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Leptospirosis Survey in a White-tailed Deer Herd in Ontario:

COMPARATIVE USE OF FLUID AND PAPER DISC-ABSORBED BLOOD*

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Abstract

White-tailed deer *(Odocoileus virginianus)* were sampled in a wilderness area of Southern Ontario in 1965, 1966, and 1967. Serological evidence of *Leptospira pomona* and *L. grippotyphosa* infection was found. Leptospirosis has not depressed the deer population. There was a positive correlation between age and reactor rate. Paper disc-absorbed whole blood has been tested comparatively with fluid serum and found to be a useful tool for field serological surveys.

Introduction

Surveillance for leptospirosis in white-tailed deer has been carried on in Southern Ontario since 1961, when the disease in cattle suddenly reached epizootic proportions[1,6,9]. Enzootic leptospirosis in a wilderness (Canonto) deer herd has been diagnosed by sampling deer at the time of the annual fall hunts.

During the years 1961 to 1965, fresh sera were collected in the field. In 1966, the method of whole blood absorption in paper discs was adopted [2,5,7,11,12].

The purposes of this paper are (1) to describe the application of this method to the annual Canonto leptospirosis surveys, and (2) to present further evidence, based on 1965-1967 studies, to show that leptospirosis does not constitute a depressing factor on the Canonto deer herd.

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Materials and Methods

The Canonto Study Area is located in the eastern part of Southern Ontario, near the edge of the Canadian Shield just west of the Ottawa Valley Lowland. It rests mainly on a granitic bedrock with a few vestiges of sedimentary limestone caught in faults.

Whole blood was collected in vials by hunters, from the thoracic cavities of shot deer, and submitted to a biologist in the field. Serum was removed and stored in ice until delivered to the laboratory. Paper disc-absorbed (PDA) blood was collected by a biologist or by hunters. Paper discs were No. 740-E, 12.7 mm. in diameter, made by Carl Schleicher and Schuell Co., Keene, N.H. Field kits, as shown in Figure 1, were distributed to hunters. Samples were prepared by applying blood with a dropper to saturate the paper discs, which held about 0.1 ml. each. They were air dried in a petri dish with the lid raised. Dried samples could be stored in the laboratory in philatelic envelopes, at room atmosphere, for an indefinite period of time before testing.

Fluid sera were tested by the microscopic agglutination test[4] using a spot plate, a three-fold dilution scheme, and a positive criterion of 50% clearance of antigen at serum dilution 1:60 or greater.

PDA samples were eluted in a test tube by soaking two discs with 1 ml. of eluent, shaking 15 seconds on a vibratory shaker, refrigerating at 4 C for about 16 hours, and separating the fluid from the paper by forceps and centrifugation. The eluate was considered equivalent to a 1:10 dilution of serum, and was tested in the same way as fresh serum. Live antigens used in all diagnostic tests were *Leptospira pomona, L. grippotyphosa,* and *L. icterohaemorrhagiae.*

Two buffers, saline phosphate at pH 7.6 and aqueous phosphate at pH 7.0, were compared as to their ability to elute *L. pomona* antibody from paper discs soaked in serum of known titer.

Reliability of the PDA method was assessed in the following way: seventeen split sample comparisons were made, using all the serologically positive deer taken in the hunt, for which both fluid and PDA blood samples were available, and 9 negative paired samples.

Results

Table 1 contains a summary of the results of deer surveys from 1965 to 1967. Although *Leptospira pomona* was clearly the predominant serotype, antibodies to *L. grippotyphosa,* were also found.

TABLE 1. *Serological Reactor Rates against* Leptospira pomona, L. icterohaemorrhagiae, *and* L. grippotyphosa, *in White-tailed Deer in the Canonto Study Area of Southern Ontario, 1965-1967*

Year	No. Tests	No. pos.*	Reactors to L. pom.	L. grippo.	L. ictero	% reactors	Method
1965	93	19	19	0	0	20	Fluid sera
1966	110	32	32	1 **	0	29	Fluid & PDA ***
1967	189	56	56	3 **	1 **	30	PDA
Total	392	107	107	4	1		

* 50 percent clearance of antigen at a serum dilution of 1:60 or greater
** Titer to *L. pomona* predominated in these reactions
*** PDA = paper disc-absorbed blood

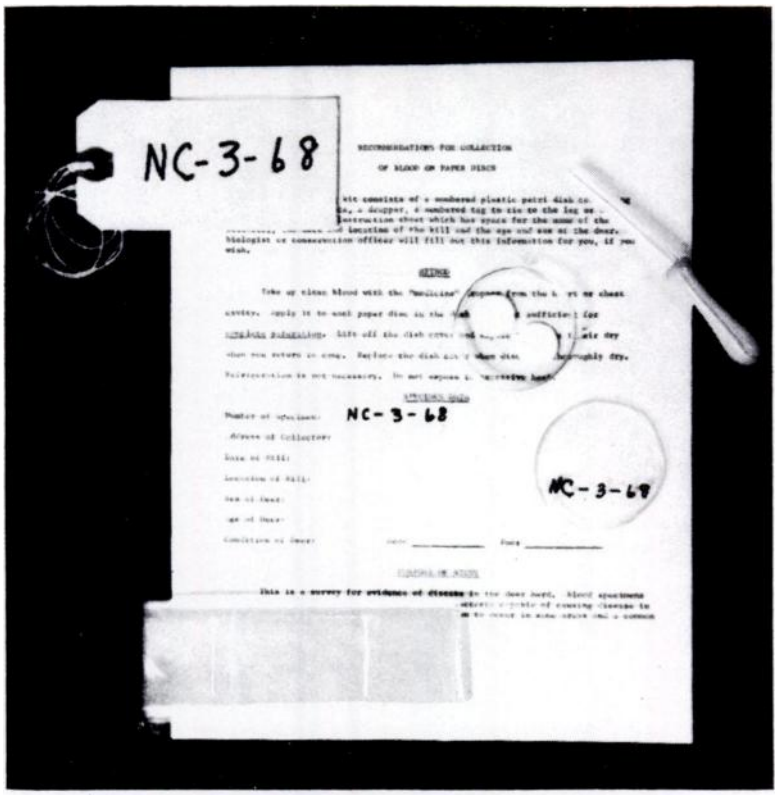

FIGURE 1. *Field kit used by hunters and biologists for the collection of PDA whole blood of deer in Ontario*

The reactor rates in lactating and non-lactating does in Canonto, based on a three year period, were 29.6% and 26.7%. The ratio of lactating to non-lactating does shot each year was about 3:1.

The Canonto deer were placed in yearly age classes, from ½ year, to 5½ years and over, and the reactor rate was calculated on each class and averaged for the three years of the study. A trend to higher rates with increasing age was shown by the results, as follows: ½ year—6.3%; 1½ year — 26.7%; 2½ year — 25.3%; 3½ year — 31.7%; 4½ year — 53.0%; 5½ year — 51.7%; 6½ year and over — 50%.

Seventeen split sample comparisons of fluid serum and PDA whole blood methods showed equivalent results in 10 tests, and a discrepancy of one three-fold dilution in 7. In assessing the performance of buffers as eluents, antibody eluted with the pH 7.0 buffer was equal in titer to the corresponding fluid serum, while antibody eluted with the pH 7.6 buffer was one three-fold dilution lower in titer.

Discussion

The continued productivity in the Canonto deer herd is evidenced by the high ratio of lactating to non-lactating adult does shot in the fall hunt. The reactor rate in the does approximated that in the whole herd, and was equal in both classes of does. Trainer et al[10] allude to a survey of gestating and lactating does in Wisconsin, the results of which showed a lack of correlation between fawnless does and *L. pomona* reactors.

The key factor in the disease pattern in does and fawns may be, as Trainer[10] suggests, the time of exposure, excepting unusual stress. He produced abortion experimentally in four of five pregnant does by exposing them to *L. pomona* in the fifth or sixth month of pregnancy. Over this interval, and throughout all but perhaps the first month of pregnancy, Canonto does are unlikely to contact water-borne leptospires, due to the winter freeze-up. By the time of spring break-up, the fawns are within a month of term. The incubation period for fetal death following exposure of the dam to virulent cultures was 12 to 25 days[10]. One can speculate that infection established in the doe during the last month of pregnancy may not result in abortion. Apparently, if infections do occur at this time, the fawns are born healthy and obtain passive immunity through the colostral antibodies[10].

The unlikelihood of direct deer-to-deer transmission[8,10] and the possibility of surface water-mediated transmission[8] have been shown experimentally.

The titers of Canonto deer sera taken in November, compared to those from experimental infections, further support the probability that deer are stimulated antigenically in summer rather than in winter. Ferris et al[3] and Trainer[10] reported peak agglutinin titers of 10^5 to 10^6 occurring from 15 to 35 days after inoculation, followed by decline to 10^2 or 10^3 at 150 days, and to negative status at 200 days. Canonto deer sera in November had antibody levels 1/20 to 1/540, except for 7 with titers of 1/800 to 1/1020, and 2 with titers indicative of active infections (1/12,800) out of the 107 reactors. The majority of the titers reflect exposure between June and July, interpolating from the experimental results cited above.

The paper disc-absorbed whole blood sample method used in current deer surveys offers convenience in the collection, holding, and shipping of samples. Reactor rates in enzootic areas correspond with rates obtained by fluid serum surveys of previous years. Hunters generally approved of the field kits, after noisiness and fragility had been eliminated.

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