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ETIOLOGY OF YELLOW FEVER.

VIII. Presence of a Leptospira in Wild Animals in Guayaquil and Its Relation to Leptospira icterohæmorrhagiæ and Leptospira icteroides.

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Leptospira icterohæmorrhagiæ was demonstrated in the kidneys of wild rats first by Ido and his associates in Japan,[1] then by Stokes, Ryle, and Tytler,[2] Courmont and Durand,[3] Martin and Pettit,[4] Renaux,[5] Coles,[6] Monti,[7] Grasso,[8] and Dalmau[9] in Europe; and by Noguchi[10] and Jobling and Eggstein[11] in North America; by Nicolle and Lebailly[12] in Tunis, and by Lhéritier[13] in Algeria. That the leptospira found in wild rats is in all probability identical with that which produces infectious jaundice in man was the conclusion of Ido and his associates[1] and later of the writer[10] from the reciprocal immunity reactions between the human and rat strains. Just how the rat strain enters the human body is still a

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[1] Ido, Y., Hoki, R., Ito, H., and Wani, H., *J. Exp. Med.*, 1917, xxvi, 341.

[2] Stokes, A., Ryle, J. A., and Tytler, W. H., *Lancet*, 1917, i, 142.

[3] Courmont, J., and Durand, P., *Bull. et mêm. Soc. mêd. hôp. Paris*, 1917, xli, 115.

[4] Martin, L., and Pettit, A., *Compt. rend. Soc. biol.*, 1917, lxxx, 10, 574; 1918, lxxxi, 697.

[5] Renaux, E., *Compt. rend. Soc. biol.*, 1917, lxxx, 405.

[6] Coles, A. C., *Lancet*, 1918, i, 468.

[7] Monti, A., *Boll. Soc. med.-chir. Pavia*, 1917, cited by Martin, L., and Pettit, A., Spirochétose ictérohémorrhagique, Monographies de l'Institute Pasteur, Paris. 1919.

[8] Grasso, G., *Pathalogica*, 1918, x, 8.

[9] Dalmau, M., *Treballs Soc. Barcelone*, 1918, cited by Martin, L,. and Pettit, A,. Spirochétose ictérohémorrhagique, Monographies de l'Institute Pasteur, Paris, 1919.

[10] Noguchi, H., *J. Exp. Med.*, 1917, xxv, 755.

[11] Jobling, J. W., and Eggstein, A. A., *J. Am. Med. Assn.*, 1917, lxix, 1787.

[12] Nicolle, C., and Lebailly, C., *Compt. rend. Soc. biol.*, 1918, lxxxi, 349.

[13] Lhéritier, A., *Bull. Soc. path. exot.*, 1918, xi, 357.

matter of conjecture, based on circumstantial evidence. It is not yet entirely clear how the leptospira happens to be carried by rats. It is probable that the infection is an accidental one, occasioned by the direct transmission of the leptospira to man through exposure of some part of the body to the water of a cesspool or to moistened ground on which the urine of an infected rat has been deposited within a few hours previously. In a longer period the organisms would be destroyed by other saprophytic bacteria.[14] The incidence of infection is much greater in countries where the body is exposed, especially the feet and hands, to ground or water infested by wild rats. The occupation of the people also plays an important part in the frequency of infection. Once a rat becomes infected it supplies the infective agent for an indefinite length of time, possibly until its death. Transmission of the leptospira from rat to rat is accomplished by infected food or drink and is particularly easy in view of their cannibalism.

The question of the presence of a leptospira in tropical countries, especially where endemic foci of yellow fever exist, has not heretofore been studied, but with the isolation of a pathogenic leptospira from certain cases of yellow fever in Guayaquil the relation between the human and animal strains demands a thorough investigation. A general survey has therefore been made to detect the presence of the leptospira among the wild animals encountered in Guayaquil.

The mode of study consisted in inoculating intraperitoneally 1 to 2 cc. of a Ringer solution emulsion of both kidneys of two animals into three guinea pigs. The emulsions were prepared, in the proportion of 1 gm. of the kidney to 10 cc. of Ringer solution, by finely grinding up the kidneys in a mortar with sterile sand. Rats, mice, bats, and an opossum were examined for the presence of the leptospira. These animals are abundant in and about the houses and buildings in Guayaquil, and they were caught and sent in alive by the Board of Health[15] of the city. Table I shows the results of the investigation.

The experiments show that eight out of twelve groups of rats carried in their kidneys a leptospira capable of producing in guinea pigs pathological changes similar to those produced by *Leptospira icteohæmorrhagiæ*. Only two groups of rats were absolutely free

[14] Noguchi, H., *J. Exp. Med.*, 1918, xxvii, 593.

[15] I am greatly indebted to Dr. León Becerra of the Board of Health, for his cooperation in the course of the work which was carried on in Guayaquil. For some specimens of bats I am also indebted to Dr. Gonzales-Rubio, Jr.

TABLE I.

Experiments with Rats.

Groups of rats.	Guinea pig No.	Results.	Remarks.
1 (doubtful).	373	Temperature 40.2°C. on 5th day. Survived.	Perhaps an abortive infection.
	374	Temperature 39.8°C. on 5th day. Survived.	" "
	375	Apparently no reaction.	Negative.
2 (positive).	376	Temperature 40.4°C. on 6th day. Epistaxis and icterus on 7th day. Killed. Lungs and stomach hemorrhagic. Liver icteric and enlarged. Acute parenchymatous nephritis. Other organs unchanged. No leptospira found by dark-field examination.	Transfer to Guinea Pig 377 A (blood 2 cc.). Temperature 40°C. on 5th day. Killed " 8th " Typical, but no leptospira found by dark-field examination.
	376 A	Apparently no febrile reaction. Animal became icteric with temperature of 36.2°C. on 8th day. Killed. Findings typical; difficult to find leptospira in blood or organs.	In a later passage the leptospira was demonstrated.
	377	Died over night.	
3 "	378	Only slight temperature rise, but typically jaundiced on 8th day and was killed. Typical changes of the organs. Leptospira difficult to find.	
	338 A	Died in 9 days without typical changes.	
	389	Died over night.	
4 "	380	Became jaundiced on 5th day. Died in 9 days. Typical findings. Leptospira not demonstrated.	
	381	Temperature 40.2° on 10th day; 40°C. on 11th day, but animal never came down with typical symptoms.	
	382	Temperature 40.5°C. on 8th day and 37.4° on 10th day, with extreme jaundice. Typical findings with leptospira in the tissues.	

TABLE I—*Continued.*

Groups of rats.	Guinea pig No.	Results.	Remarks.
5 (negative).	383	Died of intercurrent infection in 10 days.	
	384	No reaction.	
	385	Temporary rise in temperature for several days. Survived.	
6 "	386	Died in 2 days. Negative.	
	386 A	Temperature 40.3°C. on 7th day, but with ultimate recovery.	
	387	No reaction.	
7 (doubtful).	388	Nothing typical.	
	388 A	Temperature 40.2°C. on 4th day, but recovered.	Perhaps an abortive infection.
	389	Died in 14 hours. Negative.	
8 (positive).	390	Temperature 40.2° on 5th day, 38.6°C. on 7th, with marked jaundice. Killed. Typical findings in the organs; leptospira not demonstrated by dark-field examination.	Transfer to two guinea pigs (blood 2 cc.). Both became typically yellow and were killed on 10th day. Typical findings, with leptospira in liver, but none in kidney or blood, of one animal; second animal showed no leptospira.
	390 A	Highest temperature 39°C. on 5th day. Died in 9 days. Typical lesions.	
	391	Slight febrile reaction. Recovered.	
9 "	392	Highest temperature 39.4°C. on 5th day. Died on 7th day with typical lesions.	
	392 A	Highest temperature 39.6°C. on 8th day. Died on 10th day with icterus and hemorrhages.	
	393	Temperature 40.1°C. on 6th day, 39.9° on 7th day. Transient icterus on 10th day. Recovered.	

TABLE I—*Concluded.*

Groups of rats.	Guinea pig No.	Results.	Remarks.
10 (positive).	394	No high fever. Icterus in 8 days. Killed in 11 days. Lesions typical. Leptospira difficult to find.	
	394 A	Temperature 39.4°C. on 5th day. Icterus following day. Killed on 8th day. Typical lesions at autopsy. Leptospira found.	Transfer to two guinea pigs. Both died in 7 days with typical lesions and jaundice.
	395	Remained well.	
11 "	396	Temperature 39.7°C. on 4th, 40° on 5th, 36.6° on 7th day. Intensely jaundiced. Killed in 6 days. Typical changes. Leptospira difficult to find.	Transfer to two guinea pigs (blood 2 cc. each). One died in 7 and the other in 9 days—both intensely jaundiced. Typical lesions. Melena and black vomit in latter.
	396 A	Had very little fever, but died with jaundice in 9 days. Autopsy typical.	
	397	Temperature 39.6–39.8°C. on 6th, 7th, and 8th days. Suspicion of icterus. Killed for examination in 15 days. Lungs showed old hemorrhagic areas; other organs not changed.	This animal was convalescing after a mild infection.
12 "	398	Remained well.	
	399	" "	
	400	Died in 13 days, with typical jaundice and lesions.	

from the organism, while two others showed a suspicious reaction without terminating in fatal infection. In other words, about 67 per cent of the rats studied harbored the leptospira in their kidneys.

In testing mice for the same organism it was found that out of three groups of seven mice each, one produced extreme jaundice and the other typical changes in two out of three guinea pigs, one dying in 8 and the other in 9 days after the inoculation of the kidney emulsion. There was hemorrhage before death from the rectum in one

and from the nose in the other. The leptospira was difficult to find in the blood, liver, and kidney, but was found in subsequent passages.

No positive results were obtained with the kidney emulsions of eight bats and one opossum. It may be noted that Nicolle and Lebailly[12] obtained negative results with bats caught in Tunis.

The experiments show that the emulsions which produced a fatal infection in guinea pigs did not always kill all the guinea pigs inoculated with the same quantities of the same material and under the same conditions. Some died, while others had a mild abortive infection or escaped infection altogether. This seems to indicate that there exists among individual guinea pigs a considerable variation in their susceptibility to the same strain of the organism and explains why it is important to use as many guinea pigs as convenient for the purpose of transmitting the organism to this animal. There is a close analogy with the frequent abortive infections which were obtained in the attempts to transmit *Leptospira icteroides* to guinea pigs.

The relation between the rodent strains and the strains of *Leptospira icteroides* on the one hand and those of *Leptospira icterohæmorrhagiæ* on the other was next studied from the standpoint of pathogenicity and immunity. For this purpose two strains of leptospira isolated from rats and one from mice were used. The three strains, designated Groups 8, 11, and 30, have morphological features identical with those of the strains isolated from wild rats caught in the vicinity of New York,[10] and are consequently indistinguishable from the icterohemorrhagic strains derived from the Japanese and European sources. They are slightly coarser than the strains of *Leptospira icteroides*.

With regard to the pathogenicity of the Guayaquil rat leptospira, it is difficult to point out any essential difference between the symptoms and lesions that occur in guinea pigs infected with it and those in animals inoculated with the icterohemorrhagic strains of temperate climates. They all produce jaundice and hemorrhages, although with some strains hemorrhage is the outstanding feature.

Identification of the Organism by Means of Immunity Reactions.

In order to determine whether the leptospira isolated from wild rodents in Guayaquil is identical with the strains of *Leptospira icterohæmorrhagiæ* from other sources, and what relation it may have to *Leptospira icteroides* from yellow fever patients in the same city, their immunity reactions were taken into consideration in a series of experiments.

Immune Sera.—Two rabbits were immunized with each of the three strains of Guayaquil rodent leptospira by injecting intravenously 2 to 4 cc. of rich living cultures of the organisms several times at 7 to 12 day intervals. The animals were bled on the 9th day after the last injection and the effects of their sera tested not only upon the same and other strains of Guayaquil origin, but also upon the Japanese, European, and New York strains of *Leptospira icterohæmorrhagiæ*. The relation of this group of organisms to that of *Leptospira icteroides* was likewise studied and will be discussed at greater length in connection with the relation between yellow fever and infectious jaundice.

The first experiments were designed for observation of the action of each of these sera upon the organism *in vitro*. To 0.5 cc. of a rich suspension of culture in saline solution was added 0.2 cc. of the immune serum with or without the simultaneous addition of 0.2 cc. of fresh normal guinea pig serum as complement. The mixture was placed in a water bath at 37°C. for 2 hours and then examined under the dark-field microscope. The entire procedure was carried out with strict aseptic precautions, and each experiment was accompanied by a control with normal rabbit serum. The tubes were kept at room temperature for 96 hours and their contents examined again. Except for a greater amount of precipitation in some tubes the results were about the same as those observed at the end of 2 hours at 37°C. The control tubes with normal rabbit serum showed numerous active organisms after 4 days.

Pfeiffer's phenomenon was also studied by the usual procedure; that is, examination of the peritoneal fluid of guinea pigs after inoculation with a given serum and the strain in question. In this instance 0.5 cc. of rich culture was mixed with 1 cc. of the immune serum and

injected intraperitoneally, examination being made after 30 minutes and 2 hours.

As another means of identification the protective property of each immune serum (1 cc.) was tested on guinea pigs against approximately 10 minimum lethal doses of the different strains. Unfortunately

TABLE II.

Immunological Relation of the Guayaquil Rat Leptospira to Leptospira icterohæmorrhagiæ.

Immune Serum 906, produced with Group 8 strain, injected on Jan. 15, 22, Feb. 3, 15, 1919.

Cultures tested.	Effects *in vitro*.	Pfeiffer phenomenon.	Protective property against infection in guinea pigs.
Group 8	Marked precipitation, agglutination, and disintegration.	Positive.	1 cc. protected guinea pigs against about 10 minimum lethal doses.
" 11	Effects similar to the foregoing but less in degree.	"	" "
" 30	Marked agglutination and later disintegration.	"	" "
Japanese.	Similar to the foregoing but less marked.	"	" "
American No. 1	Rather marked agglutination.	"	Could not be tested because of loss of virulence.
" " 3	Effects similar to the foregoing.	"	" "
French.	" "	"	1 cc. protected guinea pigs against about 10 minimum lethal doses.

the virulence of the American and British strains of *Leptospira icterohæmorrhagiæ* was considerably attenuated during my absence of 6 months and could not be tested with reliable results. This, however, was not a serious obstacle to determining the affinity of these strains for the Guayaquil strains, because an immune serum produced in rabbits with the avirulent American strain was tested against the pathogenic Guayaquil strains. A brief summary of the foregoing experiments is given in Tables II to VI.

TABLE III.

Immunological Relation of the Guayaquil Rat Leptospira to Leptospira icterohæmorrhagiæ.

Immune Serum 914, produced with Group 11 strain, injected on Dec. 30, 1918, Jan. 6, 14, 21, 1919.

Cultures tested.	Effects *in vitro*.	Pfeiffer phenomenon.	Protective property against infection in guinea pigs.
Group 8	Moderate agglutination but no degeneration.	Positive.	1 cc. protected guinea pigs against about 10 minimum lethal doses.
" 11	Marked precipitation and agglutination; later degeneration.	"	" "
" 30	Effects similar to the foregoing but less marked.	"	" "
Japanese.	" "	"	" "
American No. 1	" "	"	Could not be tested because of loss of virulence.
" " 3	Marked agglutination and degeneration.	"	" "
French.	" "	"	1 cc. protected guinea pigs against about 10 minimum lethal doses.

TABLE IV.

Immunological Relation of the Guayaquil Rat Leptospira to Leptospira icterohæmorrhagiæ.

Immune Serum 904, produced with Group 30 strain, injected on Dec. 30 1918, Jan. 6, 14, 22, Feb. 3, 15, 1919.

Cultures tested.	Effects *in vitro*.	Pfeiffer phenomenon.	Protective property against infection in guinea pigs.
Group 8	Marked agglutination and subsequent disintegration.	Positive.	1 cc. protected guinea pigs against about 10 minimum lethal doses.
" 11	Slight agglutination, but no degeneration.	"	" "
" 30	Marked precipitation, agglutination, lysis, and degeneration.	"	" "
Japanese.	" "	"	" "
American No. 1	" "	"	Could not be tested on account of loss of virulence.
" " 3	Slight agglutination; no degeneration.	"	" "
British.	" "	"	" "

TABLE V.

Immunological Relation of the Guayaquil Rat Leptospira to Leptospira icterohæmorrhagiæ.

Immune Serum 952, produced with American Strain 1, injected on Dec. 30, 1918, Jan. 6, 14, 22, Feb. 3, 15, 1919.

Cultures tested.	Effects *in vitro*.	Pfeiffer phenomenon.	Protective property against infection in guinea pigs.
Group 8	Moderate agglutination; slight degeneration.	Positive.	1 cc. protected guinea pigs against about 10 minimum lethal doses.
" 11	Very marked agglutination and degeneration.	"	Not tested.
" 30	Slight agglutination and degeneration.	"	1 cc. protected guinea pigs against about 10 minimum lethal doses.
Japanese.	Moderate agglutination and degeneration.	"	Not tested.
American No. 1	Marked agglutination and degeneration.	"	" "
British.	Slight agglutination and degeneration.	"	" "

TABLE VI.

Immunological Relation of the Guayaquil Rat Leptospira to Leptospira icterohæmorrhagiæ.

Immune Serum 911, produced with the Japanese strain, injected on Dec. 30, 1918, Jan. 6, 12, 22, Feb. 3, 15, 1919.

Cultures tested.	Effects *in vitro*.	Pfeiffer phenomenon.	Protective property against infection in guinea pigs.
Group 8	Moderate agglutination and degeneration.	Positive.	1 cc. protected guinea pigs against about 10 minimum lethal doses.
" 11	Slight agglutination; no degeneration.	"	" "
" 30	Marked agglutination and disintegration.	"	" "
Japanese.	Very marked agglutination and lysis.	"	" "
American No. 1	Moderate agglutination and degeneration.	"	Could not be tested because of loss of virulence.
British.	Slight agglutination and degeneration.	"	" "

The tables show that while there exist undeniable variations in the intensity of the immunity reactions as manifested *in vitro* in the form of agglutination and subsequent disintegration of the organism, as well as *in vivo* in Pfeiffer's phenomenon and protection against infection with one or the other strains, the variations are nevertheless of so slight a nature as to lead one to assume that the strains isolated

TABLE VII.

Immunological Relation of the Guayaquil Rat Leptospira to Leptospira icteroides.

Immune Serum 906, produced with Group 8 strain, injected on Jan. 15, 22, Feb. 3, 15, 1919.

Leptospira icteroides strain.	Effects *in vitro*.	Pfeiffer phenomenon.	Protective property against infection in guinea pigs.
A. A.	Slight agglutination; no degeneration.	Negative.	No protection.
E. Ch.	No effect.	"	" "
A. Ce.	" "	"	" "

TABLE VIII.

Immunological Relation of the Guayaquil Rat Leptospira to Leptospira icteroides.

Immune Serum 914, prepared with Group 11 strain, injected on Dec. 30, 1918, Jan. 6, 14, 21, 1919.

Leptospira icteroides strain.	Effects *in vitro*.	Pfeiffer phenomenon.	Protective property against infection in guinea pigs.
A. A.	No effect.	Negative.	No protection.
E. Ch.	" "	"	" "
A. Ce.	" "	"	" "
M. G.	Definite agglutination, but no immobilization.	Doubtful.	1 cc. did not prevent the infection, but the animal survived.

from rats and mice in Guayaquil belong to the group of *Leptospira icterohæmorrhagiæ* now known to be widely distributed among these rodents inhabiting the temperate zone.

The results of a parallel series of experiments performed with the immune sera on different strains of *Leptospira icteroides* are given in Tables VII to IX.

Prophylactic Inoculation.—February 6, 1919. A number of guinea pigs were inoculated subcutaneously with 0.5 and 2 cc. of killed culture (heated to 60°C. for 10 minutes in the waetr bath) of the Guayaquil rat strains of leptospira, Groups 8, 11, and 30. These animals were inoculated after 15 days (February 21) with virulent cultures of the same and of other strains. At the same time some of the vaccinated guinea pigs were inoculated also with the icterohemorrhagic strains, including the American No. 1,[16] the Japanese, and the

TABLE IX.

Immunological Relation of the Guayaquil Mouse Leptospira to Leptospira icteroides.

Immune Serum 904, produced with Group 30 strain, injected on Dec. 20, 1918, Jan. 6, 14, 22, Feb. 3, 15, 1919.

Leptospira icteroides strain.	Effects *in vitro*.	Pfeiffer phenomenon.	Protective property against infection in guinea pigs.
E. Ch.	Slight agglutination without immobilization.	Doubtful.	No protection.
A. Ce.	No effect.	Negative.	" "
M. G.	" "	"	" "
A. A.	" "	"	" "

French. The guinea pigs previously inoculated with the killed cultures of the Guayaquil strains proved resistant to a subsequent infection, not only with homologous but also with the heterologous strains derived from Japanese, European, and American sources. This experiment indicates that the Guayaquil rodent strains of leptospira are identical with *Leptospira icterohæmorrhagiæ*.

SUMMARY.

By the inoculation of guinea pigs intraperitoneally with the emulsions of kidneys from wild rats and mice captured in Guayaquil, it was found that 67 per cent of the wild rats tested harbored in their kidneys a leptospira which produced in guinea pigs symptoms and lesions identical with those produced by *Leptospira icterohæmorrhagiæ* derived either from patients suffering from infectious jaundice in Japan or Europe, or from wild rats caught in New York.

[16] This strain was so attenuated that some of the guinea pigs escaped a fatal infection and could not be used in further experiments.

Immune sera were prepared in rabbits by injecting different strains of the Guayaquil leptospira. These sera had a marked agglutinating and disintegrating influence upon the homologous strains, and also, but often to a less pronounced degree, upon the strains of *Leptospira icterohæmorrhagiæ* from other sources. Pfeiffer's phenomenon was also found to be positive, and protection was demonstrated against infection with virulent cultures of strains of *Leptospira icterohæmorrhagiæ*.

The same sera had no effect, or at most a very slight one, upon *Leptospira icteroides*. Guinea pigs inoculated with *icteroides* strains were not noticeably protected by the use of the immune sera prepared with the Guayaquil rat strains.

Guinea pigs inoculated with killed cultures of the Guayaquil strains of leptospira proved to be resistant to a subsequent infection with heterologous as well as homologous strains of *Leptospira icterohæmorrhagiæ*.

It is concluded, therefore, that the leptospira isolated from the kidneys of wild rats and mice in Guayaquil belongs to the group of *Leptospira icterohæmorrhagiæ*, and differs from *Leptospira icteroides* in its immunity reactions.

No positive transmission was obtained with kidney material from bats and an opossum.