Comparison of two Methods in the Detection of Cryptosporidium in Pigs in Ogun State, Nigeria

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Abstract

Two diagnostic methods, a modified Kinyoun?s acid-fast staining technique and an enzyme-linked immunosorbent assay (ELISA), for the detection of Cryptosporidium spp. in porcine faeces were compared regarding their sensitivities. Of the 209 faecal samples examined, Cryptosporidium spp. was detected significantly higher ($p<0.05$) by ELISA (31.1

Index terms— cryptosporidium, elisa, nigeria, pigs.

1 Introduction

ryptosporidium species are ubiquitous and infect a wide range of vertebrate hosts, including humans and various domestic animals (Wang et al., 2010) and they cause enteric infections and severe diarrhoea in these host species. Cryptosporidial infections in pigs were first described by Bergeland (1977) and Kennedy et al. (1977), and in contrast to the numerous studies on bovine cryptosporidiosis (Ibrahim et al., 2007;Xiao and Fayer, 2008 In some studies, it was determined that the sensitivity of the ELISA was higher than those of various staining

2 Materials and Methods

3 a) Study period and area

A total of 209 faecal samples were obtained from five piggeries and one slaughter slab in Ogun state, southwestern Nigeria. The collection of faecal samples was initiated in September, 2012 and ended in April, 2013.

4 b) Sample collection

Faecal samples were collected per rectum from individual pigs. For pigs in which rectal sampling was not possible, such as neonates, freshly voided faeces were collected by the use of wooden tongue depressors which were used to scoop up the superficial layer of faeces without contacting the floor. The faeces were then dropped into individual universal sample bottles and labeled appropriately. These were then transported, in cold packs, to the laboratory where analysis was carried out immediately. When analysis was delayed, the samples were stored at 4oC until they were processed. c) Detection of Cryptosporidium oocysts by microscopy Faecal sample concentration: This was achieved using the formalin-ethylacetate sedimentation method as previously carried out 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 resuspended 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.

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Year 2015 d) 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 carbolfuscin for 15

minutes. The slides were then rinsed briefly with distilled water. The smears were immediately decolorized by flooding them with 10% 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. e) Detection of Cryptosporidium parvum antigens by ELISA The detection of Cryptosporidium parvum coproantigens in the samples was done using a commercially available ELISA kit for faecal samples (RIDASCREEN® Cryptosporidium; R-Biopharm AG, Germany). 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.

6 III.

7 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.

IV.

8 Results

The detection rate of Cryptosporidium in the samples was significantly higher ($p<0.05$) with ELISA, which detected the coproantigens in 31.1% (65/209), when compared to the detection rate by microscopy, which detected Cryptosporidium oocysts in 16.3% (34/209) of the samples (Table 1).

The sensitivities of the ELISA and MZN techniques were 100% and 52.3% respectively (Table1). Sensitivity: a. ELISA: (34/34) X 100 = 100% b. Microscopy: (34/65) X 100 = 52.3%

While acid-fast staining of faecal smears may help identify Cryptosporidium oocysts, there is the need for experienced staff (Kuhnert-Paul et al., 2012). In contrast, ELISA, an antigen-based technique is easy to perform and its evaluation does not require considerable experience.

The higher sensitivity of the ELISA than the modified Kinyoun's acid-fast staining technique in detecting Cryptosporidium infection in faeces of pigs As reported by Johnston et al. (2003), faecal samples containing only a few Cryptosporidium oocysts often yield a false-negative ELISA result. The lack of false-negative ELISA result observed in this study may therefore imply that the faeces of infected pigs contained at least 17.6 oocysts/µl of Cryptosporidium (Johnston et al., 2003).

The ELISA detects a high molecular, soluble glycoprotein that is secreted by the parasite during replication (Kuhnert-Paul et al., 2012). This antigen may also appear in the faeces before and after the end of patency (oocysts excretion) (Ungar, 1990). This may therefore account for the false-positive results of ELISA observed in this study. The lesser detection of oocysts in stained faecal smears may be related to several aspects of the staining procedure, especially decolourization, which causes some of the oocysts to lose their stain (Baxby and Blundell, 1983). Furthermore, storage of the samples at 4oC may reduce the sensitivity of microscopy in detecting Cryptosporidium oocysts (Kuhnert-Paul et al. 2012).

From our study, the ELISA, though more expensive than the acid-fast staining method, is more sensitive, easier to perform and evaluate, therefore more suitable for routine screening of porcine faecal samples in laboratories. It has however been suggested that ELISA should be carried out together with one of the staining techniques to increase the accuracy of diagnosis (Godekmerdan et al., 1999).

The high prevalence rate of Cryptosporidium coproantigens observed in this study necessitates routine examination of symptomatic and asymptomatic V.

9 Discussion

pigs. Thus, Cryptosporidium antigen screening of porcine stools by ELISA should be regularly carried out in laboratories in Nigeria.

10 Ethical consideration

The manuscript does not contain clinical studies or patient data.

Figure 1:

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	Microscopy Positive	Microscopy Negative	Total (ELISA)
ELISA Positive	34	31	65
ELISA Negative	0	144	144
Total (Microscopy)	34	175	209

Figure 2: Table 1 :

.1 Conflict of interest

The authors declare that they have no conflict of interest.

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