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Comparison of New Jersey and Palestine Strains of Bovine Leptospira.

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In the previous work on leptospirosis in cattle in New Jersey[1,2] the similarity to the disease reported in Palestine[3-5] was obvious, but no culture of the Palestine strain was at hand for serological comparisons. The present paper reports serological studies carried out with 6 strains of leptospira isolated in an outbreak of the disease in a New Jersey dairy herd and a strain of bovine leptospira from Palestine.

Methods. Chang's[6] semisolid and fluid media (in which rabbit serum had been substituted for horse serum and the hemoglobin omitted) were used for storing cultures and Schueffner's[7] medium was used for the serological tests. Actively growing cultures incubated 5 to 7 days at 30°C were used as antigen either as living or formalinized preparations. Equal amounts of serum dilutions and leptospira suspensions were mixed in small test tubes. For agglutination tests formalinized cultures (0.3% formalin) were incubated for 2 hours at 37° C and kept at room temperature, while with living antigen the incubation period was the same but the tubes were stored in the refrigerator. Readings were made the following morning by examining uncovered drops by dark-field illumination with objective 10 and ocular 10. The results with living cultures were recorded as +, all organisms lysed; ± many organisms lysed; and —, no lysis. With formalinized material the readings were recorded as +, nearly all leptospiras agglutinated in large clumps; ±, many leptospiras agglutinated in small clumps; and —, no agglutination.

Serological Examination. In the serological studies it was found that both living and formalinized cultures gave identical titers as a rule, yet is was useful to check the results of one method with the other. Small antibody concentrations may not be detected if living cultures are employed, as minor degrees of lysis are more difficult to read than slight agglutination reactions obtained with formalinized material. The disadvantage, however, of using live cultures is offset by the avoidance of spontaneous clumping which not infrequently occurs in formalinized cultures.

Three leptospiral strains (N.J.) were used to immunize rabbits. The rabbits received 3 intravenous injections of living cultures at weekly intervals and were bled one week after the last injection. Each New Jersey immune serum agglutinated the 3 New Jersey strains to the same titer. It was therefore assumed that the strains were identical and this was confirmed in absorption experiments. Subsequently 2 rabbits were immunized, one with a New Jersey culture, the other with the Palestine strain. Each animal received 4 injections of living culture intravenously and a week after the last injection they were bled from the heart. The results for both agglutination and

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1 Baker, J. A., and Little, R. B. Presented in abstract form before the Twenty-eighth Conference of Research Workers in Animal Diseases in North America, Chicago, Ill., Dec. 2, 1947. Also in Bovine Mastitis. A Symposium (R. B. Little and W. N. Plastridge, editors), New York, McGraw-Hill Book Co., Inc., 1946.

2 Baker, J. A., and Little, R. B., *J. Exp. Med.*, 1948, **88**, 295.

3 Bernkopf, H., Olitzki, L., and Stuczynski, L. A., *J. Infect. Dis.*, 1947, **80**, 53.

4 Ungar, H., and Bernkopf, H., *Arch. Path.*, 1947, **44**, 59.

5 Bernkopf, H., Stuczynski, L. A., Gottlieb, T., and Halevy-Katz, C., *J. Infect. Dis.*, 1948, in press.

6 Chang, Shih Lu, *J. Infect. Dis.*, 1947, **81**, 28.

7 Kelser, R. A., and Schoening, H. W., Manual of Veterinary Bacteriology, 4th Edition, Williams and Wilkins Co., Baltimore, Md., 1943, p. 411.

TABLE I.
Agglutination and Lysis Tests with Immune Sera Prepared in Rabbits Against the New Jersey and the Palestine Strains of Leptospira.

Strain	Antigen used	Antiserum to New Jersey strain 1:20	1:200	1:2,000	1:20,000	1:200,000	Antiserum to Palestine strain 1:20	1:200	1:2,000	1:20,000	1:200,000
New Jersey	Formalinized culture	+	+	+	±	—	—	—	—	—	—
	Living culture	+	+	+	±	—	—	—	—	—	—
Palestine	Formalinized culture	±	±	—	—	—	+	+	+	±	—
	Living culture	±	—	—	—	—	+	+	+	±	—
L. icterohaemorrhagiae	Formalinized culture	—	—	—	—	—	±	±	—	—	—
	Living culture	—	—	—	—	—	—	—	—	—	—
L. canicola	Formalinized culture	—	±	—	—	—	—	±	—	—	—
	Living culture	—	—	—	—	—	—	—	—	—	—

TABLE II.
Examination of Sera of Two New Jersey Cows* for Antibodies Against the New Jersey and Palestine Strains of Leptospira.

Cow No.	Date of onset of illness	Date of bleeding	Agglutination of formalinized culture: New Jersey strain, Dilution of sera 1:20	1:200	1:2000	1:20,000	Agglutination of formalinized culture: Palestine strain, Dilution of sera 1:20	1:200	1:2000	1:20,000	Lysis of living culture: New Jersey strain, Dilution of sera 1:20	1:200	1:2000	1:20,000	Lysis of living culture: Palestine strain, Dilution of sera 1:20	1:200	1:2000	1:20.000
1	12/2/46	12/2/46	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
		1/9/47	±	+	+	—	—	—	—	—	±	+	+	—	—	—	—	—
2	4/6/48	4/6/48	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
		4/26/48	+	+	+	—	+	—	—	—	+	+	+	—	—	—	—	—

* Bled at the onset of the disease and after recovery.

TABLE III.
Inoculation of the New Jersey Strain into Guinea Pigs Previously Inoculated with the Palestine Strain.

Guinea pig No.	First inoculation	Results of second inoculation with New Jersey strain
1	Palestine strain	Fever after 3 days
2	"	" " 6 "
3	"	" " 7 "
4	"	" " 7 "
5	"	No fever
6	"	" "
7	"	" "
8	Not inoculated	Fever after 2 days
9	" "	" " 3 "
10	" "	" " 3 "
11	" "	" " 3 "

lysis tests are given in Table I. Strains of *Leptospira canicola* and *Leptospira icterohaemorrhagiae* kindly supplied by Dr. R. E. Kelser were also included in this test.

It can be seen in Table I that no marked cross reactions occurred with the New Jersey and Palestine strains. Antiserum to the Palestine strain did not cross react with the New Jersey strain, while the antiserum to the New Jersey strain showed a weak cross reaction with the Palestine strain in a dilution of 1:20 (lysis) and 1:200 (agglutination). It is also clearly shown in Table I that serologically both bovine strains are different from *L. icterohaemorrhagiae* and *L. canicola*.

Examination of sera from naturally infected cows. Sera from cows of the New Jersey herd which had recovered from an attack of the disease reacted strongly with the New Jersey strain[1,2] but showed little if any reaction against the Palestine culture.[3] The reaction of the sera of 2 cows taken at the onset of the disease and 20 and 38 days later, respectively, is given in Table II. The specificity of the reaction with the New Jersey strain is clearly shown, for neither serum from the recovered animals agglutinated the Palestine culture.

The sera of 10 cows which had shown typical symptoms of the disease in May 1946 (bled 2 to 3 weeks after the onset of the illness) reacted with the New Jersey culture in dilutions up to 1:20,000. One serum agglutinated the Palestine strain in a dilution of 1:2,000. Of the bovine sera tested from both normal and recovered cases in the New Jersey herd, this was the only serum that agglutinated the Palestine culture in any dilution.

Forty-six sera from cows in a barn in which 2 typical cases of abnormal milk (bloody or thick off-colored milk) had occurred 2 months earlier were tested against both strains. Eight of the sera gave a positive reaction with the New Jersey strain in a dilution of 1:200, and 4 in a 1:2,000 dilution, thus suggesting that inapparent infections are not uncommon.

Pathogenicity of the Strains for Guinea Pigs. A cross infection experiment was set up by inoculating guinea pigs first with the Palestine strain and 3 weeks later with a New Jersey strain isolated from the milk of cow No. 1489. Each animal received intraperitoneally one ml of a leptospira culture in fluid medium. The results are given in Table III.

The guinea pigs showed no reaction following the injection of the Palestine strain, confirming earlier observations.[3] The New Jersey strain, however, produced fever in all control animals 2 to 3 days after the inoculation.[2] Only one of the 7 guinea pigs, which had received the Palestine strain before, developed fever 3 days after inoculation with the New Jersey strain. Three guinea pigs of the same group became febrile after an incubation period of 6 to 7 days (nearly twice as long as in the controls) while the remaining animals appeared normal during the entire period of observation. It is obvious therefore that, based on the development of fever, infection with the Palestine strain had produced complete immunity in 3 guinea pigs and partial immunity in 3 other animals out of a total of 7 tested.

A comparison of Tables I and III shows that the serological examination, because it is quantitative, gave a better differentiation of the strains. Schueffner and Mochtar[8] found in numerous experiments that guinea pigs that had recovered from infection with one strain of leptospira were protected against strains which serologically were unrelated.

[8] Schueffner, W., and Mochtar, A., *Z. f. Bakt. Abt. 1, Orig.*, 1926-27, **101**, 405.

Summary. A study has been made of strains of leptospira recovered from an outbreak of leptospirosis among cattle in New Jersey and a strain isolated from an outbreak of the disease in Palestine. Sera from recovered cattle as well as sera from immunized rabbits were used in agglutination and lysis tests and it was found that all the New Jersey strains reacted alike while the Palestine strain belonged to another serological group.

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Effect of Antihistamines on Loss of Adhesiveness of Corneal Epithelium After Injection of Histamine.

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In a previous paper[1] we described the effect of histamine on the adhesiveness of the corneal epithelium of excised bovine corneas. In amounts from 0.5 to 1.0 μg per cornea (600-800 mg wet weight) histamine caused a marked decrease in epithelial adhesiveness. In contrast to other agents which decrease adhesiveness by disruption at the stroma epithelium boundary, histamine seems to effect the detachment of the upper layers of the epithelium from the basal one.

In view of the relatively high amounts of histamine required for this effect (about 10 - 100 times more than for relaxation of cats intestine) and of the gradual culmination of epithelial loosening it seemed desirable to compare the action on the corneal epithelium of histamine alone and of mixtures of histamine with certain antihistamines. The same technic was used as described previously.[2] Between 0.04 to 10 μg of the antihistamines were injected together with a constant amount of 2 μg of histamine in a total volume of 0.2 ml per cornea. At this concentration the antihistamines were found to have no effect on the adhesiveness of the corneal epithelium. On injection of 50 μg per cornea or more, toxicity with loosening of the epithelium was observed for all antihistamines tested so far. The indicated amount of histamine was sufficient to cause loosening in all samples. The corneas were incubated for 12 - 15 hours at 28 - 30°C. The adhesiveness was tested with a simple scraper which allows a semi-quantitative determination of the adhesiveness, the amount of removable epithelium being a function of the adhesiveness and of the weight on the scraping blade.[2] The procedure was simplified in the present series of experiments by keeping the pressure on the scraper constant (60 g) and recording the removal of more than 2/3 and of less than 1/3 of the epithelium of the corneal test strip. After injection of histamine alone there was always under these conditions a removal of more than 2/3 of the epithelium. If antihistamines were injected simultaneously the loss of adhesiveness was reduced to less than 1/3 depending upon the concentration of the respective compound.

In Table I our results are summarized designating protection with removal of less

[1] Herrmann, H., *Bull. Johns Hopkins Hosp.*, 1948, **82**, 208.

[2] Herrmann, H., and Hickman, F. H., *Bull. Johns Hopkins Hosp.*, 1948, **82**, 182.