Epidemiology of Cryptosporidiosis in Ruminant Species in Kebbi State, Nigeria

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Background: The zoonotic, parasitic diarrhoea disease Cryptosporidiosis, is a major constraint to livestock production throughout the tropics and beyond. However, the risk factors in ruminant species have not been sufficiently established in Northern Nigeria.

Methods/procedure: A cross sectional study on the prevalence of cryptosporidiosis and the factors influencing its distribution in ruminant species was carried out in two communities in Kebbi State, Nigeria. Faecal specimens were examined for Cryptosporidium by formal-ether concentration and modified Ziehl-Neelsen staining technique.

Results and findings: A total of 900 ruminant species were tested, 178 (19.8%) were infected with Cryptosporidium. Prevalence in cattle was 28.0% (98/350), Goats 17.1% (46/260) and Sheep 11.7% (34/290). Age related differences in prevalence were observed among goats (P=0.029), sheep (P<0.001) and cattle (P<0.0001). However such variations were not gender related in Goats (P=0.658) and sheep (P=0.105) but was related in cattle (P=0.013). Logistic regression analysis showed that cattle were about three times prone to infection than sheep and goats (Odds Ratio =2.510, P-value=0.004, 95% Confidence Interval =1.612- 3.909), diarroeal ruminants were significantly prone to infection than healthy animals.

Conclusion: Prevalence of cryptosporidiosis in ruminants is high in Kebbi State. Calves and diarrhoeal condition are significant factors in the spread among ruminants and probably humans. Improved management system and hygienic practices must be embraced and promoted by owners.

Key words: Cryptosporidiosis, Ruminants, Prevalence, risk factors, Diarrhea, Kebbi State
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II. Materials and Methods

Study Area

Zuru is located in the south eastern part of Kebbi State, between longitude 11° 24'09" N and latitude 5° 15'07" E. It has an estimated population of about 165,547 thousand people living in the local government area. While, Aliero on the other hand is located in the extreme northwestern part, between latitude 12°19'06" N and 4° 30'10" E with a population of about 123,785 people. Population estimates based on the 2006 national census [20]. The two communities feature low socioeconomic status and poor environmental sanitation. Adequate water supply, sewage, and waste disposal systems are lacking. Garbage is burned or thrown away near houses and can be found deposited on several places. Zuru land supports the savannah kind of vegetation with pockets of woodland vegetation along the river basins. Grains, tubers, legumes, fruits are grown in the area. While Aliero is flat and slightly undulating with compact stony and brown soil, and has northern guinea savannah vegetation. The leading economic activity in both communities is mainly agriculture. The inhabitants therefore are mostly farmers, animal keepers, blacksmiths, traders and some are civil servants. The people of Aliero are predominantly of Hausa/ Fulani tribe, but Zuru people are Dakarkari by tribe.

Sample Collection and Handling

Visits were made to homes that gave their consent earlier before the sample collections commenced. Young and adult animals were sampled. For cattle, a calve of less than twelve (12) months was classified as young, while for Sheep and Goats, animals below six (6) months were considered to be young [21]. During the visits fresh rectal faecal samples were collected from each of the animals into a sterile, airtight, 10mL plastic tube. For animals in which rectal sampling was not possible, such as neonates, wooden tong depressors were used to scoop up the superficial layer of faeces without touching the floor. Collected faecal samples were labelled and transported in a cool box to the biology laboratory of Kebbi State University of science and Technology, Aliero (at least within 3 hours of collection) prior to dispatch in refrigerated containers for analysis.

Laboratory Analysis of Fecal Samples

In the laboratory, stool samples were concentrated by formal-ether technique. Briefly using an applicator stick, about 1 g of stool sample was placed in a clean 15 ml conical centrifuge tube containing 7 ml formalin. The sample was suspended and mixed thoroughly with applicator stick. The resulting suspension was filtered through a sieve (cotton gauze) into a beaker and the filtrate was poured back into the same tube. The debris trapped on the sieve was discarded. To this mixture, 3 ml of diethyl ether was added and hand shaken; the content was centrifuged at 2000 rpm for 3 minutes. The supernatant was poured away, leaving only the fine sediment at the bottom of the tube [22]. This was then used to prepare slides for the detection of Cryptosporidium spp.

Ziehl-neelsen acid fast technique

One to two drops of fine sediment was smeared on the slide and air dried. This was fixed with absolute methanol for 2 minutes. The slide was flooded with carbol fuchsin for 15 minutes and rinsed thoroughly with water and decolorized with 1% acid alcohol for 2 minutes after which it was rinsed with water. It was then counter stained with malachite green for 1 minute and rinsed with water. This was finally air dried and examined under the microscope using the 10x objective. To achieve better view 40x objectives using oil immersion were used. Where present, Cryptosporidium oocysts appear round and stain red against a green to purple background. Samples were considered positive if at least one morphologically distinct Cryptosporidium spp. oocyst was observed [23].

Statistical analysis

Data collated at the end of the study were subjected to statistical analysis using the version 15 Statistical package for Social Sciences (SPSS Inc, Chicago, IL) on windows 10. Prevalence rates were calculated and presented in percentages. Chi square test was used to compare differences in prevalence for variables under consideration. A logistic regression analysis was carried out to assess the occurrence of Cryptosporidium infection among ruminant species. Values at p<0.05 were considered significant.

Results

Out of the 900 ruminant animals examined, the overall prevalence of Cryptosporidium in all species was 19.8% (178/9000). Prevalence in cattle was 28.0% (98/350) while the proportion in Goats and Sheep were 17.7% (46/260) and 11.7% (34/290) respectively. Distribution of infection in cattle for the two study communities was significant (P=0.004), but infections in Sheep and Goats was comparable for the communities (Table 1).

Table1: Logistic regression analysis of species level occurrence of Cryptosporidium

DOI: 10.9790/2380-081213944 www.iosrjournals.org 40 | Page
Epidemiology of Cryptosporidiosis in Ruminant Species in Kebbi State, Nigeria

The occurrence of Cryptosporidium among the three species of study animals was compared using logistic regression analysis. A significant difference was observed among Cattle, Sheep and Goats. The likelihood of occurrence in Cattle was about three times more than its occurrence in Goats and Sheep (OR=2.510, 95% CI=1.612 – 3.909) whereas no significant difference was observed in Goats and Sheep (Table 2).

Table 2: Prevalence of Cryptosporidium in ruminant species in Aliero and Zuru, Kebbi State

<table>
<thead>
<tr>
<th>Animal species</th>
<th>Total Infection (%)</th>
<th>Aliero infected (%)</th>
<th>Zuru infected (%)</th>
<th>χ²</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sheep</td>
<td>34/290(11.7)</td>
<td>15/166(9.0)</td>
<td>19/124(15.3)</td>
<td>2.710</td>
<td>0.100</td>
</tr>
<tr>
<td>Goats</td>
<td>46/260(17.7)</td>
<td>20/114(17.5)</td>
<td>26/146(17.6)</td>
<td>0.003</td>
<td>0.956</td>
</tr>
<tr>
<td>Cattle</td>
<td>98/350(28.0)</td>
<td>44/200(22.0)</td>
<td>54/150(36.0)</td>
<td>8.333</td>
<td>0.004</td>
</tr>
</tbody>
</table>

Table 3: Relationship of age, sex and diarrhoea status with occurrence Cryptosporidium in Sheep at Aliero and Zuru (n= 290)

<table>
<thead>
<tr>
<th>Factor</th>
<th>Examined</th>
<th>Infected (%)</th>
<th>OR</th>
<th>95% CI</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Young</td>
<td>83</td>
<td>09(10.8)</td>
<td>0.514</td>
<td>0.376-0.704</td>
<td>0.0001</td>
</tr>
<tr>
<td>Adult</td>
<td>207</td>
<td>25(12.1)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diarrhoea Status</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>With diarrhoea</td>
<td>87</td>
<td>22(25.3)</td>
<td>4.318</td>
<td>3.205-5.817</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Without diarrhoea</td>
<td>203</td>
<td>12(5.9)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>152</td>
<td>14(9.2)</td>
<td>0.784</td>
<td>0.584-1.052</td>
<td>0.105</td>
</tr>
<tr>
<td>Female</td>
<td>138</td>
<td>20(14.5)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 3: Relationship of age, sex and diarrhoea status with occurrence Cryptosporidium in Sheep at Aliero and Zuru (n= 290)

The distribution of oocysts in goats is summaried in table 4. While infection in young goats was significantly higher (27.1%) than in adults (14.9%), occurrence of oocysts was significantly common in diarrhoecal than in non-diarrhoecal goats. Diarrhoeal goats were 3.432 prone to infection than the non-diarrhoeal (OR=3.432, 95% CI=1.744-6.755, P<0.0001). However, the distribution was not related to the sex of the animals.

Table 4: Relationship of age, sex and diarrhoea status with occurrence Cryptosporidium in Goats at Aliero and Zuru (n=260)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Examined</th>
<th>Infected (%)</th>
<th>OR</th>
<th>95% CI</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Young</td>
<td>59</td>
<td>16(27.1)</td>
<td>0.447</td>
<td>0.217-0.920</td>
<td>0.029</td>
</tr>
<tr>
<td>Adult</td>
<td>201</td>
<td>30(14.9)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diarrhoea Status</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>With Diarrhoea</td>
<td>69</td>
<td>22(31.9)</td>
<td>3.432</td>
<td>1.744-6.755</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Without Diarrhoea</td>
<td>191</td>
<td>24(12.0)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>130</td>
<td>24(18.5)</td>
<td>1.163</td>
<td>0.597-2.264</td>
<td>0.658</td>
</tr>
<tr>
<td>Female</td>
<td>130</td>
<td>22(16.9)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Comparism of prevalence in Cattle is presented in table 5. Occurrence of oocysts was significant in distribution (<0.0001). Infection was about two times higher in calves (42.9%) than in adult animals (22.9%).

DOI: 10.9790/2380-081213944 www.iosrjournals.org 41 | Page
Diarrhoeal animals shedded significantly higher percentage of oocysts than non-diarrhoeal animals (53.6% versus 18.2%) and were more than six times likely to be infected than healthy animals (OR=6.153, 95%CI=3.691-11.553, P<0.0001). Also, prevalence in cattle was observed to be sex dependent (p = 0.001). Female animals discharged more oocysts (36.9%) than males (20.7%).

Table 5: Relationship of Site, Age, Diarrhoea Status and Sex with occurrence Cryptosporidium in Cattle (n=350)

<table>
<thead>
<tr>
<th>Factor</th>
<th>Examined</th>
<th>Infected (%)</th>
<th>OR</th>
<th>95% CI</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Young</td>
<td>98</td>
<td>42(42.9)</td>
<td>0.246</td>
<td>0.138-0.439</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Adult</td>
<td>252</td>
<td>56(22.2)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diarrheal Status</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>With diarrhoea</td>
<td>97</td>
<td>52(53.6)</td>
<td>6.153</td>
<td>3.691-11.553</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Without diarrhoea</td>
<td>253</td>
<td>46(18.2)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>193</td>
<td>40(20.7)</td>
<td>0.515</td>
<td>0.305-0.871</td>
<td>0.013</td>
</tr>
<tr>
<td>Female</td>
<td>157</td>
<td>58(36.9)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

III. Discussion

Various epidemiological factors account for different levels of parasitic infections in different settings. Cryptosporidiosis is prevalent the world over but with variable levels of infection in different host categories and climatic environments.

Prevalence rates reported in Nigeria among cattle include; 23.4%, [24], 30.6% [3], 37.5% [25], 33.0% [26], 28.0% [27]. Others from other parts of the world include; 19.2% among calves in Zambia [28], 17.6% in central Ethiopia [29], 18.5% among cattle in Brazil [30], 24.0% among goat kids in Romania [31] and 24.5% among lambs in Australia [32]. These reports are consistent with the 28.0%, 17.7% and 11.7% prevalences in cattle, goats and Sheep observed in this study respectively. Other workers have reported higher prevalences of 35.6% in USA, 33.5% in Vietnam, 28.5% in Sri Lanka, 47.9% in Spain, 50% in Netherlands and 70% in USA by [33-38] respectively. The relatively high infection rate observed in this study may have resulted from the higher risk of infection reportedly possessed by young animals [21, 28, 32, 39, 40]. The undeveloped immune system and/or the husbandry practice on farms in Nigeria is suggested to facilitate neonatal transmission in which animals of all ages, are grazed together thereby increasing the infection rate [21, 41, 42].

Our result showed that there was no significant (>0.05) difference between the rate of infection in sheep at Aliero and those at Zuru (P=0.004), while the infection rate between goats at Aliero and Zuru was also not significant in distribution (0.100). However infection amongst cattle in Zuru was significantly higher than in those at Aliero (p=0.004). This finding corroborates a previous report from Iran where significant differences in infection were observed in sheep in six ecological zones [43].

In this study, species-specific prevalence of Cryptosporidium was higher in cattle (28.0%) than in Sheep (11.7%) and Goats (17.7%) which is in consonance with the submissions of [9, 44, 45], who noted that infection is commonly reported in calves than other ruminants, for which reason they have received extensive attention. This study reveals that infection was almost thrice likely in cattle than in sheep and goats (OR=2.510, P=0.0001, CI=1.612 – 3.909). The higher prevalence observed in cattle could be attributed to the intensive or overcrowded management system, where animals of all ages are housed together bringing infected animals together with the healthy ones including young animals whose immune level is low. Animals raised under such confinement will be susceptible to infection due to ease of oocyst contamination and transmission [36, 45, 46]. Also, the fact that Goats or sheep are not always kept together in such restriction, gives them an advantage of adequate space especially at nights resulting in minimized infection rate among small ruminants.

Several works have indicated that cryptosporidiosis is significantly associated with neonates than adult animals [21, 28, 31, 32, 36, 39, 40, 47, 48]. But on the contrary, distribution of infection between young and adult sheep in this study was not age related (P=0.100) suggesting a possible interplay of other exposure risks. This trend was also observed by [46] when they reported that there was no significant difference in infection between young and older calves.

Another feature of this study, is the significant difference in infection between male and female cattle (P=0.001). Prevalence was higher in female cattle than in males. The reason for the disparity is not well understood, though this might have resulted from the practice by farmers to retain more females than males for the advantage of breeding and milk production.

The significantly higher rates in diarrhoeic animals recorded in this study, is in line with the submissions of [10, 25, 49] reiterating the presence of diarrhoea in young animals as a significant source of oocyst contamination of the environment. Also the environment, management practices, genetics, physiology
and immune status of the animals might have contributed to such outcome. How these factors work is poorly understood and require elucidation.

It has been suggested that the pathogenesis of Cryptosporidium infection alongside other enteric infections, such as Rotavirus, Salmonella, Escherichia coli, Eimeria, etc are very likely to result in such diarrhoeic condition [50,51]. Characteristic diarrhoea is thought to result from maldigestion and malabsorption due to reduction in both enzymatic action and absorptive area in the gastrointestinal tract owing to diminution of microvilli and destruction of intestinal epithelia by Cryptosporidium. An increase in Paracellular permeability of the intestinal tract and destruction of the functional mucosal barrier system are both as a result of the damage caused by the parasite [52]. In their report, [53] had explained that C. parvum infection in calves have shown that jejunum and ileum is mainly affected and the diarrhoea occurs either due to hindrance in sodium absorption coupled with increased prostaglandin production in the intestinal mucosa or owing to increased permeability.

Furthermore, the influence of seasonal variation is likely to affect the incidence of diarrhoea in domestic ruminants. For instance, clean grazing pastures and environments are difficult to maintain by animal owners in Nigeria due to the complex nature of fecal contamination of the environment. There is the possibility of fecal contamination, when rainwater transport Cryptosporidium oocysts from faecal deposition in the environment and then into the surface water sources where animals drink freely. This could be one of the factors influencing the numerical increase of oocysts in the environment. The result of which is an increase in the spread of microbes and gastrointestinal parasites associated with diarrhoea.

This study showed that Cryptosporidium infection is important in ruminants in Nigeria, particularly calves with female animals at higher risk of infection. The study also underscores the significance of diarrhoea as a key factor in the spread of infection. Thus, given these findings, concerted efforts should be directed towards improving our management systems and diagnosis, in order to ensure healthy production of ruminants and reduce possible zoonotic transmission of the parasite in this and similar settings in Nigeria.

Conflicts of interest: None declared by the authors

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