

Prevalence of *Cryptosporidium* species copro-antigens in piglets in Kafanchan, Kaduna State, Nigeria

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ABSTRACT: Cryptosporidiosis is a zoonotic disease caused by the protozoan parasite *Cryptosporidium* spp. A cross-sectional study was conducted to determine the prevalence of *Cryptosporidium* spp. copro-antigens in piglets from Kafanchan, Kaduna State, Nigeria. A total of 185 faecal samples from piglets were collected and tested using a commercial kit (*Copro-Enzyme Linked Immunosorbent Assay*TM). An overall prevalence of 16.8% (31/185) was recorded from the study. The prevalence was relatively higher in piglets of age 5 to 6 weeks (17.8%), than age 0 to 4 weeks (15.4%), in male (17.7%) than in female (15.9%), in Land white breed (17.5%) than in Land race breed (12.0%), in piglets managed under semi-intensive (18.7%), than those managed under intensive system (10.9%). There were no statistical significant associations ($p > 0.05$) among the various parameters investigated, however, piglets with diarrhea had higher (34.5%) rate of infection than those without (13.5%) diarrhea and the association was statistically significant (OR = 3.383; 95% CI on OR: 1.385 < OR < 8.265, $p = 0.007$). Among the investigated factors, presence of diarrhea was shown to be a significant factor in the spread of *Cryptosporidium* infection in piglets. It may be concluded that such a symptom needs to be routinely investigated under the production systems practiced in the study area in view of the economic and public health importance.

Keywords: Copro-antigen, copro-ELISA, piglets, prevalence, Kafanchan.

INTRODUCTION

Cryptosporidium is an intracellular, extracytoplasmic protozoan parasite which invades the micro villous border of the gastrointestinal epithelium, causing cytopathic effect (Kuehn, 2017), thereby resulting in diarrhoea, dehydration, growth retardation and mortality in livestock (Taylor et al., 2007; Brar et al., 2017). *Cryptosporidium* infection occurs worldwide, infecting humans and various animals. This leads to self-limiting illness in immunocompetent persons to severe enteritis that could result to fatality in immunocompromised patients and neonates (Wilhelm and Yarovsky, 2014).

Various studies have identified several *Cryptosporidium* species in pigs (Yatswako et al., 2007; Maikai et al., 2009; Budu-Amoako et al., 2012; Zhang et al., 2013; Yui et al., 2014). Recent genetic characterization studies revealed

that pigs are infected with a genetically distinct and apparently host-adapted species of *Cryptosporidium* (*Cryptosporidium* "pig" genotype II) and *C. suis*. They can also be naturally infected with *C. muris*, *Cryptosporidium* mouse genotype I and the zoonotic *C. parvum* (Budu-Amoako et al., 2012).

Porcine cryptosporidiosis has been reported to occur worldwide (Alaa et al., 2015; Brar et al., 2016). Higher prevalence of *Cryptosporidium* infection in pigs has been reported in Spain (21.9%) and Trinidad (19.6%) with asymptomatic infections in most of the pigs and higher rates in 1 to 2 months old pigs (Kaminjolo et al., 1993; Quilez et al., 1996). Cryptosporidiosis has been implicated in diarrheal illness in children and immune-compromised individuals and it is increasingly becoming a major public

health threat as an opportunistic infection in immune-suppressed and immunocompromised individuals, especially in HIV/AIDS (Hunter and Nichols, 2002; Nyamwange, et al., 2012). Infections in HIV+ patients have been associated with low CD4+ cell counts (< 200 cells/mm³), lack of access to highly active antiretroviral therapy (HAART), and poor hygiene, while infections in children are associated mostly with young age and poor hygiene (Ayinmode et al., 2014).

The oocysts are source of infection for animals and humans (Singla et al., 2013) and transmission occurs by faecal-oral route (OIE, 2016).

Pig farming is a thriving business in the Southern part of Kaduna State, where Kafanchan is situated, and there is no religious or socio-cultural barrier to rearing of pigs compared to other parts of the State. Furthermore, because of the rapid turn-over rate in pig farming, most families are engaged in pig production to supplement their income. The aim of the study was to survey for *Cryptosporidium* spp. copro-antigen in piglets in Kafanchan, Kaduna State, Nigeria. This will give an insight into the prevalence of the copro-antigen in pigs and its public health importance which will be useful in creating awareness on the preventive and control measures that will disrupt the transmission cycle between animals and humans in the study area.

MATERIALS AND METHODS

Study area

The survey was conducted in Kafanchan (Figure 1), the headquarters of Jema'a Local Government Area of Kaduna State, Nigeria. It is located on the latitude 9° 34' N and longitude 8° 17' E. The highest average air temperature occurs in April (28.9°C) and the lowest in December (22.9°C) through January (23.1°C). The mean atmospheric relative humidity ranges between 70 to 90% and 25 to 30% for the rainy and dry seasons respectively. The annual average rainfall in the Southern part of the State (Kafanchan) is about 1733mm (Abaje et al., 2016). It has a population of 6,113,503 (NPC, 2006).

Sample size

Sample size was determined using the formula by Thrusfield (1997) at 95% confidence interval as follows:

$$N = \frac{Z^2 pq}{d^2}$$

Where: N = Sample size, Z = Standard deviation at 95% confidence interval (1.96), P = prevalence 13.6% (Maikai et al., 2009) = 0.136, q = 1-p, d = allowable error (0.05) and N = 180.56.

A total of 185 faecal samples were collected.

Study design

A cross-sectional study was employed. Kafanchan has 13 residential areas where pigs are kept: Ungwan Galadima, Adwan, Ungwan Binzom, Ungwan Musa, Ungwan Yashiyi, Takau, Bayan Loco, Unguwan VIO, Zakwa, Zauru, Katsit and General Hospital area.

Sample collection

A total of 185 faecal samples of piglets were collected, 14 each from Adwan, Ungwan Musa, Ungwan Yashiyi, Takau, Bayan Loco, Unguwan VIO, Zakwa, Zauru and Katsit and 15 each from Ungwan Galadima, Ungwan Mission and General Hospital area which has more pigs when compared to the other 10 areas. Convenient sampling method was employed for the selection of households that rear pigs which was based on the availability of the animal and willingness of the owners to participate in the study. Sampling was done between September to November 2016. Fresh faecal samples were collected from each animal using a disposable hand gloves and emptied into a sterile, airtight, plastic tube. Samples were transported in icebox to the Parasitic Zoonoses Laboratory of the Department of Veterinary Public Health and Preventive Medicine, Ahmadu Bello University, Zaria for processing.

Questionnaire administration

Questionnaire was administered to pig owners during sample collection. Data relating to collection site such as management practice, age, sex, breed and diarrheal status of the piglets were obtained.

Detection of *Cryptosporidium* spp. Copro-antigen using Copro-Enzyme Linked Immunosorbent Assay (Copro-ELISA) kit (Savyon™ Diagnostics Inc., Israel)

The faecal samples were examined for the presence of *Cryptosporidium* species copro-antigens by ELISA using a commercial kit (Copro-Enzyme Linked Immunosorbent Assay™ for detection of *Cryptosporidium* antigen in faeces, manufactured by Savyon® Diagnostics Limited, Israel). The test kit contains a 96-well micro titration plate coated with specific polyclonal antibodies directed against *Cryptosporidium* spp. antigen. The faecal sample to be tested was diluted in stool diluent and incubated with the pre-coated plate. After which *Cryptosporidium* spp antigen were bound to the immobilized antibodies. Non-specific antigens were removed by washing. Anti-*Cryptosporidium* monoclonal antibody conjugated to horseradish peroxidase (HRP) was added and incubated. This step

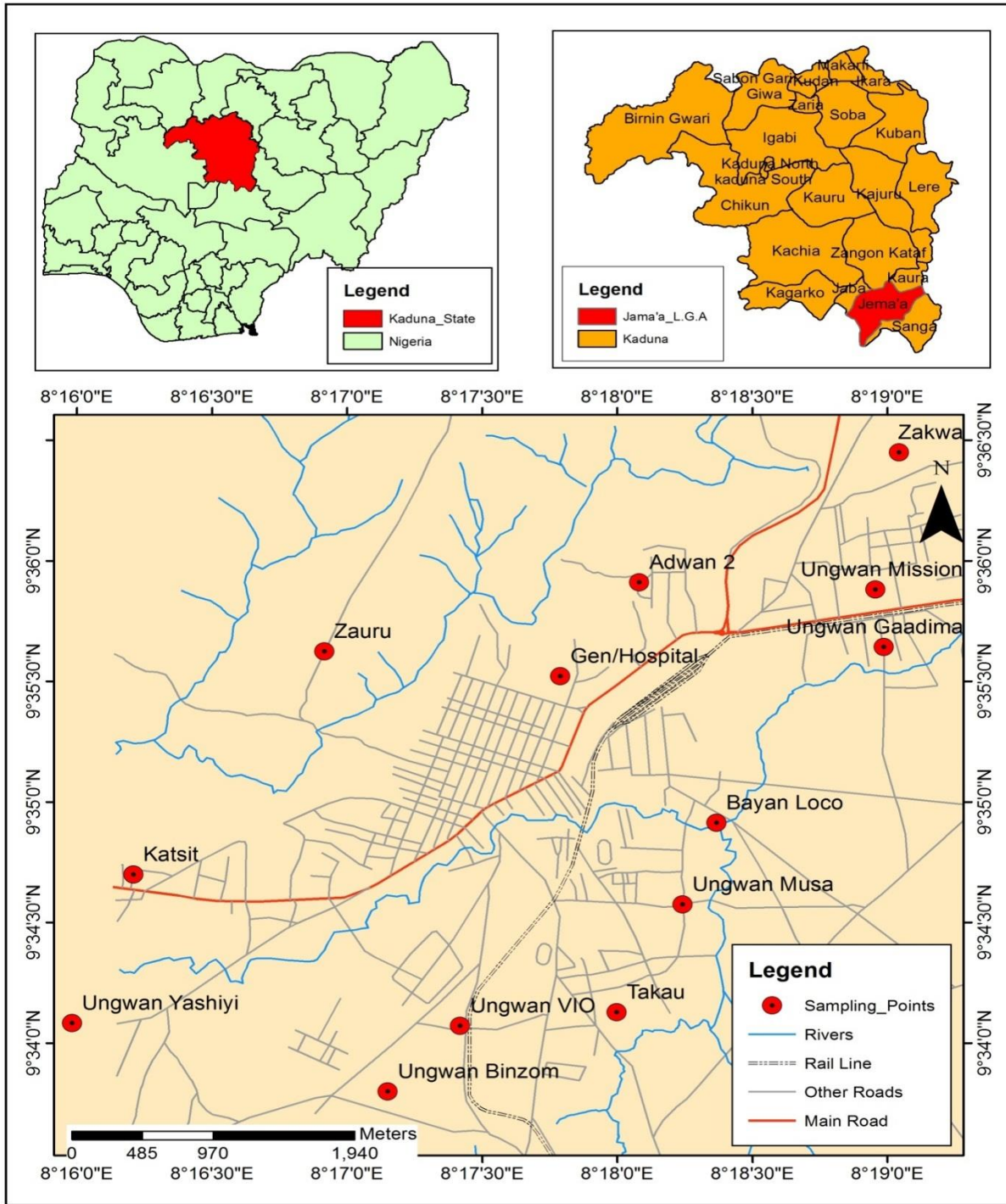


Figure 1. Map of Kafanchan showing sampling point. Source: Modified from street map of Kafanchan Town.

involves the binding of HRP-conjugate to the pre-bound antigen-antibody complex. Unbound conjugate was removed by washing. Upon the addition of TMB-substrate, the substrate was hydrolyzed by the peroxidase, yielding a blue colouration of the reduced substrate. When stop solution was added, colour changed from blue to yellow after which reading was taken by an ELISA reader at a

wavelength of 450 nm. The absorbance was proportional to the number of *Cryptosporidium* species cells sensitized by specific antibodies for an antigenic determinant of *Cryptosporidium* organism. Absorbance value of ≥ 0.3 Optical Density (OD) units indicated that the sample is positive therefore contained *Cryptosporidium* antigen while an absorbance value of < 0.3 units indicated that the

Table 1. Association between the prevalence of *Cryptosporidium* spp. copro-antigen and demographic characteristics of piglets in Kafanchan, Kaduna State, Nigeria.

Factors	No. samples examined	Positive samples	Specific rate (%)	Odds ratio	95% CI on OR	P – value
Age group (weeks)						
0-4	78	12	15.4	1.188	0.539-2.617	0.670
5-8 ^{ref}	107	19	17.8	1		
Sex						
Male	97	17	17.7	1.123	0.518-2.438	0.769
Female ^{ref}	88	14	15.9	1		
Breed						
Large white	160	28	17.5	0.643	0.180-2.297	0.493
Land race ^{ref}	25	3	12.0	1		
Diarrhoea						
Present	29	10	34.5	3.383	1.385 - 8.265	0.007*
Absent ^{ref}	156	21	13.5	1		
Management system						
Intensive	46	5	10.9	1.887	0.679-5.241	0.217
Semi-intensive ^{ref}	139	26	18.7	1		

Reference standards (ref), CI = Confidence Interval, OR =Odds ratio, *p < 0.05 is significant.

sample did not contain detectable level of *Cryptosporidium* antigen and therefore negative.

Data analyses

Statistical Package for Social Sciences (SPSS, version 20.0) (SPSS Inc. Chicago IL, USA) was used to analyze the data obtained. Odds ratio and 95% confidence interval were used to test the association between the prevalence of *Cryptosporidium* spp. copro-antigen and general animal characteristics observed. Results were presented in table. Values of $p \leq 0.05$ were considered statistically significant.

RESULTS

Of the 185 piglets faecal samples examined, *Cryptosporidium* spp. copro-antigen was detected in 31 (16.8%). The prevalence was relatively higher in piglets of age 5 to 6 weeks (17.8%) than those between 0 to 4 weeks (15.4%), in male (17.7%) than in female (15.9%), in Land white breed (17.5%) than in Land race breed (12.0%), in piglets managed under semi-intensive (18.7%), than those managed under intensive system (10.9%), but there were no significant associations ($p > 0.05$) between the prevalence of *Cryptosporidium* spp. copro-antigen with age, sex, breed and management system of piglets in Kafanchan. A statistically significant ($p < 0.05$) association

was observed between the prevalence of *Cryptosporidium* spp. copro-antigen and diarrhea in piglets. Piglets with diarrhea had more than 3 times likelihood of harboring *Cryptosporidium* spp. copro-antigen than those without diarrhea (OR = 3.383; 95% CI on OR: 1.385 < OR < 8.265, P = 0.007) (Table 1).

DISCUSSION

The 16.8% prevalence rate of *Cryptosporidium* spp. copro-antigen in piglets recorded in this study by ELISA was similar to 15.7% and 13% previously reported among pigs from Nigeria by Kwaga et al. (1988) and Maikai et al. (2009) respectively. The prevalence was relatively higher than the 10.5% recorded in pigs in Korea (Jae-Ran and Min, 2004). However, it is lower compared to 26.8% in pigs and 46.5% in piglets reported by Akinkuotu and Fagbemi (2014a) and (2014b) respectively. These differences reported by various authors may be attributed to differences in stocking rate and husbandry systems practiced in the different areas where the researches were carried out. It may also be due to differences in the level of environmental contamination with *Cryptosporidium*. The discrepancy between the sensitivity of the diagnostic tests used might also be the cause of this variation (Geurden et al., 2006). The higher detection rate in male piglets than in females in this study is similar to the report of Olabanji et al. (2016). This study shows that *Cryptosporidium* spp.

antigens can be detected in both of the two breeds of piglets common in Kafanchan. The significant association observed between the prevalence of *Cryptosporidium* spp. copro-antigen and diarrhea in piglets is similar to the report of Maikai et al. (2009) and Akinkuotu and Fagbemi (2014a) in piglets and also Abare et al. (2019) in sheep. This may be because of the pathogenesis of the parasite since it mostly affects the gastrointestinal system of the host. This implies that, diarrhoeic piglets may contribute to the spread of the parasite in the study area. Although *Cryptosporidium* spread easily among animals managed under intensive system due to faecal-oral route of transmission (Maikai et al., 2009), the relative higher detection rate in piglets managed under semi-intensive system in this study may be because of the wide environmental contamination, and that animals may ingest the oocysts as they move from one point to another as previously observed by Ceballos et al. (2009). On the other hand, *Cryptosporidium* infection is widely reported among neonatal animals of many animal species (Ramirez et al., 2004), and may indicate the futility of husbandry efforts. However, the zoonotic potential of *Cryptosporidium* indicates the importance of piggery waste management and personal hygiene among pig farmers.

Conclusion

Cryptosporidium spp. copro-antigen was detected in 16.8% of the studied population. The infection was distributed irrespective of their age, sex, and breed and management system. Diarrhea was found to be the most significant factor in the spread of *Cryptosporidium* in the study area. Close monitoring of *Cryptosporidium* infection in animals such as piglets are required, since there can be zoonotic transmission of the infection which is not only of public health but also of great economic importance.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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