Global Journal of Medical Research: G
Veterinary Science and Veterinary Medicine
Volume 15 Issue 1 Version 1.0 Year 2015
Type: Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals Inc. (USA)
Online ISSN: 2249-4618 & Print ISSN: 0975-5888

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

By Akinkuotu Olufemi Ambrose, Jacobs Eniope Bamidele & Okwelum Ngozi

Federal University of Agriculture, Abeokuta, Nigeria

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%) than the acid-fast staining method (16.3%). The sensitivities of the ELISA and acid-fast staining techniques were 100.0% and 52.3% respectively. The ELISA is therefore a preferable method than microscopy for detection of *Cryptosporidium* spp.

Keywords: *cryptosporidium, elisa, nigeria, pigs.*

GJMR-G Classification : *NLMC Code: WC 900*

Strictly as per the compliance and regulations of:

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

Akinkuotu Olufemi Ambrose [α], Jacobs Eniope Bamidele [σ] & Okwelum Ngozi [ρ]

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%) than the acid-fast staining method (16.3%). The sensitivities of the ELISA and acid-fast staining techniques were 100.0% and 52.3% respectively. The ELISA is therefore a preferable method than microscopy for detection of *Cryptosporidium* spp. in faeces of pigs and will be useful in routine diagnosis and screening of large number of samples in epidemiological surveys.

Keywords: cryptosporidium, elisa, nigeria, pigs.

I. Introduction

Cryptosporidium 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; Ayinmode and Fagbemi, 2010), there are relatively fewer epidemiological studies on porcine *cryptosporidiosis* (Chen and Huang, 2007; Kvac *et al.*, 2009; Chen *et al.*, 2011).

Different methods are used for diagnosis of *cryptosporidiosis* and these vary in their sensitivities, need for experienced staff and cost (Kuhnert-Paul *et al.*, 2012). A conventional method of identification is the microscopic examination of faecal smears stained with acid-fast stains (Yatswako *et al.*, 2007; Ayinmode and Fagbemi, 2010) and other staining methods (Mahdi and Ali, 2004; Hamedi et al., 2005; Kuhnert-Paul *et al.*, 2012). In some studies, it was determined that the sensitivity of the ELISA was higher than those of various staining methods (El-Shazly et al., 2002; Yilmaz *et al.*, 2008). El-Shazly *et al.* (2002) stated that the acid-fast staining technique showed the lowest sensitivity when compared to ELISA and the polymerase chain reaction (PCR) for diagnosis of C. parvum in cattle.

In Nigeria, very few studies have been carried out to detect *Cryptosporidium* spp. in pigs (Kwaga *et al.*, 1988; Yatswako *et al.*, 2007; Maikai et al., 2011) with the acid-fast staining method being utilized in majority of these studies. To the best of our knowledge, the comparison of an acid-fast staining technique and an ELISA to diagnose porcine cryptosporidiosis has not been previously reported in Nigeria. The results of this study will therefore highlight which of these diagnostic methods is more sensitive and suitable for routine diagnosis and epidemiological studies on *Cryptosporidium* infections in pigs in Nigeria.

II. Materials and Methods

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.

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 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.

Author α σ ρ : Federal University of Agriculture, Abeokuta, Ogun State, Nigeria. e-mails: divinvelivn@yahoo.com, akinkuotuoa@funaab.edu.ng

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.

III. 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. 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).

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

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

Sensitivity:

a. *ELISA: (34/34) X 100 = 100%*
b. *Microscopy: (34/65) X 100 = 52.3%*

V. Discussion

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 corroborates previous reports by Yilmaz *et al.* (2008), Kuhnert-Paul *et al.* (2012) and Chalmers *et al.* (2011). In contrast, similar sensitivities were reported by El-Moamly and El-Sweify (2011) and Ignatius *et al.* (1997).

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

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

Ethical consideration

The manuscript does not contain clinical studies or patient data.

Conflict of interest

The authors declare that they have no conflict of interest.

References Références Referencias

1. Ayinmode, A.B., Fagbemi, B.O. 2010. Prevalence of *Cryptosporidium* infection in cattle from southern Nigeria. *Veterinarski Archiv* . 80(6): 723-731.
2. Baxby, D., Blundell, N. 1983. Sensitive, rapid, simple methods for detecting *Cryptosporidium* in faeces. *Lancet* 2:1149.
3. Bergeland, M. J. 1977. Necrotic Enteritis in Nursing Piglets. *American Association of Veterinary Laboratory Diagnosticians* 20: 151–158.
4. Chalmers, R.M., Campbell, B.M., Crouch, N., Charlett, A., Davies, A.P. 2011. Comparison of diagnostic sensitivity and specificity of seven *Cryptosporidium* assays used in the UK. *J Med Microbiol.* 60:1598–1604.
5. Chen, F., Huang, K. 2007. Prevalence and phylogenetic analysis of *Cryptosporidium* in pigs in eastern China. *Zoonoses Public Health* 54: 393–400.
6. Chen, Z., Mi, R., Yu, H., Shi, Y., Huang, Y., Chen, Y., Zhou, P., Cai Y., Lin, P. 2011. Prevalence of *Cryptosporidium* spp. in pigs in Shanghai, China. *Veterinary Parasitology* 181: 113-119.
7. El-Moamly, A.A., El-Sweify, M.A. 2011. ImmunoCard STAT! cartridge antigen detection assay compared to microplate enzyme immunoassay and modified Kinyoun's acid-fast staining technique for detection of *Cryptosporidium* in fecal specimens. *Parasitol Res*. 78:122-128.
8. El-Shazly, A. M., Gabr, A., Mahmoud, M.S., Aziz, S.S., Saleh, W.A. 2002. The use of Ziehl-Neelsen stain, enzyme-linked immunosorbent assay and nested Polymerase Chain Reaction in diagnosis of cryptosporidiosis in immunocompetent, -compromised patients. *J. Egypt Soc. Parasitol*. 32: 155-166.
9. Godekmerdan, A., Kalkan, A., Erensoy, A., Kilic, S.S. 1999. Prevalence of Cryptosporidium spp. in children with diarrhoea. *Acta Parasitol. Turcica*. 23: 122-125. In.Yilmaz H., Zeynep, T., Mautalip, C. 2008. Investigation of cryptosporidiosis by enzyme-linked immunosorbent assay and microscopy in children with diarrhoea. *Saudi Med. J*. 29(4): 526-529.
10. Hamedi, Y., Safa, O., Haidari, M. 2005. *Cryptosporidium* infection in diarrhoeic children in southeastern Iran. *Pediatr. Infect. Dis*. J. 24: 86-88.
11. Ibrahim, U.I., Mbaya, A.W., Mahmud, H., Mohamed, A. 2007. Prevalence of cryptosporidiosis among captive wild animals and birds in the arid region of North-eastern Nigeria. *Vet. Arch*. 77: 337-344.
12. Ignatius R., Eisenblätter M., Regnath T., Mansmann U., Futh U., Hahn H., Wagner J. 1997. Efficacy of different methods for detection of low Cryptosporidium parvum oocyst numbers or antigen concentrations in stool specimens. *Eur J Clin Microbiol Infect Dis* 16:732–736.
13. Johnston, S. P., Ballard, M.M., Beach, M.J., Causer, L., Wilkins, P.P. 2003. Evaluation of three commercial assays for detection of *Giardia* and Cryptosporidium organisms in fecal specimens. *J. Clin. Microbiol*. 41:623–626.
14. Kennedy, G.A., Kreitner, G.L., Strafuss, A.C. 1977. Cryptosporidiosis in three pigs. *J.Am. Vet. Med. Assoc.* 170: 348–350.
15. Kuhnert-Paul, Y., Berit, B., Katja, D., Arwid, D., Ronald, S. 2012. Cryptosporidiosis: comparison of three diagnostic methods and effects of storage temperature on detectability of cryptosporidia in cattle faeces. *Parasitol. Res.* 111: 165-171.
16. Kvac, M., Sak, B., Hanzlikova, D., Kotilova, J., Kvetonova, D. 2009. Molecular characterization of *Cryptosporidium* isolates from pigs at slaughterhouses in South Bohemia, Czech Republic. *Parasitol. Res*. 104: 425–428.
17. Kwaga, J.K., Uzor, E.I., Umoh, J.U. 1988. *Cryptosporidium* infections in calves and piglets in some parts of Kaduna state, Nigeria. *Z. Vet*. 3: 86-89.
18. Mahdi, N. K., Ali, N.H. 2004. Cryptosporidiosis and other intestinal parasitic infections in patients with chronic diarrhoea. *Saudi Med. J*. 25: 1204-1207.
19. Maikai, B. V., Umoh, J. U., Kwaga, J.K.B., Maikai, V. A., Egege, S.C. 2011. Prevalence and risk factors associated with faecal shedding of *Cryptosporidium* oocysts in piglets, Kaduna, Nigeria. *Journal of Parasitology and Vector Biology* 1(1): 001-004.
20. Ungar, B. L. P. 1990. Enzyme-linked immunoassay for detection of *Cryptosporidium* antigens in fecal specimens. *J. Clin. Microbiol*. 28:2491-2495.
21. Wang, R., Qiu, S., Jian, F., Zhang, S., Shen, Y., Zhang, L., Ning, L., Cao, J., Qi, M., Xiao, L. 2010. Prevalence and molecular identification of *Cryptosporidium* spp. in pigs in Henan, China. *Parasitol. Res*. 107: 1489-1494.
22. Xiao, L., Fayer, R. 2008. Molecular characterisation of species and genotypes of *Cryptosporidium* and *Giardia* and assessment of zoonotic transmission. *Int. J. Parasitol*. 38:1239–1255.
23. Yatswako, S., Faleke, O.O., Gulumbe, M.L., Daneji, A.I. 2007. *Cryptosporidium* oocysts and Balantidium coli cysts in pigs reared semi-intensively in Zuru, Nigeria. *Pak. J. Biol. Sci.* 10: 3435–3439.

24. Yilmaz H., Zeynep, T., Mautalip, C. 2008. Investigation of cryptosporidiosis by enzyme-linked immunosorbent assay and microscopy in children with diarrhoea. *Saudi Med. J.* 29(4): 526-529.

Global Journals Inc. (US) Guidelines Handbook 2015

www.GlobalJournals.org