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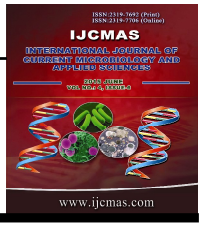

Original Research Article

The Earliest Occurrence of *Escherichia coli* in Calves in Zaria, Nigeria

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A B S T R A C T

Colibacillosis is a group of diseases caused by *Escherichia coli* in young animals. It is known to be the most important neonatal disease which causes a major economic loss in livestock production. It is also of public health significance. A study was undertaken to observe the earliest age occurrence of *Escherichia coli* in calves, in Zaria, Nigeria. Fifty rectal swabs were collected from 32 calves of ages 0–48 hours from three different locations within Zaria, National Animal Production research Institute (NAPRI) Farm, Shika; University farm and Veterinary Teaching Hospital, Ahmadu Bello University, Zaria. These were examined for *E. coli* by culture and some biochemical tests. Out of these numbers, 78% (42) of the samples were *E. coli* positive and 22% (12) were *E. coli* negative. 48% of the samples had other bacteria other than *E. coli* such as *Klebsiella*, *Proteus* and *Corynebacterium spp.*, and 3% (2) of the calves had diarrhoea. *E. coli* was isolated from of the 2 calves that had diarrhoea, one was a stillbirth and the other delivered by traction. It was observed that there were low prevalence of *E. coli* between 0–4 hours of age and higher between 6^{th}–48^{th} hours. This suggests that calves must promptly ingest colostrum within the first few hours of birth as a preventive measure against colibacillosis. Also the possibility of intrauterine infection is suggested.

Introduction

Escherichia is a large group of serologically related Gram negative non-sporing and non-acid fast rods consisting of motile and non-motile bacteria that conform to the family *Enterobacteriaceae* (Anonymous, 1958). As members of Gram-negative rods, they form part of the normal flora of the intestinal tract of man and animals (Smith and Grabb, 1961). They are aerobes and ferment a wide range of carbohydrates and possess a complex antigenic structure (Sojka, 1965a,b,c). Characteristically, most strains acidify lactose with gas formation at 37°C and 44°C including the fermentation

ofadonitol and inositol and the production of indole at 37°c and at 44°C (Wilson and Miles, 1955). *E. coli* gives a positive methyl-red reaction, a negative Voges-Proskauer reaction, and no growth in koser's citrate medium (Adetosoye, 1976a,b). *E. coli* causes a group of diseases in young animals referred to as colibacillosis, which is known to be the most important neonatal disease that causes major economic loss in livestock production (Blood *et al.,* 1983a,b). The disease is commonly seen in animals that are grouped together (Tekdek and Goje, 1986). *E. coli* produces endotoxin from complex lipopolysaccharides in their cell wall which is a major factor in a variety of pathophysiologic effects on the host (Agbonlahor and Odugbemi, 1982). The pathogenesis of colibacillosis is influenced by specific adhesive factors (fimbrae) and the production of endotoxin (Sojka, 1965a,b,c). The common sources of infection of *E. coli* are: faeces of infected animals, contaminated calf pens or farrowing barns in swine, milk from mastitic cows, skin, perineum, udder and teat of infected animals, inanimate objects like beddings, pails, boots, tools, clothing, feed and water supplies. The major routes of infection are: orally by ingestion, through the umbilical vessel and the nasopharyngeal mucosa. These routes determine the nature of clinical signs seen. Umblical and nasopharyngeal routes could result to colisepticaemia, meningitis, polyserositis, and ascites, while by ingestion it produces various degrees of diarrhoea, dehydration, metabolic acidosis, shock and septicaemia (Glantz and Rothernbacher, 1965).

Some authors have categorized *E. coli* into four different strains viz., Invasive, non-invasive, non-pathogenic and enteropthogenic (Smith, 1960; Smith and Halls, 1967; Smith and Halls, 1978; Agbonlahor and Odugbemi, 1982; Agbonlahor *et al.,* 1984). As a result of these different strains of *E. coli*, a variety of disease forms have been described in calves. Three forms are described by Gay (1965) as Septicaemic form (colisepticaemia): in which sudden death is associated with *E. coli* bacteraemia. The organism can be isolated (usually as a single serotype in pure culture) from organs and tissues throughout the body. This is seen within the first few hours or days of life. Enterotoxaemic form:- this is characterised by collapse and sudden death and is associated with proliferation of specific strains of *E. coli* in the intestines. There may be no bacteraemia. This occurs within the first week of life. The enteric form (calf diarrhoea, calf scours, white scours), where the physiological disturbances are severe, the calf may die, but it is not always fatal. This is seen between birth and first 10 days of life.

The purpose of this study is to evaluate the earliest time of appearance of *E. coli* within the first 48 hours of life in the faeces of calves in Zaria. This knowledge may be helpful in the management of the disease in calves for the purpose of prevention.

Materials and Methods

Location

The animals used for the experiment were calves aged 0 to 48 hours from three locations within Zaria, Nigeria. The locations were the dairy unit of the National Animal Production Research Institute (NAPRI) Farm, Shika, Ahmadu Bello University farm and Veterinary Teaching Hospital Animal pens, Ahmadu Bello University, Zaria. The management system in all these farms involved allowing the dams stay with the calves for at least three days to ingest colostrum. The experiment was conducted between February and May 1986.

Sample collection

The samples (rectal swaps) were collected from calves at various times, ranging from 0-48 hours after calving. The samples were collected from calves using sterile swabs at approximately two hour intervals after calving up to 48 hours of age. The rectal swabs were transported in universal bottles containing of MacConkey Broth.

Culture

The samples were incubated for 24 hours to enhance the growth of *E. coli*, after which they were sub-cultured on Eosin Methylene Blue (EMB) Agar and incubated at 37^{o}c overnight.

Colony appearance

E. coli was identified by the characteristic growth on EMB agar as discretely raised flatten top colonies with darkish centre and green metallic sheen at the periphery. Any sample that shows such characteristic growth was considered presumptive positive for *E. coli*

Biochemical tests

The presumptive isolates were identified as *E. coli* using the following biochemical tests: indole, methyl red (MR), VP, citrate, and pigmentation tests. Other biochemical tests were conducted to identify and differentiate the other bacteria according to standard procedures.

Data Analysis

The results were analysed using descriptive statistics (tables, frequencies, histogram).

Results and Discussion

A total of 32 animals (Calves) were sampled by rectal swabs within the 3 locations, between 0 and 48 hours and examined for the isolation of *E. coli*, results showed that, 78% were *E. coli* positive, 22% were *E. coli* negative and 48% had other bacteria other than *E. coli* and 3% of the calves had diarrhoea. Some animals were sampled more than others depending on location and accessibility, those at the VTH were located near the faculty and therefore more accessible. Those at the university farm were the most far and therefore less accessible. The animals were sampled as follows (look above).

Four animals were sampled at zero hour post- partum, out of which 3 were positive for *E. coli*. Four animals were also sampled at 2hours all were positive for *E. coli* except UF2. At 3hours four animals were sampled 3 were positive and one negative. Three animals were sampled at 4hours and 2 were positive for *E. coli* while one was negative. Four animals were again sampled at 5hours and all were positive for *E. coli*. At the 6hour three animals were sampled 2 were positive and one negative for *E. coli*, while at the 7^{th} hour two were sampled and all were positive for *E. coli*. At 8hours three animals were sampled one was positive and the other negative and at the 9^{th} hour one animal was sample was sampled it was positive for *E. coli*.

Two animals were sampled at the 10^{th} hour and both were positive for *E. coli*. While, one animal was sampled at the 11^{th} hour and it was negative. One was sampled at the 12^{th} hour it was positive for *E. coli*. Two animals each were sampled at the 13hour and 18^{th} hour respectively all were positive for *E. coli*. At the 19^{th} and 22^{nd} hour only one animal each was sampled, respectively. While at 19^{th} hour, it was positive for *E. coli* the one at 22hour was negative. One animal was sampled at the 23^{rd} hour it was positive three animals while, were sampled at 25^{th} hour all were positive for *E. coli*.

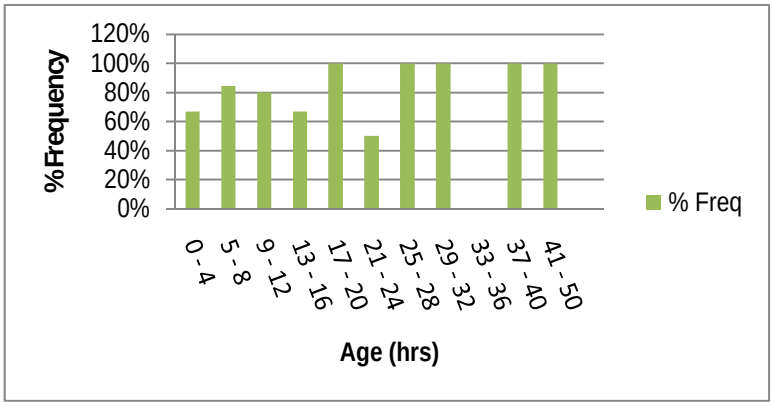
Table.1 Table showing the percentage frequency of occurrence of *E. coli* from 32 calves up to 48 hours of age

Age (hours)	# of *E. coli* positive	# Samples	% Frequencies
0 - 4	11	15	67%
5 - 8	10	13	85%
9 - 12	4	5	80%
13 - 16	2	3	67%
17 - 20	3	3	100%
21 - 24	1	2	50%
25 - 28	4	4	100%
29 - 32	1	1	100%
33 - 36	1	1	0%
37 - 40	2	2	100%
41 - 50	1	1	100%

Table.2 Biochemical test to differentiate *E. coli* from other coliform

	Indole	MR	VP	CITRATE	PIGMENT	Urease	Phenylalanine deaminase	Catalase	Gelatinase
E. coli	+	+	-	-	-				
Klebsiellaspp	-	-	+	+	-				
Proteus spp.						+	+		
Corynebacterium spp.								+	+

Figure.1 Histogram showing the percentage frequency of occurrence of *E. coli* in calves up to 48 hours of age



The same animal was again sampled at 26 and 29th hour it was positive. One animal was sampled at 35th hour, it was negative. One animal at 40th hour and the last animal at 48th hour both were positive for *E. coli*.

The presumptive *E. coli* isolates were all positive in indole and Methyl Red tests, while *Klebsiella* spp. were negative, but were positive for VP and citrate tests. *Proteus* spp. were found to be positive for urease and phenylalanine deaminase and *Corynebacterium* spp. were catalase and gelatinase positive. The other bacteria could not be identified.

The appearance of *E. coli* in the faeces of calves after birth and its gradual built up has been observed by Smith (1960). This can be seen on Table 1 and figure 1.The low prevalence within the first 4 hours of birth may be attributed to the internal environment of the gut at birth and also the exposure rate of the calf to *E. coli* sources (Blood *et al.,* 1983a,b). The internal environment of the gut of the new born calf is expected to be sterile at birth and therefore small numbers of *E. coli* are expected (Blood *et al.,* 1983a,b). The organism requires time in order to colonise the gut and overcome the host's acidic gastric environment which is destructive in nature to bacteria (Gyles, 1960). The volume of inoculum of *E. coli* in the gut is a factor in *E. coli* colonising the gut. Large numbers end up colonising the gut while small numbers at times are washed out with the faeces (Gyles, 1960). The increase in numbers of *E coli* from 6 hours of age may be due to longer exposure of the calf to its environment and also changes in diet (Blood *et al.,* 1983a,b; Wray and Thomlinson, 1975). Inglis (1960) noted that calves born weak are much more susceptible to the infection and that the vigour of the calf is influenced by the nutrition of the dam before calving. This agrees with this study where, *E. coli* was isolated at 0 hours from one of the calves which was born weak and died 9 hours later. Smith (1960) noted that *E. coli* was one of the first organisms to appear in faeces of calves while it competes with other bacteria like *Bacteroides, Lactobacilli* and *Streptococcus* spp. However other bacteria found included *Proteus, Klebsiella* and *Corynebacterium spp*., this does not agree with the observations of Smith (1960), as reflected on table 2, but this may be due to geographical differences. At 0-4 hours of age, the low infection of *E. coli* could be that calves have acquired some level of protection against colibacillosis since calves are born agamaglobullinemic (Blood *et al,* 1983a,b). This suggests that calves must promptly ingest adequate colostrum within the first hours after birth to confer some level of immunity against colibacillosis (Blood *et al.,* 1983a,b). As time went on the frequency of *E coli* continue to rise. This is in agreement with the observation of Logan (1974), who reported that the maximum efficiency of absorption of colostrum in calves occurs during the first 6–12 hours after birth. There was the case of a stillbirth and another case of a calf that was delivered by traction, both were found to be *E. coli* positive. This may suggest that other routes of infection exist, e.g. intrauterine infection, this needs further investigations.

This study showed that *E. coli* is one of the first organisms to colonise the gut of calves and reaches significant level by the 6th hour of birth. Prevention of colibacillosis from intrauterine infection needs to be investigated. It is recommended that as soon as calves are born, some form of prophylaxis could be employed to prevent colibacillosis especially if the calf is weak or does not promptly ingest enough colostrum.

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