

BOVINE LEPTOSPIRA POMONA INFECTION:  
ENVIRONMENTAL CONTAMINATION AND  
THE SPREAD OF THE DISEASE IN A  
SUSCEPTIBLE HERD

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SUMMARY

A *Leptospira pomona* outbreak was initiated and maintained in a herd of cattle pastured under simulated dairy-farm conditions. Initial attempts to spread leptospirosis from four inoculated carriers to a group of in-contact heifers, over the dry months from August to October 1963, resulted in only one heifer contracting the disease. *L. pomona* subsequently disappeared from the animals and the environment.

When three experimentally infected steers were introduced during the wet month of November the disease became established in susceptible animals in the herd. Over a 6-month period 20 of 21 heifers, 4 of 7 natural increase calves, and all of 26 young steers contracted leptospirosis due to exposure in the contaminated environment.

The 26 young steers were exposed to infection in three groups. One was run with the herd, one pastured in a separate paddock and allowed to drink from the infected water source while the herd was being sampled, and one was held in the yards and worked with the herd. Animals in these three groups became infected at approximately equivalent rates. It was considered that the water source may well have been a focus of dissemination of infection. *L. pomona* was isolated from this site when there were large numbers of excreting cattle present.

Towards the end of the experiment, when the level of environmental contamination with *L. pomona* was declining, eight groups of three to five steers were introduced into the herd in consecutive weeks and removed after 1 week. Only one of these animals developed leptospirosis and this isolated infection was associated with a single fall of heavy rain in an otherwise dry period. There was some correlation between magnitude of rainfall and incidence of infection when the number of leptospires present in the environment was relatively low. Rain fell fairly constantly over the interval when the majority of cattle contracted the disease. The incidence of infection tended to follow the level of environmental contamination with *L. pomona*.

It would seem that the incidence of infection was a function of the number of susceptible cattle, the degree of environmental contamination with *L. pomona*, and the amount and distribution of rainfall.

The disease was not detected in the 51 rats, three bandicoots, three possums, five water dragons, two cranes or one pit wit trapped on the property. It did not spread to four susceptible cattle maintained in an adjacent pasture.

## I. INTRODUCTION

A search of the literature (Doherty 1965) revealed 36 original publications describing one or more bovine *Leptospira pomona* outbreaks, both within Australia (Sutherland, Simmons, and Kenny 1949; Sutherland 1950; Peterson 1951; Wellington, Stevenson, and Ferris 1951, 1953; Spotswood 1962; Spradbrow and Seawright 1963; Emanuel, Mackerras, and Smith 1964) and in nine other countries. However, there has been no report of an intensive day-by-day study of a *L. pomona* outbreak in a herd of cattle at pasture.

In order to make such an investigation, an outbreak was initiated in a susceptible herd by introduction of cattle that had been inoculated with *L. pomona*. Previous workers have succeeded in transmitting this disease to susceptible calves by pasturing them (Webster 1959) or confining them in pens (Burnstein and Baker 1954; Morter and Morse 1956) with calves or pigs excreting *L. pomona*. Also introduction of infected cattle has been implicated as the origin of a number of naturally occurring outbreaks (Baker and Little 1948; Reinhard, Tierney, and Roberts 1950; Wellington, Ferris, and Stevenson 1953; Stoenner *et al.* 1956; Hughes and Keech 1960; Spradbrow and Seawright 1963).

This paper describes the spread of leptospirosis in the experimental herd.

## II. MATERIALS AND METHODS

*The environment:*—The site of the experiment was a 200-ac property which had been a dairy farm, 16 miles north of Brisbane. The area used in the experiment is illustrated in Figure 1. There had been no cattle in this area for more than 2 months before the experimental cattle were introduced, the only animals on the property being three horses, a cat, a dog and an unknown wildlife population.

Ten soil samples from various sites in the experimental area were predominantly acid, with an average pH of 5.9 (range 5.0 to 7.4). However, a total of six water samples from the waterhole (W), the dam (Q) and the river (R) were all between pH 7.0 and 7.4.

Daily rainfall was measured in a 5-in. rain gauge positioned on the fence between E and F (Figure 1).

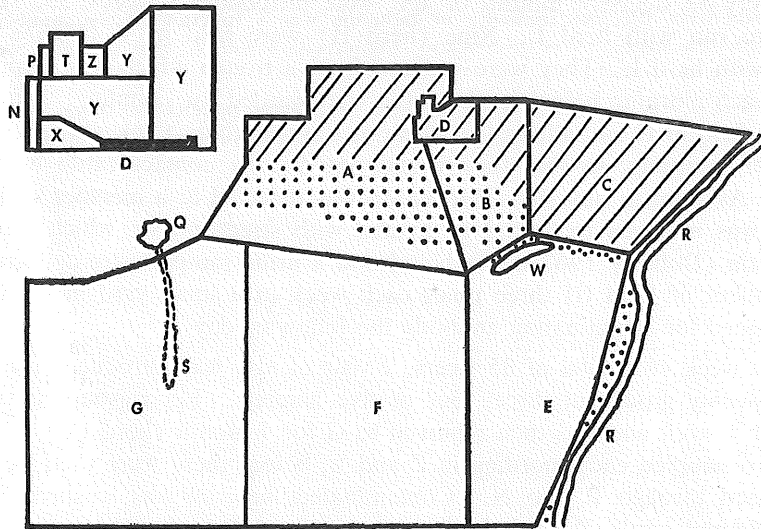


Fig. 1.—Map of the experimental site. G, F and E represent well-drained flat pastures about 20 ft above the river, R. The only water in these is a seepage, S, from the dam, Q, and a waterhole, W. The diagonally striated areas are about 25 ft above GFE and the dotted areas have a gradient. The detail of the dairy yards, D, is drawn to three times the scale of the remainder of the map. Y and N are well-grassed yards, X is a crush, Z and P are concrete-floored yards, and T the sampling area.

*Introduction and management of cattle:*—In attempts to initiate the outbreak, susceptible cattle were pastured with animals that had been inoculated with a strain of *L. pomona*. The syndrome in these inoculated cattle and the history of the strain of *L. pomona* have been described (Doherty 1967a). The time sequence used in this previous communication has been adhered to throughout the present paper. Day 1 of the experiment was May 25, 1963.

The following cattle constituted the main experimental herd (herd I) and were pastured in F, E and B (Figure 1). Twenty-one heifers, 2 years of age, were introduced in week 11. Five of these were inoculated with *L. pomona* at time of introduction on August 3, 1963. Another heifer (H7) was inoculated and four more susceptible heifers and a calf with leptospiruria (C4) were introduced in week 16. A heifer (H8) was inoculated in week 23, three inoculated steers (S1, S2 and S3) were introduced in week 24 and three susceptible heifers were introduced in week 27. One calf was born in each of weeks 21, 24, 26, 30, 31, 34 and 35.

Cattle in herd I were all yarded daily, held in the concrete-floored yard Z, sampled in T (formerly the milking-shed with four individual bays with forward opening doors) and released into P and Y (Figure 1). They were worked once weekly through the crush (X) for collection of blood samples.

Twenty-six steers, 8 months of age, were introduced in week 35. Eight of these were run with herd I. Nine (herd II) were held in Y (Figure 1) and sampled with herd I. They were watered from a trough which was emptied and inverted each morning before herd I cattle were yarded for sampling. Nine (herd III) were held in C and allowed to drink at the waterhole (W) while herd I was yarded for sampling, so that they would not directly contact cattle with leptospiruria. As soon as any sign of infection was detected in a member of herds II or III it was removed to herd I or treated with streptomycin, which terminated leptospiruria (Doherty 1967*b*). A blood and a urine sample were collected from every member of herd III three times each week in a small yard in C, and sera were screened for agglutinating antibody the following day.

After week 46 a total of 35 steers, 1 year of age, was introduced into herd I in eight weekly groups of three, four or five animals. Each group remained in herd I for 1 week and was then removed to G for 1 month (herd IV). Herd IV cattle were sampled each morning in Z and T, before these were used by herd I, and released through P into N. Contaminated material was washed from the concrete-floored yards, Z, T and N, onto the grassed yards (Y) each day after sampling was completed.

When leptospiruria had ceased each animal was removed to a remote area on the property and bled once weekly until sent for slaughter.

A group of four susceptible animals (herd V) was maintained in A from week 11. These were initially heifers but were replaced by steers in week 16.

*Sampling and laboratory examination techniques.—*

(i) *Cattle*: The techniques used with cattle have been described previously (Doherty 1966, 1967*a*). Rectal temperatures were recorded and urine samples were collected each morning. Operators wore rubber gloves, overalls, waterproof clothing and rubber boots. The outsides of the gloves and thermometers were washed through disinfectant (1:200 I.C.I. "Savlon") and rinsed through water before sampling each animal. Leptospiruria was determined by dark-ground examination of centrifuged formalinized urine samples and serum agglutinating antibody was measured by the technique of Winks (1962).

(ii) *Other animals*: Of the four domestic animals on the property before the experimental cattle were introduced, one horse had antibody to *L. pomona* but no leptospiruria, and the remainder were seronegative. The other two horses were vaccinated twice with *L. pomona* bacterin (Commonwealth Serum Laboratories, Melbourne) and the dog was confined to the house yard.

Sixty-two small wild animals (Table 1) were trapped in live-animal cages in the area shown in Figure 1, and three birds that had associated with the waterhole and the infected cattle were shot at the end of the experiment. Seven of the animals were found dead in the cages, but sera from the remaining 55 and the three birds were screened for agglutinating antibody to *L. pomona*. Warthin Starry (United States Armed Forces Institute of Pathology 1960) stained kidney sections were examined from all 65 for presence of leptospiras.

TABLE 1  
 DETAILS OF WILDLIFE TRAPPED IN THE AREA SHOWN IN FIGURE 1

| Species  | Number Trapped   |                   |                   |       |
|--|------------------|-------------------|-------------------|-------|
|  | Weeks<br>1 to 12 | Weeks<br>13 to 54 | Weeks<br>55 to 63 | Total |
| Rat ( <i>Rattus rattus</i> ) .. .. .                             | 27               | 15                | 9                 | 51    |
| Bandicoot ( <i>Thylacis macrourus</i> ) .. .. .                  | 2                | 1                 | 0                 | 3     |
| Possum ( <i>Pseudocheirus laniginosus</i> ) .. .. .              | 0                | 3                 | 0                 | 3     |
| Water dragon ( <i>Physignathus lesueurii lesueurii</i> ) .. .. . | 1                | 4                 | 0                 | 5     |
| Pit wit ( <i>Grallina cyanoleuca</i> ) .. .. .                   | 0                | 0                 | 1                 | 1     |
| Crane ( <i>Notophyx novaehollandiae</i> ) .. .. .                | 0                | 0                 | 2                 | 2     |

Because of variations in the supply of laboratory animals several different techniques were used in attempts to isolate *L. pomona* from wildlife. Macerated kidney material (Doherty and Baynes 1967) from 54 wild animals and the three birds was inoculated into single guinea pigs or groups of four weanling laboratory mice. Serum was collected from inoculated animals at autopsy 21 or 14 days later. Small pieces of kidney from eight other wild animals were cultured in six tubes of semi-solid medium incorporating Difco Stuart's medium (Roth and Galton 1960) and examined weekly for 6 weeks.

(iii) *The waterhole*: The waterhole (W) was examined for the presence of *L. pomona* at intervals throughout the experiment. From weeks 7 to 14 a total of 18 guinea pigs was exposed by the subcutaneous flow method of Thiel and Veer (1941), as used by Smith and Self (1955), or the bathing method of Appelman (1934). Because of their complexity and the occurrence of non-specific deaths, both these methods were discarded in favour of the inoculation technique used successfully by Gillespie and Ryno (1963). The bottom of the waterhole was disturbed before removing a muddy sample of water in a 4-oz bottle. This was shaken, the mud allowed to settle, and the supernate used as an inoculum. One to three millilitres of water was injected intraperitoneally into 100–300-g guinea pigs, which were killed 21 days later and their sera tested for antibody to *L. pomona*. A total of 56 guinea pigs in groups of one to four was exposed on weeks 15, 24, 27 to 33, 38 and 47 to 54.

*Analysis and expression of results*.—An attempt was made to express the amount of contamination of the environment with leptospires as distinct from the number of cattle excreting *L. pomona*. This 'magnitude of leptospiruria' was calculated by dividing by 7 the sum of the daily figures for "level of leptospiruria" (Doherty 1966) for all cattle for each day of the week and was an estimate of the mean daily excretion of leptospires for that week.

Rainfall and incidence of infection were related by a method similar to that adopted by Derrick (1956) in analysis of cases of leptospirosis in humans in North Queensland.

Cattle were considered to have contracted leptospirosis if they developed serum agglutinating antibody to *L. pomona*. Febrile reactions that were followed soon afterwards by detection of serum antibody and leptospiruria were regarded, in retrospect, as first signs of leptospirosis. If there was no febrile reaction the first sign of leptospirosis observed was leptospiruria, or presence of serum antibody in weekly blood samples.

Uninoculated cattle that contracted leptospirosis were referred to as being 'naturally infected' (N.I.).

### III. RESULTS

Four of the experimentally inoculated cattle—the heifers H2, H3, H4 and H8—did not excrete leptospiras in the urine. The remainder had leptospiruria for 10 (H5), 45 (H6), 34 (H7), 34 (C4), 46 (S1), 48 (S2) and 42 (S3) days.

The weekly incidence of leptospirosis in all naturally infected cattle, as detected by first appearance of leptospiras in the urine, is compared with the degree of contamination of the environment with *L. pomona* and weekly rainfall in Figure 2. A description of the course of the outbreak follows.

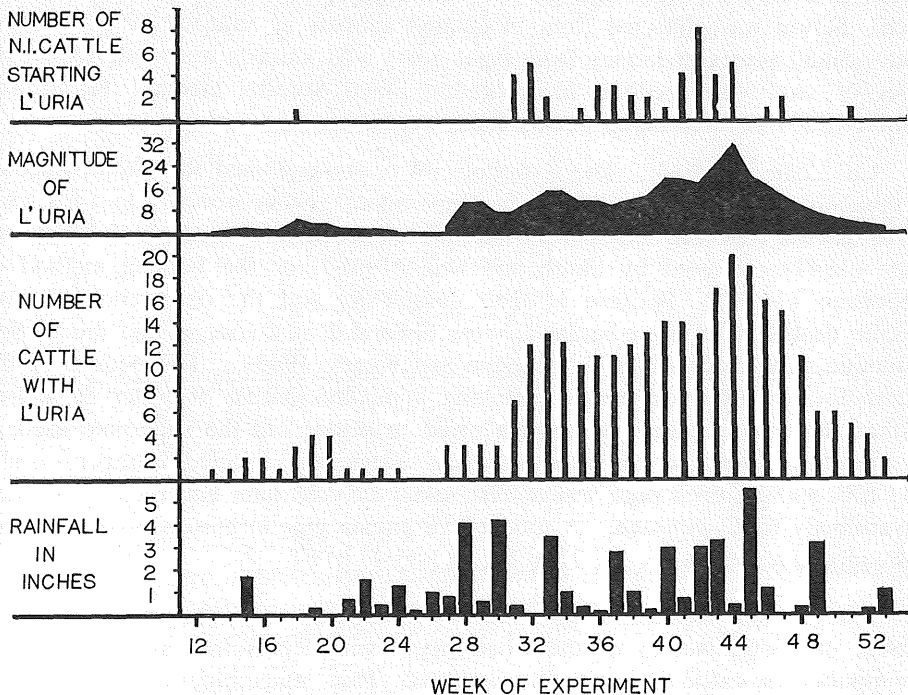


Fig. 2.—Comparison of the number of naturally infected (N.I.) cattle starting leptospiruria, the magnitude of leptospiruria, the number of cattle with leptospiruria and the rainfall for weeks 12 to 54 of the experiment. Day 1 of the experiment was May 25, 1963.

Only two of five heifers inoculated in week 11 developed leptospiruria. One naturally infected animal was febrile 24 days after the first of these commenced excretion, and 12 days after the start of a 2-day fall of 1.59 in. of rain in an otherwise dry period.

A greater number of carriers and 'magnitude of leptospiruria' were present during the subsequent dry weather which lasted until week 20, but no natural infection was observed. Rain fell each week from weeks 21 to 26 and daily falls of up to 1.42 in. were recorded, but the 'magnitude of leptospiruria' was low and no new cases of leptospirosis occurred.

In week 27 the three inoculated steers commenced leptospiruria and within 19 to 32 days eight naturally infected animals commenced a febrile reaction. A further two animals had no fever but became seropositive during this period. This incidence of infection, 40% of the susceptible herd, followed 18 to 24 days after the start of a 7-day total of 4.93 in. of rain.

No new cases were observed until six heifers, 40% of the remaining uninfected cattle, were febrile in week 35. There was 1 month between the initial reaction dates in each of these two incidences of infection. Maximum leptospiruria in the first 10 cattle would have been concurrent with a single fall of 2.10 in. of rain 9-12 days before the temperature peaks in the second six. One heifer was febrile and another was serologically positive without prior fever in week 36.

The sequence of infection of the 26 susceptible steers introduced in week 35 is detailed in Table 2. Two, from herds I and II, were febrile 14 and 15 days after introduction. Only 0.34 in. of rain fell over this interval. Two steers from herd III were serologically positive in week 39, 27 days after first exposure. They were transferred into herd I and commenced leptospiruria 4 and 7 days later. There was no marked difference in the rates of infection of these three groups of steers.

TABLE 2  
COMPARATIVE RATES OF INFECTION OF THREE GROUPS OF STEERS  
GIVEN DIFFERENT REGIMENS OF EXPOSURE TO LEPTOSPIROSIS  
FROM WEEK 35

| Week of Experiment | Number of Cattle Showing Initial Antibody Titre to <i>L. pomona</i> |  |   |
|--------------------|---|--|---|
|                    | Group Pastured with Herd I (8 animals)                              | Group Maintained in Yards (Herd II, 9 animals) | Group Exposed only to Paddock Environment (Herd III, 9 animals) |
| 37                 | 0   | 1  | 0   |
| 38                 | 1   | 0  | 0   |
| 39                 | 0   | 0  | 2   |
| 40                 | 0   | 0  | 0   |
| 41                 | 2   | 0  | 4   |
| 42                 | 2   | 0  | 2   |
| 43                 | 3   | 5  | 1   |
| 44                 | 0   | 0  | 0   |
| 45                 | 0   | 0  | 0   |
| 46                 | 0   | 2  | 0   |
| 47                 | 0   | 1  | 0   |

Over a 17-day interval, from weeks 40 to 43, 70% of the remaining susceptible cattle in herds I, II and III showed first symptoms of leptospirosis. Rainfall and 'magnitude of leptospiruria' were high when these cattle must have contracted the disease. To week 47 a total of 50 animals became naturally infected as a result of exposure to cattle excreting *L. pomona*. Four animals did not become infected and three of these were later found to be relatively resistant on challenge inoculation.

Of the eight groups of steers (herd IV) exposed weekly from week 47 only one animal, which was in the third group, developed leptospirosis. It became febrile 14 days after it was introduced and was immediately removed to herd I. Rain (0.30 in.) fell on the day of introduction of the third group and 1.13 in. fell on the following day. Only 0.13 in. was recorded from this date to the introduction of the seventh group. All, except members of the eighth group, were exposed to cattle excreting *L. pomona* while in herd I, though the 'magnitude of leptospiruria' was steadily declining.

*L. pomona* was first demonstrated in the waterhole (W) in week 33 and again in week 48. All other attempts were negative.

A period of heavy rainfall was found either 7-14 days before maximum febrile reaction, or 10-20 days before appearance of serum antibody in those animals without fever, in 93% of all naturally infected cattle. However, no great significance can be accorded to this result as a consecutive 3-day dry period was found in the same interval for 76% of these animals and rain fell on 46% of days from week 26 to week 45.

No evidence of leptospirosis was found in any of the 65 small wild animals and birds trapped on the property (Table 1), the cattle in herd V, the dog, the cat or the five operators.

#### IV. DISCUSSION

In the first studies of human *L. pomona* infection in south-eastern Queensland, Clayton, Derrick, and Cilento (1937) found that the disease was more common in the wet summer than in the dry winter. According to Seddon (1953) most cases of bovine leptospirosis occur in Queensland in the first half of the year and in the southern States chiefly in the spring, both times corresponding to the highest rainfall distribution. In New Zealand, which has a steady rainfall all year round, there is no strict seasonal incidence of this disease in cattle (Salisbury 1954). Keast, Forbes, and Wannan (1964) could not demonstrate seasonal variation in the incidence of bovine leptospirosis in New South Wales.

Because of the stress placed in previous publications on the importance of rainfall in the spread of leptospirosis, a search was made for such a relationship in the experiment reported here. However, it was not possible to definitely correlate rainfall and incidence of infection over the period when the majority of cattle were infected. 'Magnitude of leptospiruria' was high and rain fell fairly



constantly. Rain may be important in providing a suitable environmental situation for infection when 'magnitude of leptospiruria' is low. The first naturally infected heifer and the single 1-year-old steer were febrile 12 and 14 days after heavy single falls of rain in an otherwise dry period. Heavy rain was falling when the first instances of natural infection in the 10 and 6 heifers would have been expected to occur. The 'magnitude of leptospiruria' was also at a maximum. It could be postulated that these two factors combined to give a high level of natural infection, in each case 40% of the susceptible herd.

Incidence of infection tended to follow the level of contamination of the environment with *L. pomona*. In Figure 2 there is a trend for the histogram representing the number of cattle commencing leptospiruria from weeks 31 to 42 to follow the same general pattern as that for 'magnitude of leptospiruria' 3 weeks previously. This is not apparent from weeks 42 to 46, when the 'magnitude of leptospiruria' was consistently high but the number of susceptible animals had markedly decreased.

Adult carriers were frequently observed to urinate while in close contact with susceptible animals in the concrete-floored yards (Z & T). Baker and Little (1948) thought that the main method of transmission of *L. pomona* infection between cattle was by nasal inoculation of a urine droplet spray. Baker and Little (1948), Ringen *et al.* (1955), Ringen and Bracken (1956), Ringen and Okazaki (1958) and Kenzy *et al.* (1958) all infected cattle with *L. pomona* by dropping or spraying excretor urine or virulent culture into their nostrils or conjunctivae. In the experiment reported here, the small steers held in the yards and the steers and calves pastured with the main herd would have been much exposed to this possible mode of infection.

According to Hanson, Mansfield, and Andrews (1964), the major factor in the spread of leptospirosis in a beef herd, during an 11-year study, was close contact between animals in a wet pasture environment. If contact between animals and exposure to infected pastures were the only important factors in the spread of *L. pomona* infection, in the study reported here, the herd II steers would have been expected to contract the disease just as quickly as those in herd I, for the grassed yards (Y) in which the herd II steers were confined could be considered to be an intensive contaminated environment as well as a site of concentration of cattle. The herd III steers exposed only to the waterhole and surrounding pastures for 2-3 hr each day would have become infected much less rapidly. However, this was not the case. The herd III steers became infected just as quickly as those in herd I; the rate of infection in herd II steers was slightly slower.

The pasture soils were acidic, which is known to be unfavourable for the spread of bovine leptospirosis (Musaev 1960; Blood, Szyfres, and Moya 1963). The waterhole may have been a focus of infection for herds I and III. Herd I cattle that were excreting *L. pomona* were frequently observed to urinate while standing in the water. The herd III steers only watered once daily and tended

to all stand in the water and drink together as soon as they were allowed into the infected environment. This resulted in considerable stirring of the muddy pool bottom, which is thought to be important in isolation of leptospiras from ground water (Thiel 1948). However, *L. pomona* could not be readily, or consistently, isolated from the waterhole. This could have been a result of dilution (Taylor and Knowelden 1957) or due to a short survival time in this environment (Okazaki and Ringen 1957).

Ponds have been implicated as sources of spread of leptospirosis in epidemics in cattle studied in Russia (Byalik 1961) and Hungary (Fuzy and Kizsel 1962). *L. pomona* has been isolated from water associated with outbreaks in cattle in North America (Gillespie *et al.* 1957; Gillespie and Ryno 1963). Stoenner *et al.* (1956), in an extensive survey, stressed the importance of watering points as sources of dissemination of infection. According to Gillespie *et al.* (1957), *L. pomona* can only be isolated from water when contamination of the environment is at a very high level. Ringen and Bracken (1956) infected two of six 12-month-old Herefords by placing an abraded foot in a bucket of diluted urine containing *L. pomona* for a maximum of 2 min.

The failure to demonstrate *L. pomona* in the 65 wild animals and birds trapped on the property is in accord with the negative results of wildlife surveys in south-eastern Queensland (Johnson 1939; Agnew 1962). All the rats (Table 1) were *Rattus rattus*, which Emanuel, Mackerras, and Smith (1964) classified as an incidental host of *L. pomona* in North Queensland. From the results of a comprehensive epidemiological survey conducted in Malaya, Gordon-Smith *et al.* (1961) concluded that "different species, although living side by side, may have very little real contact of the sort necessary to pass on leptospirosis". This postulate is further supported by the observations that, in the experiment reported here, the four susceptible herd V cattle did not contract the disease from the infected herd in the adjacent pasture and none of the five operators became infected.

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#### REFERENCES

- AGNEW, D. W. (1962).—“Leptospirae Causing Leptospirosis in the Nambour District and a Survey of Small Wild Mammals as Carriers of Leptospirae and Mite Parasites”. Essay, University of Queensland Medical School.
- APPELMAN, J. M. (1934).—Thesis, Leiden. Cited by P. H. Van Thiel in “The Leptospiroses”. (Universitaire Pers Leiden:Leiden).
- BAKER, J. A., and LITTLE, R. B. (1948).—Leptospirosis in cattle. *J. Exp. Med.* 88:295-306.

- BLOOD, B. D., SZYFRES, B., and MOYA, V. (1963).—Natural *Leptospira pomona* infection in the pampas cavy. *Publ. Hlth Rep., Wash.* 78:537-42.
- BURNSTEIN, T., and BAKER, J. A. (1954).—Leptospirosis in swine caused by *Leptospira pomona*. *J. Infect. Dis.* 94:53-64.
- BYALIK, Z. M. (1961).—Data on the epidemiology and clinical course of leptospirosis in the Voronezh oblast. *J. Microbiol. Epidemiol. Immunobiol.* 32:1086-93.
- CLAYTON, G. E. B., DERRICK, E. H., and CILENTO, R. W. (1937).—The presence of leptospirosis of a mild type (seven-day fever) in Queensland. *Med. J. Aust.* 1:647-54.
- DERRICK, E. H. (1956).—Leptospirosis in North Queensland: an epidemiological comparison between the various leptospiral serotypes. *Med. J. Aust.* 1:281-7.
- DOHERTY, P. C. (1965).—The epizootiology of bovine leptospirosis. M.V.Sc. Thesis, Univ. of Queensland.
- DOHERTY, P. C. (1966).—Comparison of direct microscopic and guinea pig inoculation techniques for demonstrating leptospires in bovine urine. *Aust. Vet. J.* 42:466-7.
- DOHERTY, P. C. (1967a).—Bovine *Leptospira pomona* infection: the disease in inoculated cattle. *Qd J. Agric. Anim. Sci.* 24:343-50.
- DOHERTY, P. C. (1967b).—Streptomycin treatment of bovine carriers of *Leptospira pomona*. *Aust. Vet. J.* 43:138-9.
- DOHERTY, P. C., and BAYNES, I. D. (1967).—The effect of feeding oxytetracycline on leptospiuria in pigs infected with *Leptospira pomona*. *Aust. Vet. J.* 43:135-7.
- EMANUEL, M. L., MACKERRAS, I. M., and SMITH, D. J. W. (1964).—The epidemiology of leptospirosis in North Queensland 1. General survey of animal hosts. *J. Hyg., Camb.* 62:451-84.
- FUZI, M., and KISZEL, J. (1962).—Leptospirosenepidemie in einer westungarischen Gemeinde. *Acta Microbiol. Hung.* 9:167-73. (Abstracted in *Bull. Hyg., Lond.* 38:579).
- GILLESPIE, R. W. H., KENZY, S. G., RINGEN, L. M., and BRACKEN, F. K. (1957).—Studies on bovine leptospirosis. III. Isolation of *Leptospira pomona* from surface waters. *Am. J. Vet. Res.* 18:76-80.
- GILLESPIE, R. W. H., and RYNO, J. (1963).—Epidemiology of leptospirosis. *Am. J. Publ. Hlth* 53:950-5.
- GORDON-SMITH, C. E., TURNER, L. H., HARRISON, J. L., and BROOM, J. C. (1961).—Animal leptospirosis in Malaya. 2. Localities sampled. *Bull. Wld Hlth Org.* 24:23-34.
- HANSON, L. E., MANSFIELD, M. E., and ANDREWS, R. D. (1964).—Epizootic of enzootic leptospirosis in a cattle herd. *Proc. U.S. Live Stk Sanit. Ass.* 68:136-46.
- HUGHES, D. E., and KEECH, H. L. (1960).—An epizootic of leptospirosis in institutional herds of cattle and swine. *Proc. U.S. Live Stk. Sanit. Ass.* 64:143-50.
- JOHNSON, D. W. (1939).—Activities of the Mobile Unit. In *Rep. Hlth Med. Servs Qd* 1938-39.
- KEAST, J. C., FORBES, B. R. V., and WANNAN, J. S. (1964).—Bovine leptospirosis in New South Wales including the results of a 10-year survey. *Aust. Vet. J.* 40:19-26.

- KENZY, S. G., KEOWN, G. H., OKAZAKI, W., GILLESPIE, R. W. H., and RINGEN, L. M. (1958).—Detection of viable *L. pomona* in bovine kidneys after leptospirosis had apparently ceased. *Vet. Med.* 53:647-8.
- MORTER, R. L., and MORSE, E. V. (1956).—Experimental leptospirosis. II. The role of calves in the transmission of *Leptospira pomona* among cattle, swine, sheep and goats. *J. Am. Vet. Med. Ass.* 128:408-13.
- MASAEV, M. A. (1960).—Environmental factors influencing the incidence of bovine leptospirosis. *Veterinariya.* 37:22-5. (Abstract in *Vet. Bull.* (1960) 30:491).
- OKAZAKI, W., and RINGEN, L. M. (1957).—Some effects of various environmental conditions on the survival of *Leptospira pomona*. *Am. J. Vet. Res.* 18:219-23.
- PETERSON, J. E. (1951).—Leptospirosis of cattle and pigs in Western Australia. *Aust. Vet. J.* 27:40-43.
- REINHARD, K. R., TIERNEY, W. F., and ROBERTS, S. J. (1950).—A study of two enzootic occurrences of bovine leptospirosis. *Cornell Vet.* 40:148-64.
- RINGEN, L. M., and BRACKEN, F. K. (1956).—Studies on bovine leptospirosis. II. The effect of various levels of tetracycline hydrochloride on bovine leptospirosis. *J. Am. Vet. Med. Ass.* 129:266-71.
- RINGEN, L. M., BRACKEN, F. K., KENZY, S. G., and GILLESPIE, R. W. H. (1955).—Studies on bovine leptospirosis. I. Some effects of dihydrostreptomycin and terramycin on the carrier condition in bovine leptospirosis. *J. Am. Vet. Med. Ass.* 126:272-6.
- RINGEN, L. M., and OKAZAKI, W. (1958).—The prophylactic effect of chlortetracycline, given orally, on bovine leptospirosis. *J. Am. Vet. Med. Ass.* 133:214-5.
- ROTH, E. E., and GALTON, M. M. (1960).—Isolation and identification of *Leptospira hardjo* from cattle in Louisiana. *Am. J. Vet. Res.* 21:422-7.
- SALISBURY, R. M. (1954).—Leptospirosis in New Zealand livestock. *J. N.Z. Brch R. Sanit. Inst.* 15:2-12.
- SEDDON, H. R. (1953).—“Diseases of Domestic Animals in Australia” Part 5, Vol. 2. *Serv. Publs Dep. Hlth Aust. Vet. Hyg.* No. 10.
- SMITH, D. J. W., and SELF, H. R. M. (1955).—Observations on the survival of *Leptospira australia* A in soil and water. *J. Hyg., Camb.* 53:436-44.
- SPOTSWOOD, C. L. (1962).—Leptospirosis as a problem in general practice. *Aust. Vet. J.* 38:177-9.
- SPRADBROW, P. B., and SEAWRIGHT, A. A. (1963).—*Leptospira pomona* infection in calves. *Aust. Vet. J.* 39:423-33.
- STOENNER, H. G., CREWS, F. W., CROUSE, A. E., TASCHNER, L. E., JOHNSON, C. E., and WOHLER, J. Jr. (1956).—The epizootiology of bovine leptospirosis in Washington. *J. Am. Vet. Med. Ass.* 129:251-9.
- SUTHERLAND, A. K. (1950).—Diseases of calves. *Aust. Vet. J.* 26:238-47.
- SUTHERLAND, A. K., SIMMONS, G. C., and KENNY, G. C. (1949).—Bovine leptospirosis. *Aust. Vet. J.* 25:197-202.
- TAYLOR, I., and KNOWELDEN, J. (1957).—“Principles of Epidemiology”. (J. & A. Churchill: London).
- THIEL, P. H. VAN (1948).—“The Leptospiroses”. (Universitaire Pers Leiden:Leiden).

- THIEL, P. H. Van, and VEER, W. C. (1941).—Neue methodenzum isolieren von *Leptospira icterohaemorrhagiae* aus wasser. *Acta Brev. Neerl. Physiol.* 11:21-4.
- UNITED STATES ARMED FORCES INSTITUTE OF PATHOLOGY (1960).—U.S. Armed Forces Institute of Pathology. "Manual of Histologic and Special Staining Technics". 2nd Ed. (McGraw Hill:New York).
- WEBSTER, W. M. (1959).—Active immunization of young calves against *Leptospira pomona*. *Proc. 16th Int. Vet. Congr.* 2:709-10.
- WELLINGTON, N. A. M., FERRIS, A. A., and STEVENSON, W. J. (1953).—Leptospirosis amongst farm animals in a dairying district. *Aust. Vet. J.* 29:212-7.
- WELLINGTON, N. A. M., STEVENSON, W. J., and FERRIS, A. A. (1951).—Endemic leptospirosis in Victoria. *Med. J. Aust.* 2:15-8.
- WINKS, R. (1962).—Incidence of *Leptospira pomona* and *Leptospira hyos* titres in beef cattle in Central Queensland. *Aust. Vet. J.* 38:185-9.

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