

Experimental Leptospirosis Infection in Chickens.*

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While the number of mammals found to be leptospiral carriers is steadily mounting, nothing is known about such infections in domestic fowl or other birds. Cases of leptospirosis have been reported among personnel of poultry farms and among poultry dressers but rats have always been considered the source of infection, and the possibility that chickens might be infected from rats seems to have been neglected. It seemed of interest, therefore, to examine the susceptibility of chickens to experimental leptospiral infection.

Since no virulent strain of *Leptospira ictero haemorrhagiae* was available, a bovin strain of leptospira,¹ which causes severe infections in cattle and human beings, was used. A heavy growth of the organism on semisolid medium was diluted 1:3 in saline and 1 cc was inoculated subcutaneously into 32 chickens. The birds varied in age from 6 weeks to 5 months.

Twelve birds were bled from the heart daily for 10 days after inoculation and 0.2 to 0.5 cc of blood was inoculated into semisolid or Schueffner's medium or both. Cultures were incubated at 30°C and were examined weekly with the dark field microscope. Positive cultures were obtained from 2 birds, one on the fourth and one on the ninth day after injection. This low proportion of positive cultures may have been due to the fact that chicken serum when added to heavily inoculated Schueffner medium has a harmful effect on the organism and inhibits growth.

The bird with the positive blood culture on the 9th day showed no clinical signs of illness but serum taken at 3 weeks had an agglutinin titer of 1:800. When the animal was sacrificed at 4 weeks no pathological

changes were found and no organisms were seen in sections of organs stained by Levaditi's method. A calf which was injected with 1 cc of a culture of the isolated strain reacted with a short fever (41.5°C) and developed an agglutinin titer of 1:1600 against the bovine strain of leptospira.

The bird with the positive culture on the fourth day became sick on the 8th day and was sacrificed. Liver, spleen and kidney were cultured on semisolid medium and organisms were successfully cultivated from the liver where they were also demonstrated in considerable number in sections stained by Levaditi's method. The organisms were found within well preserved liver cells. In some areas the liver cells were detached from one another and numerous leucocytes were found in the capillaries. A few organisms were found in sections of the kidney but they could not be cultivated from this organ or the spleen. There were no gross pathological changes on autopsy.

Five chickens were found dead between the 9th and the 14th day after inoculation and 5 more were sacrificed between the 6th and 9th day. No specific changes were found in these birds on autopsy. No leptospiras were demonstrable with certainty in Levaditi stained organ sections although argentophile coils of undetermined nature were seen a number of times.

All of the surviving birds were bled at one to 2 week intervals for serological tests. Sera in dilutions of 1:20 and 1:200 were tested for the agglutination of formolized suspensions of *L. icterohaemorrhagiae*† and the bovine leptospiral strain. Equal volumes of serum dilution and antigen were mixed in small test tubes and incubated at 37°C for 2 hours. The results were read the following

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¹ Bernkopf, H., Olitzki, L., and Stuczynski, L. A., *J. Infect. Dis.*, 1947, **80**, 53.

† The Jackson strain kindly supplied by Dr. J. C. Broom, The Wellcome Laboratories of Tropical Medicine, London.

morning by examining uncovered drops with a low power objective with dark field illumination. All the pre-inoculation bleedings were negative and the antibody response was very variable. Of six, 6-week-old birds, 3 developed no agglutinins at all and 3 developed titers of only 1:40. Among the 2- to 5-month-old birds, levels of 1:800 to 1:8000 were reached and during a 2- to 3-month period of observation these titers did not fall appreciably.

Since the successful demonstration of infection in subcutaneously inoculated birds was demonstrated in only 2 instances the actual number of infections accomplished cannot be stated since the antibody response in older birds may have resulted from the antigenic stimulus of the inoculum. This possibility was eliminated in another experiment in which 5 birds were given the organism by feeding. Each bird received 1 cc of a fluid leptospiral culture drop by drop with a pipette, care being taken that the material was slowly swallowed and that the mucous membranes were not traumatized.

Three weeks later all 5 birds had developed specific serum antibodies in titers ranging from 1:800 to 1:3200. This established the susceptibility of domestic fowls to infection in a manner which could well occur in nature. The actual occurrence of infection in nature remains to be demonstrated.

More than 500 chicken sera, from several flocks, have been examined for their agglutinin titer with bovine leptospira and *L. icterohaemorrhagiae* and while titers of 1:20 and 1:40 were frequently observed these are not considered significant. Two sera were found with titers of 1:400 with both strains and one serum which was positive at 1:200 with the bovine strain only. These serological findings though not adequate in themselves, suggest the occurrence of leptospira infections in chickens in nature.

Summary. Domestic chickens were shown to be susceptible to experimental infection with a bovine strain of leptospira, when given subcutaneously or by mouth. The infection occurs in inapparent form, as a rule.

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Influence of Fluorine in Mottled Teeth on Dental Caries.*

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If compounds of fluorine are to be used as drugs in combating human dental caries,¹ eventually the manner of action must be explained in order to direct their most practical application. At present the literature assumes that it works (1) through a constant intake of fluorine-bearing water² or (2) by

direct chemical combination with the constituents of the tooth, introduced either prenatally³ or postnatally.⁴ The latter assumption has been contested by the presentation of data which shows that a significant effect of fluoride acquired after tooth eruption on induced rat caries is not evident.⁵ Using

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¹ Editorials, *J. A. D. A.*, 1947, **34**, 345; *J. A. D. A.*, 1947, **34**, 411.

² Dean, H. T., *Am. J. Ortho. and Oral Surg.*, 1947, **33**, 49.

³ Cox, G. J., Matuschak, M. C., Dixon, S. F., Dodds, M. L., and Walker, W. E., *J. D. Res.*, 1939, **18**, 481.

⁴ Armstrong, W. D., and Brekhuis, P. J., *J. D. Res.*, 1938, **17**, 393.

⁵ McClure, F. J., *J. Nutrition*, 1941, **22**, 391.