O. O. Oni [1]
A. A. Adesiyun [1]
J. O. Adekeye [2]
S. N. A. Sai'du [3]

Sero-prevalence of agglutinins to *Listeria monocytogenes* in Nigerian domestic animals

ONI (O. O.), ADESIYUN (A. A.), ADEKEYE (J. O.), SAI'DU (S. N. A.). Séro-prévalence des agglutinines à *Listeria monocytogenes* chez les animaux domestiques au Nigeria. *Revue Élev. Méd. vét. Pays trop.*, 1989, **42** (3) : 383-388.

Une enquête, utilisant le test d'agglutination en tube, a été conduite pour déterminer la prévalence de *Listeria monocytogenes* sérotypes 1/2a, 1/2b, 1/2c, 3a et 4b ; 1 190 échantillons de sérum de 8 espèces animales, provenant de diverses localités des États de Kano et Kaduna, Nigeria, ont été testés. Après l'absorption avec l'antigène *Staphylococcus aureus* pour éviter les réactions croisées d'agglutinines, 52 (68,4 p. 100) des sérums de chevaux étaient positifs. Ving-six (36,1 p. 100) des sérums de porcs, 52 (20,8 p. 100) de ceux des bovins, 50 (20,0 p. 100) de ceux des chèvres, 20 (20,0 p. 100) de ceux des chiens étaient aussi positifs. Les poulets élevés en liberté révélaient une prévalence des anticorps de 18 (32,1 p. 100), alors que ceux élevés en intensif n'en avaient que 3 (6,8 p. 100), une différence trouvée statistiquement significative ($P \leq 0,01$; X^2). Les sérums ovins récoltés à l'abattoir de Zaria avaient une prévalence de 30 (14,7 p. 100) alors que ceux de l'Hôpital vétérinaire de l'Université d'Ahmadu Bello avaient une prévalence de 6 (13,0 p. 100). La prévalence chez les dromadaires était de 4 (4,3 p. 100). Au total, sur les 1 190 échantillons de sérums testés, 26 (21,9 p. 100) étaient séro-positifs pour les agglutinines de *L. monocytogenes*. Chaque espèce animale testée pour *L. monocytogenes* était positive pour les 5 sérotypes, à l'exception des dromadaires, négatifs pour le sérotype 3a. Quarante-quatre (53,0 p. 100) échantillons étaient positifs à un titre ≥ 480 pour le sérotype 1/2a, 60 (58,3 p. 100) pour 1/2b, 57 (52,3 p. 100) pour 1/2c, 7 (13,7 p. 100) pour 3a et 23 (39,0 p. 100) pour 4b. Il en a été conclu que la listériose à *L. monocytogenes* est largement répandue chez les animaux domestiques au Nigeria. *Mots clés* : Animal domestique - Listériose - *Listeria monocytogenes* - Agglutinine - Sérologie - Nigeria.

INTRODUCTION

Since the first isolation of *Listeria monocytogenes* in 1926 (20), it has been isolated from a variety of sources (4, 10, 11, 24) and lately, it is being isolated with increasing frequency (7, 19). As an ubiquitous microbe, *L. monocytogenes* is often carried by both humans and animals without clinical signs until immune-compromising obvious diseases set in (15, 17). Isolations of the organism in humans have been reported in Nigeria (8, 12, 22, 23) ; however, no published report on isolation of *L. monocytogenes* in animals exist in Nigeria, although isolations have been reported in animals in other African countries (3, 16).

The serological study carried out on human patients residing in Lagos remains the only serological work on listeriosis in Nigeria, (21), however, in other African countries, different rates have been reported from serological studies carried out on prevalence of *L. monocytogenes* agglutinins in animals (2, 13). Animals (especially sick ones and carriers) have been speculated as possible sources of listeriosis (9, 26).

This study was therefore carried out to determine the prevalence of agglutinins to *L. monocytogenes* in animals with a view to ascertain the possible reservoir role of domestic animals for listeriosis in Nigeria.

MATERIALS AND METHODS

Study area

A total of 1,190 blood samples were collected from Kano and Kaduna states of Nigeria. The source of samples and species distribution are shown in table I.

Sources of type cultures

Listeria monocytogenes serotypes 1/2a (F9486), 1/2b (F9475), 1/2c (F9293), 3a (F8828) and 4b (F9499) were kindly supplied by Dr. WEAVERS of Centre for Disease Control, Georgia, USA and *Staphylococcus aureus* (F265) an enterotoxigenic strain (type A) isolated from food was kindly supplied by Prof. S. R. TATINI of University of Minessota, USA.

Sample collection

Clean rubber-stoppered glass test tubes were used in all cases to obtain blood samples prior to separation of serum.

1. Department of Veterinary Public Health and Preventive Medicine, Ahmadu Bello University, Samaru. Zaria. Kaduna State, Nigeria.

2. Department of Veterinary Pathology and Microbiology. Ahmadu Bello University, Samaru, Zaria, Kaduna State, Nigeria.

3. Agricultural Extension, Research and Liaison Services. Ahmadu Bello University, Samaru, Zaria, Kaduna State, Nigeria.

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O.O. Oni, A.A. Adesiyun, J.O. Adekeye, S.N.A. Sai'Du

TABLE I Prevalence of **Listeria monocytogenes** *agglutinins in several animal species from various sources.*

Specie	Source	Number tested	Type of sera	
			Unabsorbed	Absorbed
			No. (per cent) positive	No. (per cent) positive
Horse	Kaduna ranch	76	60 (78.9)	52 (68.4)
Pig	Kaduna abattoir	72	35 (48.6)	26 (36.1)
Cattle	Zaria abattoir	250	115 (46.0)	52 (20.8)
Goat	Zaria abattoir	250	95 (38.0)	50 (20.0)
Dog	Clinic	100	28 (28.0)	20 (20.0)
Chicken (a)	Zaria abattoir*	56	33 (58.9)	18 (32.1)
(b)	Backyard poultry	44	10 (22.7)	3 (6.8)
Sheep (a)	Zaria abattoir	204	65 (31.9)	30 (14.7)
(b)	Clinic	46	19 (41.3)	6 (13.0)
Camel	Kano abattoir	92	15 (5.4)	4 (4.3)
Total		1,190	475 (39.9)	261 (21.9)

* Free range management system.

For all samples obtained from the abattoir, the blood was collected from the animals, at point of slaughter into the test tubes. In case of samples collected from stable and clinics (live animals), sterile hypodermic needles were used to collect the samples by venopuncture. All the samples were transported to the laboratory without delay. Harvested sera were stored at -20 °C until needed.

Preparation of *Listeria* O-antigens

The procedure described by SEELIGER (25) was used to prepare *Listeria* O-antigen to all the serotypes used. The final O-antigen cell suspensions were then prepared in 0.5 p. 100 formal saline (0.5 p. 100 formaldehyde solution in 0.85 p. 100 sodium chloride) to approximate number 5 McFarland density.

Raising of positive and negative *Listeria* control antisera

Two adult New Zealand white rabbits were maintained for each serotype. The control antisera were raised using the procedure described by SEELIGER (25). The harvested sera were stored at -20 °C until needed.

Titration of *Listeria* control antisera

In order to determine the best titres at which to use the *Listeria* control antisera, titrations were carried out on each using the tube agglutination technique (25). Initial dilutions were carried out in microtitre plates (*) and final titrations done in glass test-tubes. Consequently, dilutions of 1/64, 1/64, 1/16, 1/32 and 1/32 were chosen for serotypes 1/2a, 1/2b, 1/2c, 3a and 4b respectively.

Screening of serum samples for *Listeria monocytogenes* antigens

The initial screening of all serum samples was carried out at a dilution ratio of 1:80 using the tube agglutination technique (25).

For all treatments, positive and negative controls were set up, using positive *Listeria* control antisera as well as negative *Listeria* control antisera and normal saline, respectively.

Absorption of serum samples

Somatic (O)-antigen to *Staphylococcus aureus* (F 265) was raised in a similar manner as *Listeria* O-antigen and diluted to number 7 McFarland density. This was then used to absorb each of the samples positive from the initial screening, of cross-reacting antigen, according to the procedure described by NJOKU-OBI and NJOKU-OBI (21).

(*) Cooke microtiter ™ system

Titration of absorped sera

Each absorped serum was subsequently titrated at dilutions of 1:60, 1:120, 1:240 and ⩾ 1:480, using the tube agglutination technique (25).

RESULTS

A total of 1,190 serum samples were tested for *Listeria monocytogenes* agglutinins. Table I shows the various sources and number of samples collected.

Absorption of serum samples with *Staphylococcus aureus* antigen resulted in decreased prevalence of agglutinins to all serotypes tested in all the animal species as shown in table II. After absorption, the following were the antibody prevalence obtained : horse 52 (68.4 p. 100), pig 26 (36.1 p. 100), cattle 52 (20.8 p. 100), goat 50 (20.0 p. 100), dog 20 (20.0 p. 100), chicken (a) from Zaria abattoir 18 (32.1 p. 100) and chicken (b) from backyard poultry houses 3 (6.8 p. 100). This difference was found to be statistically significant ($P \leq 0.01$; X^2), sheep (a) from Zaria abattoir 30 (14.7 p. 100) and sheep (b) from Large animal clinic, Veterinary Teaching Hospital, Ahmadu Bello University, Zaria 6 (13.0 p. 100) and from camel, 4 (4.3 p. 100).

Table II shows the agglutinin prevalence to the five *Listeria* serotypes listed. In horse, serotype 1/2b had the highest frequency of occurrence with 27 (35.5 p. 100). The serotypes with the highest frequency in the other animals were, pig 1/2b and 4b : (13.9 p. 100), cattle 1/2c : 34 (13.6 p. 100), goat 1/2c : 26 (10.4 p. 100), dog 1/2b : 16 (16.0 p. 100), chicken (a)

TABLE II Prevalence of agglutinins to five serotypes of **Listeria monocytogenes** *in various animal species.*

Specie	No. tested	Number (per cent) of samples positive to various serotypes				
		1/2a	1/2b	1/2c	3a	4b
Horse	76	24 (31.6)	27 (35.5)	16 (21.1)	20 (26.3)	6 (7.9)
Pig	72	8 (11.1)	10 (13.9)	3 (4.2)	8 (11.1)	10 (13.9)
Cattle	250	8 (3.2)	20 (8.0)	34 (13.6)	3 (1.2)	6 (2.4)
Goat	250	20 (8.0)	12 (4.8)	26 (10.4)	6 (2.4)	14 (5.6)
Dog	100	7 (7.0)	16 (16.0)	3 (3.0)	1 (1.0)	4 (4.0)
Chicken (a)	56	8 (14.3)	8 (14.3)	13 (23.2)	1 (1.9)	4 (7.1)
(b)	44	3 (6.8)	1 (2.3)	1 (2.3)	0 (0.0)	1 (2.3)
Sheep (a)	204	3 (1.5)	7 (3.4)	9 (4.4)	12 (5.9)	10 (4.9)
(b)	46	1 (2.2)	1 (2.2)	2 (4.3)	0 (0.0)	3 (6.5)
Camel	92	1 (1.1)	1 (1.1)	1 (1.1)	0 (0.0)	1 (1.1)
Total	1,190	83 (7.0)	93 (7.8)	108 (9.1)	51 (4.3)	59 (5.0)

Chicken : (a) = Abattoir (free range management) ; (b) = Backyard poultry : Sheep : (a) = Abattoir ; (b) = Clinic.

TABLE III Agglutinin titres of seropositive animals.

	Serotypes of *Listeria monocytogenes*																								
	1/2a					1/2b					1/2c					3a					4b				
Specie	No. *	60	120	240	⩾480	No. *	60	120	240	⩾480	No. *	60	120	240	⩾480	No. *	60	120	240	⩾480	No. *	60	120	240	⩾480
Horse	24	1	5	7	11	27	3	2	3	19	16	1	4	3	8	20	9	5	2	4	6	2	1	0	3
Pig	8	0	0	2	6	10	3	1	0	6	3	1	1	0	1	8	3	5	0	0	10	2	0	2	6
Cattle	8	0	1	2	5	20	2	1	0	17	34	6	1	6	21	3	2	1	0	0	6	1	1	1	3
Goat	20	3	3	2	12	12	7	2	1	2	26	6	3	3	14	6	0	2	2	2	14	5	3	1	5
Dog	7	2	1	0	4	16	1	3	3	9	3	1	1	0	1	1	1	0	0	0	4	2	2	0	0
Chicken :																									
(a)	8	2	4	1	1	8	4	1	0	3	13	0	1	4	8	1	1	0	0	0	4	0	2	1	1
(b)	3	1	1	0	1	1	0	0	1	0	1	0	0	0	1	0	0	0	0	0	1	1	0	0	0
Sheep																									
(a)	3	0	0	0	3	7	4	0	0	3	9	6	0	0	3	12	8	3	0	1	10	4	3	0	3
(b)	1	0	0	0	1	1	0	0	0	1	2	1	0	1	0	0	0	0	0	0	3	1	0	0	2
Camel	1	1	0	0	0	1	1	0	0	0	1	1	0	0	0	0	0	0	0	0	1	1	0	0	0
Total	83	10	15	14	44	103	25	10	8	60	108	23	11	17	57	51	24	16	4	7	59	19	12	5	23

Chicken : (a) = Abattoir (Free range management) . (b) = Backyard poultry . Sheep : (a) = Abattoir : (b) = Clinic : * = Number positive.

1/2c : 13 (23.2 p. 100), chicken (b) 1/2a : 3 (6.8 p. 100), sheep (a) 3a : 12 (5.9 p. 100), sheep (b) 4b : 3 (6.5 p. 100) and in case of camel, except for serotype 3a that had a prevalence of 0 (0.0 p. 100), all the others had 1 (1.1 p. 100).

The titres of agglutinins of seropositive sera are shown in table III. For the 5 serotypes used in the study, the following are the number (p. 100) positive for titres of $\geqslant$ 1:480 ; 1/2a 44 (53.0 p. 100), 1/2b 60 (58.3), 1/2c 57 (52.3), 3a 7 (13.7) and 4b 23 (39.0).

DISCUSSION

This study provides the first reported serological evidence of *Listeria monocytogenes* antibodies in animals in Nigeria. In the study, the total prevalence of *L. monocytogenes* antibodies in 8 domestic animal species from various sources before and after absorption of their sera with *Staphylococcus aureus* antigens was found to be 39.9 p. 100 and 21.9 p. 100 respectively. This finding agrees with that obtained from a similar study conducted on human patients in a hospital in Lagos area in 1965 where absorption of serum samples with *S. aureus* antigen was shown to result in reduction of observed *L. monocytogenes* antibody titres (21) and confirms the view held by SEELIGER (25) that in using tube agglutination test, there is the tendency to have pronounced agglutination between cross-reacting O-antigens of *S. aureus* and *L. monocytogenes* and therefore the need to absorb sera with some Gram-positive antigen before determining the levels of *Listeria* agglutinins in serum samples (15).

Different prevalence rates of *Listeria* agglutinins have been reported in various animal species (2, 13, 27). A prevalence of 3.2 p. 100 was obtained for serotype 1/2a in cattle, 8.0 p. 100 for 1/2b, 13.6 p. 100 for 1/2c, 1.2 p. 100 for 3a and 2.4 p. 100 for 4b ; these figures are lower than what was obtained from Kenya (12) and higher than that obtained in Senegal (2), but suggest the possibility of widespread infection by this organism in Nigerian cattle.

A prevalence of 27 p. 100 and 14.3 p. 100 was reported in horses and pigs respectively in Brazil (27), however, a higher prevalence of 68.4 p. 100 and 36.1 p. 100 respectively was detected in this study. The high prevalence of *Listeria* agglutinins observed thus indicates that Nigerian domestic animals might be reservoirs of the organism, since a variety of domestic and wild animals can serve as hosts for *L. monocytogenes* (5, 14).

Higher rates (32.1 p. 100) were obtained from free-ranging chickens in this survey than those kept in backyard poultry houses (6.8 p. 100), a difference found to be statistically significant ($P \leqslant 0.01$; X^2). A similar trend was observed in another study involving *Yersinia enterocolitica* in free-rangers and semi-intensively managed chickens (1). These findings may be explained, in part, by the fact that the free rangers have a higher exposure potential to various microbial agents than those kept under backyard (semi-intensive) system of management.

Serotype 1/2c was the most prevalent in this study (9.1 p. 100) as opposed to 7.0 p. 100, 7.8 p. 100 and 5.0 p. 100 for 1/2a, 1/2b and 4b respectively. This finding is not surprising as serological typing of large numbers of cultures indicated that type 1 was more prevalent in Europe and Africa while 4b is more prevalent in the United States (6, 18, 25). The fact that serotype 3a was found to be the least prevalent (4.3 p. 100) in the samples tested also agrees with earlier observations (15).

Suggestions as to which titres are significant status for infection, range from 1:25 to 1:800 (15), however, titres of 1:320 and 1:640 have been suggested as minimum titres indicative of recent and present infections respectively, in the absence of clinical symptoms simulating listeric infections (25). Based on these suggestions a total of 191 (16.1 p. 100) animals out of the total number of animals sampled in this study (representing titres of $\geqslant$ 480 for 5 serotypes) could be suffering from current infections, while 112 (9.4 p. 100) of them (representing titres of between 1:120 and 1:240) could become shedders of the organism with the superimposition of immunosuppressive diseases (15).

CONCLUSION

It cannot be over-emphasized therefore that with the detection of agglutinins at such high titres as obtained in this study, the risk of contracting listeriosis from animal sources in Nigeria is high as most of these animal species serve either as food or pet animals. There is therefore the need to consider listeriosis as a differential in the diagnosis of livestock diseases in this environment and also in the treatment of human ailments that mimmick listeriosis. This is due to the fact that rural people live in close proximity to their domestic animals and consume some of their products, such as milk that can be contaminated by *Listeria* organism, without further heat processing.

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ONI (O. O.), ADESIYUN (A. A.), ADEKEYE (J. O.), SAI'DU (S. N. A.). Sero-prevalence of agglutinins to *Listeria monocytogenes* in Nigerian domestic animals. *Revue Élev. Méd. vét. Pays trop.*, 1989, **42** (3) : 383-388.

A survey using tube agglutination test was conducted to determine the antibody prevalence to *Listeria monocytogenes* serotypes 1/2a, 1/2b, 1/2c, 3a and 4b in 1,190 serum samples of 8 animal species from various sources in Kano and Kaduna states of Nigeria. Following absorption with *Staphylococcus aureus* antigen to remove cross-reacting agglutinins, 52 (68.4 p. 100) of the horse samples were positive. Twenty-six (36.1 p. 100) pig, 52 (20.8 p. 100) cattle, 50 (20.0 p. 100) goat, 20 (20.0 p. 100) dog, serum samples were also positive. Free-ranging chickens had an antibody prevalence of 18 (32.1 p. 100) while those intensively managed had 3 (6.8 p. 100), a difference found to be statistically significant ($P \leq 0.01$; X^2). Sheep sera collected from Zaria abattoir had a prevalence of 30 (14.7 p. 100) while those from Ahmadu Bello University, Veterinary Teaching Hospital had 6 (13.0 p. 100) prevalence. The prevalence in camel was 4 (4.3 p. 100). Overall, of the 1,190 serum samples tested, 26 (21.9 p. 100) were sero-positive for *L. monocytogenes* agglutinins. Each specie of animal tested for *L. monocytogenes* was positive for all five serotypes, except camel which was negative for serotype 3a. Fourty-four (53.0 p. 100) samples were positive at a titre of ≥ 480 for serotypes 1/2a, 60 (58.3 p. 100) for 1/2b, 57 (52.3 p. 100) for 1/2c, 7 (13.7 p. 100) for 3a and 23 (39.0 p. 100) for 4b. It is concluded that *L. monocytogenes* infection is widespread in domestic animals in Nigeria. *Key words* : Domestic animal - Listeriosis - *Listeria monocytogenes* - Agglutinin - Nigeria.

ONI (O. O.), ADESIYUN (A. A.), ADEKEYE (J. O.), SAI'DU (S. N. A.). Sero-prevalencia de las aglutininas a *Listeria monocytogenes* en los animales domésticos en Nigeria. *Revue Élev. Méd. vét. Pays trop.*, 1989, **42** (3) : 383-388.

Se efectuó una encuesta, al utilizar la prueba de aglutinación en tubo, para determinar la prevalencia de *Listeria monocytogenes*, serotipos 1/2a, 1/2b, 1/2c, 3a y 4b, en 1 190 muestras de suero de 8 especies animales proviniendo de varias localidades de los estados de Kano y Kaduna, Nigeria. Después de la absorpción con el antigeno *Staphylococcus aureus* para evitar las reacciones cruzadas de aglutininas, 52 (68,4 p. 100) de los sueros de caballos fueron positivos. Veinte y seis (36,1 p. 100) de los sueros de cerdos, 52 (20,8 p. 100) de los de bovinos, 50 (20 p. 100) de los de cabras, 20 (20 p. 100) de los de perros fueron también positivos. Los pollos criados en libertad mostraron una prevalencia de los anticuerpos de 18 (32,1 p. 100) mientras que los mantenidos en cria intensiva no tuvieron más que 3 (6,8 p. 100), diferencia encontrada estadisticamente significativa ($P \leq 0,01$; X^2). Los sueros de ovinos recogidos en el matadero de Zaria tuvieron una prevalencia de 30 (14,7 p. 100) mientras que los de Veterinary Teaching Hospital, Ahmadu Bello University, tuvieron una prevalencia de 6 (13 p. 100). La en los dromedarios fueron de 4 (4,3 p. 100). Al total, de las 1 190 muestras de sueros probados, 26 (21,9 p. 100) fueron sero-positivos para las aglutininas de *L. monocytogenes*. Cada especie probada fué positiva para con todos los 5 serotipos, salvo los dromedarios, negativos para el serotipo 3a. Quarenta y cuatro (53 p. 100) muestras fueron positivas a un titulo ≥ 480 para el serotipo 1/2a, 60 (58,3 p. 100) para 1/2b, 57 (52,3 p. 100) para 1/2c, 7 (13,7 p. 100) para 3a y 23 (39 p. 100) para 4b. Se concluyó que la listeriosis a *L. monocytogenes* es muy generalizada en los animales domésticos en Nigeria. *Palabras claves* : Animal doméstico - Listeriosis - *Listeria monocytogenes* - Aglutinina - Serologia - Nigeria.

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