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S. N. A. Sai’du 3

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

**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 immuno-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 1.

**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* (type A) isolated from food was kindly supplied by Prof. S. R. TATINI of University of Minnesota, USA.

**Sample collection**

Clean rubber-stoppered glass test tubes were used in all cases to obtain blood samples prior to separation of serum.
TABLE 1  Prevalence of Listeria monocytogenes agglutinins in several animal species from various sources.

<table>
<thead>
<tr>
<th>Specie</th>
<th>Source</th>
<th>Number tested</th>
<th>Type of sera</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Unabsorbed</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>No. (per cent) positive</td>
</tr>
<tr>
<td>Horse</td>
<td>Kaduna ranch</td>
<td>76</td>
<td>60 (78.9)</td>
</tr>
<tr>
<td>Pig</td>
<td>Kaduna abattoir</td>
<td>72</td>
<td>35 (48.6)</td>
</tr>
<tr>
<td>Cattle</td>
<td>Zaria abattoir</td>
<td>250</td>
<td>115 (48.0)</td>
</tr>
<tr>
<td>Goat</td>
<td>Zaria abattoir</td>
<td>250</td>
<td>95 (38.0)</td>
</tr>
<tr>
<td>Dog</td>
<td>Clinic</td>
<td>100</td>
<td>28 (28.0)</td>
</tr>
<tr>
<td>Chicken</td>
<td>Zaria abattoir*</td>
<td>50</td>
<td>33 (66.0)</td>
</tr>
<tr>
<td></td>
<td>Backyard poultry</td>
<td>44</td>
<td>10 (22.7)</td>
</tr>
<tr>
<td>Sheep</td>
<td>Zaria abattoir</td>
<td>204</td>
<td>65 (31.9)</td>
</tr>
<tr>
<td></td>
<td>Clinic</td>
<td>46</td>
<td>19 (41.3)</td>
</tr>
<tr>
<td>Camel</td>
<td>Kano abattoir</td>
<td>92</td>
<td>15 (5.4)</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>1,190</td>
<td>475 (39.9)</td>
</tr>
</tbody>
</table>

* 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

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 absorbed sera

Each absorbed 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 (20.8 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 ≤ 0.01; χ²).
- 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) 108 (9.1).

<table>
<thead>
<tr>
<th>Specie</th>
<th>No. tested</th>
<th>1/2a</th>
<th>1/2b</th>
<th>1/2c</th>
<th>3a</th>
<th>4b</th>
</tr>
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<tbody>
<tr>
<td>Horse</td>
<td>76</td>
<td>24 (31.6)</td>
<td>27 (35.5)</td>
<td>16 (21.1)</td>
<td>20 (26.3)</td>
<td>6 (7.9)</td>
</tr>
<tr>
<td>Pig</td>
<td>72</td>
<td>8 (11.1)</td>
<td>10 (13.9)</td>
<td>3 (4.2)</td>
<td>8 (11.1)</td>
<td>10 (13.9)</td>
</tr>
<tr>
<td>Cattle</td>
<td>250</td>
<td>20 (8.0)</td>
<td>12 (4.8)</td>
<td>26 (10.4)</td>
<td>6 (2.4)</td>
<td>14 (5.6)</td>
</tr>
<tr>
<td>Goat</td>
<td>100</td>
<td>7 (7.0)</td>
<td>16 (16.0)</td>
<td>3 (3.0)</td>
<td>1 (1.0)</td>
<td>4 (4.0)</td>
</tr>
<tr>
<td>Dog</td>
<td>56</td>
<td>8 (14.3)</td>
<td>8 (14.3)</td>
<td>13 (23.2)</td>
<td>1 (1.9)</td>
<td>4 (7.1)</td>
</tr>
<tr>
<td>Chicken (a)</td>
<td>44</td>
<td>3 (6.8)</td>
<td>1 (2.3)</td>
<td>1 (2.3)</td>
<td>0 (0.0)</td>
<td>1 (2.3)</td>
</tr>
<tr>
<td>Sheep (a)</td>
<td>204</td>
<td>3 (1.5)</td>
<td>7 (3.4)</td>
<td>9 (4.4)</td>
<td>12 (5.9)</td>
<td>10 (4.9)</td>
</tr>
<tr>
<td>Camel</td>
<td>92</td>
<td>1 (1.1)</td>
<td>1 (1.1)</td>
<td>1 (1.1)</td>
<td>0 (0.0)</td>
<td>1 (1.1)</td>
</tr>
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</table>

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

<table>
<thead>
<tr>
<th>Specie</th>
<th>No. tested</th>
<th>1/2a</th>
<th>1/2b</th>
<th>1/2c</th>
<th>3a</th>
<th>4b</th>
</tr>
</thead>
<tbody>
<tr>
<td>Horse</td>
<td>24</td>
<td>5 (20.8)</td>
<td>3 (12.5)</td>
<td>2 (8.3)</td>
<td>1 (4.1)</td>
<td>6 (24.0)</td>
</tr>
<tr>
<td>Pig</td>
<td>8</td>
<td>5 (62.5)</td>
<td>3 (37.5)</td>
<td>3 (37.5)</td>
<td>1 (12.5)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Cattle</td>
<td>20</td>
<td>7 (35.0)</td>
<td>6 (30.0)</td>
<td>4 (20.0)</td>
<td>3 (15.0)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Goat</td>
<td>20</td>
<td>4 (20.0)</td>
<td>6 (30.0)</td>
<td>4 (20.0)</td>
<td>3 (15.0)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Dog</td>
<td>7</td>
<td>1 (14.3)</td>
<td>1 (14.3)</td>
<td>1 (14.3)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Chicken (a)</td>
<td>0</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>1 (100)</td>
</tr>
<tr>
<td>Sheep (a)</td>
<td>3</td>
<td>1 (33.3)</td>
<td>1 (33.3)</td>
<td>1 (33.3)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Camel</td>
<td>1</td>
<td>1 (100)</td>
<td>1 (100)</td>
<td>1 (100)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
</tr>
</tbody>
</table>

**Table III Agglutinin titres of seropositive animals.**

<table>
<thead>
<tr>
<th>Specie</th>
<th>No. tested</th>
<th>1/2a</th>
<th>1/2b</th>
<th>1/2c</th>
<th>3a</th>
<th>4b</th>
</tr>
</thead>
<tbody>
<tr>
<td>Horse</td>
<td>24</td>
<td>5 (20.8)</td>
<td>3 (12.5)</td>
<td>2 (8.3)</td>
<td>1 (4.1)</td>
<td>6 (24.0)</td>
</tr>
<tr>
<td>Pig</td>
<td>8</td>
<td>5 (62.5)</td>
<td>3 (37.5)</td>
<td>3 (37.5)</td>
<td>1 (12.5)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Cattle</td>
<td>20</td>
<td>7 (35.0)</td>
<td>6 (30.0)</td>
<td>4 (20.0)</td>
<td>3 (15.0)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Goat</td>
<td>20</td>
<td>4 (20.0)</td>
<td>6 (30.0)</td>
<td>4 (20.0)</td>
<td>3 (15.0)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Dog</td>
<td>7</td>
<td>1 (14.3)</td>
<td>1 (14.3)</td>
<td>1 (14.3)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Chicken (a)</td>
<td>0</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>1 (100)</td>
</tr>
<tr>
<td>Sheep (a)</td>
<td>3</td>
<td>1 (33.3)</td>
<td>1 (33.3)</td>
<td>1 (33.3)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Camel</td>
<td>1</td>
<td>1 (100)</td>
<td>1 (100)</td>
<td>1 (100)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
</tr>
</tbody>
</table>
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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 (0.5 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 $\geq 1 : 480$ : 1/2a 44 (53.0 p. 100), 1/2b 60 (58.3), 1/2c 5/ (52.3), 3a / (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 testing (9.4 p. 100) of them (representing titres of between I:20 and I:240) could become shedders of the organism with the superimposition of immunosuppressive diseases (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 ($P < 0.01 ; \chi^2$). 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 1:000 (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 $\geq 480$ 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.

**ACKNOWLEDGEMENT**

The technical assistance rendered by Mr. MOSES IBRAHIM is acknowledged. This project was funded by Ahmadu Bello University, Board of Research and Wellcome Nigeria fund.
A survey using tube agglutination test was conducted to determine the antibody prevalence to *Listeria monocytogenes* serotypes 1/2a, 1/2b, 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) pigs, 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 (36.1 p. 100) of the sera samples were also positive. *Staphylococcus aureus* was used to avoid the reactions caused by agglutinins, 52 (68.4 p. 100) of the sera of calves were positive. In the dairy cows were 50 (20.8 p. 100) of the sera of bovines, 20 (20 p. 100) of the sera of calves. Sheep sera collected from Zaria abattoir had a prevalence of 30 (14.7 p. 100) while those from Ahmadu Bello University, Veterinary Teaching Hospital, Ahmadu Bello University, had a prevalence of 32.1 p. 100) of the sera 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 positive. 

Se efectuó una encuesta, al utilizar la prueba de aglutinación en tubo, para determinar la prevalencia de *Listeria monocytogenes*, serotipos 1/2a, 1/2b, 3a y 4b, in 1,190 muestras de suero de 8 especies animales proviniendo de varias localidades de los estados de Kano y Kaduna, Nigeria. Después de la absorción con el antígeno *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, 20 (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 cría intensiva no tuvieron más que 3 (6,8 p. 100), diferencia encontrada estadísticamente significativa (P < 0,01 ; X²). 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 *Listeria monocytogenes*. Cada especie probada fue positiva para con todos los 5 serotipos, salvo los dromedarios, negativos para el serotipo 3a. Cuarenta y cuatro (53 p. 100) muestras fueron positivas a un título = 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 (29,9 p. 100) para 4b. Se concluyó que la listeriosis a *L. monocytogenes* es muy generalizada en los animales domésticos en Nigeria. 

Key words: Domestic animal - Listeriosis - *Listeria monocytogenes* - Agglutinin - Nigeria.


Se efectuó una encuesta, al utilizar la prueba de aglutinación en tubo, para determinar la prevalencia de *Listeria monocytogenes*, serotipos 1/2a, 1/2b, 3a y 4b, in 1,190 muestras de suero de 8 especies animales proviniendo de varias localidades de los estados de Kano y Kaduna, Nigeria. Después de la absorción con el antígeno *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, 20 (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 cría intensiva no tuvieron más que 3 (6,8 p. 100), diferencia encontrada estadísticamente significativa (P < 0,01 ; X²). 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 fue positiva para con todos los 5 serotipos, salvo los dromedarios, negativos para el serotipo 3a. Cuarenta y cuatro (53 p. 100) muestras fueron positivas a un título = 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 (29,9 p. 100) para 4b. Se concluyó que la listeriosis a *L. monocytogenes* es muy generalizada en los animales domésticos en Nigeria.


