PREVALENCE OF ANTIBODIES TO LEPTOSPIRAL SEROVARS IN FARM RUMINANT ANIMALS IN THE MARKHAM VALLEY, PAPUA NEW GUINEA

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ABSTRACT

A study on the prevalence of antibodies to leptospiral serovars in adult farm animals was carried out in vaccinated (n = 47) and non-vaccinated (n = 47) cattle, and non-vaccinated sheep (n = 21) and goats (n = 39) in the Markham valley of Papua New Guinea (PNG) during the period between June and July 2006. Sera were separated from all the randomly selected animals and subjected to the Microscopic Agglutination Test (MAT) against a reference panel of 22 live leptospira serovars. A titer of (³1:400) was considered as positive and accordingly 12.76% vaccinated as well as 17.02% non-vaccinated cattle had positive titer to leptospira infection. This indicates leptospirosis is prevalent in PNG and vaccination has limited role in immune response. This study also confirms hardjo, tarassovi and topaz as the predominantly occurring leptospiral serovars in ruminant food animal populations with topaz as a serovar never previously recorded in PNG. The seroresults of the tested sheep and goats showed none had positive titer (³ 1: 400) to leptospirosis. This indicates small ruminants currently may not be important host of leptospirosis in PNG. It may be concluded from the results of this study that leptospirosis is an important disease in ruminant farm animal herds in the Markham valley of PNG which needs attention for further study on it's zoonotic aspects and control in human and animal populations.

Keywords: *Ruminant, leptospirosis, serovars, Microscopic Agglutination Test (MAT)*

INTRODUCTION

Leptospirosis is a worldwide bacterial zoonotic disease, caused by Spirochetes of the genus *Leptospira* that affects humans and a wide range of animals, including mammals, birds and reptiles. All the pathogenic leptospiras were formerly classified as members of the species *Leptospira interrogans*, however the genus has recently being recognized and pathogenic leptospiras are now identified in several species of leptospira (Yasuda *et al.* 1987; Ramadass *et al.* 1992). Internationally there are more than 200 distinct leptospiral serovars recognized within the seven species of pathogenic leptospira (Marshall and Manktelow, 2002) and these are arranged in 23 sero-groups (Veloso *et al.* 2000). Serovars are identified based on antigens on the surface of the organism (Bolin 2003).

A recent study in Trukai farm has confirmed the occurrence of 15 leptospiral serovars and established that hardjo as the dominant serovar maintained in beef herds in Papua New Guinea (PNG) (Wai'in *et al.* 2003). Sub-optimal fertility is an ongoing problem in beef herds and the role of leptospirosis in sub-fertility is not clear in PNG. Although Trukai uses a bivalent vaccine regularly containing the serovars hardjo and pomona as antigens, the problems often persist, which might be due to either leptospirosis or other factors. Diagnosis of leptospirosis can be broadly divided into those that detect bacteria, their antigens or genomic material and those that detect antibody of the infecting bacteria. A variety of serological techniques are available for diagnosis of leptospirosis like Microscopic agglutination test (MAT), Enzyme linked immunosorbent assay (ELISA) and the fluorescent antibody test (FA). Among the DNA based assays the polymerase chain reaction (PCR) test is used to detect leptospiral DNA from tissues or body fluids in clinical cases. In general the PCR test has specificity and reliability but can not determine the infecting serovar and the process can be exquisitely sensitive to contamination with exogenous leptospiral DNA and therefore maybe prone to false-positive reactions. The MAT therefore; remains the only serological assay

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widely accepted as capable of producing serovar-specific results (Smith *et al.* 1994; Cumberland *et al.* 1999; O'Keefe 2002). This paper describes the sero status of leptospiral serovars in vaccinated and non-vaccinated beef cattle and non-vaccinated small ruminant farm animals in PNG.

MATERIALS AND METHODS

Cattle (cows) destined for the slaughter house from Trukai farm (vaccinated against leptospirosis with serovar hardjo and pomona) and Markham farm (non-vaccinated) were randomly selected for this study. Forty seven cows from each farm were selected for blood collection. The sheep (n=21) and goats (n=39) were also randomly selected from the Department of Agriculture and Livestock demonstration flock at Erap for this study. All the selected small ruminant animals were adults and sex was not considered. Blood samples of at least 10mls in sheep and goats were collected from the jugular vein with vacutainers and a similar volume of blood was collected from cows slaughtered at the abattoir. Sera were separated from blood clots by centrifugation as per conventional method and stored in sterile vials at –20 °C until shifted to the testing laboratory. The samples were then packed and sent to the WHO / FAO / OIE Leptospirosis Reference Laboratory in Brisbane for the microscopic agglutination test (MAT). Each serum sample was tested for antibodies to 22 live leptospira serovars as described by Stallman (1984) with some modifications. The serovars included: (1) Pomona, (2) Tarassovi, (3) Celledoni, (4) Australis, (5) Robinsoni, (6) Kremastos, (7) Medanensis, (8) Cynopteri, (9) Bataviae, (10) Javanica, (11) Shermani, (12) Hardjo, (13) Grippotyphosa, (14) Copenhageni,(15) Zanoni,(16) Canicola, (17) Szwajizak, (18) Bulgarica, (19) Aborea, (20) Djasiman, (21) Panama and (22) Topaz.

MODIFIED TEST PROCEDURE

Doubling dilutions of the sera from 1: 50 to 1: 6400 were prepared in 96 well microtiter plates using phosphate buffered saline (PBS) pH 7.4. An equal volume of live leptospira culture containing approximately 2-4 x 10^8 cells / ml was added to each dilution. After 90 minutes at 30 °C, the trays were examined by dark filled microscopy for agglutination of leptospira cells. The degree of agglutination was assessed in terms of the proportion of free leptospires. The reported titer was the reciprocal of the highest dilution of serum that agglutinated at least 50% of cells for each serovar used; as compared with the control culture diluted at 1: 2 PBS. A single titer of ^{3} 1: 400 was accepted as the cut off value for this study and was considered as positive. The results were analyzed in terms of percentage, and the difference in leptospira infection between vaccinated and non-vaccinated cattle and small ruminants was analyzed.

RESULTS AND DISCUSSION

The present study on the comparative leptospiral antibody prevalence in vaccinated and non-vaccinated cattle and small ruminants was carried out for the first time in PNG. Wai'in *et al.* (2003) reported the prevalence of leptospiral serovars in only non-vaccinated cattle from PNG. The overall prevalence of leptospirosis in ruminant farm animals is presented in Table 1.

It appears that there is no significant difference on the overall sero-prevalence of leptospira infection between Trukai and Markham farms. Of the cattle tested 25.53 % of cows from Trukai and 27.66% from Markham farm did not demonstrated any antibodies. There was however; a slightly high number of suspect (1:50-1:200) animals on Trukai

Table 1. **Seroprevalence of leptospirosis in adult farm ruminant animals**

MAT titer	Interpretation	Sero-results in different farms, No. (%)			
		Trukai farm	Markham farm	DAL Erap Farm	
		Cattle (n = 47)*	Cattle (n = 47)	**Sheep (n = 21)	**Goats (n = 39)**
< 1: 50	Negative	12 (25.53)	13 (27.66)	12 (57.14)	35 (89.74)
1:50-1:200	Suspect	29 (61.70)	26 (55.32)	09 (42.86)	04 (10.26)
? 1: 400	Positive	06 (12.76)	08 (17.02)	0	0

farm (61.70%) compared to Markham farm (55.53%). The elevated titers from Trukai may be the result of the vaccination program under taken in the farm and a valid analysis was not possible in this study. However, the Markham farm had a high number of positive (e"1:400) animals (17.02%) compared to Trukai farm (12.76%). In general high titers are suggestive of recent clinical disease and this work indicates Markham farm may have problems. Between the small ruminants, goats (89.74%) had negative titers for leptospira antibodies compared to sheep (57.14%). However, leptospira is widespread in sheep and goats with suspect antibody titers of (42.86%) and (10.26%) respectively. There is no evidence to suggest sheep and goats are reservoir hosts for hardjo and tarassovi, the main serovars responsible for clinical leptospirosis in cattle.

The results on the prevalence of antibodies to leptospiral serovars in vaccinated and non-vaccinated cattle is shown in Table 2. It appears that the PNG cattle had leptospira antibody titer at different levels against 12 serovars. However, the positive level of antibodies was only found against hardjo (8.51%), and tarassovi (2.13%) and topaz (2.13%) serovars respectively in vaccinated cattle, whereas non-vaccinated cattle had positive titer only against hardjo (14.89%) and tarassovi (2.13%). The dominance of hardjo, szwajizak and tarassovi in PNG cattle has been reported by Wai'in *et al.* (2003). Of the dominant serovars, L. interrogans topaz has not been screened before.

It is possible for cross reactions to occur among serovars with similar antigenic component at higher titers but this study was not able to determine cross reactivity. A positive result indicates exposure of animals in the herd to infection, but there are no published data available at present, which allow correlation of antibody level with the probability of active versus chronic infection. Nevertheless the results presented here confirm that exposure to infection is widespread in the unvaccinated cattle herds in PNG cattle. The Trukai cattle were vaccinated against hardjo and pomona serovars but the antibody detected only against hardjo, not against pomona serovar. This may be due to several factors including negligible immune response and the varying effect on the host with the inoculated antigen which occurs due to relatively low initial development of agglutinating antibody titers. The low immune response however does not mean the absence of the antibodies but the serological test employed merely could not detect the pomona antibodies. Although, a high cut off titer (e"1:400) was used for this study and Trukai vaccinated against leptospirosis, antibodies to prominently occurring serovars in the two cattle farms are essentially the same.

Table 2. **Prevalence of Leptospira serovars in vaccinated (n = 47) and non-vaccinated (n = 47) beef cattle detected by MAT**

Serovars	MAT titer with No. of cases												Total positives (≥ 1: 400) No. (%)	
	1: 50		1: 100		1: 200		1: 400		1: 800		1: 1600			
	V	NV	V	NV	V	NV	V	NV	V	NV	V	NV	V	NV
Hardjo	9	3	8	7	2	7	3	5	1	2	-	-	04 (8.51)	7 (14.89)
Szwajizak	9	13	6	10	5	-	-	-	-	-	-	-	0	0
Arborea	4	2	1	-	-	-	-	-	-	-	-	-	0	0
Topaz	7	8	8	4	3	-	-	1	-	-	-	-	1 (2.13)	0
Kremastos	1	6	5	3	-	-	-	-	-	-	-	-	0	0
Tarassovi	8	5	3	6	-	2	-	1	-	-	1	-	1 (2.13)	1 (2.13)
Medanensis	7	5	2	3	3	2	-	-	-	-	-	-	0	0
Pomona	4	-	-	2	-	-	-	-	-	-	-	-	0	0
Bataviae	2	1	1	-	-	-	-	-	-	-	-	-	0	0
Australis	-	1	1	-	-	-	-	-	-	-	-	-	0	0
Shermani	2	-	-	-	-	-	-	-	-	-	-	-	0	0
Panama	-	-	-	2	-	-	-	-	-	-	-	-	0	0
Total													6 (12.76)	8 (17.02)

V = Vaccinated with serovars hardjo and pomona, NV = Non-vaccinated, - = Negative

The prevalence of antibodies to leptospiral serovars in small ruminant animal species is presented in Table 3. Although none of the sheep and goats showed positive level of antibody titer (≥1: 400) against all tested serovars, the suspected level of antibodies (1: 50 - 1: 200) was detected against arborea and topaz serovars. The result shows that three (14.29%) and six (28.57%) sheep had antibodies to arborea and topaz, respectively, whereas only topaz serovar was recorded in four (10.26%) goats (Table 3).

Although more works needs to be done topaz seemed to be a dominant serovar maintained in ruminant farm animals in PNG.

This result could not be compared due to lack of similar inland reports on leptospirosis in small ruminants from PNG. Elsewhere however, Batra *et al.* (1990); observed pomona as a dominant serovar in Haryana. Ciceroni *et al.* (2000) documented catellonis as the highest serovar in South Tyrol and in an earlier study in South–East Bolivia Ciceroni (1992) reported poi as the dominant serovar in sheep and goats. A recent review by Levett (2001) observed pomona and hardjo as dominant serovars in sheep.

This variation in dominance might be due to the varying epidemiological andmaintenance-host relationship; characteristic of the agro ecological systems in different parts of the world. Data on clinical leptospirosis in food animals and its significance on productivity is scarce in PNG. However, this work confirms earlier studies by Wai'in *et al.* (2003) that leptospirosis is prevalent in PNG beef cattle herds. There are 15 (Wai'in, *et al.* 2003) leptospira serovars known to be prevalent in cattle. In addition this work confirms L. interrogans topaz as a new serovar never previously recorded in PNG. The results also indicate small ruminants may not be important hosts of hardjo and tarassovi the main serovars responsible for clinical leptospirosis in PNG. There is a significantly high proportion of cattle with active infection in Markham farm compared to Trukai farm.

Further work is required to determine the source and extend of leptospira infection in animal and human populations and to determine the role of vaccine in the control of leptospirosis in experimental and field conditions in PNG.

ACKNOWLEDGEMENTS

The authors wish to acknowledge Dr. Nime Kapo and National Agriculture Quarantine and Inspection Authority's Veterinary field staff, in liaising with producers and collecting the samples. The generous assistance of producers in the three farms whose herds were included in the study is also acknowledged. Thanks are also extended to Professor Barry Norton of the ACIAR for providing fellowship to the first author for post-graduate studies in the Department of Agriculture, PNG University of Technology and also to Dr. Lee Smythe (Chief Scientist) of the WHO /FAO/OIE Leptospirosis Reference Laboratory for conducting the MAT test.

REFERENCES

Batra, H. V., Chandiramani, N. K., and Usha, M. V. (1990). Prevalence of leptospirosis in farm animals in Haryana. *Indian Journal of Animal Sciences*, 60: (7) 55 – 760.

Bolin, C. A., (2003). Diagnosis and control of bovine leptospirosis. Department of Pathobiology and Diagnostic Investigation, College of Veterinary Medicine, Michigan State University, Proceedings of the 6th Western Diary Management Conference, March 12-14 Reno USA

Table 3. **Leptospira serovar status in small ruminants**

Serovars (n = 22)	Animals species	No. of animals tested	No. of animals with antibody titer		
			1: 50	1: 100	Total , No. (%)
Arborea	Sheep	21	2	1	3 (14.29)
	Goats	39	0	0	0
Topaz	Sheep	21	5	1	6 (28.57)
	Goats	39	4	0	4 (10.26)

n= No. of Serovars tested

Ciceroni, L., Lombardo, D., Pinto, A., Ciarrocchi, S., and Simeoni, J. (2000). Prevalence of antibodies to leptospira serovars in sheep and goats in Alto Adige-South Tayrol. *Journal Veterinary Medicine* Series B (47) 3 217

Ciceroni, L., Bartoloni, H., Pinto, A., Gugliemetti, P., Valdez, V.C., Gamboa, B.H., Roselli, M., Giannico, F., and Paradisi, F. (1997). Serological survey of leptospiral infections in sheep, goats and dogs in Cordillera Province, Bolivia. *Journal of New Microbiology* (20) 1 77-81

Cumberland, P., Everard, C. O. R., and Levett, P. N. (1999). Assessment of the efficacy of IgM – ELISA and Microscopic Agglutination Test (MAT) in the diagnosis of acute leptospirosis. *American Journal of Tropical Medicine & Hygiene* 61 (5) 731 – 734.

Levett, P. N. (2001). Leptospirosis, *Clinical Microbiology Review,* 14: (2) 296-326

Marshall, R. B., and Manktelow, B. W. (2002). Fifty years of leptospira research in New Zealand – a perspective. *New Zealand Veterinary Journal,* 50: 61 – 63.

O'Keefe, J. S. (2002). A brief review on the Laboratory diagnosis of leptospirosis. *New Zealand Veterinary Journal,* 50: (1) 9 – 13.

Ramadass, P., Jarvis, B. D. W., Corner, R. J., Penny, D., and Marshall, R. B. (1992). Genetic characterization of pathogenic Leptospira spp. by DNA hybridization. International Journal of Systematic Bacteriology, 42: 215 – 219.

Smith, C. R., Ketterer, P. J., McGowan, M. R., and Corney, B. G. (1994). A review of laboratory techniques and their use in the diagnosis of leptospira interrogans serovar hardjo infection in cattle. Australian Veterinary Journal, 71: (9) 290 – 294.

Stallman, N. D. (1984). International Committee on Systematic Bacteriology. Sub-committee on the taxonomy of leptospira. International Journal of Bacteriology, 34: 258 – 259.

Veloso, I., Lopes, M.T.P., Salas, C. E., and Moreira, E. C. (2000). A comparison of three DNA extractive procedures with leptospira for polymerase chain reaction analysis. *Mem Inst Oswaldo Cruz, Rio de Janeiro,* 95 (3) 339-343.

Wai'in, P., Robertson, I., Reid, S., Feinwick, S., and Smythe, L. (2003). The Epidemiology of Leptospirosis in PNG. *Proceedings of the 2nd Australia/Asia/Oceania CVA Workshop & Meeting 28th October -2nd November, Lae International Hotel,* Lae PNG.

Yasuda, P.H., Steigerwalt, A. G., Sulzer, K. R., Kaaufman, A. F., Rogers, F., and Brenner, D. J. (1987). Deoxyribonucleic acid relatedness between serogroups and serovars in the family *Leptospiraceae* with proposals for seven new Leptospira species. *International Journal of Systematic Bacteriology,* 37: 407 – 415.