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A serological survey of leptospirosis in cattle of rural communities in the province of KwaZulu-Natal, South Africa

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ABSTRACT

A serological survey of leptospirosis in cattle originating from rural communities of the province of KwaZulu-Natal (KZN) in South Africa was carried out between March 2001 and December 2003. The survey was designed as a 2-stage survey, using the local diptank as the primary sampling point. In total, 2021 animals from 379 diptanks in 33 magisterial districts were sampled and tested with the microscopic agglutination test (MAT). The apparent prevalence at district level was adjusted for clustering and diagnostic test sensitivity and specificity and displayed in maps. The prevalence of leptospirosis in cattle originating from communal grazing areas of KZN was found to be 19.4 % with a 95 % confidence interval of 14.8–24.1 %. At district level the prevalence of leptospirosis varied from 0 to 63 % of cattle. Bovine leptospirosis was found to occur in communal grazing areas throughout the province with the exception of 2 districts. The southeastern regions showed a higher prevalence than other areas of the province; while in some of the northern and western districts a lower prevalence was noted. Several serovars were detected by the MAT and although *Leptospira interrogans* serovar *pomona* occurred most frequently, serovars *tarrasovi, bratislava, hardjo, canicola* and *icterohaemorrhagica* were also frequently identified. The findings of the survey are discussed.

Key words: Africa, rural communities KwaZulu-Natal, leptospirosis, prevalence, zoonosis.

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INTRODUCTION

The rural areas of the coastal province KwaZulu-Natal (Fig. 1) are generally characterised by small-scale, extensive farming of multiple species, which often include cattle, chickens, goats, pigs and donkeys. Dogs are also commonly found in the rural areas. Information on the prevalence of infectious diseases of importance with regard to their zoonotic potential and impact on production parameters is often not available or of very poor quality. To improve this knowledge and consequently increase the ability to prioritise diseases in need of control, the Veterinary Services of KwaZulu-Natal initiated a large-scale serological survey between 2001 and 2003 that included leptospirosis amongst other diseases.

Leptospirosis is a worldwide zoonotic infection caused by spirochaetes, occurring in domestic and wild mammals, but the agents have also been isolated from other vertebrates such as birds and amphibians[1].

Clinical presentation following acute infection can range from transient febrile episodes to life-threatening acute haemolytic disease. Where a maintenance host of a serovar is involved, acute infections may be mild and can result in the establishment of chronic infections, which may result in intermittent, sometimes lifelong, shedding of leptospires in the urine and from the genital tract[25].

Production losses in cattle as a result of leptospirosis occur mainly as a result of decreased reproductive performance and decreased milk production. The disease is known to occur in South Africa, especially in the coastal high rainfall areas such as KwaZulu-Natal, and while several serovars (*Leptospira interrogans* serovars *pomona, mini, canicola* and *hardjo*) have been isolated from cattle[14,23,24,30], it appears that serovar *hardjo* is the most important cause of bovine leptospirosis in South Africa[16]. In the southern African context the occurrence of the disease does not appear to be significantly different between commercial and communal farms[12]. As a zoonosis the risk of *Leptospira* infection in humans is closely related to humans coming into contact with tissues or urine from infected animals. Contact with mud or water contaminated with animal excreta constitutes an important route of infection, the risk of which is increased in the presence of skin lesions or abrasions[18].

The most frequent clinical sign of leptospirosis in humans is a self-limiting systemic illness, although in a smaller percentage of cases infection can lead to severe multi-systemic disease[19]. Since most human cases present with flu-like

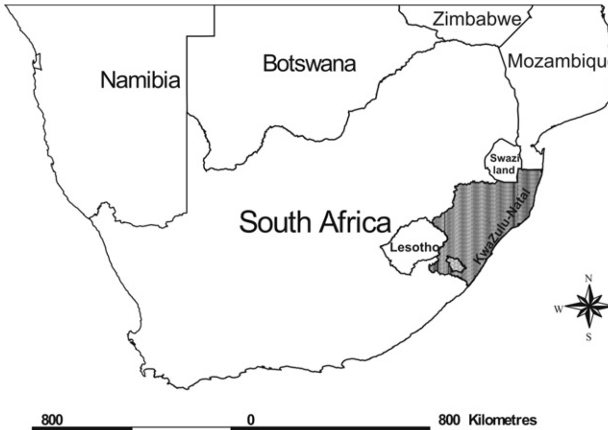

Fig. 1: **Location of the province of KwaZulu-Natal.**

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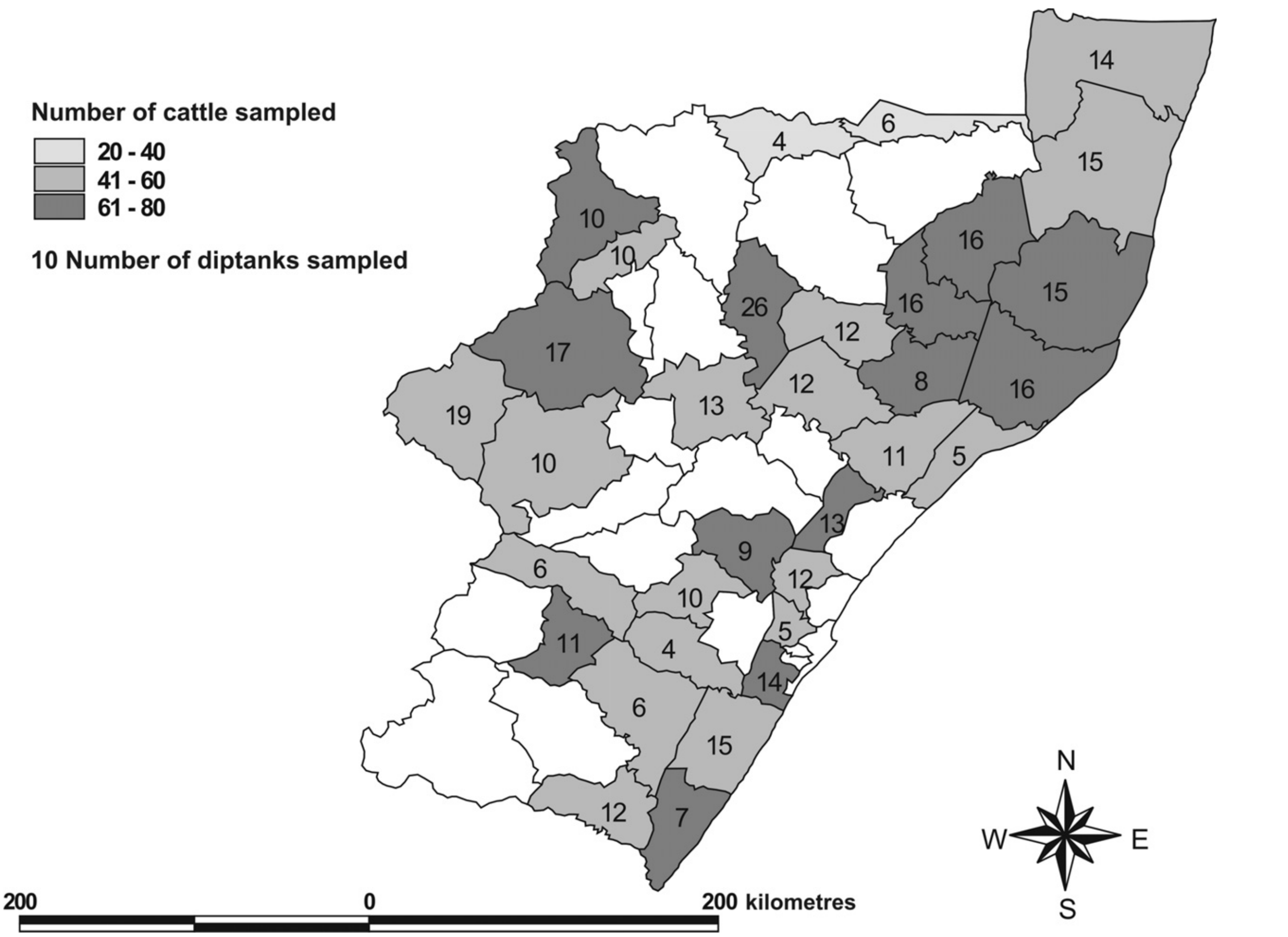

Fig. 2: **Number of animals** (shading) **and diptanks sampled** (numbers) **for bovine leptospirosis in a serological survey of KwaZulu-Natal in 2001–2003.**

symptoms, the disease is often misdiagnosed or ignored.[18] It has been suggested that outbreaks of the disease are likely to go unnoticed in rural settings[12].

MATERIALS AND METHODS

Serological samples were taken from March 2001 until December 2003. At the time of the survey the province of KwaZulu-Natal was divided into 52 magisterial districts according to the existing political boundaries. This division has been applied to the design of the survey and required sample sizes were calculated on a district basis. Since rural communities are not present in all districts, only 33 districts were included. The sample sizes and strategy were developed in consultation with a statistician. Diptanks were the primary sampling point and approximately 60 cattle were sampled in each district at between 4 and 26 diptanks, depending on the number of diptanks present in the district. The number of diptanks and the number of animals sampled in each district are given in Fig. 2.

Diptanks were selected at random by drawing numbers from a hat. The sampling design intended animals to also be selected on a random basis provided their owners approved of the collection of samples, which most (>80 %) did. Samples were only taken from fully-grown animals with no stratification according to age or sex. Animal health technicians bled the cattle according to standard procedures from the jugular vein into serum tubes and the samples were sent to the Onderstepoort Veterinary Institute for testing with the microscopic agglutination test (MAT).

Once all samples had been tested, the results were entered into a spreadsheet (Excel 97, Microsoft Corporation, USA)[20]. Results at diptank level were linked to a database on the geographic location of diptanks, which was collated by the State Veterinary Services in KwaZulu-Natal. For the creation of diptank maps, only diptanks recorded in the results tables that could be matched with certainty in the GIS database were used. All maps were created in ArcView 3.3[2].

For districts where serological reactions were found, the apparent prevalence was adjusted for diagnostic test sensitivity and specificity according to Rogan and Gladen[28]. To account for the clustering effect of sampling at diptanks the 95 % confidence intervals around the mean true prevalence were adjusted according to Thrusfield[31]. To allow for uncertainty about the test sensitivities, these were entered as a probability distribution. The sensitivity and specificity were entered as a uniform distribution (Uniform 0.9, 0.98)[3,6] and the above described parameters were obtained through the outputs of a simulation with 10000 iterations, using the mean for the true prevalence and the relevant confidence intervals for the 95 % confidence interval of the prevalence.

For districts where no serological reactions were found, calculations were carried out using the program Survey Toolbox[5] to evaluate how certain one can be about the absence of the disease. The software allows for integration of test sensitivity and specificity, and uses a hypergeometric distribution function for assessing freedom from disease. These calculations were done assuming a minimum leptospirosis prevalence of 1 % and a Type I and II error of 5 %.

RESULTS

The mean provincial prevalence of leptospirosis in communal cattle of KZN was found to be 19.4 % (lower 95 % confidence limit (LCL) = 14.8; upper 95 % confidence limit (UCL) = 24.1 %). At district level the mean prevalence varied from 0 to 61.8 %. Fig. 3 displays the mean

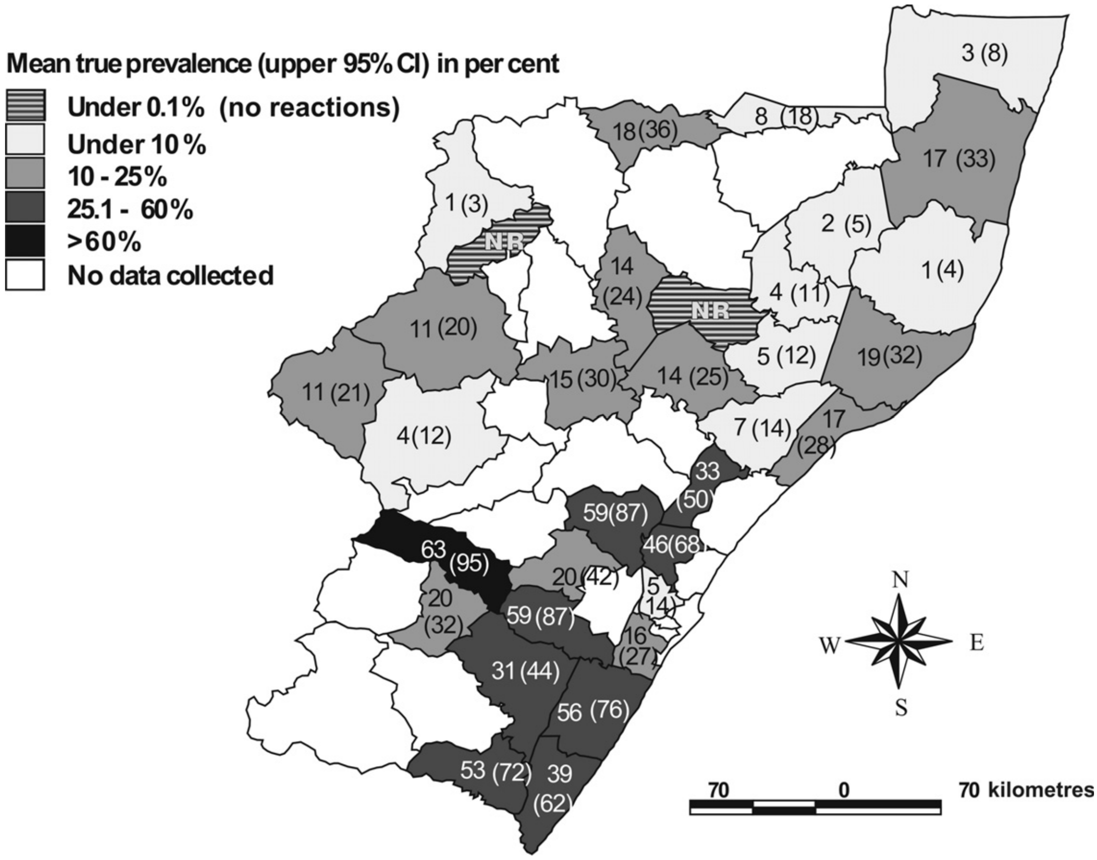

Fig. 3: **Mean seroprevalence and upper 95 % confidence interval** (in brackets) **of the prevalence of bovine leptospirosis in communal areas of KwaZulu-Natal in 2001–2003 at district level.**

prevalence and the UCL of bovine leptospirosis for each district surveyed.

Bovine leptospirosis was found in all districts surveyed with the possible exception of Babanango and Dannhauser (indicated in Fig. 3 by 'NR'). The results that these 2 districts were free of disease were inconclusive ($P = 0.014$) at the 99 % confidence level due to the relatively small sample size (60 and 50 cattle respectively). The southeastern regions showed a higher prevalence than other areas of the province, while in some of the northern and western districts a lower prevalence was noted. The mean adjusted prevalence for each dip tank surveyed is displayed in Fig. 4.

Several serovars were detected by the MAT and although serovar *pomona* occurred most frequently, serovars *tarrasovi, bratislava, hardjo, canicola* and *icterohaemorrhagica* were also frequently identified. The proportions of detected serovars are displayed in Fig. 5.

DISCUSSION

This survey is the first large-scale serological study of leptospirosis in cattle in the rural areas of South Africa. Figs 3 and 4 illustrate that the disease occurs throughout the province, although it was not detected at all diptanks. The northeastern region appears to be less affected and 2 districts (Dannhauser and Babanango) may be free of the disease.

The true prevalence of leptospirosis in this survey needs to be interpreted with care, as a high uncertainty exists around the mean, and the confidence intervals are very large (up to 67 %). This is mainly an effect of the number of cattle sampled at each dip tank (around 5–10 animals). The sample size of 10 animals at the diptanks is an indicator of the presence or absence of the disease in the herd rather than an estimate of the underlying prevalence[13]. This is because antibody levels decline fast and remain above the diagnostic threshold only for a limited time,[13] so the prevalence may be underestimated. However, a sample size of 5 or 10 animals allows the minimum prevalence of the disease to be 20 or 10 %, respectively, at diptank level and this gives the impression on a map that the prevalence is higher than it actually may be. Additional widening of the confidence intervals was caused by uncertainty about the sensitivity of the MAT.

Another confounding factor in the serological interpretation of survey results can be vaccination against leptospirosis[15,29], but this is thought to have played a minimal or no role in this survey, as vaccination is not usually practised for this disease in the communal areas.

The widespread presence of the infection reflects the results of other studies that found leptospirosis in cattle, pigs and game in South Africa[8,16,22,27] and the overall prevalence of 19.4 % agrees with that of rural areas in Zimbabwe[12].

The highest density of affected diptanks occurs in the southeastern region of the province, while the northeastern region appears to be less affected by the disease. Considering that *Leptospira* spp. are excreted in the urine and can survive especially well under moist conditions (up to 94 days in river water)[9,17,26] and that exposure to contaminated water sources as well as inadequate husbandry[1] are important risk factors, conditions for the occurrence of leptospirosis are very favourable in rural communities in KZN, especially in the southeastern part of the province where high humidity and mist combined with high rainfall prevail. The favourable conditions in the southeast of the province are enhanced by the frequent practice by local commercial farmers of irrigating pastures with slurry and the predominance of mixed farming units, many of which include pigs. In contrast, few pigs are kept in the northern region, where mainly beef and game farming under extensive conditions is practised.

The main serovar identified in cattle in this survey was *pomona* (22 %), but a wide variety of serovars was detected: (*tarassovi* (19 %), *bratislava* (15 %), *canicola* (13 %),

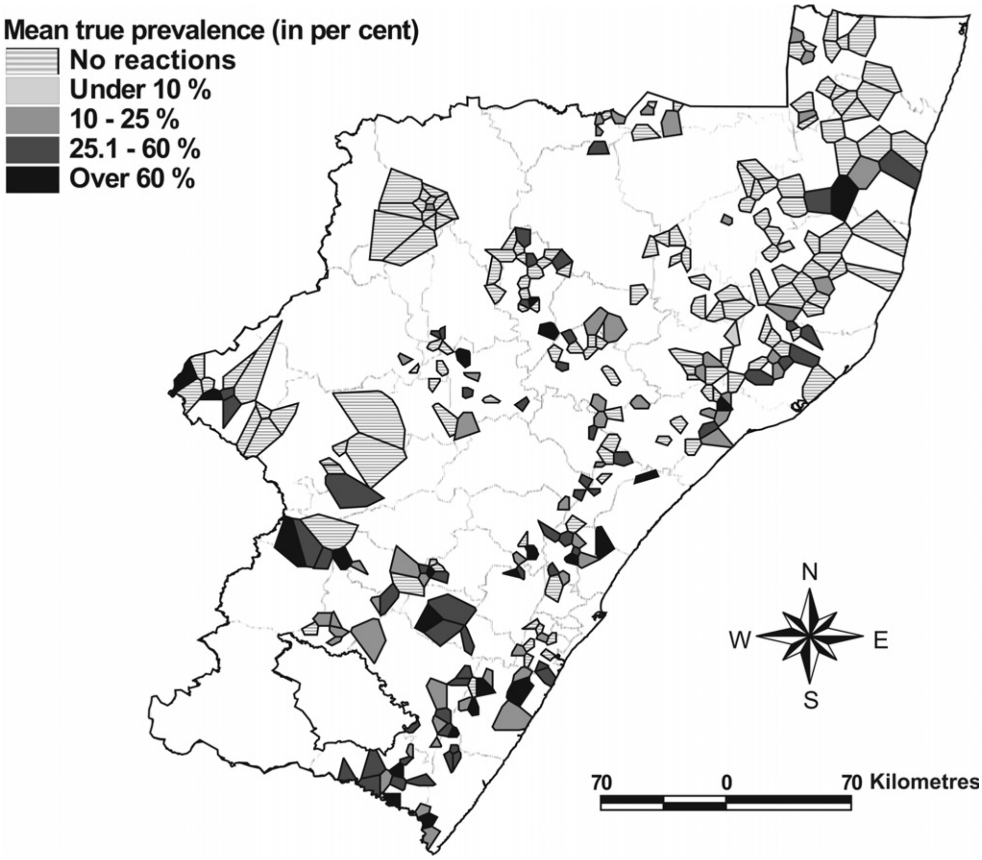

Fig. 4: **Mean true prevalence of serovars of *Leptospira interrogans* detected in cattle in the 2001–2003 survey of the communal grazing areas in KwaZulu-Natal.**

hardjo (13 %), *icterohaemorrhagica* (12 %), *szwajizak* (4 %) and *grippotyphosa* (2 %)). Generally serovar *pomona* is thought to cause only sporadic infections in cattle in South Africa, while *hardjo* is considered the most important cause of bovine leptospirosis[24]. The second most frequently identified serovar was *tarassovi* which is maintained by pigs[1] but was not detected in a previous study on South African pigs[27].

However, *tarassovi* is also known to occur in cattle and along with with *hardjo* and *wolffi* was among the most frequent serovars in a survey on bovines carried out in Mexico[21]. It also had a high prevalence in cattle in Zimbabwe[12].

The serovar *bratislava,* which was found to be the cause of 15 % of the serological reactions, has frequently been identified to be an important serovar in infections in pigs.[4,7] This serovar is, however, maintained by many free-living species[1,13], and pigs[1,10], horses[1,11] and probably dogs[32] can serve as maintenance hosts. The serovar *hardjo,* which is adapted to and maintained by cattle[1], accounted for 13 % of the serological reactions. However, the weighting of serovars should take into account that that cross-reactions between the serovars are common in the MAT test[19].

The wide variety of serovars and the widespread prevalence of leptospirosis in KZN suggest that the disease is endemic.

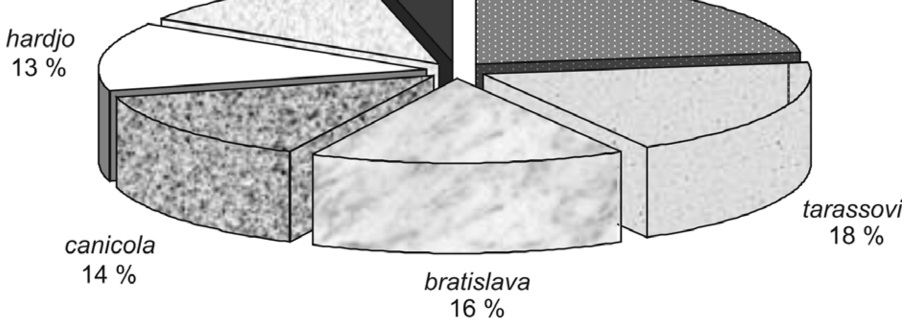

Fig. 5: **Prevalence of *Leptospira interrogans* serovars at communal dip tanks surveyed in a serological survey of KwaZulu-Natal in 2001–2003.**

The involvement of many species in transmission combined with the frequent mixing of species on the communal lands, the role of carrier animals in the infection, the warm and humid climate and the husbandry practices that do not prevent contact with rodents, dogs and other animals, are likely to maintain the disease in KZN and render control of the disease complicated. To decrease the zoonotic impact, a campaign is needed to inform owners of the routes of infection and useful protective measures, such as wearing footwear, using disinfectants when in contact with animal excreta, and covering abrasions when handling animals[18]. In addition, it might be useful to heighten the awareness of health service practitioners with regard to the presence of the disease in order to reduce its misdiagnosis as other conditions with similar flu-like or jaundice-like symptoms, such as malaria.

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REFERENCES

1. Alonso-Andicoberry C, Garcia-Pena F J, Pereira-Bueno J, Costas E, Ortega-Mora L M 2001 Herd-level risk factors associated with *Leptospira* spp. seroprevalence in dairy and beef cattle in Spain. *Preventive Veterinary Medicine* 52: 109–117
2. Arcview 3.3, ESRI, California, USA
3. Bajani M D, Ashford D A, Bragg S L, Woods C W, Aye T, Spiegel R A, Plikaytis B D, Perkins B A, Phelan M, Levett P N, Weyant R S 2003 Evaluation of four commercially available rapid serologic tests for diagnosis of leptospirosis. *Journal of Clinical Microbiology* 41: 803–809.
4. Boqist S, Chau B L, Gunnarsoson A, Olsson Engvall E, Vagsholm I, Magnusson U 2002 Animal- and herd-level risk factors for leptospiral seropositivity among sows in the Mekong delta, Vietnam. *Preventive Veterinary Medicine* 53: 233–245
5. Cameron A R, Baldock F C 1998 Two-stage sampling in surveys to substantiate freedom from disease. *Preventive Veterinary Medicine* 34: 19–30
6. Chappel R J, Prime R W, Millar B D, Mead L J, Jones R T, Adler B 1992 Comparison of diagnostic procedures for porcine leptospirosis. *Veterinary Microbiology* 30: 151–163
7. Cisneros-Puebla M A, Moles-Cervantes L P, Gavaldon-Rosas D 2002 Serologica diagnostica de leptospirosis porcina en Mexico 1995-2000. *Revista Cubana de Medicina Tropical* 54: 28–31
8. De Lange J F, Gummow B, Turner G V, Redman A R 1987 The isolation of *Leptospira interrogans* serovar *pomona* and related serological findings associated with a mixed farming unit in the Transvaal. *Onderstepoort Journal of Veterinary Research* 54: 119–121
9. Ellis W A 1986 Leptospirosis. *Journal of Small Animal Practice* 27: 683–691
10. Ellis W A 1989 *Leptospira australis* infection in pigs. *Pig Veterinary Journal* 22: 83–92
11. Ellis W A, O'Brien J J, Cassells J A, Montgomery J 1983 Leptospiral infection in horses in northern Ireland: serological and microbiological findings. *Equine Veterinary Journal* 15: 317–320
12. Feresu S B 1987 Serological survey of leptospiral antibodies in cattle in Zimbabwe. *Tropical Animal Health and Production* 19: 209–214
13. Hathaway S C, Little T W, Pritchard D G 1986 Problems associated with the serological diagnosis of *Leptospira interrogans* serovar *hardjo* infection in bovine populations. *Veterinary Record* 119: 84–86
14. Herr S, Riley A E, Neser J A, Roux D, De Lange J F 1982 *Leptospira interrogans* serovar *pomona* associated with abortion in cattle: isolation methods and laboratory animal histopathology. *Onderstepoort Journal of Veterinary Research* 49: 57–62
15. Hodges R T, Day A M 1987 Bovine leptospirosis: the effects of vaccination on serological responses as determined by complement fixation and microscopic agglutination tests. *New Zealand Veterinary Journal* 35: 61–64
16. Hunter P, Flamand J R, Myburgh J, van der Merwe S M 1988 Serological reactions to *Leptospira* species in game animals of northern Natal. *Onderstepoort Journal of Veterinary Research* 55: 191–192
17. Kaasschieter G A, de Jong R, Schiere J B, Zwart D 1992 Towards a sustainable livestock production in developing countries and the importance of animal health strategy therein. *Veterinary Quarterly* 14: 66–75
18. Leal-Castellanos C B, Garcia-Suarez R, Gonzalez-Figueroa E, Fuentes-Allen J L, Escobedo-de la Penal J 2003 Risk factors and the prevalence of leptospirosis infection in a rural community of Chiapas, Mexico. *Epidemiology and Infection* 131: 1149–1156
19. Levett P N 2003 Usefulness of serologic analysis as a predictor of the infecting serovar in patients with severe leptospirosis. *Clinical Infectious Diseases* 36: 447–452
20. Excel 97, Microsoft Corporation, USA
21. Moles-Cervantes L P, Cisneros-Puebla M A, Gavaldon-Rosas D 2002 Estudio serologico de leptospirosis bovina en Mexico. *Revista Cubana de Medicina Tropical* 54: 24–27
22. Myburgh J G, Otto Q T 1990 Serological survey for bovine leptospirosis in the Volksrust district. *Journal of the South African Veterinary Association* 61: 172–173
23. Myburgh J G, Otto Q T 1990 Serological survey for bovine leptospirosis in the Volksrust district. *Journal of the South African Veterinary Association* 61: 172–173
24. Norval R A I , Horak I G 2004 Vectors: ticks. In Coetzer J A W, Tustin R C (eds) *Infectious diseases of livestock* Vol. 1 . Oxford University Press Cape Town: 3–42
25. O'Keefe J S 2002 A brief review on the laboratory diagnosis of leptospirosis. *New Zealand Veterinary Journal* 50: 9–13
26. Okazaki W, Ringen L M 1957 Some effects of various environmental conditions on the survival of *Leptospira pomona*. *American Journal of Veterinary Research* 18: 219–223
27. Potts A D, Lotter C, Robinson J T 1995 Serological prevalence of leptospiral antibodies in pigs in South Africa. *Onderstepoort Journal of Veterinary Research* 62: 281–284
28. Rogan W J, Gladen B 1978 Estimating prevalence from the results of a screening test. *American Journal of Epidemiology* 107: 71–76
29. Stringfellow D A, Brown R R, Hanson L E, Schnurrenberger P R, Johnson J 1983 Can antibody responses in cattle vaccinated with a multivalent leptospiral bacterin interfere with serologic diagnosis of disease? *Journal of the American Veterinary Medical Association* 182: 165–167
30. Te Brugge L A, Dreyer T 1985 *Leptospira interrogans* serovar *hardjo* associated with bovine abortion in South Africa. *Onderstepoort Journal of Veterinary Research* 52: 51–52
31. Thrusfield M 2005 Surveys. In Thrusfield M (ed.) *Veterinary epidemiology.* Blackwell, Oxford: 228–242
32. Van der Broek A H M, Thrusfield M V, Dobbie G R, Ellis W A 1991 A serological and bacteriological survey of leptospiral infections in dogs in Edinburgh and Glasgow. *Journal of Small Animal Practice* 32: 118–124