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Colonial, biochemical and serological characteristics of *Yersinia* species isolated from animals in Nigeria

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RÉSUMÉ

AGBONLAHOR (D. E.), ADESIYUN (A. A.), KWAGA (J. K. P.), LOMBIN (L. H.). — Caractéristiques sérologiques, biochimiques et culturales de certaines espèces de *Yersinia* isolées sur des animaux au Nigeria. *Rev. Elev. Méd. vét. Pays trop.*, 1985, **38** (4) : 416-422.

Les auteurs ont étudié les caractéristiques culturales, biochimiques et sérologiques de 14 souches de *Yersinia,* isolées à partir de 2 029 prélèvements obtenus sur des bovins, des porcs, des moutons et des chèvres apparemment sains, au Nigeria.

Neuf souches de *Y. enterocolitica,* 4 de *Y. intermedia* et 1 de *Y. frederiksenii* ont été isolées de bovins et de porcs. Il n'y a eu aucun isolement sur moutons et sur chèvres.

Les 3 espèces de *Yersinia* rencontrées ont toutes révélé les mêmes caractéristiques de culture sur milieux au citrate désoxycholate, de MacConkey et *S.S.* Le sérotype prédominant isolé était le 0 : 12, 26, trouvé dans 5 (38 p. 100) des 13 souches typables de *Yersinia.*

Les 5 souches (0 : 12, 26) ont toutes été isolées sur bovins, et leur identification a été confirmée par la biochimie comme étant *Y. enterocolitica.*

Trois souches de *Y. enterocolitica* répondaient au sérotype 0 :8 ; 2 ont été isolées de bovins, 1 de porc. Deux souches de *Y. intermedia* répondaient au sérotype 0 :52, 53, tandis que les 3 dernières répondaient respectivement aux sérotypes 0:36, 0:16 et 0:3.

Le seul sérotype 0:3 isolé (*Y. enterocolitica*) a été classé comme biotype 4, tandis que les 8 autres appartenaient au biotype 1.

L'isolement du sérotype 0:8 de *Y. enterocolitica,* à partir de porcs et de bovins au Nigeria, revêt une importance significative, parce que ce sérotype n'avait encore jamais été signalé sur des animaux en dehors des Etats-Unis.

De plus, l'isolement des sérotypes 0:3 et 0:8 (dont le pouvoir pathogène est connu pour l'homme), à partir d'animaux destinés à la consommation humaine au Nigeria, semble indiquer que les bovins et les porcs pourraient jouer un certain rôle dans les infections à *Y. enterocolitica* chez l'homme, dans cet environnement.

Mots clés : Bovin - Porc - *Yersinia enterocolitica* - *Yersinia intermedia* - *Yersinia frederiksenii* - Nigeria.

SUMMARY

AGBONLAHOR (D. E.), ADESIYUN (A. A.), KWAGA (J. K. P.), LOMBIN (L. H.). — Colonial, biochemical and serological characteristics of *Yersinia* species isolated from animals in Nigeria. *Rev. Elev. Méd. vét. Pays trop.*, 1985, **38** (4) : 416-422.

The colonial, biochemical and serological characteristics of 14 strains of *Yersinia* isolated from 2,029 samples obtained from apparently healthy cattle, pigs, goats, and sheep in Nigeria were studied.

Nine strains of *Yersinia enterotocolitica,* 4 of *Yersinia intermedia,* and 1 of *Yersinia frederiksenii* were isolated from cattle and pigs. No isolations were made from sheep and goasts.

All 3 species of *Yersinia* encountered exhibited identical colonial characteristics on desoxycholate citrate, MacConkey and *Salmonella-Shigella* media.

The predominant serotype isolated was 0:12, 26 found in 5 (38.5 p. 100) of 13 typable strains of *Yersinia.* All 5 strains (0:12, 26) were isolated from cattle and were confirmed biochemically to be *Yersinia enterocolitica.*

Three strains of Y. enterocolitica were serotype 0:8, 2 isolated from cattle and 1 from a pig.

Two strains of *Y. intermedia* were serotype 0:52, 53 while 1 strain each was of serotype 0:36, 0:16 and 0:3.

The only *Y. enterocolitica,* serotype 0:3 isolated was classified as biotype 4 while the other 8 strains of *Y. enterotocolitica* were biotype 1.

The isolation of *Y. enterocolitica* serotype 0:8 from pigs and cattle in Nigeria is of significance because this serotype has not been reported in animals outside the United States of America. Furthermore, the isolation of serotypes 0:3 and 0:8, known as human pathogens, from food animals in Nigeria suggests that cattle and pigs may play some role in *Y. enterocolitica* infections in human beings in this environment.

Key words : Cattle - Pig - *Yersinia interocolitica* - *Yersinia intermedia* - *Yersinia frederiksenii* - Nigeria.

INTRODUCTION

Isolations of *Yersinia enterocolitica* have been reported from farm animals like pigs (28), cattle (14, 23), chickens (4), goats (20), sheep (21), and horses (23) in several countries, particularly in Europe and North America. Is is also believed that these animals may serve as reservoir of human infections by *Y. enterocolitica* (13, 15, 16). This is because the serotypes associated with human diseases have been isolated from animals in the same environment (12, 24).

For sometime, it was believed that *Y. enterocolitica* infections in humans and animals were restricted to temperate countries since the micro-organism has an affinity for low temperatures (25). Furthermore, most reports of isolations of any of the 4 recognised species of *Yersinia,* namely, *Y. enterocolitica sensu stricto, Y, intermedia, Y. fredericksenii,* and *Y. kristensenii* (6, 7, 9, 30) have come from temperate countries.

However, of recent, human infections by species of *Yersinia* have been reported in tropical and subtropical countries specifically, Nigeria and Bangladesh (2, 10). To date, information is not available on the prevalence and characteristics of *Yersinia* spp. infections in livestock in Nigeria.

This paper reports the colonial, biochemical and serological characteristics of strains of *Yersinia* spp. isolated from livestock in Nigeria.

MATERIALS AND METHOD

Source of animals

Dairy cows : a total of 46 dairy cows at 2 institutional and governmental farms were rectally swabbed fortnightly for a period of 3 months. A total of 276 samples were collected.

Slaughter cattle : caecal samples from 287 bulls slaughted at the National Animal Production Research Institute (NAPRI) abattoir were collected. In addition, 237 and 93 caecal contents of cattle from Zaria and Sabon Gari abattoirs, respectively, were taken. A total of 617 caecal contents from cattle were processed. Slaughter sheep and goats : caecal contents of 294 sheep and 66 goats slaughtered at the Zaria abattoir, 26 sheep and 132 goats slaughtered at Sabon Gari abattoir were sampled. Three hundred and twenty sheep caecal contents and 198 from goats were tested.

Live pigs : rectal and tongue swabs of each of 309 apparently healthy pigs kept at institutional and private (backyard) farms were studied. A total of 618 samples from pigs were tested.

Overall, 2,029 samples were processed for *Yersinia* isolation.

Sample collection

Rectal swabs were obtained from dairy cows and pigs by the use of sterile swabs inserted into the rectum of each animal beyond the anal sphincter and gently rotated several times to obtain adequate faeces.

To obtain tongue swabs, the dorsum and ventrum of the tongue of each pig was sampled with a sterile swab.

Caecal contents of slaughtered cattle, sheep and goats were sampled using sterile swabs, after an incision was made into each caecum.

Each swab taken from the animals was immediately returned into 5 ml of sterile M/15 phosphate buffered saline (PBS), pH 7.6 and refrigerated in the laboratory.

Enrichment of swab samples

All samples in PBS were cold-enriched at 4 °C for 3 weeks. Subcultures were made onto desoxycholate citrate agar (DCA) and MacConkey agar (Mac) after 1 week and 3 weeks, respectively.

Isolation and identification of *Yersinia*

Tiny to medium lactose non-fermenting colonies growing after 48 h at 25 °C on DCA and Mac plates were inoculated into triple sugar iron (TSI) agar slants. Cultures that produced acid slant over acid butt without H_2S but with or without gas production were inoculated into urea agar slants. All urease-positive suspect cultures were further biochemically characterized (see table n° II) by standard methods (11, 22). Speciation of the isolates was as earlier recommended (6, 7, 9, 30).

Determination of colonial morphology

Cultures that were biochemically demonstrated to be *Yersinia* were streaked for isolation on DCA, Mac and *Salmonella-Shigella* (SS) agar. Inoculated plates were incubated at both 25 °C and 37 °C for 24-48 h. The color and size of each culture on these media under the

TABLE N° I - Colonial characteristics of *Yersinia* spp. from Nigerian cattle and pigs on 3 commonly used enteric media

Medium	Colour	Colonial characteristics*			
		24 hours		48 hours	
		25°C (mm)	37°C (mm)	25°C (mm)	37°C (mm)
Salmonella-Shigella (SS)	Colourless	Pinpoint	Pinpoint	0.5-2.0	1.0-2.0
MacConkey	Pale	Pinpoint	1.5-2.0	1.0-1.5	2.0-2.5
Desoxycholate citrate agar (DCA)	Colourless	Pinpoint	0.5-1.0	1.5-2.0	2.0-2.5

* 3 species of *Yersinia* encountered and studied i.e. *Y. intermedia*, *Y. enterocolitica* and *Y. frederiksenii* exhibited identical colonial characteristics.

TABLE N° II - Biochemical characteristics of 14 *Yersinia* spp. isolated from animals in Nigeria

Characteristic	Number of isolates positive*		
	Y. enterocolitica (n=9)	*Y. intermedia* (n=4)	*Y. frederiksenii* (n=1)
Mobility at 25°C	9	4	1
at 37°C	0	0	0
Indole	8	4	1
Voges-Prouskauer at 25°C	9	4	1
at 37°C	0	0	0
Urease** at 25°C	9	4	1
at 37°C	9	4	1
Citrate (Simmon's)	0	4	0
Phenylalanine deaminase	0	0	0
Hydrogen sulphide (SIM)	0	0	0
Nitrate reduction	9	4	1
Lysine decarboxylase	0	0	0
Arginine dihydrolase	0	0	0
Ornithine decarboxylase	9	4	1
Oxidase	0	0	0
B-Galactosidase (ONPG)	9	4	1
Acid from glucose	9	4	1
Sucrose	9	4	1
D-Cellobiose	9	4	1
L-Arabinose	9	4	1
D-Trehalose	9	4	1
L-Rhamnose	0	4	1
D-Raffinose	0	4	0
D-Melibiose	0	4	0
α-methyl-D-glucoside	0	4	0
Maltose	9	4	1
Dulcitol	0	0	0

* Positive reactions were recorded generally after 48 hours incubation at 25°C except where 37°C is indicated.
** Urease detection was generally quicker at 37°C.

growth conditions mentioned earlier were determined.

Determination of serotypes of *Yersinia* isolates

The slide agglutination test described by WAUTERS (31) was employed to determine the serotypes, using O-antisera 1 to 49 kindly provided by WAUTERS and S. WINBLAD.

Biotype determination of isolates

The *Yersinia* isolates were classified into biotypes as described by WAUTERS (31).

RESULTS

The colonial characteristics of *Yersinia* spp. strains isolated from pigs and cattle in Nigeria on SS, DCA and Mac are shown in table n° I. The 3 species of *Yersinia* isolated, namely, *Yersinia enterocolitica, Yersinia intermedia* and *Yersinia frederiksenii* exhibited identical characteristics.

Table n° II shows the biochemical characteristics of 9 strains of *Y. enterocolitica,* 4 of *Y. intermedia* and 1 of *Y. frederiksenii* isolated in Nigerian livestock. All 9 strains of *Y. enterocolitica* isolated gave typical reactions except a strain that was indole-negative. None of the 9 strains was rhamnose-positive. *Yersinia intermedia,* and *Y. frederiksenii* strains also gave typical biochemical reactions.

The serotypes and biotypes of *Yersinia* isolates are shown in table n° III. The predominant serotype was 0:12,26 which constituted 5 (38.5 p. 100) of the 13 typable strains of *Yersinia* isolated. All 5 isolates (0:12,26) were *Y. enterocolitica* and were all obtained from cattle.

Three strains of *Y. enterocolitica* were serotype 0:8, 2 of these isolated from cattle while 1 was from a pig.

Only 1 strain of *Y. enterocolitica* isolated was serotype 0:3 (biotype 4) and it came from a pig. All other *Y. enterocolitica* strains were classified as biotype 1.

Serotype 0:52,53 was detected in 2 of the *Y. intermedia* strains while the only *Y. frederiksenii* strain was serotype 0:16.

Overall, of 2,029 samples from 4 species of animals tested, 14 (0.69 p. 100) *Yersinia* species were isolated. Out of 893 and 618 samples examined for cattle and pigs respectively, the frequency of isolation of species of *Yersinia* were 9 (1.01 p. 100) and 5 (0.81 p. 100). *Yersinia* was not isolated from sheep and goats.

DISCUSSION

This is considered the first comprehensive study on *Yersinia* spp. infections in livestock in a tropical country. The frequency of isolation (0.69 p. 100) appears low when compared to studies in temperate countries (21, 23,

TABLE N° III- Serotypes and biotypes of *Yersinia* spp. isolated from pigs and cattle in Nigeria

Strain N°	Source	Species	Serotype	Biotype
YFR 175	Pig[a]	*Y. intermedia*	0 : 52,53	NA
YFR 168	Pig[a]	*Y. intermedia*	0 : 52,53	NA
YFT 86	Pig[b]	*Y. intermedia*	0 : 36	NA
YFT 12	Pig[b]	*Y. enterocolitica*	0 : 3	4
YFT 283	Pig[b]	*Y. enterocolitica*	0 : 8	1
B23	Cattle[c]	*Y. intermedia*	NT	NA
B94	Cattle	*Y. enterocolitica*	0 : 12,26	1
B95	Cattle	*Y. enterocolitica*	0 : 12,26	1
B17 (1) A	Cattle	*Y. enterocolitica*	0 : 12,26	1
B17 (1) B	Cattle	*Y. enterocolotica*	0 : 12,26	1
B282	Cattle	*Y. enterocolitica*	0 : 12,26	1
B262 A	Cattle	*Y. enterocolitica*	0 : 8	1
B262 B	Cattle	*Y. enterocolitica*	0 : 8	1
BSG 37	Cattle	*Y. frederiksenii*	0 : 16	NA

a = Pig rectum; b = Pig tongue; c = All isolations made from cattle came from caecal contents; NT = not typable with 49 antisera available; NA = not applicable.

28). The finding in the present study is however in agreement with the relatively low isolation frequency for *Yersinia* spp. of 1.3 p. 100 from human acute gastroenteritis cases in Southern Nigeria (2).

Yersinia spp. infections were not until recently first confirmed in Bangladesh, a subtropical country, despite several earlier attempts to demonstrate the presence of the organism in that country (25). Earlier failures to isolate *Yersinia* spp. from tropical and subtropical countries have been attributed to high temperatures in these areas (25).

Three of the 4 recognized species of *Yersinia,* namely, *Y. enterocolitica, Y. intermedia* and *Y. frederiksenii* were isolated from livestock in Nigeria. These 3 species have earlier been isolated from human cases in Southern Nigeria (2). Although the pathogenicity of strains of *Y. enterocolitica* have been ascertained, it is not established yet what role other species of *Yersinia* play in infection despite reported isolation in diarrheal cases, either as only pathogen present or in association with other pathogens (2, 10, 17). It is therefore presently impossible to assess the risk to human beings, of isolation of *Y. intermedia* and *Y. frederiksenii* from food animals in Nigeria.

The finding that 5 strains of *Y. enterocolitica* isolated from cattle were serotype 0:12, 26, representing 38.5 p. 100 of typable strains isolated is of significance. It conflicts with a recent impression given by ALEKSIC and BOCKEMUHL (3) that serotype 0:12,26 exclusively belongs to *Y. kristensenii* and did not occur in any other species. All our 5 strains which were serotype 0:12,26 did not belong to *Y. kristensenii* as they repeatedly fermented sucrose, a feature not exhibited by *Y. kristensenii* (7). Contrary to their findings, all our 5 strains were *Y. enterocolitica* biotype 1 (table n° II).

Of public health significance was the isolation of *Y. enterocolitica* serotypes 0:3 and 0:8, 2 known human pathogens, from apparently healthy cattle and pigs. In an earlier study in human cases in Southern Nigeria, 4 of 6 serotyped strains of *Y. enterocolitica* confirmed to cause acute gastroenteritis were serotype 0:3 (2). In the same environment where the present study was conducted, AWUNOR-RENNER and LAWANDE (5) serologically detected a predominance of *Y. enterocolitica* serotype 0:8 infection in chronic glumerulopathy patients, over serotypes 0:3 and 0:9. Pigs have been demonstrated to be reservoirs of *Y. enterocolitica* serotype 0:3 infection in humans in some countries (23, 29). Deer have been suspected as a reservoir of serotype 0:8 infections in humans in the United States of America (18). Therefore cattle and pigs may serve as reservoirs of human infections by *Y. enterocolitica* serotypes 0:3 and 0:8.

Of relevance to the debate on the unsolved mystery of the geographical distribution of *Yersinia* spp. serotypes was the isolation of serotype 0:8 from cattle and pigs in Nigeria. The occurrence and distribution of the serotypes of *Yersinia* have been reported to differ not only between continents but also between geographically adjacent countries (8). Except for a short report (1) on our first isolate of *Y. enterocolitica,* serotype 0:8 recovered from an apparently healthy piglet, this serotype had never been reported in animals outside the United States of America. The isolation of this serotype from cattle agrees with the report of SHAYEGANI *et al.* (26) where apart from some other serotypes, 2 strains of *Y. enterocolitica* serotype 0:8 were isolated from 352 cows examined in the United States of America.

SHAYEGANI *et al.* (27) had earlier reported that occasionally, serotypes 0:8 recovered from animal and environmental sources, were biochemically atypical from human strains of the serotype. All our 3 isolates typed as 0:8 appeared to be biochemically typical for *Y. enterocolitica* although it might be necessary to further characterize these isolates as to their virulence, pathogenicity and enterotoxigenicity before making any comparison to isolates from humans.

In view of the fact that no specific animal reservoirs for human infections by *Y. enterocolitica* have been identified, it is imperative to further study the predominant serotype (0:12, 26) isolated from cattle. The risk of beefborne yersiniosis amongst abattoir workers and consumers of beef products in Northern Nigeria, should they be pathogenic, can then be assessed.

It was of interest to observe that no *Yersinia* spp. was isolated from all the 320 sheep and 198 goats sampled. *Yersinia enterocolitica* strains have been isolated from these animal species in other countries (20, 21). KROGSTAD (19) in Norway also failed to isolate *Y. enterocolitica* from 190 goats, although 16.3 p. 100 of these

animals were found to be seropositive for *Y. enterocolitica*. There is a need to conduct a serological investigation of sheep and goats to several serotypes of *Yersinia* in Nigeria. This will reveal the infection status of these animals to the micro-organism.

In conclusion, the demonstration of *Yersinia* spp. infections in livestock in Nigeria is an evidence that infection by members of this genus occurs in livestock in the tropics. However, before the health hazard to human beings by these strains of *Yersinia* in livestock can be assessed, it is imperative to determine their virulence and pathogenicity.

RESUMEN

AGBONLAHOR (D. E.), ADESIYUN (A. A.), KWAGA (J. K. P.), LOMBIN (L. H.). — Caractéristicas serológicas, bioquímicas y de cultivo de algunas especies de *Yersinia* aisladas en animales de Nigeria. *Rev. Elev. Méd. vét. Pays trop.*, 1985, **38 (4)** : 416-422.

Los autores estudiaron las características serológicas, bioquíminas y de cultivo de 14 cepas de *Yersinia,* aisladas a partir de 2 029 muestras sacadas de bovinos, cerdos, carneros y cabras aparentemente sanos, en Nigeria.

Aislaron 9 cepas de *Y. enterocolitica,* 4 de *Y. intermedia* y 1 de *Y. frederiksenii* de bovinos y cerdos.

No se hizó ningún aislamiento en carneros y cabras.

Las 3 especies de *Yersinia* observadas mostraron las mismas características de cultivo sobre medios de citrato desoxilato, de MacConkey y *Salmonella-Shigella.* 0:12,26 era el serotipo predominante encontrado en 5 (38 p. 100) de las cepas tipables de *Yersinia.* Todas estas fueron aisladas de bovinos, y se confirmó bioquimicamente su identificación como *Y. enterocolitica.*

Tres cepas de *Y. enterocolitica* correspondian al serotipo 0:8 ; de las cuales 2 provenían de bovinos y 1 de cerdos. 2 cepas de *Y. intermedia* correspondían al serotipo 0:52,53 mientras que las 3 últimas correspondían respectivamente a los serotipos 0:36, 0:16 y 0:3.

El solo serotipo 0:3 aislado (*Y. enterocolitica*) fué clasificado como biotipo 4, mientras que los 8 demás pertenecían al biotipo 1.

El aislamiento del serotipo 0:8 de *Y. enterocolitica,* a partir de cerdos y de bovinos en Nigeria, tiene una importancia significativa, porque no se había nunca notado dicho serotipo en animales fuera de los Estados-Unidos.

Además, el aislamiento de los serotipos 0:3 y 0:8 (cuyo poder patógeno para el hombre es conocido a partir de animales para el consumo humano en Nigeria, parece indicar que los bovinos y los cerdos podrían desempeñar un papel cierto en las enfermedades con *Y. enterocolitica* en el hombre.

Palabras claves : Bovino - Cerdo - *Yersinia enterocolitica* - *Yersinia intermedia* - *Yersinia frederiksenii* - Nigeria.

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