SEROLOGICAL AND CULTURAL EVIDENCE OF LEPTOSPIRAE IN CAPE TOWN DOGS

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Following the recent isolations of *Leptospira icterohaemorrhagiae* locally from wild rats,[1] it was decided to extend the search for leptospirae to other animal carriers. A serological survey of Cape Peninsula dogs was thus undertaken, as well as an attempt to isolate these organisms from them.

AGGLUTINATION TESTS

Material

Sera from 100 dogs were kindly supplied by local veterinary surgeons.

The sera were collected at clinics from a cross-section of dogs of all ages and breeds, seen as a routine, regardless of the diagnosis in each case.

Most of the animals came from a poor Cape Coloured community on the Cape Flats where the standard of hygiene leaves much to be desired and where the children and their pets live in very close contact with each other.

A small percentage of the dogs came from the Southern Suburbs and from Durbanville.

It was ascertained that none of these animals had ever been inoculated against leptospirosis.

Method

Each serum was serially diluted with normal saline to final dilutions ranging from 1/20 to 1/20,000. If agglutination was still found to be present at 1/20,000, further dilutions up to 1/160,000 were made.

Ten to fourteen-day-old stock cultures of leptospirae in Korthoff's medium[2] following Steytler's modifications with iron[3] were used as live antigens, 6 different serotypes in all. These were:

L. icterohaemorrhagiae A.B.,
L. canicola,
L. pomona,
L. sejroe,
L. australis A.B.,
L. ballum.

Controls were set up for each batch of tests. The tubes were incubated at 37°C for 3 hours and then left at room temperature for a ½-hour before being examined by dark-field microscopy for agglutination and lysis. Table I shows a typical reaction.

TABLE I. TYPICAL AGGLUTINATION TEST WITH LIVE ANTIGEN

Live antigens	*Serum dilutions*							
	1/20	1/200	1/2,000	1/20,000	1/40,000	1/80,000	1/160,000	*Control*
L. canicola	+	+	+	+	+	−	−	−
L. weil	+	+	+	−	−	−	−	−
L. pomona	−	−	−	−	−	−	−	−
L. sejroe	−	−	−	−	−	−	−	−
L. australis	−	−	−	−	−	−	−	−
L. ballum	−	−	−	−	−	−	−	−

Discussion

Agglutination of leptospirae by specific antibodies in serum is such a marked and striking feature that the evaluation of the antibody titre becomes quite simple with a little practice.

At low dilutions positive sera will show gross clumping and flocculation of the immobilized leptospirae, giving the appearance of ragged balls of shiny white cottonwool when examined with the dark-ground microscope. These agglutination balls have to be distinguished from 'breeding nests' of actively dividing organisms which may at times be seen in the control tubes. Usually the presence of these nests will be obviated by using 10 - 14-day-old stock cultures.

With successively higher dilutions agglutination would become less marked, and the serum's titre in each case was taken as being that dilution where two-thirds or more of the organisms were completely unaffected by agglutination.

Where lysis of the leptospirae had apparently taken place, it was invariably found that heavy flocculation had actually taken place and that the apparent lysis was in fact the false picture seen when a loopful of clear supernatant fluid from above the flocculation was examined.

Results

Of the 100 sera examined (Fig. 1), 46 showed no agglutination at all with any of the serotypes used, while 11 had relatively low titres of below 1/2,000 for *L. icterohaemorrhagiae* A.B. or *L. canicola* or for both. 43 sera had agglutination titres ranging from 1/2,000 to 1/160,000 and the details of these can be seen in Fig. 2.

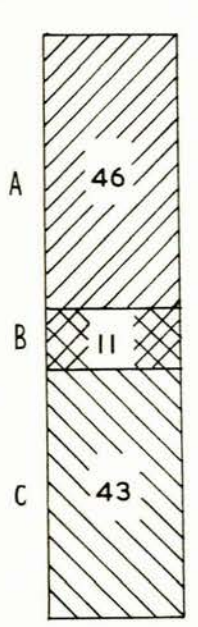

Fig. 1

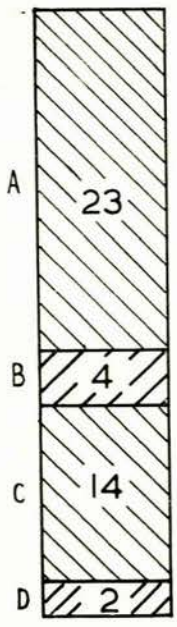

Fig. 2

Fig. 1. Leptospira titres in 100 dogs. A = Negative, B = titres below 1/2,000, C = titres 1/2,000—1/160,000.
Fig. 2. 43 titres of 1/2,000 and higher. A = *L. canicola* predominant, B = *L. canicola* only, C = *L. weil* predominant, D = *L. weil* only.

The same pattern of cross-agglutination was seen in this high titre group: 23 gave agglutination with *L. canicola* at high dilutions and showed cross-agglutination with *L. icterohaemorrhagiae* in lower dilutions; 4 gave agglutination with *L. canicola only;* 14 showed agglutination with *L. icterohaemorrhagiae* predominantly and cross-agglutinated with *L. canicola* in lower dilutions; 2 sera had antibodies against *L. icterohaemorrhagiae* only.

The well-known cross-agglutination reactions between *L. canicola* and *L. icterohaemorrhagiae* are clearly shown here.

FIRST ISOLATION OF A CANICOLA SEROTYPE IN SOUTH AFRICA

Material

Two dogs which were clinically diagnosed as possibly being in the terminal stages of leptospiral infection with nephrosis were sacrificed and autopsies performed.

One was a 3-year-old fox terrier which showed some interesting postmortem findings: (1) The kidneys were large, pale and oedematous, there were (2) interstitial haemorrhages in the lungs, and (3) ascites and (4) anaemia were present. There was no jaundice.

Methods

Under sterile conditions renal tissue was ground up in Korthoff's medium. This suspension was immediately examined by dark-ground microscopy and showed numerous active leptospirae.

One ml. of renal suspension was inoculated into 8 ml. of modified Korthoff's medium, and 1 ml. of inactivated rabbit serum was added. It was incubated at 37°C for 2 days and then at 28°C. A strong culture of leptospirae was obtained 14 days later.

Guinea-pig inoculation was performed with this culture. After 3 weeks the animals had lost condition, but were still alive. This was much in favour of a canicola infection, since test animals are almost invariably killed by the 14th day or earlier by *L. icterohaemorrhagiae*.

The animals were sacrificed during the 4th week of infection and showed some haemorrhagic lesions of the lungs and internal organs, but these were much less marked than those seen in a Weil's infection.

A Golden Syrian hamster was inoculated with the culture and died on the 10th day, as is usual with a canicola infection,[5] also showing the typical lesions.

Histology of the infected animals' kidneys showed gross interstitial nephritis and Levaditi staining showed numerous leptospirae in and around the convoluted tubules.

Discussion

Evidence was therefore much in favour of this being a canicola serotype. Agglutination tests of the dog's serum (which was obtained before sacrificing it) were unfortunately of no help in distinguishing the specific serotype since they showed titres of 1/160,000 for both *L. canicola* and *L. icterohaemorrhagiae*. A subculture was sent to the WHO leptospirosis reference laboratories in Amsterdam, where Professor Wolff confirmed this organism as *L. canicola*.

Human Epidemiological Importance

While the serological survey was being conducted, a 13-year-old Coloured boy from the same area where most of the dogs had been obtained, died soon after admission to our hospital of what had appeared to be an infectious hepatitis.

An autopsy was performed and the following striking features were seen: (*a*) Marked jaundice; (*b*) lungs with gross interstitial haemorrhages; (*c*) liver slightly enlarged, with haemorrhagic foci; and (*d*) kidneys enlarged with marked subcapsular haemorrhages.

Dark-field microscopy of the serum and of ground-up kidney tissue showed numerous leptospirae. Attempts at culturing this organism were unsuccessful owing to gross contamination of the already autolytic postmortem specimen.

Histology of the organs was compatible with a Weil's infection.

SUMMARY

A high percentage of 100 dogs from the Cape Peninsula, most of which came from a very poor community, showed strong agglutination titres against either *L. canicola, L. icterohaemorrhagiae* or both.

Of 2 sick dogs sacrificed, one yielded a culture of *L. canicola,* confirmed by the WHO reference laboratory. This is the first isolation of *L. canicola* in South Africa.

While the serological survey was being conducted, a fatal case of Weil's disease in a young boy from the same area was seen in our hospital.

I wish to thank the veterinary surgeons for supplying the serum samples, and Prof. H. D. Brede for his help and advice.

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