Prevalence of antibodies to *Leptospira* serovars in beef cattle in central Queensland

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Objective To obtain up-to-date data on the prevalence of antibodies to *Leptospira* serovars in central Queensland beef herds preliminary to assessing their role in bovine subfertility and the role of cattle as a zoonotic reservoir.

Design Sera from 2857 female cattle in 68 central Queensland beef herds were tested for antibodies to 14 *Leptospira* serovars using the microscopic agglutination test. Vaccination use and age of cattle were collected to enable the calculation of crude and age-stratified seroprevalences.

Results The most commonly detected antibodies were to serovars hardjo (15.8% crude seroprevalence), tarassovi (13.9%), pomona (4.0%) and szwajizak (2.2%). Vaccinates were omitted from the hardjo and pomona seroprevalence data. The seroprevalence for hardjo and pomona tended to increase with age of the animals.

Conclusion These results are broadly similar to those of previous serological surveys. The data suggest that serovars other than hardjo, pomona and tarassovi, are unlikely to have a significant role in bovine subfertility and that cattle are unlikely to be a source of human infection with them in central Queensland.

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Key words: Cattle, leptospirosis, survey, serology, microscopic agglutination test.

MAT	Microscopic agglutination test.
PCR	Polymerase chain reaction

Sub-optimal fertility is an ongoing problem in many beef and dairy herds. The role of leptospirosis in subfertility in Australian cattle is not clear. Although many graziers and farmers vaccinate regularly against *Leptospira* serovars hardjo and pomona, problems often persist, and may be due to infection with other *Leptospira* serovars or to factors other than leptospirosis. Serovars other than hardjo and pomona that have been isolated from cattle in Queensland and New South Wales include australis,[1] zanoni,[2] celledoni[3] and grippotyphosa.[4]

Furthermore, leptospirosis is an occupational hazard for meatworkers, dairyfarmers and others in animal associated occupations and frequently involves serovars other than hardjo and pomona.[5] This, and the recent isolation of other serovars from cattle, suggested the need for up-to-date seroprevalence data as a preliminary to assessing the roles of other serovars in bovine subfertility and the role of cattle as a zoonotic reservoir.

Early serological surveys found that the major serovars in Australian cattle were hardjo, pomona, and tarassovi.[6,7] Later surveys of Atherton Tableland (north Queensland) cattle[8] and of Victorian cattle[9] found that antibodies to serovar pomona were less common in those regions. This paper reports the results of a survey of beef herds in central Queensland. The last published survey for this region is that of Winks from 1962,[10] who screened cattle passing through Rockhampton and Gladstone meatworks for antibodies to serovars hardjo, pomona and tarassovi.

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Materials and methods

Study area

Central Queensland is an administrative region of the Queensland Department of Primary Industries (QDPI). The region (Figure 1) included the local government areas (shires) of Livingstone, Fitzroy, Calliope, Banana, Duaringa, Bauhinia, Emerald, Jericho, Peak Downs, Belyando and Broadsound.

Sample collection

As part of the QDPI active surveillance project, 30 sera were collected from each of 38 central Queensland beef herds in 1995 and one herd in 1996; 45 sera were collected from each of 44 central Queensland beef herds in 1996. For disease control purposes, all beef herds in Queensland with more than 10 head of cattle are required to be registered with the QDPI. Properties are allocated a property number that includes a field, which denotes the local government area in which the property is located. This database of properties formed the list frame from which a stratified random sample of herds was selected. To be eligible for inclusion in the active surveillance project, beef herds needed to have at least 50 breeders and stock to be tested needed to be homebred. The number of herds selected per local government area was directly proportional to the total number of herds with more than 50 breeders. This selection process also ensured that there was a reasonable geographic spread of properties within the central Queensland region (Figure 1). Although fifteen of the herds sampled in 1995 were included in the 1996 sample, different animals from each of these herds were bled in each year. There were about 2330 herds with more than 50 breeders in the region and 68 (about 3%) of these herds were sampled. The female cattle population was estimated to be about 1.6 million and 2857 (about 0.18%) of these animals were sampled.

Each herd was visited by a government field officer. During the visit, a sample of stock was examined and blood specimens were collected. On the same day, a questionnaire dealing with property management issues was completed. The use of bivalent (hardjo and pomona) *Leptospira* vaccine in the total herd and each animal was recorded as vaccinated or not. In 1995, 15 animals aged between 1 and 2 years and 15 animals older than 2 years were sampled from each herd. During 1996, 15 animals aged between 1 and 2 years and 30 animals older than 2 years from each herd were sampled. In one herd in 1996, only the 30

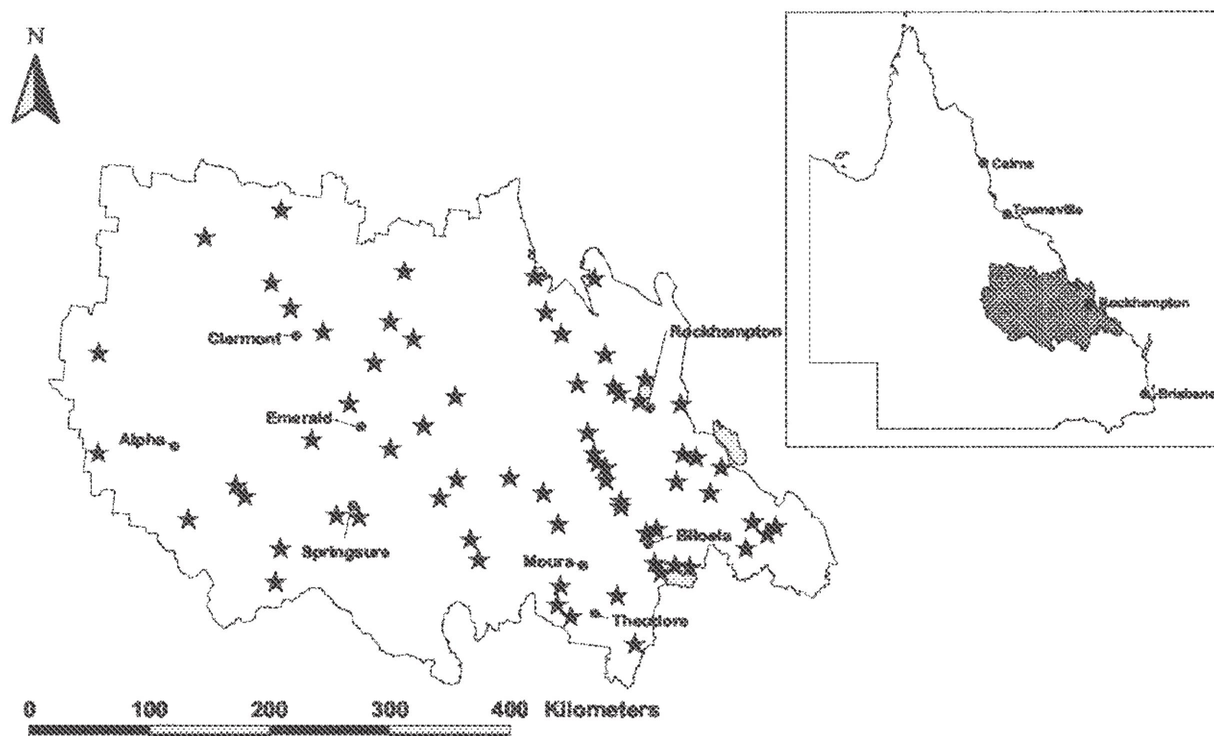

Figure 1. Location of beef herds sampled in central Queensland in 1995/1996.

Table 1. Crude seroprevalences in female cattle for 14 *Leptospira* serovars among central Queensland beef herds in 1995 and 1996.

Serovar	Serogroup	Positive sera	Sera tested	Crude seroprevalence (%)	Crude seroprevalence (95% CI)
hardjo (nonvaccinates only)	Sejroe	242	1533	15.8	4.0 – 17.7
pomona (nonvaccinates only)	Pomona	62	1533	4.0	3.1 – 5.2
tarassovi	Tarassovi	398	2857	13.9	12.7 – 15.3
szwajizak[a]	Mini	62	2857	2.2	1.7 – 2.8
medanensis[a]	Sejroe	19	2857	0.7	0.4 – 1.1
kremastos[a]	Hebdomadis	1	2857	0.0	0.0 – 0.2
celledoni	Celledoni	36	2857	1.3	0.9 – 1.8
zanoni[b]	Pyrogenes	16	2857	0.6	0.3 – 0.9
robinsoni[b]	Pyrogenes	3	2857	0.1	0.0 – 0.3
grippotyphosa	Grippotyphosa	9	2857	0.3	0.2 – 0.6
australis	Australis	3	2857	0.1	0.0 – 0.3
canicola	Canicola	3	2857	0.1	0.0 – 0.3
copenhageni	Icterohaemorrhagiae	1	2857	0.0	0.0 – 0.2
bulgarica	Autumnalis	0	2857	0.0	0.0 – 0.2

[a]Cross reacting serovars. When sera had titres to more than one of these serovars, the highest titre was regarded as the correct one in both vaccinated and nonvaccinated animals. When titres were equal to a hardjo titre, the sample was recorded as a hardjo positive. In one case, the titres were equal for both szwajizak and medanensis. This sample was recorded as positive for both.
[b]Cross reacting serovars. When sera had titres to more than one of these serovars, the highest titre was regarded as the correct one.

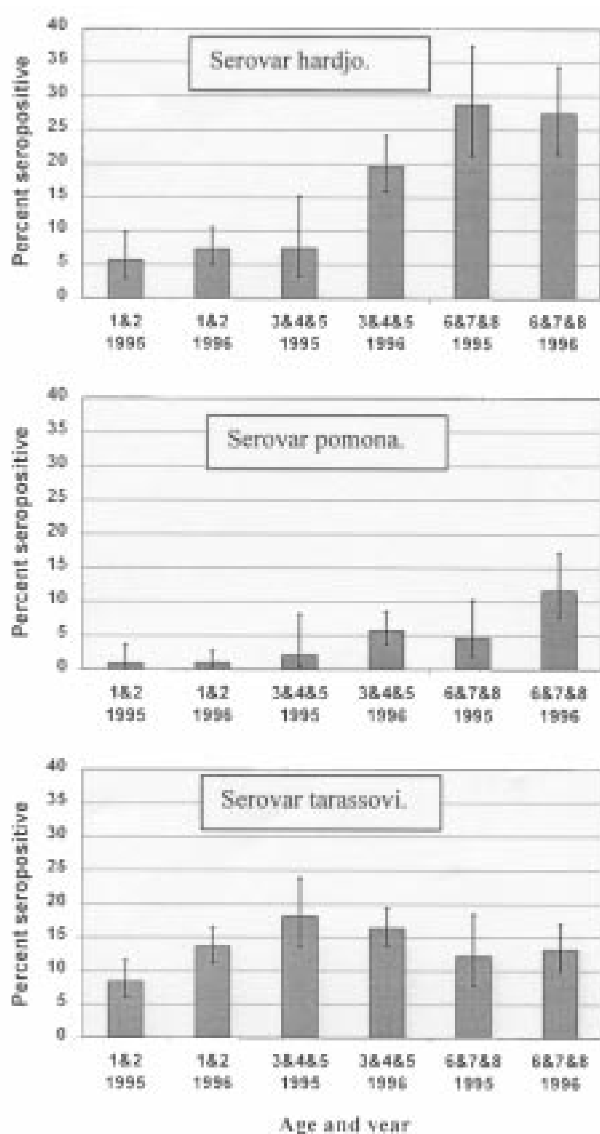

Figure 2. Age stratified seroprevalences with 95% confidence intervals in female cattle for three *Leptospira* serovars among central Queensland beef herds in 1995 and 1996.

older animals were sampled. The preference was to sample female animals if possible because they made up a larger percentage of the total cattle population, were more likely to satisfy the homebred requirement and were the major population of interest with regards to potential reproductive failure associated with leptospirosis infection. Blood samples of at least 10 mL were collected from either the jugular vein or coccygeal vein/artery, depending on the animal handling facilities available. Sera were removed from blood clots and tested for the presence of antibody as described below. Excess sera were stored for possible future retrospective analysis.

Serology

The sera were tested for antibodies to 14 *Leptospira* serovars using the MAT as described by Stallman[11] with modifications as follows. Doubling dilutions of the sera from 1:50 to 1:6400 were prepared in 96-well trays using phosphate buffered saline pH 7.4. An equal volume of live *Leptospira* culture containing approximately 2 to 4 x 10^8 cells/mL was added to each dilution. After 90 min at 30°C, the trays were examined by dark field microscopy for agglutination of the *Leptospira* cells. The titre was the highest dilution showing 50% agglutination.

Samples with insufficient sera, contamination or gross haemolysis and samples from non-female animals were excluded from the analysis. There were 219 such samples in 1995 and 74 in 1996. Results were obtained for 2857 sera (921 sera in 1995 and 1936 in 1996). Animals with titres of 50 or more to the MAT were regarded as seropositive.

For serovars hardjo and pomona, only sera from herds in which *Leptospira* vaccine had not been used were included in the seroprevalence calculations. Sera with titres to more than one serovar were regarded as seropositive for all of those serovars unless the serovars were known to cross react, in which case the sera were scored as positive to the serovar with the highest titre. The 14 serovars tested (Table 1) included two groups of cross-reacting serovars; hardjo, szwajizak, medanensis, and kremastos in one group and zanoni and robinsoni in the other.

Analysis

Crude seroprevalences were calculated for all 14 serovars. Crude rates do not take account of ages of the animals sampled and are of limited use when comparing results from other studies. Age stratified seroprevalence rates were calculated for the three most common serovars in this study. Microsoft Excel (Microsoft®Excel 97) was used for descriptive data analysis and Epi Info Version 6[12] was used to calculate the 95% Fleiss Quadratic Confidence Intervals.[13]

Results

Use of vaccine

Of the 2,857 sera, 1,324 (46.3%) were from herds where *Leptospira* vaccine had been used and 1,533 (53.7%) were from herds with no use of vaccines.

Crude seroprevalence

Table 1 shows the crude seroprevalence data for the 2,857 sera for 1995 and 1996. Serovar hardjo had the highest crude seroprevalence (15.8%), followed by tarassovi (13.9%), pomona (4.0%), and szwajizak (2.2%). The other serovars had crude seroprevalences of around 1% or less.

For the three most common serovars, an age-stratified analysis was performed. The results of the analysis are shown in Figure 2. The seroprevalences for hardjo and pomona tended to increase as age increased, but this trend was less clear for tarassovi.

Discussion

This study estimated, by MAT, the prevalence of antibodies to 14 *Leptospira* serovars in the female cattle population of central Queensland in 1995 and 1996. The major serovars in order of decreasing crude seroprevalence were hardjo (15.8%), tarassovi (13.9%), pomona (4.0%) and szwajizak (2%). Antibodies to all other serovars were rarely detected.

However, detection of antibodies does not indicate clinical disease and failure to detect antibodies does not guarantee lack of exposure to leptospire serovars. Additionally, the sensitivity and specificity of the MAT have not been described due to the difficulty of establishing a suitable gold standard for comparison with the MAT. Because culture is often difficult and therefore not to be relied on, it is unsuitable for use as a 'gold standard'

for the MAT. Despite these problems, the MAT has been the test of choice to indicate exposure to *Leptospira*.

Furthermore, the relative prevalence of antibodies to the different serovars can only be interpreted if one assumes that exposure to all serovars is equally likely to result in the production of detectable antibodies, and that detectable antibodies persist for similar periods for all serovars. These assumptions can not be tested at present.

Antibodies were most frequently detected to hardjo, and this serovar has been isolated from the urine of infected Queensland cattle on many occasions.[14-16] Hardjo is also the most commonly implicated serovar in leptospirosis in dairy farmers, meatworkers and other animal-associated occupations, including graziers,[5] which is to be expected given its presence in the urine of infected animals. Hence it is widely accepted that infected cattle are a significant source of human infection with hardjo, and vaccination of susceptible herds is recommended to reduce the risk of infection to humans. The role of hardjo in subfertility in Australian cattle is still not resolved.

Antibodies to tarassovi are also relatively common in the cattle population. However, recent attempts to isolate tarassovi from the urine of seropositive cattle in Australia were unsuccessful (BG Corney unpublished). Clinical cases attributed to tarassovi in humans are not unusual. In fact, 6% (13/231) of human cases reported by Smythe et al[5] were attributed to tarassovi and several of these occurred in graziers and one in a meatworker (L Smythe personal communication). Our failure to isolate tarassovi from the urine of cattle may have been due to deficiencies in the culture system. However, it is also possible that cattle rarely excrete this serovar. This raises the possibility that cattle are not the major source of human infection with tarassovi. Pigs, which are the preferred host for this serovar and are known to excrete the organism in their urine,[17,18] should be considered as a potential source of infection for humans. Information on whether human cases participate in activities such as shooting or hunting feral pigs, would assist in assessing whether feral pig exposure is a more significant risk factor than being a grazier or meatworker.

From our seroprevalence data, the other serovars commonly implicated in human leptospirosis in Queensland, zanoni and australis,[5] appear to be rare in central Queensland cattle. Therefore, cattle are unlikely to be a significant source of human infection for these serovars in central Queensland. Each *Leptospira* serovar has a preferred ecological niche. The reservoir hosts for many serovars have been described.[19] Cattle are generally accepted as a reservoir host for hardjo.[20] Apparently, for most of the serovars examined in this survey, either a suitable ecological niche is not present in central Queensland, or central Queensland cattle are not in close contact with a reservoir host. Therefore, it seems reasonable to conclude that serovars other than hardjo, pomona and tarassovi, are unlikely to have a major role in bovine subfertility in central Queensland.

Previous surveys of Queensland cattle[8,10,21-23] also found high seroprevalences of hardjo, tarassovi and, in the earlier surveys, pomona, although the order and percentage varied. However, these surveys targeted different regions in Queensland and did not select herds and or animals at random for sampling. Although the serological test was always the MAT, formerly known as the agglutination - lysis test, the titre used to classify a sample as positive varied from 1:30 to 1:100. The study by Winks[10] involved predominantly 3- to 5-year-old bullocks from the central Queensland area killed at Rockhampton and Gladstone meatworks in 1961. None of the other cited studies have detailed the age and sex composition of the sample. The estimates of seroprevalence are therefore biased and need to be interpreted with caution especially with reference to specific populations or when comparing seroprevalences from one study to another.

Limited previous studies[10] have not detected a difference between the crude seroprevalence in male and female cattle, although figures need to be interpreted with caution because the median and mean age of male cattle is younger than female cattle in central Queensland. Also, male cattle are often depastured on ‘better country’ than breeders in some areas of central Queensland and hence the risk of infection with various serovars may therefore differ between male and female cattle.

None of the other studies have reported age-stratified seroprevalences. This study shows that the seroprevalences for hardjo and pomona tended to increase with age. This is consistent with other studies, which have shown that antibodies persist to some degree in the exposed population.[24,25] For tarassovi, the less clear trend could be due to the persistence of antibody being more variable or to sampling variation.

This study highlights the difficulties involved in studying leptospirosis due to the deficiencies in the tests currently available. Further progress in understanding the epidemiology of leptospirosis in farm animals, and how it relates to human leptospirosis, will require the use of new technologies for detecting current infections and identifying the serovars involved. PCR-based technologies have the potential to fulfil these needs as they offer rapid and sensitive detection of leptospires in urine, tissues and the environment.[26]

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BOOK REVIEW

Pathology of laboratory rodents and rabbits. *2nd edn. Percy DH, Barthold SW, Iowa State University Press, Ames, 2001, 315 pages. Price USD99.95. ISBN 0 8138 2551 2.*

This book discusses the pathology of mice, rats, hamsters, gerbils, guinea pigs and rabbits. Since the first edition in 1993 mouse pathology has undergone the greatest change and so is emphasised in this edition although all sections have been fully updated. Both authors are very experienced veterinary pathologists and have spent a professional lifetime working, researching, teaching and writing in the field of laboratory animal pathology. Both are practical, hands-on, diagnostic pathologists and have passed on a great deal of their acquired knowledge, which is supplemented fully by published information.

The book is designed as a general reference for veterinary pathologists, laboratory animal veterinarians and students. It would also be very useful for veterinary practitioners who see rodents and rabbits in their practice. A good understanding of pathology is most useful for diagnosis of disease in these species, which show few, often vague, clinical signs. The book is aimed at the diagnostician and is not intended to be a detailed source of information on all aspects of laboratory animal pathology. It presents key diagnostic features, differential diagnoses and the significance of diseases in the most commonly used laboratory animals. The reader can source more information through the detailed references presented at the end of each section.

Information on each species is divided into anatomical features useful for diagnosticians, viral, bacterial, parasitic, and mycotic infections, nutritional and metabolic disorders, aging and degenerative disorders and neoplasms. There is considerable emphasis on infectious diseases since these can be very significant in the laboratory animal environment. The section on the mouse, which takes up one third of the book, also has a concise but very comprehensive section on the pathology of genetically engineered mice, a subject area that is expanding rapidly and is unlikely ever to be up to date in texts.

The book is well written and is easy to read, as the flow of the text is not broken by references. The text is appropriately illustrated and the quality of the illustrations is good although in the current A5 format the photos are smaller than in the original A4 format and in some cases have lost a degree of contrast.

Overall the book is a very useful text and should be accessible to all those involved in the veterinary care of laboratory animals. The book meets the ultimate purpose of the authors, which is to 'assist our colleagues in improving the health and welfare of these species that serve such a vital role in biomedical research'.

D Pass

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