Leptospira icterohaemorrhagiae* as the causative factor. In fact, a careful analysis of the clinical cases, the incubation time of from 3 to 4 weeks, and the obvious contagious character lead to the conclusion that these outbreaks were indistinguishable from the well known epidemic catarrhal or infective hepatic jaundice previously described by Blumer [1] and others for this country and well known in Great Britain through the publications by Pickles,[2] Morgan and Brown,[3] Findlay, Dunlop, and Brown,[4] and in Syria by Yenikomshian and Dennis.[5]

Thus far, all experiments to reproduce this disease in a great variety of animals have furnished little tangible evidence concerning the etiologic agent. An ultramicroscopic agent transmissible to man only is suspected. Sporadic, abortive or latent cases may be the sources of the infection. On the other hand, it must be recognized that single cases of febrile jaundice may be typical cases of Weil's disease. Relatively little attention has been paid to these infections in the United States; in 1937, Packchanian [6] places on record about 32 proved cases. More recently, 2 sporadic cases have been diagnosed in Rochester, N. Y.,[7] several in Detroit, and at least 5 have been studied in San Francisco and vicinity. Examinations of serum specimens and sections of organs derived from guinea pigs injected with urine of patients have proved the existence of Weil's disease in the Hawaiian Islands.[8] There is every reason to believe that these figures merely represent a small fraction of the actual cases.

Experience both in England, particularly London, and in Holland has shown that the incidence in proved diagnosis rapidly increases, provided reliable diagnostic methods are available. Thus,

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previous to 1933, less than 4 cases of Weil's disease were reported in London for an entire year. With facilities to test the sera of suspected cases at the disposal of the physicians, the same number of infections are now seen within a month. Since the first case of Weil's disease was diagnosed in 1924 in the Netherlands with the help of a bacteriological examination, not less than 756 cases had been detected by the end of 1936. In Rotterdam, the rate was 56 cases per 400,000 population. Likewise in France between 1924 and 1932, 263 cases were entered in the official records.

Furthermore, it is now recognized that only two-thirds of the patients infected with the leptospira show an icterus. The symptom of jaundice arouses suspicion to such an extent that an examination for leptospira is usually indicated. The relative ease with which a few of these infections have been recognized in California as soon as proper diagnostic methods were available encourages the belief that human leptospirosis may be masquerading under a diversity of diagnoses.

LABORATORY AIDS

The methods of bacteriological diagnosis are delicate and require considerable experience. It is well to remember that the parasites can be detected only in blood plasma specimens concentrated by centrifugation during the first 4 days of illness. Darkfield examinations are very misleading and have not infrequently tempted the examiners to make erroneous diagnoses. The inoculation of guinea pigs with the patients' blood again proves successful only during the early days of the illness, rarely after the 8th day. The animal test is not dependable since certain leptospira strains have a low pathogenicity even for very young guinea pigs. Perhaps, the use of deer-mice (*Peromyscus*) as suggested by Packchanian or the Richardson squirrels as recommended by Syverton, Stiles, and Berry [9] may improve the diagnostic yield. These procedures may be supplemented by cultures of the blood but they presuppose a close coöperation between the physician and the laboratory. Experience has shown that the serological tests—agglutination and lysis—followed by animal inoculations with urine or spinal fluid are the most dependable tools in epidemiological work.

The serologic technic developed by Schüffner [10] using living and formalinized (pure formalin) antigens prepared from leptospira cultures in Verwoort media has proved highly satisfactory. With this procedure, the diagnosis of Weil's disease is greatly simplified provided several strains of leptospira are used as antigens, and that titers under 1:300 are not accepted as diagnostically decisive. Esseveld,[11] in a careful study on a large series of cases (87), stresses the fact that any titer above 1:300 usually $>$ than 1:10,000 may be safely interpreted as undeniable proof of Weil's disease. In view of the difficulties inherent to these serological tests, it is advisable to centralize the examinations in state or research laboratories with trained personnel.

EPIDEMIOLOGIC OBSERVATIONS IN CALIFORNIA

Since 1927, at least 5 laborers connected with the cleaning and the maintenance of the sewers in San Francisco developed a febrile jaundice resembling Weil's disease. A definite serological diagnosis was made on one case in 1936 and on another in 1937. During the same year, a fatal Weil infection was recognized in a 61 year old Japanese who had worked in his rat infested garden, and an anamnestic diagnosis was made in a veterinarian who serves at the hospital of the Society for the Prevention of Cruelty to Animals and

handles a great many jaundiced dogs. In July, 1938, a filling station operator, aged 27, developed Weil's disease following a fishing trip along the Napa River. He also waded in the mud beds along the wharves and had eaten his lunch in the same area. At the same time, a 28 year old assistant to a veterinarian at Stockton, Calif., with an influenza-like febrile disease was diagnosed as an interesting case of Weil's disease due to *Leptospira canicola*. Investigation of this limited number of human leptospirosis disclosed the existence of two different sources of infection. Nine of the 11 cases had in one way or another been in contact [1] with water or sewage, while two were [2] exposed to dogs.

Since the degree of endemicity of Weil's disease is influenced by the murine infection of the rat population, the hydrogen ion concentration of the surface water, a low salinity and a hardness not exceeding 19 to 21, a number of preliminary surveys have been instituted.

With the assistance of the San Francisco Department of Public Health and the Laboratory for Plague Suppressive Measures of the U. S. Public Health Service, both random and spot sampling of 467 rats (*Mus norvegicus, Mus alexandrinus,* and *Mus rattus*) yielded in darkfield examinations renal leptospira findings in 176, or 35 per cent. In the vicinity of an animal stable, 42 of 44 old Norvegicus rats have been found infected. Single or pooled specimens of the rat kidneys on inoculation into guinea pigs produced the lesions typical for leptospirosis on the first, second, or third passage through these rodents. Twenty-three strains were isolated in culture and by serological tests identified as *Leptospira icterohaemorrhagiae,* generally known as the classical rat strain. Murine leptospirosis had been previously recognized by J. R. Ridlow in San Francisco. Renal leptospirosis infections have been definitely established in Washington (10 per cent), Nashville (10 per cent), New York (17.2 to 21.9 per cent), Albany (40 per cent), Baltimore (7 per cent), Chicago (3 to 52 per cent), Rochester (38 per cent) and Detroit (16 per cent). The contaminations of water, soil, and food by the urine of these rodents and possibly mice, gophers, or other rodents furnish the seeds for the human infections. Since the intact epidermis is quite resistant to the penetration of the leptospira, the contaminated water must by necessity be brought in contact with the mucous membrane.

In the city with a continuous control of the rat population as a plague preventive measure, the exposures of man to the classical rat leptospira are limited to water accidents, contact with sewage or mud and soil in the vicinity of rodent harborages.

Although the serological tests of the rat strains thus far isolated identify the organisms as indistinguishable from the classical strain studied throughout the world, it is important to remember that other types, probably antigenic variants, may be encountered. The diagnosis of human leptospirosis must employ the serological tests reservedly; just as in typhoid fever the ultimate proof of clinical Weil's disease depends on the isolation of the causative spirochete.

CANINE LEPTOSPIROSIS

The blood serum of a veterinarian, who had an attack of undiagnosed malady complicated by icterus and nephritis 8 months before the test was made, agglutinated *Leptospira canicola* in a dilution of 1:300. More recently, this spirochete has been isolated from the urine of an assistant veterinarian, whose blood clumped and lysed the *Leptospira canicola* in a dilution of 1:10,000 (4+) and 1:30,000 (3+) and *Leptospira icterohaemorrhagiae*

1:100 (4+). In both cases, the epidemiological inquiries strongly incriminated the handling of dogs suffering from "yellow" or Stuttgart's disease. It may, therefore, be appropriate to review the essential facts which have been collected concerning these canine diseases during the past 2 years.

For some time, the hospital of the Society for the Prevention of Cruelty to Animals in San Francisco has noted a high mortality among dog patients suffering from jaundice or severe hemorrhagic gastroenteritis with or without icterus. At the request of Dr. S. T. Michael, 87 dogs, either dead or moribund, have been submitted for examination in order to determine the cause of the illness or death. The prominent autopsy lesions classed the dogs into two types—hemorrhagic and icteric. The former, seen in 38 dogs, resembles Stuttgart's disease or canine typhus, while the latter noted in 42 animals is indistinguishable from "yellows" or canine jaundice. Seven dogs had passed through both types, death occurring while they were in the icteric stage.

Since the gross lesions are in every respect similar to those described by Okell, Dalling, and Pugh,[12] Wirth,[13] Klarenbeek,[14] and others, and since they will be detailed elsewhere, suffice it to record that leptospira have been demonstrated in the organs of the cadavers of both types in darkfield preparations and properly stained sections prepared from the kidneys, occasionally spleen and liver.

The isolation of these spirochetes offered many difficulties but to date 11 cultures have been obtained from California dogs. Nine were secured directly by culturing the blood from 2 days to a few hours before death or from the clotted blood at the time of autopsy. In two instances, the strains were cultured from the peritoneal fluid or heart blood of very young guinea pigs injected with the emulsion of kidneys of infected dogs. Failure to culture the leptospira is largely attributable to the heavy bacterial contaminations of the tissues secured from the moribund dogs, and the low pathogenicity of the organisms for guinea pigs. Repeated passages through these rodents are required to induce fatal infections; as a rule, hemorrhages on the viscera and the lungs are noted and a general icterus is very rare.

The isolated leptospira strains are serologically identical with *Leptospira canicola* recognized by Klarenbeek and Schüffner in 1934 in Holland as a specific spirochete of the dog. The independent position of this leptospira has been fully established by Walch-Sorgdrager and Schüffner,[15] who kindly identified the California strains. *Leptospira canicola* is widely distributed and has been found, aside from Holland, in Germany, Austria, and Denmark. Since it has never been isolated from rats, it is generally recognized that this rodent plays no rôle in the dissemination of Canicola infections.

Canine leptospirosis affects all breeds of dog, but rarely under 1 year of age. In California, the highest incidence is noted during the summer with the peak in July and August. The mortality is generally considered high, although accurate figures are not available. In San Francsico, the hospital furnishing the dogs estimates the losses at 80 to 90 per cent. Annually, approximately 150 cases are brought to the clinic for treatment. Fatal infections have been prominent among males; the ratio of the dogs autopsied and sex noted in the histories was males : females 67:18. According to the statistics of Kok in Holland, male dogs are three times more frequently infected than females.

Since it has been impossible to judge the incidence of canine leptospirosis, in particular the Canicola infection in San Francisco and Northern California, a series of serological tests have been un-

dertaken. Of 47 normal dogs obtained from one source, 16 or, 34 per cent, gave serum reactions (1:100 to 1:300,000) indicative of a passed or subclinical leptospirosis infection. The dogs of a rural community showed a slightly lower percentage—14.3 per cent or 4 in 28 canines—of latent infections.

Through the courtesy of Dr. Norman J. Pyle of the Lederle Laboratories, Pearl River, New York, the blood sera of 111 dogs, comprising 13 different breeds ranging in age from 1 month to 12 years, were subjected to agglutination and lysis tests with leptospira strains. Ten dogs (9 per cent) agglutinated *Leptospira canicola* in dilutions 1:100 to 1:300,000, while 3, or 2.7 per cent, reacted with *Leptospira icterohaemorrhagiae*.

The existence of canine leptospirosis in the East had been suspected, but it is now proved that both types of leptospira may cause clinical and latent infections in a manner and distribution somewhat similar to that reported from Holland. The observations of Molner and Kasper [16] in Detroit would indicate the existence of canine Weil's disease in the Middle West. As a rule, the Canicola infections overshadow those due to the classical strains. According to Klarenbeek, in 1937, 31 dogs reacted with the *Leptospira canicola* and 21 with the *Leptospira icterohaemorrhagiae* strains. Previous data indicate the following relationship: *Leptospira canicola : Leptospira icterohaemorrhagiae* 125:89. On the other hand, in a Danish village, Borg and Petersen and Jacobsen [17] found the sera of 18 in a series of 53 dogs (33 per cent) to give serum reactions indicative of latent classical Weil's disease (*Leptospira icterohaemorrhagiae* serum titers 1:300 to 1:30,000). Only 1 dog of the series reacted with *Leptospira canicola;* he shed the specific spirochete in the urine. Thus 19 dogs, or nearly one-third of the dog population examined, suffered or had passed through leptospira infection.

The absence of classical strain infections among the canines of San Francisco and California is interesting, and may in some way be associated with the rodent control measures which prevent the hunting of rats by dogs.

The incidence of latent infections increases with age. In dogs over 6 years of age, nearly 60 per cent show the residuals of a latent infection (Kok). The California Canicola leptospirosis is complicated by icterus, and in this respect differs from that seen in Holland. The renal lesions are, however, remarkably similar to those described by Dhont, Klarenbeek, Schüffner, and Voet.[18] In fact, the nephritis, which may lead to acute or subacute fatal uremia, persists following recovery and forms the basis for a leptospiruria for several months.

It is this shedder stage with the *Leptospira canicola* which doubtless maintains the epidemization of the dog population in form of acute and fatal, atypical, abortive, and latent infections. Since the dog exhibits great interest for the urine and the genitalia of its own species, the infection is doubtless spread by contact directly through the tongue or nose. This behavior is preëminent among male dogs and thus explains the higher incidence. Sexual intercourse may equally be a factor. General uncleanliness may lead to the infection of an entire dog family and to clinical or latent human cases.

HUMAN "LEPTOSPIROSIS CANICOLARIS" OR CANICOLA FEVER

The two observations on Canicola fever in California may be classed with the occupational diseases. Both the veterinarian and, in the second case, an assistant in a dog pound handled a great many (about 150 annually) dogs suffering from leptospirosis. However, it is not unlikely that Canicola fever

is more widely prevalent than is indicated from the available records. Mild cases are difficult to diagnose and Canicola infections, without any icterus, prevalent among dogs may readily cause atypical or abortive cases in the owners (Roos, Walch-Sorgdrager and Schüffner).[19]

Without a special bacteriological examination, an influenza or undulant fever-like disease or a meningitis may be suspected as a Canicola infection provided the inquiry discloses an association with dogs. During the past 3 years, the diagnosis of Canicola fever has been established for 12 human beings living in Holland. At least 6 cases have been recognized in Denmark. Brammer, Petersen, and Scheel-Thomsen[20] traced the infection of a 25 year old housemaid with signs suggestive of undulant fever or miliary tuberculosis, but with a Canicola serum titer of 1:300, to a 7 month old puppy which lived in the house and suffered from an acute attack of nephritis. The persistent dysuria which followed forced the maid to mop up after the dog several times a day.

WEIL'S DISEASE TRANSMITTED FROM DOGS

Aside from the 20 cases of Canicola fever thus far reported from Holland, Denmark, and the United States, human Weil's disease caused by the classical strain transmitted from the dog to man have been reported. The two infections described by Krumbein and Frieling[21] and one by Montagu Lawrence and Okell[22] have not been proved bacteriologically. On the other hand, the fourth case seen by Borg and Petersen and Jacobsen in a plumber was traced to his pet dog, an 8 month old "Rattler Pinscher" with serum titer for the classical strain of 1:30,000 and positive findings of spirochetes in the urine. Two cases of Weil's disease in Detroit are doubtless of canine origin.

LATENT LEPTOSPIROSIS

In view of the widespread and regionally high incidence of murine and canine leptospirosis, it is reasonable to suspect that aberrant human infections due to water accidents, etc., and due to association with diseased subclinically infected dogs may be more frequently encountered than the present reports would indicate. The finding of leptospira antibodies by Mason[23] in the sera of 6 out of 11 rat catchers, none of whom recollected having been jaundiced, strongly suggests the existence of latent infections.

In San Francisco, 1 of 10 sanitarians engaged in rodent control work agglutinated *Leptospira icterohaemorrhagiae* in a dilution 1:300.

Roos,[19] in an intriguing report on Canicola fever in a family, discovered with the aid of the serologic tests two additional contact infections in a young adult and child, who apparently had passed through a leptospirosis diagnosed as influenza. The original case, which led to the discovery of the focus, suffered from meningitis. These and other less carefully investigated mysterious illnesses rather forcibly indicate that human Canicola disease may masquerade in a community in form of atypical, abortive, and subclinical infections. It is, therefore, recommended that in future all sera submitted to public health laboratories for the Widal or Brucella reaction be subjected to tests with different leptospira strains.

Since at least 40 per cent of the human Weil's infections may not be accompanied by an icterus, the extent of this disease of murine or even canine origin requires further investigation. However, in this connection, it is imperative to extend a word of warning. The bacteriological diagnoses of leptospirosis, in particular the serological tests, require considerable experience and judicious interpretation. They

should be, therefore, entrusted to a few properly qualified laboratories.

The facts here recorded amply attest to the increasingly important fact that the animal kingdom is a reservoir of disease.

REFERENCES

1. Blumer, G. *J.A.M.A.*, 81:353, 1923.
2. Pickles, W. N. *Brit. J. Child. Dis.*, 33:192, 1936.
3. Morgan, M. T., and Brown, H. C. *Rep. Pub. Health & Med.* Subj. No. 42, London, 1927.
4. Findlay, G. M., Dunlop, J. L., and Brown, H. C. *Tr. Roy. Soc. Trop. Med. & Hyg.*, 25:7, 1931–1932.
5. Yenikomshian, H. A., and Dennis, E. W. *Tr. Roy. Soc. Trop. Med. & Hyg.*, 32:189, 1938.
6. Packchanian, A. *Bull. Office internat. d'hyg. pub.*, 29:2350, 1937.
7. *J. Bact.*, 36:37, 1938.
8. *Proceedings of the Staff Meetings of the Clinic of Queen's Hospital, Honolulu, T. H.*, March, 1938, Vol. 4, No. 3. Personal Communication.
9. Syverton, J. T., Stiles, W. W., and Berry, G. P. *J. Bact.*, 36:37, 1938.
10. Schüffner, W. *Arch. f. Schiffs.-u. Tropen-Hyg.*, 36:239, 1932.
Schüffner. W., and Walch-Sorgdrager, B. (Mme.). *Bull. Office internat. d'hyg. pub.*, 29:297, 1937.
11. Esseveld, H. Thesis. Amsterdam, 1937.
12. Okell, C. C., Dalling, T., and Pugh, L. P. *Vet. J.*, 81:3, 1925; *Brit. Med. J.*, 1:34, 1925; and *Proc. Roy. Soc. Med.*, 18:17, 1925.
13. Wirth, D. *Wien. klin. Wchnschr.*, 50:1115, 1937.
14. Klarenbeek, A. *Tijdschr. v. Diergeneesk.*, 62:1182, 1935, and *Twelfth Int. Vet. Congress*, New York, 3:349, 1934.
15. Walch-Sorgdrager, B., and Schüffner, W. *Zentralbl. f. Bakteriol.*, I. Abt., Orig., 141:97, 1938.
16. Molner, J. G., and Kasper, J. A. *J.A.M.A.*, 110:2069, 1938.
17. Petersen, C. B., and Jacobsen, E. *Compt. rend. Soc. de biol.*, 126:797 and 799, 1937.
18. Dhont, C. M., Klarenbeek, A., Schüffner, W. A. P., and Voet, J. *Nederl. Tijdschr. v. geneesk*, 78:5197, 1934.
19. Roos, C. J., Walch-Sorgdrager, B., and Schüffner, W. A. P. *Nederl. Tijdschr. v. geneesk.*, 81:3324, 1937.
20. Brammer, E., Petersen, C. B., and Scheel-Thomsen, A. *Ugesk. f. laeger*, 100:419, 1938.
21. Krumbein, R., and Frieling, B. *Deutsch. med. Wchnschr.*, 42:564, 1916.
22. Montagu Lawrence, C. J., and Okell, C. C. *Lancet*, II; 327, 1929.
23. Mason, W. N. M. *J. Path. & Bact.*, 46:631, 1938.