VETERINARSKI ARHIV 79 (1), 87-96, 2009

Seroprevalence of leptospiral serovars other than Canicola and Icterohaemorrhagiae in dogs in the Southwestern Nigeria

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OKEWOLE, E. A., M. O. AYOOLA: Seroprevalence of leptospiral serovars other than Canicola and Icterohaemorrhagiae in dogs in the Southwestern Nigeria. Vet. arhiv 79, 87-96, 2009.

ABSTRACT

Serum antibody titres against the prevailing serovars of *Leptospira interrogans* were evaluated in 52 dogs that were clinically and epidemiological screened for leptospirosis by the microscopic agglutination test in three ecologically distinct veterinary clinics in the Southwestern Nigeria. New non-vaccinal serovars of grippotyphosa, pomona and bratislava were of higher ($P<0.01$) prevalence than the old vaccinal serovars of canicola and icterohaemorrhagiae due to growing pet-wildlife contact in urbanization that exposes the pets to these former animal serovars. Grippotyphosa was again singularly higher ($P<0.02$) in prevalence in the large adult male dogs among the local and exotic breeds tested in the coastal urban clinic than in the arid suburban clinic with an over-representation of the German Shepherd breed (46.2%). Aetiological serovars were diagnosed as grippotyphosa in both the rain forest belt clinic A and the arid savannah belt clinic C and mixed grippotyphosa, bratislava and pomona in the urban coastal clinic B. Heavy coastal rains that promote spirochetes survival in dirty flood waters, the contaminating vaccination-induced "carrier" state that exposes dogs to new and more pathogenic serovars, use of abattoir-offal as dog foods and the fast urbanization in city clinics that permits more dog-wildlife contacts, which also promotes contact with more pathogenic wild serovars, were responsible for the change in epidemiology. Use of new polyvalent subunit vaccines against the new serovars with an improved protocol, good kennel hygiene, including watering and feeding and environmental sanitation, including wildlife control, are recommended for control.

Key words: leptospira, serovars, titres, grippotyphosa, serum, antibody

Introduction

Leptospirosis is a zoonotic disease caused by antigenically distinct serovars of *Leptospira interrogans*. As recent as the 1980's, *L.* Icterohaemorrhagiae and *L.* Canicola were identified as the most prevalence serovars causing leptospirosis in the

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ISSN 0372-5480
Printed in Croatia

canine species worldwide (GREENE and SHOTTS, 1990). Infections with these serovars typically cause a hepatonephric syndrome (GREENE and SHOTTS, 1990), characterized by acute haemorrhagic diathesis, subacute icterus or subacute uremia in the dog (ADIN and COWGILL, 2000). Worldwide use of different brands of commercial bivalent vaccines against these two serovars has led to decreased incidence of leptospirosis in dogs (RENTKO et al., 1992). In the last ten years, however, veterinarians have become aware that a number of newly identified serovars can cause clinical disease in dogs. (RENTKO et al., 1992; HARKIN and GARTRELL, 1996; BROWN et al., 1996).

In Nigeria a total of 32, 45 and 54 valuable adult dogs (including referrals) died of a severe clinical syndrome pathologically attributable to acute renal failure associated with leptospirosis in 2001, 2002 and 2003 respectively at the Small Animal Clinic of the Veterinary Teaching Hospital of this University.

Clinical manifestations in these cases were more of renal than hepatic involvement before death. Yet, the available bivalent commercial vaccines are not only serovars-specific in their protection but also protects against clinical diseases only (RENTKO et al., 1992).

Given the zoonotic risks constituted by the persistence of the causal bacteria in both wide and domestic animals and its ability to change host-specificity and virulence in response to selective pressures in the environment (GREENE and SHOTTS, 1990), the changing trend becomes more worrisome from both zoonotic and animal health viewpoints.

This sero-epidemiological survey was undertaken to identify the current prevailing serovars of *Leptospira interrogans* in dogs in Southwestern Nigeria with a view to formulating appropriate control measures against the zoonosis.

Materials and methods

Study place and time. This survey was done at the three most patronized and strategically located veterinary clinics in the Southwestern Nigeria viz. clinic A-the Small Animal clinic of the Veterinary Teaching Hospital of the University of Ibadan, Ibadan Nigeria, clinic B-the Metropolitan Veterinary clinic, Ikoyi, Lagos. Nigeria and clinic C-the Petworld Veterinary clinic, Ilorin, Kwara State of Nigeria. It lasted from May 2005 to July 2006 (15 months).

Study design. A prospective serological survey for the antibodies against the prevailing serovars of leptospires of dogs in the Southwestern Nigeria. Case records of dogs who came regularly to these clinics were reviewed for signalments and other information relating to housing, feeding, watering and vaccination records. Questionnaires were administered to clients presenting dogs and questions included signalment: the number of dogs owned, type of household, type of food and water, regular or occasional walking

distances on-lead or freely, opportunity to scavenge, possible wildlife around kennels, ectoparasitism, recent change in food or water source, change in residence etc.

Inclusion criteria. (a) Dogs of all breeds and of both sexes that were over 8 weeks of age, (b) those with or without leptospirosis vaccination history, (c) those that had not changed their households or residences in the last one year, (d) those presenting all or at least three of the following symptoms, fever/hypothermia, muscular weakness, haematochezia, jaundice, melena, epistaxis, dehydration, extensive petecheation, coughing, dyspnoea, oliguria, polyuria or anuria, (e) those with the microscopic agglutination test (MAT) titres of over 1:100 against the vaccinal serovars (BADWIN and ATKINS, 1987) and a four-fold rise 10-14 days later or a single serum titre of between 1:800 to 1:3,200 to the non-vaccinal serovars (BIRNNAUM et al., 1998).

Serum sample collection. Blood samples were aseptically collected from all selected dogs into sterile plain tubes. These were allowed to clot at room temperature, rim-clotted and centrifuged at 650 gm for 10 minutes. Serum was pipetted into separate tubes for preservation at -20 °C until tested. When practically feasible, a second blood sample was collected from the same dog 10-14 days later, to provide for comparison.

Serology. Sera were screened initially at 1:100 dilution before the positives were retested by serial two-fold dilutions to their maximum dilutions against serovars: pomona, grippotyphosa, canicola, icterohaemorrhagiae and bratislava, using the commercial antigens: (4-day old leptospire cultured in Stuart (EMJH) medium-Difco laboratories, Detroit, Michigan) and the improved microtechnique for the leptospiral MAT as modified by COLE et al. (1973). The test was done on plastic trays for dilution and incubation, and was subsequently transferred to a glass slide for reading of agglutination on a long-working-distance 10 × objective and 10 × ocular - dark-field condenser microscope (Leitz-E, Inc, Rockleigh, N.J).

Urinalysis. Urine samples were taken by cytocentesis from all selected dogs. Urine specific gravity, total protein glucose, bilirubin etc were determined by the use of a hand-held refractometer (Goldberg refractometer, American optical Corp., Chicago, IL.), while the microscopic sediment examination was done on a dark field microscope that uses fluorescent antibody stains (Difco laboratories, Inc. Detroit, Mich).

Routine treatments. These consisted of initial ampicillin or amoxicillin or enroflaxacin oral or parenteral treatments, rehydration therapy with normal saline or balanced electrolyte solutions, Diuretics such as mannitol, furosemide or dopamine were administered as dictated by the degree of azotemia. Gastric protectants like cimetidine and famotidine were given to some ureamic dogs especially at the clinic B in Lagos.

Statistical analysis. Descriptive statistics were mainly used, but decay of vaccinal immunity and aetiological serovars were compared for the clinics by the Student's *t*-test for significance at 5% level.

Results

Study sample and epidemiology data. A total of 52 dogs satisfied the inclusion criteria in the three clinics viz. 17 (12 males, 5 females) of age range 12-36 months (mean 22 months) in the VTH Clinic, Ibadan (A), 27 (19 males and 8 females) of age range 10-30 months (mean 19 months) in the Metropolitan Clinic in Lagos (B) and 8 (6 males, 2 females) of age range 12-25 months (mean 17 months) in the Petworld Clinic C. Breeds included German Shepherds, Rottweilers, Poodles, Doberman pinschers, Chow-chow, Collies, Scottish terriers, Dalmatians, Dachshunds, Rhodesian ridgebacks, local breeds and their crosses, with an over-representation of the German Shepherd breed, 24 (46.2%), Rottweilers 14 (26.9%) and their crosses with the local breeds, 9 (17.0%). Clinic A in Ibadan falls within the rain forest belt with fairly high annual rainfall, clinic B in Lagos falls with the heavy-rainfall coastal swamp forest belt, while clinic C in Ilorin falls within the arid savannah belt with relatively low annual rainfall. There were more outdoor males 37 (71.2%) than females, 15 (28.8%). Fifty-one (98.0%) of the 52 dogs were primarily vaccinated and boostered variously with commercial bivalent vaccines including Progand®-Intervet, Vanguard®-Pfizer, Galaxy®-Schering-Plough etc containing serovars canicola and icterohaemorrhagiae. Thirty-seven (71.2%) of the 52 were from multiple-dog households (with dogs ranging from 2 to 8) while 15 (28.8%) were from single-dog households. Forty-two (80.8%) were on home-made foods dominated by abattoir offals; 4 (7.7%) were on commercial dog foods and only 6 (11.5%) regularly scavenged for food. The 46 (88.5%) on home-made foods and commercial dog foods were also on treated pipe-borne waters, with only 6 (11.5%) scavengers on the untreated urine-contaminated gutter waters sometimes containing abattoir effluents. Only 22 (42.3%) of the 52 had opportunities for regular/occasional walking distances freely or on leads, and they were all in-door exotic breeds viz. German Shepherds 11, Rottweilers 9 and Scottish terriers 2. Twenty-four respondents (68.6%) agreed that *Rhipicephalus sanguineus* ticks were abundantly present in their kennels; while 40 (76.9%) of the 52 had recently changed their residences (kennels) to newly acquired areas that had more wildlife access e.g hedgehogs, brown rats, jackals, bobcats, field mice, foxes etc.

Clinical manifestations. The commonest clinical signs in the selected dogs, especially from the urban clinic B, included sudden illness (18), anorexia (42), vomiting (30), depression (40), subnormal rectal temperature ($<37 \pm 1.2$) (32), shock (22), dehydration (45), oliguria (41), anuria (12), renomegaly of the palpable left kidney (37) and uremia (18) resulting from severe azotemia.

Urinalysis. The urine specific gravity was between 1.004 to 1.010 (mean, 1.008) in 47 (90.4%) of the 52 dogs, with 37 of these 47 being isosthenuric, with specific gravity range of 1.004 to 1.008. Only 5 (9.6%) of the 52 retained their urine-concentrating ability with values ranging from 1.022 to 1.024.

Table 1. Serum antibody profile of a selected group of dogs with leptospirosis

Sampling Clinic and vegetational cover	Seropositive					Aetiologic Serovar
	Nº/sex	%	Reacting Serovar	Nº	Mean MAT Titres	
Clinic A.	17	32.7	Grippotyphosa	11	1:12,800	Grippotyphosa
V.T.H Clinic	M = 12		Bratislava	7	1:3,600	
Ibadan	F = 5		Pomona	6	1:3,800	
			Canicola (V)	5	1:1,000	
Urban Rain forest			Icterohaemorrhagiae (V)	6	1:800	
Clinic B.	27	52.0	Grippotyphosa	22	1:6,400	(Mixed)
Metropolitan	M = 19		Bratislava	9	1:8,800	Grippotyphosa
Clinics, Lagos	F = 8		Pomona	14	1:6,600	Bratislava
			Canicola (V)	6	1:5,600*	Pomona
Urban Swamp forest			Icterohaemorrhagiae (V)	8	1:4,800*	
Clinic C.	8	15.4	Grippotyphosa	6	1:6,400	Grippotyphosa
Pet-world clinic,	M = 6		Bratislava	4	1:3,400	
Ilorin, Nigeria	F = 2		Pomona	3	1:4,200	
			Canicola (V)	4	1:600	
(Arid Savannah)			Icterohaemorrhagiae (V)	3	1:200	
Total	52	100				

V = vaccinal serovars; * effect of booster vaccination

Analysis of urine by dipstick method revealed, +++ occult blood in 32 (61.5%) of 52, + bilirubin in 27 (51.9%) and a trace of protein in only 6 (11.5%). Urinary sediments included granular casts (27), erythrocytes (31) and bacterial cocci and rods in 15 (28.8%) of the 52 dogs.

Serology. Single serum samples were obtained in 14 (26.9%) and paired samples from 38 of the 52 dogs. Serum antibody titres were highest to Grippotyphosa in 11 (64.7%) of 17 dogs in clinic A, mixed serovars of Grippotyphosa, Bratislava and Pomona in the 27 (52.0%) dogs from clinic B and grippotyphosa in the 8 (15.4%) dogs in clinic C. (Table 1). Lower titres to serovars bratislava and pomona in clinics A and C were interpreted as cross-reactivity between serovars (RENTKO et al., 1992; ADIN and COWGILL, 2000) while the lower titres to serovars canicola and icterohaemorrhagiae reflected waning vaccinal immunity at different booster vaccination levels, being significantly higher ($P<0.01$) in the clinic B dogs with two boosters (Table 2).

Table 2. Summary of the rate of decay of serum antibodies against vaccinal serovars Canicola and Icterohaemorrhagiae in dogs

Clinic	Reacting serovars	N°/ serovar/ clinic	Mean age in months	N° and % with N° primary vaccination	N° and % with only primary vaccination	N° and % with only primary vaccination and 1 booster	N° and % with primary vaccination and 2 boosters	Mean titre P-value A vs B	Mean titre P-value B vs C	Mean titre P-value A vs C
A Urban and rain forest clinic	Canicola	5	27 ± 3	0	4 (80%)	1 (20%)	0	<0.02	-	<0.03
	Icterohaemorrhagiae	6	21 ± 2	1 (16.7%)	5 (83.3%)	0	0	<0.01	-	<0.02
B Urban and coastal clinic	Canicola	6	18 ± 3	0	2 (33.3%)	0	4 (66.7%)	<0.02	>0.05	-
	Icterohaemorrhagiae	8	20 ± 2	0	1 (12.5%)	1 (12.5%)	6 (75.0%)	<0.01	<0.01	-
C Suburban and arid clinic	Canicola	4	19 ± 3	0	3 (75.0%)	1 (25.0%	0	-	>0.05	<0.03
	Icterohaemorrhagiae	3	18 ± 4	0	3 (100%)	0	0	-	<0.01	<0.02

Treatment outcome. Three dogs (1 from clinic A and 2 from B) died on day 3 of treatment. Necropsy revealed ulcerative haemorrhagic gastritis with intestines filled by haemorrhagic fluid in two. Additionally, thick tan foci were seen in the renal cortices in the third dog. Large multifocal coalescing foci of plasmacytic inflammation were seen in the renal interstitium and also at the cortico-medullary junctions. A few tubules contained necrotic debris and neutrophils. Van orden silver staining (VAN ORDEN and GREENE, 1977) revealed globular debris and intact spirochetes within tubules in the 3 dogs.

Discussion

The preponderance of the large breed males in the positive samples was probably because they are likely to spend more time outside and are therefore exposed to leptospiral organisms in the environments. This result is consistent with the earlier ones by RENTKO et al. (1992) and WARD et al. (2002). Similarly, there seemed to be a positive correlation between the annual rainfall (as reflected by the vegetation type) and the number of cases seen in these clinics, being highest in the coastal clinic B and least in clinic C with the lowest annual rainfall (savannah vegetation). This is also in agreement with earlier report of ADIN and COWGILL (2000) and HARTMANN and GREENE (2005). Also the higher prevalence in the urban clinic B supports the earlier description of leptospirosis as an urban disease (ADIN and COWGILL, 2000; BARR, 2002) and also implies the possibility of a rising prevalence with growing urbanization that promotes dog-wildlife contacts.

The results of this survey are convincing enough that leptospirosis is still endemic in Southwestern Nigeria and that the aetiological serovars had changed from the previously predominant serovars of canicola and icterohaemorrhagiae (for which there were several bivalent commercial vaccines) to new serovars of grippotyphosa, pomona and bratislava. Although hepatic disease has been associated with the older serovars canicola and icterohaemorrhagiae infections in dogs (RENTKO et al., 1992), renal involvement with attendant azotemia, including uremia, appeared to be a major component of the newer serovars even here in Nigeria as BROWN et al., (1996) observed in the USA. Similar trends have also been reported from The Netherlands (HARTMAN, 1984), USA (THIERMANN, 1980; NIELSEN et al., 1991; ADIN and COWGILL, 2000) and Canada (PRESCOTT et al., 1991), with the change accompanied by change in the clinical syndrome from previous hepatic insufficiency and coagulation abnormalities to those relating to acute or subacute renal failure with attendant azotemia, including uremia. In the current survey, clinical, epidemiological, urinalytic data, as well as the histopathological lesions in the dead dogs, were consistent with renal rather than hepatic involvement, especially in clinic B, to further align the similarities with those reported from other parts of the world. To the best of our knowledge, this is the first report of these serovars in Nigeria.

Two factors were identified as being responsible for the changing trend. Firstly, the previous preventive vaccinations with the different brands of commercial bivalent vaccines containing serovars canicola and icterohaemorrhagiae are serovar-specific in protection

and do not protect against newer emerging serovars like grippotyphosa bratislava and ponoma that are abundant in the neighbouring wildlife.

Moreover, these vaccinations only prevent clinical diseases, with an increasing number of "carrier" dogs, that shed contagion to other dogs that are not vaccinated or in which vaccination for these old serovars has been discontinued. This situation has produced an increasing number of susceptible dogs to the new and more pathogenic serovars; like those from wildlife that are unadapted to the canine species. Secondly, the geographical extension of the urban (Lagos) and suburban (Ibadan) clinical areas into previously rural forest areas might have increased exposure of susceptible pets to wildlife that was already infected with leptospiral serovars not adapted to dogs and which are therefore highly pathogenic. For example BROWN et al. (1996) identified field voles (*Microtus lagrestis),* wood mice (*Apodemus sylvaticus*) and bank voles (*Evotomys glareolus)* as the reservoir hosts for serovar Grippotyphosa, while FRANTZ et al., (1988) associated serova bratislava with swine reproductive failures including abortions. MICHNA (1970) also associated serova pomona with renal infections of pigs with attendant urine-contaminating tendencies. Given the highest prevalence in clinic B and the high popularity of abattoir-offal dominated home-made foods among these indoor dogs, the chances of infection by these animal serovars through ingestion is very high. According to MICHNA (1970), kidneys of carrier animals and meat from animals slaughtered during leptospiraemia and occasionally from diseased or convalescent cattle play an important role in the epidemiology of leptospirosis (BARR, 2002). The scavenging outdoor dogs in clinic C were likely infected through their sharing of urine and faecal-contaminated pond waters, especially during the long dry season, characterized by water scarcity in the arid area.

While the results of this survey in no way suggest that serovars icterohaemorrhagiae and canicola are no longer prevalent in the Southwestern Nigeria, it has presented a host range of prevailing serovars against which vaccinal protection should be directed. Epidemiologically, current leptospiral vaccination with the commercial bivalent vaccines (Canicola and Icterohaemorrhagiae) is bedeviled with two problems: it has a shorter duration of immunity (about 6 months, GREENE, 1998) than the viral antigens in-combination with it and also has a high risk of post-vaccinal hypersensitivity. Yet, it is only commercially available in this combination with the distemper antigen in particular that has recently been recommended for fewer vaccination protocols (OLSON et al., 1997). Should this be widely adopted, a growing subpopulation of unprotected dogs and wildlife to all serovars would emerge in both urban and suburban cities.

The most effective control measure, therefore, would be to eliminate the "carrier state". Unfortunately, wild animal reservoirs and the subclinically infected animals, including vaccinated dogs, continue to harbor and shed organisms. Also, control of rodents, rats and mice in kennels, as well as the maintenance of environmental conditions that exclude the survival of leptospiral organisms, are all part of the effective control methods.

Furthermore, prompt isolation of infected dogs, good kennel hygiene that stresses clean food and water, and prompt doxycycline treatment of the isolated cases further enhance control. In view of the emerging new serovars, a polyvalent commercial envelope vaccine like Duramune® (Fort-Dodge, USA) or the pentavalent-outer envelope vaccine (BEY and JOHNSON, 1978) that includes the new serovars might be more protective than the old bivalent types.

Acknowledgements

The technical assistance and full cooperation of Drs, L. A. H. Cole, T. Afolayan and T. Laba of Ibadan, Lagos and Ilorin Clinics respectively are thankfully acknowledged. The technical assistance of Dr. L. K. Harrison of the Bacteriology Diagnostic laboratory of the Obafemi Awolowo University, Ile-ife, Nigeria is also acknowledged.

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Received: 30 May 2007
Accepted: 21 December 2008

OKEWOLE, E. A., M. O. AYOOLA: Serološko istraživanje leptospiroze u pasa u Nigeriji. Vet. arhiv 79, 87-96, 2009.

SAŽETAK

Titar serumskih protutijela za najčešće serovarove vrste *Leptospira interrogans* određivan je mikroskopskom aglutinacijom u uzorcima seruma 52 psa s triju ekološki različitih područja jugozapadne Nigerije. Prevalencija je bila veća ($P<0,01$) za novodokazane serovarove Grippotyphosa, Pomona i Bratislava protiv kojih psi nisu bili cijepljeni, nego za serovarove Canicola i Icterohaemorrhagiae koji se rabe za proizvodnju cjepiva. To se događa zbog sve češćeg dodira između kućnih ljubimaca i divljih životinja pa su ljubimci izloženiji serovarovima iz divljih životinja. Veća prevalencija ($P<0,02$) dokazana je za serovar Grippotyphosa u populaciji odraslih mužjaka velikih pasmina u klinikama priobalnih gradova nego u klinikama sušnih prigradskih naselja s najvećom učestalošću u pasmine njemački ovčar (46,2%). Serovar Grippotyphosa bio je dokazan i u vlažnom šumskog pojasu A i u suhom savanskom pojasu C dok su mješovite zaraze serovarovima Grippotyphosa, Bratislava i Pomona bile dokazane u klinikama priobalnih gradskih područja. Promjeni epizootiološkog stanja leptospiroze doprinijele su jake priobalne kiše koje pogoduju preživljavanju leptospira u površinskim vodama, kliconoštvo nakon vakcinacije što omogućuje izloženost pasa novim virulentnijim serovarovima, upotreba klaoničkih otpadaka za pasju hranu te brza urbanizacija koja doprinosi prijenosu bolesti s divljih životinja na pse. Uporaba novih polivalentnih podjediničnih cjepiva protiv novih serovarova na tom području s poboljšanim protokolom cijepljenja, dobra higijena štenara, uključujući pranje, hranidbu i sanitarne mjere te kontrola kretanja divljači preporučuju se kao kontrolne mjere za suzbijanje leptospiroze.

Ključne riječi: leptospire, serovarovi, protutijela, titar, serum, Grippotyphosa