# COMPARISON OF ENZYME-LINKED IMMUNOSOR-BENT ASSAY WITH ACID-FAST STAINING TO DETECT *CRYPTOSPORIDIUM* OF CATTLE FAECES

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## ABSTRACT

Cryptosporidiosis is a major cause of diarrhoea, weight loss and low productivity in various domestic animals. The acid-fast staining method used to detect the parasite in cattle may be responsible for the low prevalence rates recorded in previous studies in Nigeria. A comparison of the efficacy of an enzyme-linked immunosorbent assay (ELISA) and acid-fast staining techniques in detecting *Cryptosporidium* spp. in bovine faeces was carried out in this work. A total of 200 faecal samples were collected from cattle of different age groups by the use of a simple random sampling technique. The samples were analyzed microscopically using formalin-ethylacetate sedimentation method followed by modified Kinyoun's acid-fast staining technique. All the samples were later tested for *Cryptosporidium* coproantigens by the use of a commercially available ELISA test kit. Microscopy detected *Cryptosporidium* oocysts in 24.0% while ELISA detected the antigens in 37.5% of the samples. The ELISA, with a sensitivity and specificity of 72.9% and 73.7% respectively, had a significantly higher (p<0.05) rate of detection of *Cryptosporidium* spp. than microscopy with a sensitivity and specificity of 46.7% and 89.6% respectively. The ELISA is therefore a preferable method than microscopy for detection of *Cryptosporidium* in faecal specimens and will be useful in routine diagnosis and screening of large number of samples in epidemiological surveys.

**Keywords**: Acid-fast staining, Bovine, *Cryptosporidium*, ELISA.

## INTRODUCTION

*Cryptosporidium* species are very small intestinal protozoa of the phylum Ampicomplexa, infecting humans and animals globally (Angus, 1990). Infection with *Cryptosporid-* *ium* plays an important role in animal production as it can negatively influence growth, impair feed conversion and reduce milk production (Thompson *et al.*, 2005). The lack of strict host specificity and the ubiquitous

presence of the oocysts in the environment make *Cryptosporidium* an important zoonotic pathogen (Xiao *et al.*, 2004).

It has been established that bovine cryptosporidiosis is a major cause of diarrhoea in different age groups of cattle with neonates and immunocompromised animals being the most susceptible to the infection. Cattle also serves as important reservoir for human cryptosporidial infections (Xiao and Fayer, 2008; Ayinmode and Fagbemi, 2010). The common *Cryptosporidium* species infecting cattle include *C. parvum, C. andersoni, C. bovis* and *C. ryanae* (Fayer *et al.*, 2008; Ayinmode and Fagbemi, 2010).

Transmission of the parasite could be through water, food or by direct contact with infected animals or man (Fayer *et al.*, 2006). The severity of cryptosporidiosis is increased in neonates of most mammalian species (Fayer *et al.*, 2006).

Cryptosporidiosis was initially diagnosed in intestinal biopsy specimens by electron microscopy or with various stains (Janoff and Reller, 1987). Diagnosis is now largely coprologic, although not all routine techniques have been found to be effective. A conventional method of identification is the examination of faecal smears with acid-fast stains (Morgan *et al.*, 1998). Various staining methods have been used in various studies to detect *Cryptosporidium* species (Kaur *et al.*, 2002; Mahdi and Ali, 2004; Hamedi *et al.*, 2005; Seyrafian *et al.*, 2006). However, in some of the studies, it was determined that enzyme-linked immunosorbent assay (ELISA) was preferable to the staining methods in many ways (El-Shazly *et al.*, 2002; Yilmaz *et al.*, 2008). According to these researchers, detection of *Cryptosporidium* coproantigens by ELISA has a high sensitivity (70-100%) and specificity (70-100%) when compared to acid-fast staining. El-Shazly *et al.* (2002) stated that the Ziehl-Neelsen staining showed the lowest sensitivity in relation to ELISA and the Polymerase Chain Reaction (PCR) for diagnosis of *C. parvum*.

In Nigeria, studies on bovine cryptosporidiosis from various parts of the country were based on microscopic examination of stool samples (Ayeni *et al.*, 1985; Ayinmode and Fagbemi, 2010). Much fewer, however, are the studies that used ELISA (Ayinmode and Fagbemi, 2011). This study was therefore conducted to compare the efficacy of microscopy and ELISA methods in detecting *Cryptosporidium* spp. in stools of cattle in Ogun State, Nigeria.

## MATERIALS AND METHODS
### Study area and period
The study was carried out in Ogun state, southwestern Nigeria. Ogun state is located between latitude 6.2oN to 7.8oN and longitude 3.0oE to 5.0oE. The faecal samples were collected between August and November, 2010, during the wet season.

### Sample collection
Sampling was carried out by the use of a simple random technique. The samples were obtained from four different cattle farms and two major abattoirs in the study area. A total of 200 faecal samples were collected from cattle. 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 transported, in cold packs, to the laboratory where they were analyzed immediately. If analysis was not done immediately, the samples were preserved at 4oC until use.

### Detection of Cryptosporidium oocysts by microscopy
### Faecal sample concentration

This was achieved using the formalin-ethylacetate sedimentation method as previously described by Ayinmode and Fagbemi (2010) with few modifications. Briefly, 1g of solid faeces or 3ml of watery stool was washed in 8ml of 10% formalin and centrifuged at 650x g for 10 minutes. The supernatant was decanted, after which the sediment was re-suspended with 7ml of 10% formalin. 3ml of ethylacetate was thereafter added, the mixture vigorously shaken and allowed to stand for 3 minutes. This was then centrifuged at 650x g for 10 minutes and the supernatant discarded. A small portion of the sediment was evenly spread on a microscopic slide and air dried for acid-fast staining.

### Acid-fast staining

Modified Kinyoun's acid-fast staining method was carried out. Briefly, the faecal smears were fixed with absolute methanol for 1 minute after which they were flooded with Kinyoun's carbolfuscin for 15 minutes. The slides were then initially rinsed briefly with 50% ethanol and with distilled water. The smears were immediately decolorized by flooding them with 1% sulphuric acid for 1 minute and then rinsed with distilled water. Counterstaining of the smears was done by flooding the smears with 0.4% Malachite green for 1 minute and rinsing with distilled water. The smears were air dried and examined initially at x400 and then at x1000 magnification for confirmation of the oocyst morphology.

### Detection of Cryptosporidium antigens by ELISA

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

The optical densities (OD) of the samples were read at 450nm using an ELISA reader (Model: ELx800, Biotex Instruments, USA). Samples were analyzed using the manufacturer's cut-off calculations in the instruction manual.

### Statistical analysis

Data were analyzed on Statistical Package for Social Sciences (SPSS) on Windows 7. The Chi-squared test was used to compare the detection rates of the ELISA and microscopy at 5% level of significance and 95% confidence interval.

## RESULTS

The detection rate of *Cryptosporidium* in stools was significantly higher ($p < 0.05$) with ELISA, which detected the antigens in 37.5% (75/200) of stools sampled, when compared to the detection rate by microscopy, which detected *Cryptosporidium* oocysts in 24.0% (48/200) of the samples.

The sensitivity and specificity of the ELISA technique were 72.9% and 73.7% respectively. The positive predictive value (PPV) and the negative predictive value (NDV) of the ELISA were 46.7% and 89.6% respectively. The sensitivity and specificity of the microscopy were 46.7% and 89.6% respectively (Table1).

**Table 1: Comparable performance of ELISA and microscopy for the diagnosis of *Cryptosporidium* in cattle**

|  | Microscopy Positive | Microscopy Negative | Total (ELISA) | p-value | 95% Confidence Interval | |
|---|---|---|---|---|---|---|
|  |  |  |  |  | Lower | Upper |
| ELISA Positive | 35 | 40 | 75 | <0.001 | -0.488 | -0.265 |
| ELISA Negative | 13 | 112 | 125 |  |  |  |
| Total (Microscopy) | 48 | 152 | 200 |  |  |  |

ELISA technique:
Sensitivity: (35/48) x 100= 72.9%
Specificity: (112/152) x 100= 73.7%

Microscopy method:
Sensitivity: (35/75) x 100= 46.7%
Specificity: (112/125) x 100= 89.6%

# DISCUSSION

The significantly higher rate of detection of *Cryptosporidium* coproantigens and the higher sensitivity (72.9%) of the ELISA technique over microscopy observed in this study corroborates results from previous investigations which compared immunoassays with acid-fast staining in detection of *Cryptosporidium* spp. in stools (Harrington and Kama, 2002; Yilmaz *et al.*, 2008; Yvonne *et al.*, 2012).

As reported by Ungar (1990), false positive results in ELISA, as recorded in this study, could result from the ability of ELISA to detect products of disintegrated organisms or antigens from extracellular life cycle stage. The false negative results could also be as a result of inaccessible antigens or inability of the antibody to detect the antigen, possibly due to low numbers of oocysts in the sample. The reduced capacity of ELISA to detect low numbers of oocysts as well as presence of false positive test results have also been reported in studies involving human stools (Johnston *et al.*, 2003). The ELISA was repeated for samples in this study having false negative and false positive ELISA results.

It has been stated that the acid-fast staining method has a higher diagnostic value when compared to other microscopic methods. However, ELISA is preferred in most laboratories of developing countries due to its sensitivity, easy usage, fast application and scoring and easy standardization for determination of *Cryptosporidium* coproantigens in stool samples (Yilmaz *et al.*, 2008). It has also been suggested that ELISA should be carried out together with one of the staining methods to increase the accuracy of diagnosis (Godekmerdan *et al.*, 1999).

In our study, modified acid-fast staining method was used due to its easy application, the permanency of its preparations and its low cost. It is also the most commonly em-

ployed method for detection of *Cryptosporidium* in stools of various animals and man in most studies in Nigeria. The result of the present study indicates that rapid ELISA is preferred to microscopy and also has the advantage of minimizing reporting time and manpower hours.

## CONCLUSION

From this study, it is believed that *Cryptosporidium* antigen screening in stools by ELISA should be included in laboratories where no PCR application is possible for routine examination of bovine faeces. Various studies have established the possibility of zoonotic transmission of *C. parvum* from bovine stools, therefore, measures to prevent zoonosis, especially via water contamination, should be implemented.

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