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Small Ruminant Research 37 (2000) 215–221

Small Ruminant
Research

www.elsevier.com/locate/smallrumres

Bioserogroups of *Campylobacter* species isolated from sheep in Kaduna State, Nigeria

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Received 21 January 1999; accepted 24 December 1999

Abstract

Sheep *Campylobacter* isolates from Kaduna State were characterized into their species and bioserogrouped. A total of 1100 samples were collected from Kaduna abattoir and National Animal Production Research Institute (NAPRI), Shika. The samples were from 250 gallbladder, 250 intestinal contents, 100 fetal stomach contents all from Kaduna abattoir while 250 rectal swabs and 250 vaginal swabs were from the NAPRI Small Ruminant Programme. Of a total of 1100 samples, 39 (3.54%) yielded *Campylobacter* organisms. The highest isolation rate (6.8%) was from samples of intestinal contents followed by those from gall bladders (4.0%). Samples from the vaginal and fetuses had the lowest isolation rates (2.80%) and (0%), respectively. Of the 39 *Campylobacter* isolates from all the sources, (79%) were characterized as *C. fetus subsp jejuni*, *C. coli* (13%) and *C. laridis* (8.0%). while *C. coli* and *C. laridis* were isolated from gall bladder and intestinal contents only. *Campylobacter fetus subsp jejuni* biotype 1 accounted for 40.3% of the total isolates. *C. laridis* biotypes I and II were also isolated and accounted for 5% and 3% of the isolates, respectively. 5% of the isolates were not typeable. The serogroups 4 (13%), 36 (10%), 9 (10%), 84 (8%), 29 (5%) and 20 (8%) were the commonest serogroups identified in sheep at two locations surveyed. The isolation of *Campylobacter* organisms from rectum, vagina, gallbladder, and intestinal contents is a clear indication that sheep serves as a reservoir of this organisms in Nigeria. Similarities between documented human *Campylobacter* isolates in Nigeria and those in the present study raised the possibility of cross-transmission between sheep and man. It is concluded that biotyping and serotyping can be used for epidemiological study of campylobacteriosis due to *Campylobacter jejuni* in sheep in Kaduna State of Nigeria. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: *Campylobacter*; Nigeria; *C. coli*

1. Introduction

Campylobacter organisms are a leading cause of gastroenteritis in man and animals throughout the

world (Lior, 1994). In sheep, campylobacteriosis is characterized by abortion, still births, and birth of weak lambs during late pregnancy (Kimberling, 1988). *Campylobacter jejuni* and *Campylobacter fetus* are the causative agents of this disease. The infection is highly contagious and may cause up to 70% of ewes to abort when the organisms are newly introduced into

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the flock (Dennis, 1990). Susceptible ewes may acquire infection through ingestion of contaminated *Campylobacter* organisms with fetal material or uterine discharge. Other sources of infection may include feces of carrier sheep and other mammals (Smibert, 1969).

Several epidemiological studies in developed countries have identified sources of *Campylobacter* enteritis in man to include animals, food, water, and milk products (Khan, 1982; Skirrow, 1982). Although there are sporadic reports of *Campylobacter* enteritis in developing countries particularly Nigeria (Olusanyo et al., 1983; Alabi et al., 1986; Ani et al., 1988), very little is known concerning its mode of spread.

A proper understanding of the epidemiology of *Campylobacter* infections is necessary for the planning of effective prevention and control measures (Adegbola et al., 1990; Raji et al., 1997). Moreover, previous observations of the prevalent biotypes and serogroups of *Campylobacter* isolates from humans (Alabi et al., 1986), and animal sources, especially poultry and pigs (Olubunmi and Adeniran, 1986; Adegbola et al., 1990), have encouraged us to examine the role of sheep in human *Campylobacter* enteritis in Nigeria. This paper, therefore, document the isolation, biotyping and serotyping of *Campylobacter* species isolated from sheep in Nigeria.

2. Materials and methods

A total of 1100 samples were examined from periods of September 1997 to December 1998. These samples were derived from live sheep originating from the National Animal Production Research Institute (NAPRI) Sika-Zaria, and slaughtered sheep from the Kaduna abattoir. The samples comprised of 250 gall bladder contents, 250 intestinal contents, and 100 fetal stomach contents swabs from Kaduna abattoir as well as 250 each of rectal and vaginal swabs from 250 healthy sheep at NAPRI Sika-Zaria.

The samples obtained were inoculated into sterile ferrous bisulphate pyruvate (FBP) broth containing GIBCO nutrient broth which served as transport enrichment medium (TEM). The TEM was brought to the laboratory within 4 h of collection. Butzler's medium (Oxoid SR 85) supplemented with 7% sheep blood was used as primary isolation medium. After

inoculation the plates were incubated using the candle jar extinction method at 37°C for 48–72 h. The suspected colonies were identified by Gram's stained and biochemical methods as described earlier by Morris et al. (1985) and Billingham (1981).

The colonies were Gram stained and showed small spiral gram-negative bacteria. Organisms were considered to be *Campylobacter* species if they were motile, catalase positive, oxidase positive, reduced nitrate to nitrite, grew at 37, 42°C, but not at 25°C (Coker and Adefoso, 1994).

Biotyping of *Campylobacter* isolates was done using the new extended scheme described by Lior (1984). The scheme employs three chemical reactions: rapid hydrogen sulphide production; hippurate hydrolysis; and detection of DNA hydrolysis to differentiate *C. jejuni* into four biotypes (I, II, III, IV), *C. coli* into biotypes I and II and *C. laridis* into biotypes I and II.

Similarly, serotyping of the isolates was done using the scheme of Lior et al. (1982), based on a rapid slide agglutination technique with live bacteria and absorbed antisera for detection of heat labile antigenic factor. The 21 antisera used for this study were supplied by National Reference Service for *Campylobacter*, Ontario, Ottawa, Canada. The antisera for the *C. laridis* were not included in the pack.

3. Results

The isolates that were motile gram-negative curved to spiral rods suspected to be *Campylobacter* were catalase positive, oxidase positive, reduced nitrate to nitrite, did not produce acid or H₂S in Triple sugar irons. They grew well at 37 and 42°C but not at 25°C, and also grew in 1% glycine, but not in 3.5% sodium chloride. Those isolates that were hippurate positive but H₂S and deoxyribonuclease (DNase) negative were characterized as *C. jejuni*, while isolates identified as *C. coli* were hippurate, H₂S, and DNase negative. *C. laridis* isolates were H₂S positive, but hippurate and DNase negative.

Table 1 shows the species and biotypes of *Campylobacter* isolates obtained from different anatomical sites in sheep in Kaduna State.

Of a total of 1100 samples, 39 (3.54%) yielded *Campylobacter* organisms. The highest isolation rate (6.8%) was from samples of intestinal contents

Table 1

Biotypes of *Campylobacter* species from different anatomical sites of sheep in Kaduna States

Anatomical site	Number of sample	Number positive	Isolation rate (%)	<i>Campylobacter</i> species	Different biotypes	Percentage of biotypes
Gall bladder	250	10	4	<i>C. jejuni</i> (8)	Biotype I	50
					Biotype II	10
					Biotype III	20
				<i>C. coli</i> (1)	Biotype I	10
				<i>C. laridis</i> (1)	Biotype II	10
Intestinal contents	250	17	6.8	<i>C. jejuni</i> (11)	Biotype I	34
					Biotype II	12
					Biotype III	6
					Biotype IV	12
				<i>C. coli</i> (4)	Biotype I	12
					Biotype II	12
Rectum	250	7	2.8	<i>C. laridis</i> (2)	Biotype I	12
				<i>C. jejuni</i> (7)	Biotype I	28
					Biotype II	57
					Biotype IV	15
Vagina	250	5	2.0	<i>C. jejuni</i> (5)	Biotype I	60
					Biotype II	20
					Biotype III	20
Fetuses	100	0	0	–	–	–
Total	1100	39	3.54	–	–	–

followed by those from gall bladders (4.0%). Samples from the vagina and fetuses had the lowest isolation rates (2.80%) and (0%), respectively.

Of the 39 *Campylobacter* isolates from all the sources, (79%) were characterized as *C. fetus subsp jejuni*, *C. coli* (13%) and *C. laridis* (8.0%). Isolates of *C. coli* and *C. laridis* were obtained from gall bladder and intestinal contents only.

C. jejuni isolates from the vagina, gall bladder and intestinal contents were more of biotype I. Whereas, those of rectal swabs were mostly of biotype II. There

was no *C. jejuni* biotype IV in the gall bladder, rectal swabs and intestinal contents.

Table 2 shows distribution of different biotypes of *Campylobacter* spp isolated from sheep in Kaduna state. *C. jejuni* biotype 1 was the most prevalent biotype accounting for (40.3%) of the total isolates while biotype II represented 20.5%. Biotype III 10.2% and biotype IV the least at 8.0%. *C. coli* isolates were of biotypes I and II almost of equal numbers (8%) and (5%), respectively. *C. laridis* were also typed into biotype I and II representing (5%) and (3%),

Table 2

Percentage of different biotypes of *Campylobacter* species isolated from sheep, all the anatomical sites combined

<i>Campylobacter</i> species	Different biotypes	Number	Percentage
<i>C. jejuni</i>	Biotype I	16	40.3
	Biotype II	8	20.3
	Biotype III	4	10.2
	Biotype IV	3	8.0
<i>C. coli</i>	Biotype I	3	8
	Biotype II	2	5
<i>C. laridis</i>	Biotype I	2	5
	Biotype II	1	3

Table 3

Distribution of different serogroups of *Campylobacter* species isolated from various anatomical sites in sheep in Kaduna State^a

Type <i>Campylobacter</i>	Serogroup	As % of anatomical site isolates				As % of total isolates
		Gall bladder	Intestine	Rectum	Vagina	
<i>C. jejuni</i>	4	2 (20)	–	1 (14.29)	–	7.69
	36	–	2 (11.77)	1 (14.29)	1 (20)	10.26
	53	1 (10)	–	–	–	2.56
	20	1 (10)	2 (11.77)	–	–	7.69
	9	1 (10)	2 (11.77)	–	–	7.69
	84	1 (10)	1 (5.88)	–	–	5.13
	6	–	1 (5.88)	–	–	2.56
	29	–	1 (5.88)	–	1 (20)	5.13
	8	–	–	–	2 (40)	5.13
	24	1 (10)	–	–	–	2.56
	21	–	1 (5.88)	–	–	5.88
	45	–	–	1 (14.29)	–	2.56
	5	–	–	1 (14.29)	–	2.56
	18	–	–	1 (14.29)	–	2.56
	55	–	–	–	1 (20)	2.56
	1	–	1 (5.88)	–	–	2.56
<i>C. coli</i>	4	1 (10)	2 (11.77)	–	–	7.69
	53	–	–	–	–	0.00
	9	–	–	–	–	0.00
	6	–	1 (5.88)	–	–	2.56
	84	–	1 (5.88)	–	–	2.56
<i>C. laridis</i>	ANIP	1 (10)	2 (11.77)	–	–	7.69
<i>C. jejuni</i>	NT	1 (10)	–	1 (14.29)	–	7.69

^a ANIP=antsera not included in the pack sent from Canada; NT=non-typable isolates of *Campylobacter* species.

respectively, from gallbladder and intestinal content only.

Table 3 shows the distribution of different serogroups of *Campylobacter* species isolated from various anatomical sites of sheep in Kaduna State. For *C. jejuni* serogroups 36 and 4 were the commonest serogroups representing (10.3%) and (7.7%), respectively. Other serogroups were 29 (5.1%) and 55 (2.6%).

Campylobacter coli were in serogroups 4 (7.7%), and 6 and 84 each representing 2.56%. *C. laridis* isolates were not serotyped. There was no significant difference in the different anatomical sites for the different serogroups, although serogroup 36 occurred almost in all anatomical sites except in the gall bladder. The serogroups 4 and 9 were the commonest serogroups found in the gall bladder representing 20% each. Other serogroups 53, 20 and 24 occurred in equal proportional 10% each in the gall bladder. Serogroups from the intestinal contents were 20, 84,

9, 36, 4 and 6 representing 11.77% each. The prevalent serogroups from rectal swabs were 18, 36, 22, 45 and 5 representing 14.29% each. Vaginal serogroups were 55, 36 and 29 which occurred in equal proportions of 10% each.

4. Discussion

The result of this study indicate the overall isolation rate of ovine *Campylobacter* isolates to be 3.54% from all the samples collected. The isolation rate of 2.8% from the rectal swabs of healthy sheep in Zaria were similar to those of Turkson et al. (1988), with the prevalence rate of 2.0% from rectal swabs of healthy sheep in Kenya. Olubunmi and Adeniran (1986) reported a prevalence rate of 6.25% from western Nigeria, and 6.9% prevalence rate reported by Abraham et al. (1990) in healthy Ghanaian sheep.

The results were different from those of Adegbole et al. (1991) and Adetosoye and Adeniran (1987) who

failed to isolate any *Campylobacter* organism from healthy sheep and goats in their studies in Ile-Ife, Nigeria.

Similarly, all the rectal *Campylobacter* isolates in this study were *C. jejuni* with biotype II the commonest representing 57%. This disagreed with the finding of Olubunmi and Adeniran (1986), in which *Campylobacter* biotype I accounted for 58% of their isolates. The present study agree with Abraham et al. (1990) in which biotype II predominated in their studies.

The isolation of *Campylobacter* from intestinal contents of sheep at Kaduna abattoir indicated that *Campylobacter* colonize intestinal mucosa of healthy animals as earlier reported by Butzler and Skirrow (1979). This may serve as a source of infection to other animals in the environment.

The isolation rate of 6.8% is close to the 4.9% reported by Koides (1991) for healthy sheep in Turkey. The two results however differed in the percentages of *C. jejuni* isolates. In this study, the overall percentage isolation of *C. jejuni* 64% was much lower than in Koides (1991) work which was 93%. In both findings, other *Campylobacter* such as *C. coli* was also isolated from this samples.

The isolation rate of *Campylobacter* from the vaginal swabs of sheep sampled at the NAPRI farm in Shika Zaria was 2.0%. Although, abortion cases were not reported, the birth of weak lambs was reported by the herdsmen. Presence of infection in the vaginal swabs of healthy sheep after parturition was earlier reported by Montagna et al. (1988). In their study just like the present finding, *C. jejuni* was the major *Campylobacter* species identified in the vagina of ewes.

Gall bladder regarded as a choice for the isolation of *Campylobacter* organisms (Firehammer et al., 1962), had a low 4.0% isolation rate in this work. A similar (3.3%) rate was found in the work by Marsh and Firehammer, 1953. This tended to show that most gall bladders of sheep from different geographical locations may have low isolation rate of *Campylobacter* organisms. In sheep slaughtered at Kaduna abattoir, *C. jejuni* biotype 1 accounted for 50% of the isolates from the gall bladder.

Most *Campylobacter* isolates from gall bladder, (67%) in Marsh and Firehammer's work, 1953 classified as *C. fetus subsp jejuni* whereas the remaining 33% were *C. fetus subsp fetus*. In the present study,

most gall bladder isolates (80%) were *C. jejuni*. The others were either *C. coli* or *C. laridis*. The differences are not easy to account for but the type of *Campylobacter* in the gall bladder may be a reflection of the type most commonly isolated in other anatomical parts of the animal. In this study the most common *Campylobacter* species was *C. jejuni*.

Failure to isolate *Campylobacter* from the fetuses examined in this study may be attributed to the fact that the fetuses were not obtained from clinical cases of epizootic abortion outbreaks. The most common biotype of *C. jejuni* found in the present study was biotype I accounting for 40.3% isolates. This is in agreement with the finding of Adegbola et al. (1990) in Ile-Ife, Nigema, and Varga et al. (1990) in Hungary. It is different from what Adesiyun et al. (1992) reported. They found *C. coli* biotype I as the major biotype from the healthy sheep and other farm animals in Trinidad and Tobago. The differences might be due to a number of factors. Part of this may be related to ecologic differences in the areas where the studies was conducted. Similarly, the laboratories methods used in different studies could have influenced results obtained as shown by the works of Adetosoye and Adeniran (1987), and Adegbola et al. (1991) as compared with that of Olubunmi and Adeniran (1986), in the same locality but in different laboratories.

Campylobacter fetus known to colonize the intestinal tract of domestic animals (Doyle, 1981; Manser and Dalziel, 1985), were not isolated in the present study. This may be due to hot climatic conditions in this part of the country, since *Campylobacter fetus* survive well in areas with lower temperature, especially at 25–37°C.

The isolation of *Campylobacter* organisms from rectal, vaginal, intestinal content, gall bladder swabs in this study is a clear indication of the presence of *Campylobacter* in sheep in Zaria and Kaduna. *Campylobacter* organisms must be considered as potential agents of Ovine enteritis and abortion in Nigeria.

The most common serogroups of *Campylobacter* sp. isolated from sheep in this present study were 4, 36, 20, 84, 9, 29, 6, and 8 accounting for 64% of the total isolates. Adegbola et al. (1991) reported serogroups 2, 4, 29 and 36 which accounted for 61.7% of all the serogroups as the major ones found in animals in Ile-Ife. In another study, Adegbola et al. (1990) reported serogroups 2, 4, 20, 29, 36 and 45 to be

predominant among animals in Ile-Ife. In that study they also found serogroups 2, 4, 29, 36, 45 and 53 to be the most common in humans. Recently, Coker and Adefoso (1994) reported that during a 10 years study, serogroups 29 and 36 were the commonest serogroups in human in Lagos.

The serogroups found in sheep in this study were similar to those reported in domestic animals previously in Nigeria (Adegbola et al., 1990, 1991) and in humans (Alabi et al., 1986; Coker and Adefoso, 1994).

During slaughter of sheep, goats and cattle, intestinal feces often contaminate meat. Meat and meat products should therefore, be properly cooked to destroy the organism so as to prevent possible transmission to man.

It is well established that sheep are natural reservoir of *Campylobacter* organisms (Firehammer et al., 1962). The serotypes identified in sheep in this study are similar to those reported previously in humans in Nigeria, which suggests the possibility of cross-transmission of *Campylobacter* from sheep to man. To our knowledge this may be the first time common serotypes are found in the northern part of Nigeria from sheep.

5. Conclusion

Results from this study indicate that *C. jejuni*, *C. coli* and *C. lariidis* are the most common species of *Campylobacter* organisms isolated from healthy sheep in Zaria and Kaduna. It is concluded that biotyping and serotyping can be used for epidemiological study of *Campylobacteriosis* due to *Campylobacter* species in sheep in Kaduna State, Nigeria.

Acknowledgements

We thank Mr. Makailu and other staff of the department of Microbiology and Pathology who help in one or the other way during the course of laboratory analyses. We are also grateful to the University Board of Research for the grant.

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