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BOJAAS

RESEARCH ARTICLE

Cryptosporidium infection in pigs determined by two different methods and its impact on farm environment in southern Botswana.

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ABSTRACT

The objective of this study was to determine cryptosporidium infection in pigs in southern Botswana. During October 2009 to April 2012, fecal samples collected from 407 animals of 12 commercial piggeries were screened for Cryptosporidium species oocysts by microscopy using Modified Ziehl-Neelsen technique and *C. parvum* coproantigen in Enzyme linked immunosorbent assay. The overall infection rates in pigs were 31 and 23% by Enzyme linked immunosorbent assay and Modified Ziehl-Neelsen technique, respectively. Enzyme linked immunosorbent assay was significantly more sensitive than Modified Ziehl-Neelsen technique in detecting cryptosporidium infection (P = 0.01). All farms and 12 of 33 litters (36%) had at least one animal harboring Cryptosporidium species organisms. Age-wise prevalence rates in suckling piglets, weaners, sows and boars were 31.6, 34.7, 27.9 and 22%, respectively by Enzyme linked immunosorbent assay. Though the highest infection rate was reported in animals aged 6 to < 20 weeks, there was no statistically significant difference in the infection rates between suckling and post-weaned piglets, and male and females (P = 0.67). There was no association of neonatal diarrhea and the prevalence of cryptosporidium infection indicating its asymptomatic nature. Of 12 sampled piggeries, the highest prevalence rate of 44.8% was recorded in animals of UL farm followed by 39.7% and 32.9% on QL and BL farms, respectively. Cryptosporidium infection rate on the remaining nine farms varied from 5% to 25%. Of the 81 environmental samples collected from all 12 piggeries, 13% soil, 12% water and 24% liquid manure samples from seven farms were positive for Cryptosporidium species oocysts. No significant association was observed between the presence of oocysts in the environmental samples and the infection rates detected in animals of seven piggeries. The present study demonstrated that cryptosporidium infection is highly prevalent in pigs in southern Botswana and underlines the importance of creating awareness among animal handlers on the risks of acquiring this zoonotic infection from infected animals and environmental contamination of swine barns. Therefore, hygiene in farms is important in preventing infections.

Keywords Botswana, Cryptosporidium, ELISA, oocysts, pigs, prevalence

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INTRODUCTION

Cryptosporidium infection is not only of public health significance but also of economic importance as it may induce severe gastroenteritis, inflicting heavy mortality among younger animals. *Cryptosporidium parvum* is considered among the top four entero-pathogens involved in inducing neonatal diarrhea (Castro-Hermida *et a*l., 2002). World-wide prevalence of porcine cryptosporidiosis varied from 1.4 to 100% (Guselle *et al*., 2003; Siwila, and Mwape 2012). Driesen *et a*l. (1993) and De Graaf *et al*. (1999) reported Cryptosporidium spp. induced diarrhea in pre-weaning piglets, but several other studies demonstrated asymptomatic nature of this infection in both piglets and adult pigs (Casemore *et al*., 1997; Ryan *et a*l., 2003). Three species of Cryptosporidium that infect pigs are *C. suis* (formerly pig genotype I), pig genotype II and *C. parvum* (Ryan *et al*., 2003). Johnson *et al.* (2008) identified pig genotype II and *C*. *suis* species. Transmission of cryptosporidiosis to animals, and even to people is through ingestion of oocysts from infected individuals via contaminated food, water and pasture. Oocysts are already sporulated when shed in faeces and therefore immediately infective. Close proximity of humans and livestock as well as the ability of the run-off from livestock production operations to contaminate ground and surface water supplies represent an ever present public health risk of transmission of cryptosporidial infection. Several water and foodborne outbreaks of human cryptosporidiosis reported world-wide have been

associated with contamination by cow dung and human stools (Lengerich *et al*., 1993; Fayer *et al*., 2000; Rose *et al.*, 2002). In 2006, a severe water-borne outbreak caused by a mixed infection of Cryptosporidium species and *Escherichia coli* organisms killed more than 500 children in Botswana. Majority of the infected children were enrolled under HIV/AIDS's Prevention of mother to child transmission, popularly called PMTCT program (Creek *et al*., 2010). Alexander *et al*. (2012) reported Cryptosporidium and Giardia species organisms as important waterborne etiological agents of recurring diarrhoeal outbreaks in children less than 2 years of age in Chobe district of Botswana. Sharma (2006), Sharma and Machete (2009) recorded prevalence of *C. parvum* infection in bovine calves and small stock in Botswana, but similar information concerning pigs is not available. The present cross-sectional survey was undertaken to determine Cryptosporidium species infection in the fecal samples of nursing piglets, post-weaned and adult pigs of 12 semi-intensively managed swine herds. Water, soil and manure samples collected from these herds were tested for the presence of Cryptosporidium species oocysts and to assess their potential role in the environmental contamination.

MATERIALS AND METHODS

Sample Collection and Farming System

During October 2009 to April 2012, fecal samples were collected from 407 animals belonging to 12 commercial pig enterprises located in South East and Kgatleng districts of Botswana. The names of the farms in this study are not revealed for ethical reasons and only code names used. The animals were sampled once in the study period and included 133 suckling piglets of 1 week to < 6 weeks, 147 weaners aged 6 weeks to <20 weeks, 86 sows and 41 boars. Twenty to fifty grams faecal samples were collected either directly from the rectum or a portion of freshly deposited faeces that did not have contact with the floor from each animal into sterile plastic containers without any preservative, placed on ice packs and transported to Parasitology Laboratory of the Botswana University of Agriculture and Natural Resources. Faecal consistency was recorded at the time of their collection. These were categorized as either diarrheic, passing soft to liquid faeces or /non-diarrheic with normal formed stools. Fecal samples were brought to the laboratory on ice packs in cooler boxes and kept in the refrigerator till their processing within a week. The selected pig farms were categorized either into small-scale with 15 to 30 animals or medium scale comprising of more than 30 and up to ~200 animals. Pig production systems adopted at these farms were feeder operation or weaner scheme and farrow to finish production that involved raising of pigs from birth until they were ready for market. Weaning of piglets was carried out between 6 to 8 weeks. The animals were kept in open pens with porous concrete floors. Animals were reared under semi-intensive system where pigs were allowed to move outside their pens in the farm premises for wallowing and rooting the soil. Borehole water was used for drinking, washing pens and hosing the pigs down. Deworming of sows was carried out 7-14 days before moving to farrowing pens and iron preparations were administered to piglets intramuscularly in the first week after birth in order to prevent the occurrence of iron-deficiency anemia.

Microscopic examination of faecal smears

Cryptosporidium species oocysts were detected in faecal smears microscopically after staining them with Modified Ziehl-Neelsen (MZN) stain as described by O. I. E (2004). Malachite green was used as counterstain in place of Methylene blue. Examination of the slides was carried out using calibrated light microscope at x1000 magnification under oil immersion objective. The cryptosporidial oocysts appeared light to bright red spherules with refractive walls measuring 4-5 µm in diameter on a green background. Soil and liquid manure samples were processed by standard Centrifugal flotation technique using Sodium chloride solution. Smears were made by taking a drop of supernatant, fixed them with methanol and stained with MZN stain. Water samples were filtered through a 47 mm diameter, 0.45 ± 0.02 µm pore size membrane filter. Material retained by filters were examined microscopically as a 0.9% saline smear at magnification of 400x for Cryptosporidium oocysts following the technique adopted by Bakir *et a*l. (2003).

Detection of Cryptosporidium coproantigen

A commercial RIDASCREEN Cryptosporidium (C1201) Enzyme-linked immunosorbent assay (ELISA) diagnostic kit (R-Biopharm AG, Darmstadt, Germany) was used to detect C. parvum antigen in faecal samples. ELISA was carried out following the technique described by the manufacturer of the kit. Multiskan microplate reader (Lab Systems Oy, Helsinki, Finland) was used for photometric measurements at 450nm wavelength.

Data analysis

To assess whether unpaired observations on two variables expressed in a contingency table are independent of each other, the test of independence was performed. Chi square values were calculated using 2x2 contingency table. P values (right-tailed probability of chi-squared distribution) were determined using CHIDIST function in Microsoft Excel. The relationships between two variables were assessed at 5% level of significance ($P < 0.05$). Results take into account the infection rates detected by ELISA that was found significantly more sensitive than MZN. In view of this, the significance levels among piglets, among gilts/sows and among boars comparing both ELISA and MZN were not considered. However, significance levels between groups i.e suckling and weaners and between gilts/sows and boars are presented.

RESULTS

Out of 407 faecal samples tested by ELISA and MZN, Cryptosporidium coproantigen and oocysts were detected in 126 and 93 specimens denoting infection rates of 31% and 22.9%, respectively. On examination of the stained faecal smears, the average size of light to bright red sub-spherical to spherical oocysts ranged between 4 to 5µm and was identical to those of *Cryptosporidium parvum* morphologically. It was evident from Tables 1 and 2 that ELISA was more sensitive in detecting *C*. *parvum* infection in comparison to MZN and the difference was statistically significant (P = 0.011).

Because of the greater sensitivity of ELISA over MZN, the percent infection rates and comparisons between different age groups, sexes and faecal consistencies in this study were from analysis from ELISA only. Mean infection rates were 33% in younger animals including nursing and post-weaned piglets (93 out of 280) and 26% (33/127) in sows and boars. Though pre- and post-weaned piglets appeared to be more susceptible to cryptosporidiosis than adults, the differences in their infection rates were not significant (P = 0.67). There was no significant difference in the infection rates among male (32.1%; 54/168) and female pigs (30.1%; 72/239) (P = 0.75). Of 65 diarrheic animals excreting loose to liquid feces, 18 (27.7%) were found positive for Cryptosporidium species infection. However, animals with normal faeces demonstrated a higher infection rate of 31.6%; (108/342).

All the sampled 12 piggery farms had at least one animal harboring Cryptosporidium parasites. UL Farm demonstrated highest prevalence rate of 44.8% (26/58) followed by QL and BL Farm with 39.7% (52/131) and 32.9% (23/70), respectively (Table 2). Contrary to this, the infection rates on the remaining nine farms were lower, ranging from 5% to 25%. The difference between aforementioned three severely infected piggeries in comparison to mildly infected nine piggeries was significant (P = 0.001). Of the 81 environmental samples taken, Cryptosporidium oocysts were detected in 13% (5/38) soil, 12% (3/26) water and 24% (4/17) liquid manure samples. Five positive soil samples included one each from UL, EU, QL, FV, and BL farms. Of three positive water samples, two were from QL and one from FN. Two manure samples from BL farm and one sample each from QL and TB farms were positive for Cryptosporidium species oocysts.

DISCUSSION

The present study has demonstrated the prevalence of cryptosporidium infection in domesticated pigs for the first time in Botswana. The overall prevalence rate of 31 % in pigs from 12 piggery units is considered significant since cryptosporidiosis caused by *C*. *parvum* organisms is both zoonotic and carries potent public health risk. Our results indicated less sensitivity of MZN technique in comparison to ELISA in detecting cryptosporidium infection. This may possibly be on account of not employing the concentration step in processing the faecal samples prior to microscopic examination of MZN stained smears in conjunction with low and intermittent pattern of excretion of oocysts. Our observations are similar to those of Katanika *et al*. (2001) and Marks *et al*. (2004) who also reported reduced sensitivity of MZN technique in comparison to commercial enzyme immune-assays. Morphologically, all the oocysts detected in the stained faecal smears in this study were indistinguishable from those of *C. parvum*. However, Santin *et al*. (2004) recommended that the identification of oocysts based solely on morphological characteristics must be reassessed using molecular techniques to validate species of Cryptosporidium. Pre-and post-weaned animals showed higher percent prevalence rate of 33.2% compared to adult boars and sows with 26%. These differences were non-significant and might likely to be due to wide variation in animals sampled (280 animals < 20 weeks-old versus 127 adults). Lack of active immunity in piglets could also be the reason for difference between the young and adults. This finding is in contrast to that of Olson *et al*. (1997) who recorded higher rate of infection in animals older than six months than younger animals. Weaners with higher infection rate of 35% in the present investigation corroborated the findings of Kim (1990) who reported significantly higher (59.2%) in weaned and 1-2 months old piglets at 59.2% *versus* 34.3% in fattening 2 to 6 months old pigs. Ryan *et al*. (2003) found 69.2% infection rate in pigs aged between 5-8 weeks in comparison to younger ones (P <0.0001). Johnson *et al*. (2008) recorded prevalence rate of 32.7% in postweaned pigs in comparison to 10.6% in preweaned pigs. According to Graczyk *et al*. (1999) young infected animals produced large number of oocysts, sometimes exceeding 1010 oocysts per day that may lead to an increased environmental contamination.

Of the 116 infected pigs, 84% (342/407) excreted normal solid faeces and these results were consistent with the research reports of Ramirez *et al*. (2004) and Siwila and Mwape (2012) indicating sub-clinical or asymptomatic nature of porcine cryptosporidiosis. However, it is difficult to distinguish whether the pigs that were tested positive with normal faeces were actually asymptomatic or had diarrhea prior to sampling and were in the process of recovery. Johnson *et al*. (2008) reported asymptomatic nature of cryptosporidiosis in pre-weaned animals infected with *C. suis* and Cryptosporidium species pig genotype II in post-weaned pigs. However, Morgan *et al*. (1999) from Australia recorded both *C*. *parvum* cattle genotype and pig genotype and found an association with acute diarrhea, chronic ill thrift and death in pigs.

Cryptosporidium infection rates were probably underestimated in this study because of the collection of only one faecal sample per animal that may be found negative microscopically and serologically on account of intermittent excretion and passing of few cryptosporidium oocysts.

Table 1: *Cryptosporidium parvum* infection in pigs of different age groups detected by Enzyme-linked immunosorbent assay (ELISA) and Modified Ziehl-Neelsen (MZN) staining technique

Age groups	Animals Tested (N)	Animals positive (N)		% Prevalence		P-Value
		ELISA	MZN	ELISA	MZN	
Suckling piglets (1-6 weeks)	133	42	28	31.6	21	
Weaners (6-<20 weeks)	147	51	39	34.7	26.5	0.18
Gilts/Sows	86	24	19	27.9	2.1	
Boars	41	9	7	22	17.1	0.62
Total	**407**	**126**	**93**	**31***	**22.9***	

Table 2: Prevalence of *Cryptosporidium parvum* infection in piggeries using Enzyme-linked immunosorbent assay (ELISA) and Modified Ziehl-Neelsen (MZN) staining technique

Names of farms	Animals tested (N)	Animals positive (N)		% Prevalence	
		ELISA	MZN	ELISA	MZN
UL	58	26	19	44.8	21
QL	131	52	41	39.7	31.3
BL	70	23	17	32.9	24.3
MG	26	5	4	19.2	15.4
EU	20	3	1	15	5
AT	24	6	3	25	12.5
FN	14	2	2	14.3	14.3
BK	20	1	0	5	0
TB	21	3	2	14.3	9.5
MR	10	2	2	20	20
FV	5	1	1	20	12.5
MP	8	2	1	20	12.5
Total	**407**	**126**	**93**	**31**	**22.9**

Temperature extremes and dry weather conditions prevailed throughout southern Botswana during the study period and might have also contributed to relatively lower infection rate since such climatic conditions were reported to have adverse impact on the viability of Cryptosporidium species oocysts by Anderson, (1985) and Walker *et al*., (2001). Van Gool *et al*. (2003) suggested consecutive collection and testing of three faecal specimens per animal. A wide range of cryptosporidium infection rates from 1.4 to 100% were recorded in pigs from different countries (Wieler *et al*., 2001; Zintl *et al*., 2007, Johnson *et al.*, 2008). Higher cryptosporidium infection rates of 45.5% and 44.2% were recorded by Mišić *et al*. (2003) and Siwila (2012) in Belgrade district of Serbia and Lusaka, Zambia, respectively and lower infection rates of 10.5%, 6.03% and 2.02% in pigs by Yu and Seo (2004) from Korea, Ryan *et al.* (2003) from Australia and Fiuza *et al*. (2011) from Brazil, respectively. The variations in the infection rates could be attributed to several factors which may include sanitary conditions at the farms, the frequency and season of sampling, age groups of animals, differences in the sample sizes, degree of sensitivity and modifications in the techniques used for detection of antigen or oocysts in faeces.

The high mean cryptosporidium infection rate observed at UL, QL and BL piggeries than those of remaining nine farms may be indicating environmental risk factors. Risk factors like higher stocking density and poor sanitary conditions observed in farrowing and weaner pens with porous concrete floors might have increased the chances of contamination at the aforementioned three farms. This is in support of the findings of Xiao et al. (1994) who recorded a significantly higher cryptosporidium infection rate in a piggery with porous concrete floors than a unit with slotted and wire floors in Ohio State, USA. Cryptosporidium oocysts were detected in 14.8% soil, water and liquid manure samples from 58% sampled piggeries in the current investigation. We could not find any significant relationship between the presence of oocysts in the environmental samples and the magnitude of infection in animals at the infected units. This may possibly be explained by the fact that animal sampling was undertaken from the animals directly [but soil if contaminated would be reflective of what happened in the past and collecting faecal samples directly would not affect that associations if there were previous contaminations] and the soil sampling was cumulative and this finding is in agreement with that of Barwick *et al*. (2003). Barwick *et al* (2003) recommended that testing of individual soil samples from different farm sites instead of examining the pooled samples would be helpful to establish any association between prevalence rates in animals and environmental samples. In the present investigation we could not conduct tests to check the viability of oocysts nor the contaminating

sources of water, soil and manure. It may be possible that human excreta, livestock and wild animals could have contaminated these sources. In Botswana, Creek *et al*. (2010) identified the presence of Cryptosporidium species predominantly and other enteropathogenic bacteria and viruses in the stool samples of diarrhoeic children. The swine herds found infected with Cryptosporidium species organisms in the current study could be one of the important vehicles through which oocysts may travel into water sources especially during the rainy season with the possibility of contaminating public water supplies. This was evident from an outbreak that inflicted deaths of more than 500 diarrheic children in 2006 in Botswana (Creek *et al*., 2010). This warrants further studies to determine the status of infection in pigs from different regions of Botswana including molecular characterization of Cryptosporidium species organisms

CONCLUSION

Cryptosporidium infection was highly prevalent in swine herds of southern Botswana. It emphasize the importance of creating awareness among animal handlers and farm owners living in close proximity to naturally infected pigs and contaminated farm environment of the possibility of acquiring this zoonotic infection. Due to non-availability of effective drugs for treatment of cryptosporidiosis and semi-intensive nature of the pig industry and current practices of effluent disposal on the farm premises in Botswana, adoption of good farm management practices may help in reducing cryptosporidium infection rates in pigs and dissemination of Cryptosporidium species oocysts into public water supplies.

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Conflict of Interest

None

REFERENCES

Alexander, K. A., Herbein, J. and Zajac, A. (2012). The occurrence of Cryptosporidium and Giardia infections among patients reporting diarrheal disease in Chobe district, Botswana. *Advances in Infectious Diseases* **2**:143-147

Anderson, B. C. (1985). Moist heat inactivation of Cryptosporidium sp. *American Journal of Public Health* **75**:1433

Bakir, B., Tanyuksel, M., Saylam, F., Tanriverdi, S., Araz, R. E., Hacim, A. K. and Hasde, M. (2003). Investigations of waterborne parasites in drinking water sources of Ankara, Turkey. *Journal of Microbiology* **41**: 148-151

Barwick, R. S., Mohammed, H. O., White, M. E. and Bryant, R. B. (2003). Factors associated with the likelihood of Giardia spp. and Cryptosporidium spp. in soil from dairy farms. *Journal of Dairy Science* **86**: 784-791

Creek, T. L., Kim, A., Lu, L., Bowen, A., Masunga, J., Arvelo, W., Smit, M., Mach, O., Legwaila, K., Motswere, K., Zaks, L., Finkbeiner, T., Povinelli, L., Maruping, M., Ngwaru, G., Tebele, G., Bopp, C., Puhr, N., Johnston, S. P., Dasilva, A. J., Bern, C., Beard, R. S. and Davis, M. K. (2010). Hospitalization and mortality among primarily non-breastfed children during a large outbreak of diarrhea and malnutrition in Botswana, 2006. *Journal of Acquired Immune Deficiency Syndromes* **53**: 14-19

Casemore, D. P., Wright, S. E. and Coop, R. L. (1997). Cryptosporidiosis-human and animal epidemiology. In: Cryptosporidium and cryptosporidiosis (Ed. R. Fayer). CRC Press, Boca Raton, Florida, USA. pp. 65-92

Castro-Hermida, J. A., González-Losada, Y. A. and Ares-Mazás, E. (2002). Prevalence and risk factors involved in spread of neonatal bovine cryptosporidiosis in Galicia (NW Spain). *Veterinary Parasitology* **106**: 1-10

De Graaf, D. C., Vanopdenbosch, E., Ortegia-Mora, L. M., Abassi, H. and Peeters, J. E. (1999). A review of the importance of cryptosporidiosis in farm animals. *International Journal for Parasitology* **29**: 1269-1287

Driesen, S. J., Cartland, P. G. and Fahy, V. A. (1993). Studies on pre-weaning piglet diarrhea. *Australian Veterinary Journal* **70**: 259-262

Fayer, R., Morgan, U. and Upton, S. J. (2000). Epidemiology of Cryptosporidium: Transmission, detection and identification. *International Journal for Parasitology* **30**: 1305-1322

Fiuza, V. R. S., Gallo, S. S. M., Frazao-Teixeira, E., Santin, M., Fayer, R. and Oliveira, F. C. R. (2011). Cryptosporidium pig genotype II diagnosed in pigs from the state of Rio De Janeiro, Brazil. *Journal of Parasitology* **97**:146-147

Guselle, N. J., Appelbee, A. J. and Olson, M. E. (2003). Biology of *Cryptosporidium parvum* in pigs: From weaning to market. *Veterinary Parasitology* **113**: 7-18

Graczyk, T. K., Cranfield, M. R., Fayer, R. and Bixler, H. (1999). House flies (*Musca domestica*) as transport hosts of *Cryptosporidium parvum*. *American Journal of Tropical Medicine and Hygiene* **61**: 500-504

Johnson, J., Buddle, R., Reid, S., Armson, A. and Ryan, U. M. (2008). Prevalence of Cryptosporidium genotypes in pre and post-weaned pigs in Australia. *Experimental Parasitology* **119**: 418-421

Katanika, M. T., Schneider, S. K., Rosenblatt, J. E., Hall, G. S. and Procop, G. W. (2001). Evaluation of ColorPac Giardia/ Cryptosporidium rapid assay and ProsPec T Giardia/Cryptosporidium microplate assay for detection of Giardia and Cryptosporidium in faecal specimen. *Journal of Clinical Microbiology* **39**:4523-4525

Kim, C. W. (1990). Cryptosporidiosis in pigs and horses. CRC Press, Boca Raton, Florida, USA. pp. 105

Lengerich, E. J., Addiss, D. G., Marx, J. J., Ungar, B. L. P. and Juranek, D. D. (1993). Increased exposure to cryptosporidia among dairy farmers in Wisconsin. *Journal of Infectious Diseases* **167**: 1252-1255

Marks, S. L., Hansen, T. E. and Melli, A. C. (2004). Comparison of direct immuno-fluorescence, modified acid-fast staining and enzyme immunoassay techniques for detection of Cryptosporidium spp. in naturally exposed kittens. *Journal of American Veterinary Medical Association* **225**:1549-1553

Mišić, Z., Katic-Radivojević, S. and Kulšić, Z. (2003). Cryptosporidium infection in nursing, weaning and post-weaned piglets and sows in the Belgrade district. *Acta Veterinaria (Belgrade)* **53**:361-366

Morgan, U. M., Buddle, R., Armson, A. and Thompson, R. C. A. (1999). Molecular and biological characterization of Cryptosporidium in pigs. *Australian Veterinary Journal* **77**: 44-47

O. I. E, (2004). Manual of diagnostic tests and vaccines for terrestrial animals. Chapter 210.9 (Cryptosporidiosis) http://www.oie.int/eng/normes/manual/A_00135.htm.

Olson, M. E., Thorlakson, C. L., Deselliers, L., Morck, D. W. and McAllister, T. A. (1997). Giardia and Cryptosporidium in Canadian farm animals. *Veterinary Parasitology* **68**: 375-381

Ramirez, N. E., Ward, L. A. and Sreevatsan, S. (2004). A review of the biology and epidemiology of cryptosporidiosis in humans and animals. *Microbes and Infection* **6**:773-785 http://dx.doi.org/10.1016/j.micinf.2004.02.021, PMid: 15207825

Rose, J. B., Huffman, D. E. and Gennaccaro, A. (2002). Risk and control of waterborne cryptosporidiosis. *FEMS Microbiology Review* **26**:113-123

Ryan, U. M., Samarasinghe, B., Read, C., Buddle, J. R., Robertson, I. D. and Thompson, R. C. A. (2003). Identification of a novel Cryptosporidium genotype in pigs. *Applied and Environmental Microbiology* **69**:3970-3974

Santin, M. J. M., Trout, L., Xiao, L., Zhou, L., Greiner, E. and Fayer, R. (2004). Prevalence and age related variation of Cryptosporidium species and genotypes in dairy calves *Veterinary Parasitology* **122**:103-117

Sharma, S. P. (2006). Cryptosporidium infection in cattle in southern Botswana. *Botswana Journal of Agriculture and Applied Sciences* **2**:83-89

Sharma, S. P. and Machete, J. B. (2009). Prevalence of Cryptosporidium infection in goats and sheep in Gaborone area, Botswana. *Botswana Journal of Agriculture and Applied Sciences* **5**:11-18

Siwila, J. and Mwape, K. (2012). Prevalence of Cryptosporidium spp. and Giardia duodenalis in pigs in Lusaka, Zambia. *Onderstepoort Journal of Veterinary Research* **79**(1) E1-5 doi: 10.4102/ojvr.v79i1.404.

Van Gool, T., Weijts, R., Lommerse, E. and Mank, T. G. (2003). Triple feces test: An effective tool for detection of intestinal parasites in routine clinical practice. *European Journal of Clinical Microbiology and Infectious Diseases* **22**:284-290

Walker, M., Leddy, K. and Hagar, E. (2001). Effect of combined water potential and temperature stressors on Cryptosporidium parvum oocysts. *Applied and Environmental Microbiology* **67**:5526-5529

Xiao, L., Herd, R. P. and Bowman, G. L. (1994). Prevalence of Cryptosporidium and Giardia infections on two Ohio pig farms with different management systems. *Veterinary Parasitology* **52**: 331-336

Yu, J. R. and Seo, M. (2004). Infection status of pigs with *Cryptosporidium parvum*. *Korean Journal of Parasitology* **42**:45-47

Wieler, L. H., Ilieff, A., Herbst, W., Bauer, C., Vieler, E., Bauerfeind, R., Failing, K., Klös, H., Wegert, D. and Baljer, G. (2001). Prevalence of enteropathogens in suckling and weaned piglets with diarrhea in Southern Germany. *Journal of Veterinary Medicine B* **48**:151-159

Zintl, A., Neville, D., Maguire, D., Fanning, S., Mulcahy, G. and Smith, H. V. (2007). Prevalence of Cryptosporidium species in intensively farmed pigs in Ireland. *Parasitology* **134:**1575-1582