Fifty years of leptospirosis research in New Zealand: a perspective

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Introduction

It is not known when pathogenic leptospires first became established in New Zealand. The first land mammals to arrive in this country about 1,200 years ago were human beings, dogs and the kiore, *Rattus exulans*. It cannot be determined if leptospirosis also arrived at that time. It is more likely that most, if not all pathogenic leptospires arrived with intentional and unintentional mammalian imports in the late 18th and 19th centuries.

The history of leptospirosis research in New Zealand is one which has traced the pattern of infection from overt animal and human disease through to the epidemiology of inapparent carrier states in both domestic and feral animals. We now know that these carriers represent reservoirs of infection. The causal organisms are fragile and can be difficult to grow. Serological testing has sometimes been useful in diagnosis but in earlier days serology created confusion because of cross-reactions among types (serovars) of leptospires. For example early studies suggested that the hedgehog, *Erinaceus europaeus occidentalis*, might be a carrier of infection by serovar *pomona*, but subsequent work showed that serovar *ballum* was the most common serovar in that host. Another very practical deficiency of reliance on serological diagnosis was the absence of antibody in the early stages of acute disease.

The first confirmed occurrence of leptospirosis in domesticated animals in New Zealand was in 1950 when *Leptospira pomona* (later known as *L. interrogans* serovar *pomona*) was isolated from a calf with haemoglobinuria at Wallaceville Animal Research Station (Anonymous 1951), but 1953 was the seminal year for publications on leptospirosis. At a meeting of the Northland Branch of the New Zealand Veterinary Association, Ensor (1953) reported that in Northland during the 1952 season, 76 farms reported outbreaks of redwater which were attributed to leptospirosis. At the same meeting McClure (1953) reported the clinical and postmortem characteristics of the disease in young calves. He also gave the opinion that "Investigation into the relationship of *Leptospira pomona* and bovine abortion in New Zealand would be of value." There was not long to wait because 7 months later Te Punga and Bishop (1953) recorded an outbreak of bovine abortion in the Waikato district which was established as being due to serovar *pomona*. Interestingly, the paper contains the earliest colour illustrations to appear in the New Zealand Veterinary Journal, including an excellent photomicrograph showing a silver-stained leptospiral organism in a foetal kidney. The colour plates also illustrate the contrast between the clear-cut severe lesions of placentitis due to *Brucella abortus*, which was present in New Zealand at that time, and the mildness of changes in the placenta associated with leptospiral abortion. We now know that the latter "lesions" are largely autolytic or putrefactive in nature and associated with intrauterine death of the bovine conceptus.

In 1958 Russell and Hansen extended interest in leptospirosis from cattle to pigs. They examined the sera of 1,125 healthy adult pigs and found 4% had titres of 1:200 or higher to serovar *hyos* (later renamed *tarassovi*) and 5% had similar titres to serovar *pomona*. It was not until 1975 that Ryan and Marshall reported the first isolation in culture of serovar *tarassovi* in the course of a survey for leptospirosis in pigs being submitted to an abattoir. The survey was of 80 pigs and from these, one isolation of serovar *tarassovi* was made, in contrast to serovar *pomona* which was recovered from 38. As indicated by both serological and cultural studies in this country the overall level of infection of serovar *tarassovi* in deer, goats and horses is of the order of 1–6%. It has also been isolated from dogs (Mackintosh et al 1980) and cases of human infections with this serovar have also been reported (Wilks and Humble 1997).

Human leptospirosis

Even in the early days of interest in leptospirosis as an animal disease, the phenomenon of zoonotic infection of human subjects was fully appreciated. The advent of the Accident Compensation Scheme, under which leptospirosis was classified as an occupational disease of farmers and meat industry workers, gave research in this field considerable impetus. Blood cultures of the organism became promoted as the "gold standard" for diagnosis of human leptospirosis but that required good clinical skills and early suspicion of the disease during differential diagnoses. It also required ready access to laboratories with the ability to provide the specialised services needed for the culture and identification of leptospires. The septicaemic phase of the disease is relatively short (4–7 days) and more data were needed on the dynamics of antibody levels in patients if serology was to be useful in diagnostic and epidemiological studies. Serum antibody begins to be detectable during the second week of illness and should peak about 2 weeks later but treatment with antibiotics can confuse the clarity of both serological and cultural approaches to diagnosis. In spite of this potential difficulty, in suspect cases it is important to institute antibiotic treatment at high dosage as early as possible. The antibiotic of choice is penicillin, given intravenously at the rate of 2 mega-units 6-hourly for 7 days. Oral doxycycline, 100 mg 12-hourly, is an effective alternative in patients who are allergic to penicillin (Wilks and Humble 1997). In epidemiological studies the rate of decline or half-life of leptospiral antibodies becomes an important theoretical variable to be accounted for in population studies. However, studies over 53 months of 69 meat inspectors with a range of leptospiral titres showed considerable variation in magnitude and rates of decay. Furthermore, some individuals with previously confirmed leptospirosis had titres of 1:192, seven years after infection (Blackmore et al 1984). Cross-reactivity between serovars can also create difficulty in establishing which serovars a host may have experienced.

Taxonomy of serovars present in New Zealand

The taxonomy of leptospiral organisms has been improved considerably since the first reports in this journal of the diseases they caused. Although internationally there are some 180 serovars recognised within the seven species of pathogenic leptospires, only eight of these serovars within two species have been isolated and confirmed as being present in this country. These are serovars *australis*, *canicola*, *copenhageni* and *pomona* within the species *L. interrogans*, as well as serovars *balcanica*, *hardjobovis*, *tarrasovi*

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and *ballum* within the species *L. borgpetersenii*. Serovars *canicola* and *australis* have only been isolated from human patients in this country and thus cannot be ascribed endemic status.

Important to the understanding of the epidemiology of the various leptospiral types was the advent of typing by using endonucleases to study the genomes of isolates (Marshall et al 1981; Robinson et al 1982).

Maintenance hosts and renal localisation of infection

The presence of maintenance hosts is pivotal to the endemic existence of the disease. For the optimum persistence of leptospiral infection in a maintenance host the infection should not kill the host and the shedding of viable organisms in the urine should last for a relatively long period. Recognised maintenance hosts for various serovars are cattle for serovar *hardjobovis*, pigs for serovars *pomona* and *tarassovi*, brushtail possums *(Trichosurus vulpecula)* for serovar *balcanica*, the black rat (*Rattus rattus)* for serovar *ballum*, and the brown or Norway rat *(Rattus norvegicus)* for serovar *copenhageni*. Wilson et al (1998) have also suggested that farmed deer may act as maintenance hosts for some serovars. Characteristically the maintenance relationship between organism and host is most evident in sexually mature animals. In this context horses, dogs, human beings, goats, sheep and camelids have not been identified as regular maintenance hosts and usually become infected by direct contact with infected maintenance host species, their urine, or effluent. The common natural routes of infection are believed to be via the conjunctiva, oral or nasal mucosae, or damaged skin.

The significance of localisation of infection in the kidney cannot be over-emphasised because of the importance of this phenomenon as a stage of the disease that follows the septicaemic component. The duration and intensity of leptospiruria are key factors. The localisation of leptospires in the proximal convoluted tubules of the kidney is assumed to afford them some degree of protection from humoral antibody. This protection permits replication and persistence of the organisms within the tubular lumen. Nonetheless there is an interstitial cellular reaction in infected kidneys that in some cases may give rise to focal lesions which, if severe, may be seen with the naked eye and are almost always detectable by histopathology.

In maintenance hosts, leptospiruria persists for long periods, and may even last for a lifetime. Detection of leptospiruria by either culture or direct microscopy can be used to confirm endemicity of infection in a herd or the diagnosis of subclinical disease in individual animals. Dark field microscopy will detect leptospiral organisms in urine if they are present at a concentration of at least 1,000 organisms per ml of urine. That concentration is commonly achieved in maintenance hosts and may go as high as 100,000 per ml. Cultural techniques unfortunately take up to 3 months to complete and isolation may be frustrated by the presence of other bacteria which overgrow leptospires in the non-selective, specialised media which they require for growth.

Vaccination and control

Soon after reports of the disease associated with serovar *pomona* in cattle, attempts were made to produce a suitable vaccine, using a heat-killed whole culture (Webster and Reynolds 1955). The size and recognition of the problem of serovar *pomona* infection at that time is illustrated by the fact that in the period 1952–5, 13,000 bovine sera were examined at the Wallaceville laboratory for antibodies to leptospires (McDonald and Rudge 1957). The same paper by the latter two authors reported on the preparation and testing of two vaccines prepared from a virulent field isolate of serovar *pomona*. One of these was a formalinised, alum-precipitated vaccine, the other was freeze-dried. The former induced the highest and most persistent antibody response. Vaccination was successfully used to prevent leptospirosis of young calves by a double dose vaccination of their dams in late pregnancy. Since the early work on vaccination there have been improvements in vaccines but more importantly an improved understanding of the epidemiology of the various serovars. This understanding has greatly facilitated the elaboration of effective vaccination strategies. Such strategies are now firmly established for both cattle and pig industries.

In countries where the major maintenance hosts are among wildlife, control of human leptospirosis by control of the disease in animals is not a feasible option and aggressive reduction of hosts such as rodents, plus possible vaccination of at-risk persons, is accepted as the best approach. In New Zealand, the vast majority of cases of human infection are derived from domestic animals and as a consequence there is a golden opportunity to control the economic effects of disease in animals and concurrently reduce the exposure to infection of people, especially those in high-risk occupations. Members of the Women's Division of Federated Farmers saw the toll that was caused by leptospirosis in rural workers and raised a considerable amount of money to fund research into the problem at Massey University. The main research priorities were the identification of maintenance hosts and the elaboration of the best strategies for control. The majority of human cases of leptospirosis were in dairy farmers. The belief that the number of organisms required to infect a human being was less if the route was through abraded skin or mucous membranes, as opposed to intact skin, had long been a basis for public health recommendations for workers at risk from infected animals. Application of these theoretical measures aimed at protecting people milking cows from urine splashes, and shielding skin and mucosae by mechanical barriers, or appropriate changes in work practices, were considered unlikely to be widely adopted. After dairy farmers, meat industry workers and pig keepers were the next most commonly affected groups. Vaccination of maintenance hosts using an appropriate strategy to control animal infection and thereby human disease, was soon identified as the best approach. There became apparent a strong will to tackle the problem on a broad front. The industry, Massey University, government agencies, including the Accident Compensation Commission, vaccine manufacturers and, most importantly, the veterinary profession joined in the battle. At the same time steps were taken to increase the awareness of the disease among rural medical practitioners.

Control programmes

Improvements in the performance of vaccines allowed industry-wide control programmes based on a full understanding of the disease in the maintenance species (Marshall 1987). Vaccination of adult females leads to the acquisition of passive immunity in young animals. This has the advantage of protecting the otherwise susceptible young but may interfere with production of active immunity if first vaccination occurs while humoral antibody is still present. The period during which the susceptible infected host sheds leptospires in the urine, and the concentration of those organisms, determine the chance of infection being passed to another animal of the same or different species. The dose of organisms required for a new host to become infected depends upon the species being infected, the characteristics of the invading leptospires and the route of entry. Nevertheless current vaccine-based control programmes have been extremely successful. Human beings acquire their leptospiral infections almost exclusively from animal sources. They are therefore a very useful sentinel species for success or otherwise of control of infection in animals.

A bivalent animal vaccine against serovars *pomona* and *hardjobovis* was vigorously introduced to the market at the end of 1979. Notifications of human infection dropped from 677 in 1979, to 325 in 1981. In the following year the number fell to 179 cases and remained at <200 for another 5 years with further falls later (Marshall and Chereshsky 1996). This was a very pleasing outcome.

Conclusion

Confusion and uncertainty have often occurred as a consequence of published research, surveillance, and individual diagnosis when they have relied exclusively upon serological data. In spite of the practical difficulties associated with cultural isolation of pathogenic leptospires, significant advances in an understanding of the epidemiology of leptospiral infections must still rely heavily on the "gold standard" of isolation and identification of the causal organism. The knowledge base so derived may be further enhanced by the use of more recently developed molecular biological techniques such as those pioneered by Marshall et al in 1981.

The New Zealand experience is that sound and comprehensive research must precede the formulation of control strategies. Multifaceted communication and cooperation between researchers, veterinary clinicians, and private, voluntary and statutory interests have been key ingredients of the successful control programme that is in place in this country.

References

Anonymous. *Annual Report of the New Zealand Department of Agriculture*, 1950–1

Blackmore DK, Schollum LM, Moriarty KM. The magnitude and duration of titres of leptospiral agglutinins in human sera. *New Zealand Medical Journal* 97, 83–6, 1984

Ensor CR. Bovine leptospirosis in Northland. 1. Incidence in animals and man. *New Zealand Veterinary Journal* 1, 47, 1953

Mackintosh CG, Blackmore DK, Marshall RB. Isolation of *Leptospira interrogans* serovars *tarassovi* and *pomona* from dogs. *Zealand Veterinary Journal* 28, 100, 1980.

Marshall RB. A nationwide experiment to control human leptospirosis by vaccinating dairy cattle. *Israel Journal of Veterinary Medicine* 43, 194–7, 1987

Marshall RB, Chereshsky A. Vaccination of dairy cattle against leptospirosis as a means of preventing human infections. *Surveillance* 23(1), 27–8, 1996

Marshall RB, Wilton BE, Robinson AJ. Identification of leptospiral serovars by restriction-endonuclease analysis. *Journal of Medical Microbiology* 14, 163–6, 1981

McClure TJ. Bovine leptospirosis in Northland. 2. Some clinical aspects of bovine leptospirosis. *New Zealand Veterinary Journal* 1, 47–50, 1953

McDonald NR, Rudge JM. Prevention of leptospirosis in young calves by vaccinating their dams in late pregnancy. *New Zealand Veterinary Journal* 5, 83–92, 1957

Robinson AJ, Ramadass P, Lee A, Marshall RB. Differentiation of subtypes within *Leptospira interrogans* serovars *hardjo*, *balcanica* and *tarassovi* by bacterial restriction-endonuclease DNA analysis (BRENDA). *Journal of Medical Microbiology* 15, 331–8, 1982

Russell RR, Hansen NF. The incidence of *Leptospira hyos* and *Leptospira pomona* infection in pigs in New Zealand. *New Zealand Veterinary Journal* 6, 50–1, 1958

Ryan TJ, Marshall RB. Isolation of a leptospire belonging to the serogroup tarassovi. *New Zealand Veterinary Journal* 24, 212–3, 1975

Te Punga WA, Bishop WH. Bovine abortion caused by infection with *Leptospira pomona*. *New Zealand Veterinary Journal* 1, 143–9, 1953

Webster WM, Reynolds BA. Immunisation against *Leptospira pomona*. *New Zealand Veterinary Journal* 3, 47–9, 1955

Wilks CR, Humble MW. *Zoonoses in New Zealand*. 2nd Edtn. Pp 19–23. Publication No. 178, Foundation for Continuing Education of the New Zealand Veterinary Association, Massey University, Palmerston North, 1997

Wilson PR, McGhie J, Marshall RB, Audigé LJ-M, Collins-Emerson J, Quankai W, Alley MR. Observations of leptospirosis in farmed deer. *New Zealand Veterinary Journal* 46, 131–9, 1998

Selected further reading

Blackmore DK, Schollum LM. The occupational hazards of leptospirosis in the meat industry. *New Zealand Medical Journal* 95, 494–7, 1982

Blackmore DK, Schollum LM. Risks of contracting leptospirosis on the dairy farm. *New Zealand Medical Journal* 95, 649–52, 1982

Blackmore DK, Marshall RB, Mackintosh CG. Alternative strategies for the control of leptospirosis in dairy herds. *New Zealand Veterinary Journal* 29, 19–20, 1981

Blackmore DK, Bahaman AR, Marshall RB. The epidemiological interpretation of serological responses to leptospiral serovars in sheep. *New Zealand Veterinary Journal* 30, 38–42, 1982

Bolt I, Marshall RB. The epidemiology of *Leptospira interrogans* serovar *pomona* in grower pig herds. *New Zealand Veterinary Journal* 43, 10–15, 1995

Brockie RE. Leptospiral infections of rodents in the North Island. *New Zealand Veterinary Journal* 25, 89–91, 1977

Chereshsky A, Cameron G, Marshall RB. A case of human *Leptospira canicola* infection in New Zealand. *New Zealand Veterinary Journal* 41, 101, 1993

Day TD, Waas JR, O'Connor CE. Effects of experimental infection with *Leptospira interrogans* serovar *balcanica* on the health of brushtail possums (*Trichsurus vulpecula*). *New Zealand Veterinary Journal* 45, 4–7, 1997

Fairley R. Porcine leptospirosis in New Zealand. *Surveillance* 24(4), 15, 1997

Fairley RA, Cooper DR, Guilford WG, Schollum LM. Haemolytic disease associated with *Leptospira interrogans* serovar *pomona* in red deer calves (*Cervus elaphus*). *New Zealand Veterinary Journal* 34, 116–7, 1986

Flint SH, Corner RJ, Marshall RB. A serological study of leptospirosis in domestic deer in New Zealand. *New Zealand Veterinary Journal* 36, 151–2, 1988

Hathaway SC. Leptospirosis in New Zealand: an ecological point of view. *New Zealand Veterinary Journal* 29, 109–12, 1981

Hathaway SC, Blackmore DK. Ecological aspects of the epidemiology of infection with leptospires of the *ballum* serogroup in the black rat (*Rattus rattus*) and the brown rat (*Rattus norvegicus*) in New Zealand. *Journal of Hygiene, Cambridge* 87, 427–36, 1981

Hathaway SC, Blackmore DK, Marshall RB. Leptospirosis in free-living species in New Zealand. *Journal of Wildlife Diseases* 17, 489–96, 1981

Hillbink F, Penrose M, McSporran K. Antibodies in dogs against *Leptospira interrogans* serovars *copenhageni*, *ballum* and *canicola*. *New Zealand Veterinary Journal* 40, 123–5, 1992

Hodges RT, Young GW, Thompson JTM. The efficacy of a leptospirosis vaccine in preventing leptospiruria in pigs. *New Zealand Veterinary Journal* 33, 31–4, 1985

Horner GW, Heath DD, Cowan PE. Distribution of leptospirosis in possums from New Zealand and offshore islands. *New Zealand Veterinary Journal* 44, 161, 1966

Mackintosh CG, Marshall RB, Manktelow BW. Vaccination of cattle in the face of a *pomona* abortion storm. *New Zealand Veterinary Journal* 28, 196, 1980

Mackintosh CG, Marshall RB, Thompson JC. Experimental infection of sheep and cattle with *Leptospira interrogans* serovar *balcanica*. *New Zealand Veterinary Journal* 29, 15–19, 1981

Marshall RB, Manktelow BW, Ryan TJ, Hathaway SC. *Leptospira interrogans* serovar *balcanica* from a possum. *New Zealand Medical Journal* 84, 74–5, 1976

Marshall RB, Broughton ES, Hellstrom JS. Protection of cattle against natural challenge with *Leptospira interrogans* serovar *hardjo* using a hardjo–pomona vaccine. *New Zealand Veterinary Journal* 27, 114–6, 1979

Marshall RB, Schollum LM, Dymock BL. Prevention of *Leptospira interrogans* serovar *pomona* infection in cattle. *New Zealand Veterinary Journal* 30, 177–9, 1982

O'Keefe JS. A brief review on the laboratory diagnosis of leptospirosis. *New Zealand Veterinary Journal* 50, 9–13, 2002

Schollum LM, Blackmore D K. The serological and cultural prevalence of leptospirosis in a sample of goats. *New Zealand Veterinary Journal* 29, 104–6, 1981

Schollum LM, Marshall RB. Age and ability of calves to respond to a leptospiral vaccine. *New Zealand Veterinary Journal* 33, 146–7, 1985

Thornton R. Leptospirosis in New Zealand sheep. *Surveillance* 21(2), 13–4, 1994

Vermunt JJ, West DM, Cooke MM, Alley MR, Collins-Emerson J. Observations on three outbreaks of *Leptospira interrogans* serovar *pomona* infection in lambs. *New Zealand Veterinary Journal* 42, 133–6, 1994