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Leptospirosis in water buffalo (*Bubalus bubalis*) in Trinidad

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ABSTRACT

The seroprevalence of leptospirosis in water buffalo (*Bubalus bubalis*) reared for meat in semi-intensive and extensive managed farms in Trinidad was determined. All sera were tested for specific antibodies against 17 internationally recognized serovars of *Leptospira* using the microscopic agglutination test (MAT). Animals with titres greater or equal to 100 were considered as seropositive indicating exposure to *Leptospira* and those with titres greater or equal to 800 were interpreted as cases of acute leptospirosis. Of a total of 226 apparently healthy water buffalo from five major farms in Trinidad tested, 33 (14.6%) were seropositive with titres ranging from 100 to 400. Three (60.0%) of 5 farms had seropositive animals with seropositivity rates ranging from 2.0% (1 of 50) on Farm A to 32.7% (16 of 49) on Farm B. The difference was statistically significant ($P < 0.05$; X^2). Age and sex of animals had no significant ($P > 0.05$; X^2) effect on infection rate. The prevalent antibodies to serovars of *Leptospira* were farm-specific with specific antibodies to serovars Copenhageni and Georgia being predominant on Farm B having been detected in 10 (62.5%) and 9 (56.3%) respectively of 16 seropositive animals. On Farm D however, also with 16 seropositive animals, specific antibodies to serovars Patoc and Bratislava were most frequently detected, found in 11 (68.8%) and 5 (31.3%) respectively of seropositive animals. This is the first documentation of leptospirosis in water buffalo in the Caribbean region and the health risk posed to farm workers, abattoir workers and veterinarians cannot be ignored.

Key words: leptospirosis, water buffalo, Trinidad

Introduction

Leptospirosis is a well documented zoonosis worldwide (SZYFRES, 1976; THIERMANN and FRANK, 1980; LEVETT, 2001; LEVETT, 2004) and it has been serologically demonstrated in livestock such as cattle, sheep and goats amongst others (BAHAMAN et

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al., 1987; BURRIEL et al., 2003). Clinical disease as well as chronic and asymptomatic infections occur in livestock (FERESU et al., 1999; OCHOA et al., 2000; BURRIEL et al., 2003; CERRI et al., 2003). In dogs the disease can be fatal, warranting routine vaccination using prevalent serovars in the various areas (BENGIS et al., 2004; BIRNBAUM et al., 1998; WARD et al., 2002).

Leptospira infections have been reported in water buffalo in Afghanistan (SEBEK et al., 1978), Italy (CICERONI et al., 1995) and in Malaysia (BAHAMAN et al., 1987). DURFEE and PRESIDENTE (1979) however failed to detect *Leptospira* infection in water buffalo studied in Australia. In Trinidad and Tobago and the Caribbean region, leptospirosis has been reported in dogs (EVERARD et al., 1989a), cattle (DAMUDE et al., 1979; EVERARD et al., 1985; GRANT et al., 1988; LEVETT et al., 1996), wildlife (DOWNS et al., 1962; EVERARD et al., 1976) and in farm workers, either healthy or febrile (EVERARD et al., 1989a; EVERARD et al., 1989b; EVERARD et al., 1982; EVERARD et al., 1990; EVERARD and EVERARD, 1993). Most recently, ADESIYUN et al. (2006) detected clinical leptospirosis in vaccinated dogs in Trinidad and Tobago and they suspected vaccine failure due to different serovars occurring locally. To date, the status of *Leptospira* in the water buffalo population in the Caribbean is unknown. The water buffalo (*Bubalus bubalis*) in Trinidad, with a majority of its population under extensive management system, attracted attention recently when a high seroprevalence of brucellosis was detected in two herds (FOSGATE et al., 2002; FOSGATE et al., 2003).

This study was therefore conducted to determine the prevalence of leptospirosis on selected water buffalo farms in Trinidad.

Materials and methods

Water buffalo farming in Trinidad. Water buffalo (*Bubalus bubalis*) were introduced into Trinidad in the 1900s by the East Indian indentured workers who used them to supply power for ploughing fields and transportation, mainly in sugar cane production. Over the years, through decades of selective breeding, the features of the original breed developed into one that is now locally referred to as ‘buffalypso’ (DIPTEE, 2004). With the mechanization of the sugar industry in the country and the new features, the water buffalo is mainly bred for meat. Originally, there were only three major herds. Two farms belonged to a government-owned company rearing the animals extensively on 1000 acres for each farm and the third was owned by the veterinarian involved in the early selective breeding of water buffalo in the country. Subsequently, two other relatively large farms came into existence rearing water buffalo primarily for meat. A sixth farm, a government experimental/research station, reared water buffalo along with dairy cattle, semi-intensively, on a closed farm. The farm serves as a source of water buffalo for farmers with small holdings, usually with less than 10 animals in their herds scattered across the country.

The brucellosis outbreak in the country was believed to have originated from the two government-owned water buffalo farms. Animals from these farms were sent for slaughter in an abattoir at another farm, where, upon investigation, the prevalence of brucellosis was found to exceed 40% (FOSGATE et al., 2002; DIPTEE, 2004). The investigation led the government to initiate a 'test and slaughter' hoping to regain the brucellosis-free status previously enjoyed. This policy resulted in a considerable reduction of the water buffalo population on the two government-owned farms.

Source of serum samples. Serum samples used in the present study were collected for the purpose of an investigation to compare the sensitivity and specificity of serological tests to detect brucellosis in water buffalo and cattle in Trinidad (FOSGATE et al., 2002; FOSGATE et al., 2003). Samples were randomly taken from sera collected from animals of farmers who agreed to participate in that study. All serum samples were stored at -40 °C before they were sent to the Regional Leptospirosis Reference Laboratory in Barbados for testing.

Detection of antibodies to Leptospira serovars. At the laboratory, all sera were stored at -20 °C until examined. Paired serum samples were not available for testing. The microscopic agglutination test (MAT) was performed by standard method (FAINES, 1982; ANONYMOUS, 2003) using a battery of 17 serovars which included reference serovars and local isolates. The serogroups, serovars and strains used in the study were as follows; 1-Bratislava (Jez-Bratislava); 1-Australis (Ballico); 2-Autumnalis (Akiyami A); 2-Bim (1051); 3-Ballum (S102); 4-Bataviae (Van Tienen); 5-Canicola (Ruebush); 9-Grippotyphosa (Andaman/Moskva V.); 11-Copenhagen (16441); 11-Mankarso (Mankarso); 11-Icterohemorrhagiae (RGA); 16-Georgia (LT 117); 18-Pomona (Pomona); 19-Pyrogenes (Salinem); 22-Wolffi (3705); 24-Tarassovi (Perpelitsin) and 28-Patoc (Patoc 1). Briefly, the method used included successive qualitative (screening to determine the response to serogroups) and quantitative (to determine the serum titre) parts. The cultures for the 17 *Leptospira* serovars were kindly supplied by the *Leptospira* Reference Laboratory in Barbados. Cultures were grown in Ellinhausen-McCallough-Johnson Modified Harris Medium (EMJH) and diluted 1:2 using phosphate buffered saline (PBS) to obtain a density of $1.0-2.0 \times 10^8$ leptospira per mL. The serum samples (50 microlitres) diluted 1:25 in saline were added to 50 microlitres of antigens in flat-bottom 96-well microtitre plates (Nunc™, Nalge Nunc International, Rochester, New York, U.S.A.). The plates were incubated at 30 °C for 2 h, after which sterile Pasteur pipettes were used to remove portions of the contents which were placed on microscopic slides. The slides were thereafter examined under a dark-field microscope ($\times 10$ magnification). Any sample where at least 50% of the leptospiraes agglutinated was considered positive for specific antibodies to that serovar. All positive sera were then diluted (1:10 to 1:10240) and the MAT repeated to determine the titres. A single titre of 100 or greater was considered

seropositive and indicative of exposure to leptospirosis and a single titre of 800 or greater in the MAT was indicative of acute leptospirosis.

Statistical analysis. The prevalence of leptospirosis and the serovars were compared across farms and management systems using the chi-square and Fisher's Exact tests. All statistical analyses were two-sided and interpreted at the 0.05 level of significance.

Results

Of a total of 226 apparently healthy water buffalo tested from 5 major farms across the country, 33 (14.6%) were seropositive for specific antibodies to the serovars *Leptospira* tested (Table 1). On 3 (60.0%) of 5 farms seropositive animals were detected; and on these farms, seropositivity rates varied from 2.0% (1 of 50) in Farm A to 32.7% (16 of 49) in Farm B. The difference was statistically significant ($P < 0.05$; X^2). The seropositivity rate in animals from semi-intensively managed farms was 14.3% (17 of 119) which was not statistically significantly ($P > 0.05$; X^2) and from those animals from farms managed extensively, 15.0% (16 of 107).

The sex and age of the animals did not significantly ($P > 0.05$; X^2) affect the seropositivity rate. Across farms, the seropositivity rate amongst female animals was 21.2% compared with 15.4% found amongst male animals. In adult animals (>2 years old) antibodies to *Leptospira* were detected in 23.0%, while 12.2% of young animals tested were seropositive.

Table 1. Prevalence of *Leptospira* antibodies in water buffalo (*Bubalus bubalis*) by farm

Farm identification	Management system	N ^o of animals tested	N ^o (%) positive ^a for <i>Leptospira</i>
A	Semi-intensive ^b	50	1 (2.0)
B	Semi-intensive ^c	49	16 (32.7)
C	Semi-intensive ^c	20	0 (0.0)
D	Extensive ^d	69	16 (23.2)
E	Extensive ^d	38	0 (0.0)
Total	---	226	33 (14.6)

^aA titre of 100 or higher was considered as evidence of infection; ^bA government-owned research /experimental farm rearing dairy cows and water buffalo; ^cSemi- intensively managed farm, animals reared primarily for meat; ^dTwo large extensively large farms located at different locations belonging to a government-owned company.

Table 2. Frequency of detected serovars of *Leptospira*

Farm identification	N° of <i>Leptospira</i> seropositive animals	Serovar of <i>Leptospira</i> (N° of animals)
A	1	Autumnalis/Tarassovi (1)
B	16	Copenhageni (5) Georgia (4) Mankarso (1) Copenhageni/Georgia (3) Bratislava/Georgia /Copenhageni (1) Bratislava/ Georgia (1) Bratislava/Icterohemorrhagiae/Copenhageni/Georgia (1)
C	16	Patoc (7) Bratislava (3) Icterohemorrhagiae (1) Patoc/Copenhageni/Mankarso (1) Bratislava/Patoc (1) Wolffi/Patoc (1) Patoc/Autumnalis/Icterohemorrhagiae (1) Patoc/Bratislava/ Copenhageni/ Autumnalis/Mankarso (1)

The predominant seropositivity to serovars of *Leptospira* in seropositive animals on Farm B were to Copenhageni and Georgia, each having been detected in 10 (62.5%) of the 16 seropositive animals (Table 2). Of the 17 serovars investigated, specific antibodies were detected to only 5 (29.4%) serovars on this farm. On Farm D however, the most prevalent antibodies detected were against serovars Patoc and Bratislava, with 12 (75.0%) and 5 (31.3%) respectively from the 16 seropositive animals. On this farm antibodies to 8 (47.1%) of 17 serovars tested were detected. The only seropositive animal on Farm A had antibodies to serovars Autumnalis and Tarrasovi.

Table 3 shows the serovars of *Leptospira* in infected animals. Seropositivity in animals to a single serovar was common on Farms B and D. On Farm B, 5 (31.3%), 4 (25.0%) and 1 (6.3%) animals had antibodies to Copenhageni, Georgia and Mankarso respectively, while on Farm D, of the 16 seropositive animals, 7 (43.8%) and 3 (18.8%) were only positive for antibodies to serovar Patoc and Bratislava respectively. The predominant titre in seropositive animals was 100, although 1 animal had a titre of 400 with Icterohemorrhagiae. Animals seropositive for more than one serovar were detected on all three farms.

Table 3. Titres of detected serovars of *Leptospira* in water buffalo

Farm identification	Types of infection	Serovar detected	Titres ^a			
			50	100	200	400
A ^b	Multiple ^c	Autumnalis	-	1	-	-
		Tarassovi	-	1	-	-
B ^d	Single ^e	Georgia	-	4	-	-
		Mankarso	-	1	-	-
		Copenhageni	-	5	-	-
	Multiple	Copenhageni /Georgia	1	4	-	-
		Bratislava/Copenhageni/ Georgia	1	2	-	-
		Bratislava/Georgia Bratislava/ Icterohaemorrhagiae/Copenhageni/Georgia	1	1	-	-
D ^f	Single	Patoc	-	6	1	-
		Bratislava	-	3	-	-
		Icterohemorrhagiae	-	-	-	1
	Multiple	Patoc/Copenhageni/Mankarso	-	3	-	-
		Bratislava /Patoc	-	2	-	-
		Wolffi/Patoc Patoc/Autumnalis/ Icterohemorrhagiae Patoc/ Bratislava/ Copenhageni/Autumnalis/ Mankarso	-	1	1	-
		-	-	2	1	-
		-	-	3	1	1

^aNone of the seropositive animals was considered to have acute leptospirosis; ^bOne animal was seropositive; ^cAntibodies to more than one serovar of *Leptospira* detected; ^dSixteen animals were seropositive; ^eAntibodies to only one serovar were detected; ^fSixteen animals were seropositive.

Discussion

This is, to our knowledge, the first seroprevalence report on leptospirosis in water buffalo (*Bubalus bubalis*) in the Caribbean region. However, the prevalence of 14.6% found in the present study is lower than that reported in water buffalo from other areas, ranging between 31% to 55% (SEBEK et al., 1978; BAHAMAN et al., 1987; CICERONI et al., 1995). In addition, water buffalo in Australia tested negative for the presence of antibodies to *Leptospira* (DURFEE and PRESIDENTE, 1979). It is however important to remember that the seroprevalence of leptospirosis is affected by the number of serovars used in the testing and the test procedures. Earlier reports in Trinidad and Tobago have reported

Leptospira infection in livestock, wildlife and humans (EVERARD et al., 1976; EVERARD et al., 1985; EVERARD et al., 1989a; EVERARD et al., 1990).

It was a surprise that the seropositivity rate for leptospirosis was not significantly different between semi-intensively and extensively managed farms, since in animal husbandry, closeness of animals and herd size have been reported to facilitate the spread of *Leptospira* in herds (BAHAMAN et al., 1987). It is possible that the fact that animals in extensively managed farms were essentially on a range with increased exposure to wildlife which have been documented as important sources of infection for livestock and dogs (FOSGATE et al., 2002; BENGIS et al., 2004).

Different serovars of *Leptospira* appear to be widespread in the water buffalo population since specific antibodies were detected on the three farms with seropositive animals reacting to as many as 8 (47.1%) of the 17 recognized serovars assayed for. To date in Trinidad, serovars Georgia, Wolffi and Patoc, to which specific antibodies were detected in water buffalo in the current study, have not been earlier documented from any other sources in the country (EVERARD et al., 1982; EVERARD et al., 1985; EVERARD et al., 1989a, 1989b; EVERARD et al., 1990). The potential for these serovars to infect other livestock, dogs, wildlife and humans in the country can therefore not be ignored.

The detection of farm-specific predominance of antibodies to some serovars of *Leptospira* was of epidemiological significance. Antibodies to serovar Copenhageni were detected in as many as 62.5% of seropositive animals on Farm B, while antibodies to serovar Patoc were most frequently detected on farm D, where 75.0% of the seropositive animals were infected by the serovar. This is an indication that these serovars are established on the respective farms, infecting animals more readily than other serovars. It is however difficult to assess the significance of detecting a predominance of antibodies to serovars Copenhageni and Patoc in our current study because there is a worldwide dearth of information on *Leptospira* infection in water buffalo, particularly the important serovars. BAHAMAN et al. (1987) reported that the most prevalent serovar detected in water buffalo infected with *Leptospira* in Malaysia was Sejroe and to a lesser extent Tarassovi and Pomona. In our study however, only one animal was infected by serovar Tarassovi, while all the animals tested were negative for serovar Pomona. The various serovars of *Leptospira* detected from several sources in the country have been variable. It was reported that the prevalent serovars infecting livestock in Trinidad were Icterohemorrhagiae, Autumnalis, Hebdomadis and Panama (EVERARD et al., 1985). Amongst school children studied, the predominant serovar was Autumnalis (EVERARD et al., 1989b) and serovar Bavatae was reported to be most common in apparently healthy individuals living in urban and rural communities (EVERARD et al., 1990). In acute human leptospirosis cases associated with piggery workers in the country, the predominant serovars were Icterohemorrhagiae, Canicola, Pyrogenes and Grippotyphosa (EVERARD et al., 1989a)

while in patients in hospitals and clinics across the country, EVERARD et al. (1982) found serovars Icterohaemorrhagiae, Hebdomadis and Autumnalis to be most frequently encountered. The possibility therefore exists that the serovars of *Leptospira* detected in apparently healthy water buffalo in the country could also infect humans exposed to these infected animals. It is however pertinent to mention that since most publications on the serovars of *Leptospira* infections in humans and animal sources in Trinidad and the Caribbean region at large, were conducted over a decade ago, the possibility exists of changing patterns of serovar involvement in animal and human infections in the country.

It was concluded that infection by *Leptospira* in the water buffalo population in Trinidad occurs primarily subclinically but still poses a health risk to humans exposed to them on the various farms.

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SAŽETAK

Određivana je serološka prevalencija leptospiroze u indijskog bivola (*Bubalus bubalis*) uzgajanoga za meso u poluintenzivnoj i ekstenzivnoj proizvodnji na Trinidadu. Uzorci seruma bili su pretraženi mikroskopskom aglutinacijom na specifična protutijela za 17 međunarodno važnih serovarova leptospira. Životinje s titrom $\geq 1:100$ smatrane su serološki pozitivnima što znači da su bile izložene infekciji leptospirama, dok su one s titrom $\geq 1:800$ smatrane akutno zaražene leptospirama. Ustanovljeno je da su 33 (14,6%) bivola bila serološki pozitivna u titru od 1:100 do 1:400 od ukupno 226 pretraženih klinički zdravih indijskih bivola podrijetlom s pet velikih farmi na Trinidadu. Serološki pozitivne životinje ustanovljene su na tri od pet pretraženih farmi sa stopom serološke pozitivnosti od 2% (1 od 50) na farmi A, do 32,7% (16 od 49) na farmi B. Razlika je bila statistički značajna ($P < 0,05$; X^2). Dob i spol nisu imali nikakva utjecaja na stopu zaraženosti ($P > 0,05$; X^2). Nalaz protutijela za određeni serovar leptospira bilo je specifičan za farmu. Tako su protutijela za serovarove Kopenhagen i Georgia pretežito dokazana na farmi B, s tim da su za serovar Kopenhagen bila dokazana u 10 (62,5%), a za serovar Georgia u 9 (56,3%) od 16 serološki pozitivnih životinja. Međutim, na farmi D gdje je također ustanovljeno 16 serološki pozitivnih životinja, najčešće su dokazana protutijela za serovarove Patoc i Bratislava. Protutijela za serovar Patoc dokazana su u 11 (68,8%), a za serovar Bratislava u pet (31,3%) bivola. Ovo je prvi dokaz leptospiroze u indijskih bivola na Karibima što predstavlja rizik za infekciju u farmskih i klaoničkih radnika te veterinaru.

Ključne riječi: leptospiroza, indijski bivol, Trinidad
