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Cryptosporidium infection in cattle in Ogun state, Nigeria

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Abstract

The prevalence of *Cryptosporidium* spp. in cattle faeces in Ogun state, Nigeria was determined by a commercially produced enzyme-linked immunosorbent assay kit. Out of a total of 200 samples, 37.5% were positive for *Cryptosporidium* coproantigens. The highest rate of infection (78.1%) was observed in calves up to 3 months of age while adult cattle over 4 years of age had the lowest rate of infection (25.0%). There were significant differences ($p<0.05$) between the infection rates of the different age groups of cattle sampled. There was however no significant difference ($p>0.05$) between the infection rates in males (41.2%) and females (33.6%). Furthermore, the infection rate in diarrhoeic cattle (43.2%) was not significantly higher ($p>0.05$) than in non-diarrhoeic cattle (32.4%). The result of the study showed that the prevalence of cryptosporidiosis is high in cattle in southwestern part of Nigeria with calves being at the highest risk.

Keywords: Cattle, *Cryptosporidium*, ELISA,Ogun state Nigeria.
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Introduction

Cryptosporidium is a coccidian parasite found infecting a wide range of mammals (including man), birds and lower vertebrates. Cryptosporidiosis is considered to be of socioeconomic and/or public health importance (OIE, 2004). The infection is self-limiting except in the immunodeficient hosts, such as those with the acquired immunodeficiency syndrome (AIDS) in humans, tuberculosis in cattle and neonates of various animal hosts (Current & Garcia, 1991) in which it may cause protracted diarrhoea.

It is now recognized that *Cryptosporidium* species differ principally in their host range (Adriana *et al*., 2010). While some *Cryptosporidium* species appear to be restricted to particular types of hosts, others have broad host range, including man and are therefore of zoonotic significance (Thompson *et al*., 2008). *Cryptosporidium bovis*, *C. ryanae* and *C. andersoni* are the commonly encountered species affecting cattle in Nigeria (Ayinmode & Fagbemi, 2010; Maikai *et al*., 2011). *Cryptosporidium parvum* is however recognized as an important agent in life-threatening neonatal diarrhoea in calves (Current & Garcia, 1991; Miron *et al*., 1991). Transmission of infection to humans and other mammals is thought to be caused by ingestion of the oocysts of *C. parvum* (Fayer *et al*., 2000). These oocysts are resistant to most common disinfectants and are not readily killed by routine chlorination of water supplies (LeChevallier *et al*., 1991).

Various staining methods have been used in different studies to identify *Cryptosporidium* spp. (Kaur *et al*., 2002; Mahdi & Ali, 2004; Hamedi *et al*., 2005). However, some studies, demonstrated that enzyme-linked immunosorbent assay (ELISA) had higher sensitivity and specificity than the staining methods (Kehl *et al*., 1995; Graczk *et al*., 1996; El-Shazly *et al*., 2002).

In Nigeria, the few available reports on bovine cryptosporidiosis were based on microscopic detection of the oocysts on acid-fast stained faecal smears with reports of prevalence rates of 23.4% in Oyo State (Ayinmode & Fagbemi, 2010), 28.0% in Plateau State (Pam *et al.*, 2013) and 33.0% in Sokoto State (Faleke *et al*., 2014). Fewer reports on the use of immunological methods of detection (especially

ELISA) of *Cryptosporidium* in cattle have been documented (Ayinmode & Fagbemi, 2011) with no published report on Ogun state, southwestern Nigeria. This study therefore was carried out to determine the prevalence of *Cryptosporidium* spp. in cattle in Ogun State using a commercially available *Cryptosporidium*-specific ELISA kit.

Materials and methods

Study area

The study was carried out in Ogun state, southwestern Nigeria. Samples were collected from four cattle farms and two major abattoirs in the four geopolitical zones (Egba, Yewa, Remo and Ijebu) of the state.

Sample collection: A total of 200 faecal samples were collected from different cattle. The cattle were classified on the basis of their age range: 1 day-3 months, >3 months-1 year, >1year- 4 years and > 4years.

A single faecal sample was taken from the rectum of each animal with a disposable rubber hand glove and emptied into a universal sample bottle and labeled appropriately. The samples were thereafter transported, in cold packs, to the laboratory where ELISA was performed on them. If analysis was not to be carried out immediately, the samples were preserved at 4^{o}C for a maximum of 24 hours within which the ELISA was carried out.

Detection of Cryptosporidium antigens by ELISA

The detection of *Cryptosporidium* spp. coproantigens in the samples was done using a commercially available ELISA kit for faecal samples (*Cryptosporidium* (faecal) ELISA kit, Diagnostic Automation Inc., Canada). The procedure carried out was according to manufacturer's instruction.

Briefly, 0.1g of each faecal sample was homogenized in 0.3ml of sample dilution buffer and centrifuged. 100µl of the resulting supernatant was dropped into allocated wells in the microtitre plate and incubated at room temperature for 60 minutes. The plate was thereafter washed with the wash buffer. 100µl of the conjugate was added to each well, incubated for 30 minutes and washed. 100µl of the chromogen was then added to each well and incubated at room temperature for 10 minutes. The reaction was stopped by adding 50µl of stop solution to each well and read using the ELISA reader (BIOTEX; Model: ELx800, Biotex Instruments, USA) at 450nm.

Samples with OD higher than 0.15 were reported as positive while those with OD lesser than 0.15 were reported as negative for *Cryptosporidium* coproantigens.

Statistical analysis

Chi-square test was used to compare the differences in prevalence of *Cryptosporidium* sp. coproantigens between the age groups, sexes and stool consistencies of cattle at 5% level of significance. The analysis was done using the Statistical Package for Social Sciences (SPSS).

Results

Overall prevalence of Cryptosporidium spp.

The enzyme immunoassay showed that 37.5% (75/200) of the samples analyzed were positive for *Cryptosporidium* coproantigens.

Prevalence of Cryptosporidium coproantigens in relation to age of cattle

The highest rate of infection, 78.1% was observed in suckling calves up to 3 months of age. The rate of infection decreased with increasing age of cattle with the lowest rate, 25.0%, being recorded in adult cattle above 4 years of age (Table 1). The infection rate was significantly higher ($p<0.05$) in suckling calves than those above 3 months to 1 year ($p<0.001$; Odds Ratio (OR)=2.15), young adults up to 4 years ($p<0.001$; OR=2.67) and adults above 4 years of age ($p<0.001$; OR=3.12).

Prevalence of Cryptosporidium coproantigens in relation to sex of cattle

Both sexes of cattle were infected with *Cryptosporidium* spp. with males having a higher rate of infection (41.2%) than females (33.6%). However, there was no significant difference ($p>0.05$) between both sexes in their rates of infection (Table 2).

Prevalence of Cryptosporidium coproantigens in relation to stool consistency of cattle

Both symptomatic (diarrhoeic) and asymptomatic (non-diarrhoeic) cattle were positive for *Cryptosporidium* coproantigens. No significant difference ($p>0.05$) was observed between the prevalence of *Cryptosporidium* coproantigens between diarrhoeic (43.2%) and non-diarrhoeic ones (32.4%) (Table 2).

Table 1: Comparison of the infection rates of *Cryptosporidium* coproantigens in different ages of cattle

Age group	Number of cattle (infected/sampled)	Percentage infected	Odds Ratio	p-value
1 day-3 months	25/32	78.1	2.15	<0.001
>3 months-1 year	16/44	36.4		
1 day-3 months	25/32	78.1	2.67	<0.001
>1 year-4 years	21/72	29.2		
1 day-3 months	25/32	78.1	3.12	<0.001
>4years	13/52	25.0		

Table 2: Comparison of the infection rates of *Cryptosporidium* coproantigens between the sexes and stool consistencies of cattle

Parameters		Number of cattle (infected/sampled)	Percentage infected	Odd Ratio (OR)	P-value
Sex	Male	42/102	41.2	1.23	0.199
	Female	33/98	33.6		
Stool consistency	Diarrhoeic	41/95	43.2	1.33	0.117
	Non- diarrhoeic	34/105	32.4		

Discussion

The overall prevalence (37.5%) of *Cryptosporidium* coproantigens observed in this study is consistent with the 32.3% prevalence in Oyo state, Nigeria reported by Ayinmode & Fagbemi (2011) and higher than the reports in other parts of the world: 11% of calves in Sweden (Bjorkman *et al*., 2003), 14% of cows in Denmark (Maddox-Hyttel *et al*., 2006) and 19.7% in Tanzania (Swai *et al*., 2007). This suggests that prevalence of Cryptosporidium is high in cattle reared in southwestern Nigeria. This observation may be associated with the high rainfall and relative humidity characteristic of the southwestern part of the country which favours the survival of the oocysts in the environment.

The observation that suckling calves had a higher rate of infection in this study supports the reports of Fayer *et al*. (1998), Lefay *et al*. (2000), Maddox-Hyttel *et al*, (2006) and Ayinmode & Fagbemi, (2011) who observed that the prevalence of *Cryptosporidium* spp. in pre-weaned calves is usually high. This may be attributed to higher susceptibility of calves to infection due to lack of previous exposure to the parasite. It may also suggest that the management practices by pastoralists in Nigeria, where calves are grazed with the adults, may increase their risk of infection by consumption of contaminated feed and water by the calves (Ayinmode & Fagbemi, 2010). An acquired immunity towards *Cryptosporidium* in adults due to infections in early life (Harp *et al*., 1990) may account for the low incidence of clinical condition and thus the low shedding of *Cryptosporidium* oocysts in this age group. The high rate of infection in calves may also be of zoonotic concern as calves have been reported to be important reservoirs of the infection (Barrington *et al*., 2002; Mallon *et al*., 2003a; Mallon *et al*., 2003b; Santin *et al*., 2004; Fayer *et al*., 2007; Xiao, 2010), serving as important source of contamination of water with *C. parvum* oocysts (Xiao, 2010).

The high rate of infection observed in male cattle is in tandem with reports of Maikai *et al*. (2011) but contrary to the findings of Ayinmode & Fagbemi (2010). The reason for this observation is not known. Further research is required to elucidate this and other possible reasons for the differences in infection rates between the sexes of cattle.

The lack of significant association between diarrhoea and presence of *Cryptosporidium* coproantigens observed in this study supports a similar observation by Ayinmode & Fagbemi (2010). This may be attributed to subclinical chronic phases of infection with the parasite which may not have the clinical sign of diarrhoea in the affected cattle (Ralston *et al*., 2003; Olson *et al*., 2004; Fayer *et al*., 2006).

The ELISA employed in this study, although reported to be highly sensitive in the detection of *Cryptosporidium* spp. (El-Shazly *et al*., 2002), have the risk of producing false positive results. It is therefore important to utilize molecular techniques to detect and characterize the *Cryptosporidium* spp. found in cattle in the study area as this method has

been reported to possess very high sensitivity and specificity when compared to the ELISA and microscopy (Ayinmode & Fagbemi, 2011). This study thus indicates that cryptosporidiosis is common in cattle in the southwestern part of Nigeria with suckling calves being at a higher risk of infection than older ones.

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CASE REPORT

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Diagnosis and management of sand impaction of the large intestine in an Alsatian puppy

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Abstract

A four-month-old, male German Shepherd puppy previously (six weeks ago) managed for babesiosis and toxocariasis was re-presented with complaints of weakness and poor growth. Physical examination revealed body weakness, emaciation, rough hair coat and dried muzzle. Empty rectum and caudal abdominal pain with crepitating hard masses were felt on palpation. Plane lateral thoraco-abdominal radiograph revealed gas-trapped (impacted) radio-opaque masses in the transverse and descending colon. Oral administration of vegetable oil (total of 300ml) given to effect was employed to soften and evacuate bowel content (within 3 hours of treatment). Radiographic examination 14 hours post management revealed a completely empty bowel. Compounded cereal paste-like diet was given for 5 days and the puppy was discharged 6 days after presentation.

Keywords: Colon, Sand impaction, Diagnosis, Management, Puppy
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Introduction

Sand impaction may be regarded as a focal accumulation of sand in the intestinal tract resulting in the dilatation and mechanical obstruction of the impacted intestinal segment, and it is an uncommon condition in dogs (Moles *et al*., 2010). Ingestion of materials other than normal food (called allotriophagia or pica) varies from licking to actual eating and drinking, and it is due in most cases to dietary deficiency, either of bulk or of individual nutrients such as cobalt, phosphorus, etc (Radostits *et al*., 1994). Allotriophagia is considered to be a normal behavior in rabbits and foals (Radostits *et al*., 1994). Chronic abdominal pain or gastritis and central nervous system disturbances are often accompanied by pica in animals (Radostits *et al*., 1994). Pica may have serious consequences: poisoning, foreign body lodgement in the alimentary tract, accumulations of sand which may cause obstruction, perforation of the oesophagus, stomach or intestine may result from ingestion of sharp and/or coarse foreign bodies and feeding time is often reduced (Radostits *et al*., 1994).The possible or common causes of intestinal blockage due to foreign objects swallowed by dogs are bones, stones, marbles and buttons (Sherding & Johnson, 1994; Anon., 2012a), while medical conditions causing intestinal obstruction include tumor, hernias, intussusceptions and mesenteric torsion (Anon., 2012b). Diagnosis of allotriophagia involves general history, physical examination (with emphasis on abdominal palpation) and laboratory (ultrasonography, radiography, complete blood cell count and serum biochemistry) evaluations of the patient (Sherding & Johnson, 1994). Both medical and surgical managements of intestinal sand impaction in dogs can be effective and often have a good prognosis with the medical approach being the primary treatment but, surgical intervention can be employed when necessary (Moles *et al*., 2010).

Case report

Case history of the puppy

A four-month-old male German Shepherd puppy weighing 4.5 Kg was re-presented to the Small Animal Unit of the Veterinary Teaching Hospital, Ahmadu Bello University, Zaria with complaints of weakness and poor growth. The puppy had been presented six weeks earlier, with complaints of inappetance and weakness. It was then managed for babesiosis, where blood was transfused and intramuscular injection of diminazene aceturate (Diminazene(R) - Vetinda Pharmaceuticals Ltd, India) was administered at 4mg/Kg; and dewormed with pyrantel pamoate (Pyranthrin(R) - Niemath International Pharmaceuticals plc, Oregun, Lagos, Nigeria) at 10mg/kg for toxocariosis. The puppy had been vaccinated against canine distemper, hepatitis, leptospirosis, parvo virus and para-influenza (DHLPP: Biocan(R) - Bioveta, a. s. Czech Republic). The vaccine was administered on second presentation (2 weeks prior to this presentation).

Physical examination

The body temperature was 38.9^0 C, pulse and respiratory rates were 140 beats/min. and 40 cycles/min. respectively. Weakness, rough hair coat, dried muzzle and emaciation were observed. Ventral retroperitoneal to pelvic abdominal palpation elicited pain in the colon which contained a hard crepitating mass and the rectum was empty.

Laboratory Investigation

A number of investigations involving: ultrasonographic and radiographic examinations; whole blood and serum for complete blood count and serum chemistry respectively, were conducted to ascertain what went wrong (diagnosis) with the puppy. The results of the investigations were:

Ultrasonographic and radiographic examinations: - Transabdominal ultrasonographic scan revealed normal abdominal organs, except for multilayered hyperechoic structure at the caudal abdominal region. Lateral thoraco-abdominal radiograph revealed gas-trapped radio-opaque substances impacted in the transverse and descending colons with empty rectum (Plate I).

Clinical pathology result - Complete blood count revealed severe anaemia (Table 1); and the blood chemistry showed azotaemia, hypophosphataemia and decrease in the serum bicarbonate (Table 2).

Table 1: Haematology results

Parameter	Patient's values	Reference values *
Packed cell volume (%)	20	37-55
White blood cells count (10^9/L)	8.0	6-17
Neutrophils (10^9/L)	4.2	3-12
Lymphocytes (10^9/L)	3.36	1-5
Monocytes (10^9/L)	0.24	0.2-1.5
Eosinophils (10^9/L)	0.16	0.1-0.8
Basophils (10^9/L)	0.0	Rare

*Source: Sastry, 1983

Table 2: Results of Serum chemistry and enzymes

Parameters	**Patient's Values**	**Reference Values**
Urea (mmol/L)	10.00	*6-25
Creatinine (mmol/L)	153.00	*44-138
Sodium (mmol/L)	141.00	#140-155
Potassium (mmol/L)	4.80	#3.6-5.8
Chloride (mmol/l)	101.00	#100-120
Bicarbonate (mmol/L)	12.00	#17-24
Calcium (mmol/L)	2.21	#2.0-3.0
Phosphorus (mmol/L)	0.80	*1.0-2.0
Glucose (mmol/L)	5.80	*3.9-7.8
Uric acid (mmol/L)	0.2	*0.2-0.9
Aspartate aminotransferase (mmol/L)	13	*5-55
Alanine aminotransferase (mmol/L)	4	*5-107
Alkaline phosphatase (mmol/L)	57	*10-150

Source: * Anon, 2014
Awasum, 2010

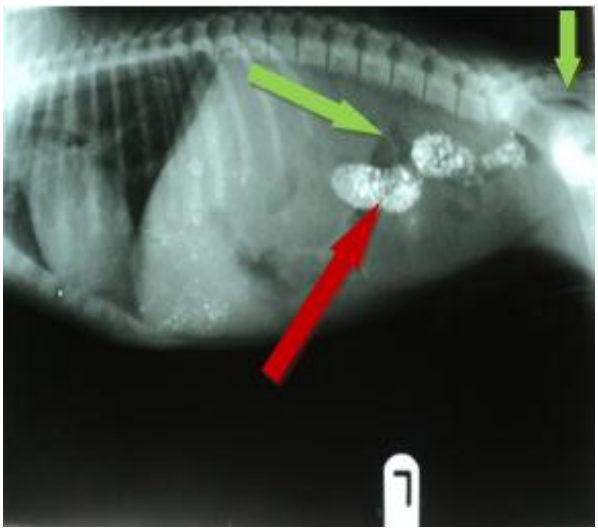

Plate I: Plane lateral radiography of thorax and abdomen showing radio-opaque substance impacted in the colon (red arrow) with gas wrapped in an empty rectum (green arrows)

Plate II: Drenching the puppy with vegetable (groundnut) oil

Plate III: The impacted sand particles voided

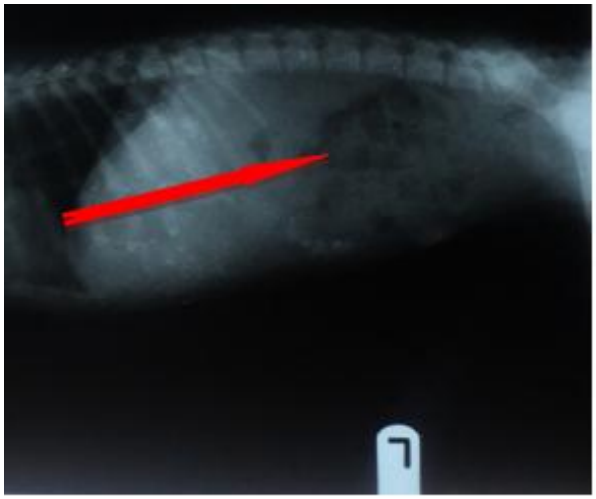

Plate IV: Lateral thoraco-abdominal plane radiograph showing clear intestinal passage to the rectum (arrowed) 14 hours after expulsion of sand particles

Case management

Three hundred milliliters of vegetable (groundnut) oil were administered orally to effect, over a period of 10 minutes (Plate II). The puppy was then walked a distance of 100 meters within 10 minutes after which it was allowed to rest and observed closely in the intensive care unit of the outpatient department of the Veterinary Teaching Hospital. Three hours later, the drenched vegetable oil began to be voided through the anus, followed by the impacted sand particles (50 g) in a projectile form (Plate III). Lateral thoraco-abdominal radiograph was then taken 14 hours after expulsion of the impacting sand particles (Plate IV). The puppy was placed on compounded paste-like diet (a paste composed of water, grounded millet and guinea corn, sugar and fried fish) for 5 days with 2.5% ciprofloxacin (CIPCON$^{(R)}$ – Concept Pharmaceuticals Limited, Mumbai India) oral suspension at 7mg/kg x $^{5}/_{7}$ and multivitamins (Vetzyme$^{(R)}$ – Bob martin (UK) Ltd, Wemberham Lane Yatton, North Somereset, UK) at 1400mg x $^{1}/_{12}$ p.o.

Discussion

The occurrence of large intestinal sand impaction in dogs generally is very rare worldwide (Moles *et al*., 2010). The condition is the first encounter in our practice and from the literature search there is no available information of such report, except that on eight (experimental) dogs with small intestinal sand impaction (Moles *et al*., 2010) managed surgically. The suggestive predisposing factor in this case that led to the puppy consuming sand particles is attributable to the previous medical conditions (babesiosis and toxocariosis) that caused anemia (that could have led to azotaemia) and metabolic acidosis because of anaerobic glycolysis following haemolysis, thereby increasing cellular production of hydrogen ion- H^+; this results in loss of bicarbonate leading to reduction in the buffering capacity of the body and thus allowed H^+ to accumulate); and mineral deficiency (hypophosphataemia due to prolonged inappetance) (Steven & Michael, 2008). Complete evaluation (diagnostic techniques) was employed to ascertain the cause of the condition. Depending on the patient's condition, medical management generally involves the use of intravenous fluid therapy, gastrointestinal protectants, analgesics and a combination of laxatives and cathartics with frequent radiographic monitoring (Anon., 2012c). The vegetable (groundnut) oil administered was to act as gastrointestinal protectant, laxative and cathartic; the exercise (walking) was to aid relaxing the gastrointestinal tract for the oil to mix and flow together with the sand; the diet was a source of nutrient with little disturbance to the alimentary tract; ciprofloxacin was to take care of possible bacterial infection in the colon; and multivitamins was to supplement some minerals and vitamins.

In conclusion, the case reported here happens to be unique in puppies because, the available literatures attributed the occurrence of sand impaction is in adult dogs (if it does) and surgical intervention had been advocated (Sherding & Johnson, 1994).

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