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Research Article

The Occurrence of Cryptosporidium Species in Soil and Manure in Jos and Environs, Plateau State, Nigeria

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ABSTRACT

This study investigated the mean occurrence of *Cryptosporidium* oocysts in soil and manure samples, samples were collected from some of the areas visited in Jos North & South LGA of Plateau State, Nigeria. Samples were analysed for the presence of *Cryptosporidium* species. *Cryptosporidium* species were determined by parasitological techniques (Formol-ether and floatation concentration techniques Staining was done using Zielh Neelsen technique, Mac-Master counting technique was used for the determination of the frequency of occurrence). Our results revealed higher means in the dry season with means of 19.2, 13.4 and 12.6 recorded for the months of January, February and March respectively than the rainy season that recorded the means of 6.8, 5.8, and 4.2 for the months of June, July and August. These results are discussed in relation to the epidemiology, distribution and significance of *Cryptosporidium* to the farmers that are constantly in contact with the samples and the impact of the contagious effect of these parasites in the environment on humans and animals.

Keywords: Occurrence, Cryptosporidium, species, Soil, Manure, Jos and Environs.

INTRODUCTION

Organisms of the genus *Cryptosporidium* species are small coccidian parasites that infect the microvillous border of the epithelium cells lining the digestive and respiratory organs of vertebrates (Current, 1983). Recognized and named over 80 years ago, (Tyzzer, 1910), these small (2 to 6μm depending on the stage of the life cycle) obligate, intracellular protozoan's remained until recently nothing more than a biomedical curiosity. Prior to 1986, infections with species of *Cryptosporidium species* were considered rare in animals and in humans. They were thought to be the result of a little known opportunistic pathogen of immune deficient individuals outside its normal host. Beginning in 1982, the concept of these protozoan parasites changed to the consideration that they are important, widespread causes of diarrhoeal illness in humans and some domesticated animals (Tzipori, 1983b; Tyzzer, 1912).

Members of the genus *Cryptosporidium species* are parasites of the intestinal tracts of fish, reptiles, birds and mammals. It seems that members of this genus do not display a high degree of host specificity, so, the number of species in this genus remains a matter of some discussion. *Cryptosporidium* isolated from humans is now referred to as C*. parvum. Cryptosporidium* infections have been reported from a variety of wild and domesticated animals, and in the last six or seven years, literally hundreds have been reported, including epidemics in several major urban areas in the United Kingdom (Ayeni, 1985; Angus, 1933).

In looking for causes of cryptosporidiosis, numerous studies of outbreaks and sporadic disease have been conducted and parasitological evidence has been obtained. The evidence can provide information on risk factors that indicate likely transmission pathways as well as the sources of contamination and possible public health breakdowns that have contributed to the infections. A case-control study of sporadic cryptosporidiosis found differences in risk factors between *C. parvum* and *C. hominis*, the former being associated with animal contact and the latter with changing diapers (Bomfim et al., 2005). The study also found a negative association with eating ice cream and raw vegetables (Dela-Feunte et al., 1999, Castro-Hermida et al., 2002).

Evidence for the origin of environmental oocysts is more by implication than scientific demonstration. Because oocysts are only produced naturally within the gastrointestinal and respiratory tracts of vertebrates, the sources are thought to be feces of human, agricultural and domestic animals. The use of genetic typing has aided and for the most part confirmed it, but there are a small but significant number of cases that may derive from other sources. Sub typing environmentally derived oocysts and those from animals may provide evidence of possible sources of fecal contamination. While animal strains can be derived from human as well as animal feces it is generally thought that *C. hominis* infections derive from human feces. Although experimental and natural infections of animals with *C. hominis* have been reported there is no evidence that this occurs commonly.

Cryptosporidium can be transmitted from animals to humans through direct contact. This has occurred with veterinary workers (Bradford and Schijven, 2002, Cicek et al., 2006) and other people exposed to animals particularly those whose jobs are associated with agricultural animals and children who visit farms (Bjorkman et al., 2003, Finch et al., 1993). Educational farms need to provide hand washing facilities for children and adults (Grimason et al., 1993, Epe et al., 2004). An outbreak was associated with an animal nursery in a fair in Tasmania (Davies et al., 2003, Current, 1990). Infections deriving from agricultural animals are predominantly *C. parvum*. An outbreak of Foot and Mouth Disease in the UK in 2001 led to a reduction in people's access to the countryside and there was a reduction in cases of cryptosporidiosis that may have been linked to this (Caroline et al., 2006, Baris et al., 2009, Appelbee et al., 2005).

MATERIALS AND METHOD

Soil and manure collection

Soil and manure samples were collected from five different sites visited (Yanshanu, Abattior, Faringadan, Kasuwa Doki and Gada-Biu). At each sampling site, soil and manure samples were randomly collected from the areas visited (10g each for soil and manure from different points of collection) into plastic containers and transported to the parasitology and diagnostic laboratory of the national Veterinary Research Institute, Vom, Nigeria for analysis. The sampling was carried out for a period of 12 months (January to December, 2007).

Extraction procedure used for soil and manure Microscopic Examination to Detect Oocysts (Ewakochzynska and Daniel, 1999)

Demonstration of *Cryptosporidium* oocysts was done by the microscopic examination of smears made after the sucrose gradient sedimentation technique after each Eppendorf tube was thoroughly vortexed, about 10µl of the sediment were pipetted into the slide wells, dried with a slide warmer and stained using the modified Ziehl Neelsen staining technique described by Henricksen and Pholenz (1981). The stained slides were examined under a microscope under the x10 and x40 objectives.

Identification and Counting of Oocysts (Jorgen and Brian, 1999)

The oocysts were identified by their smooth oval shape, thick-walled containing when fully sporulated four elongated, naked red sporozoites within an unstained oocyst wall and a cytoplasmic residual body. The modal size measurement of *Cryptosporidium* sp oocysts is 4.5 x5.0µm (range 4-6µm), this was confirmed by the presence of atlas presented by (WHO, 1991). The MacMaster counting technique was used for counting the frequency of occurrence of oocysts.

RESULTS

Occurrence of Oocysts in Manure Samples

Occurrence of oocysts in manure samples in the different sampling sites is represented in Figure 1. Cattle manure has the highest frequency among all the five sampling sites. *Cryptosporidium* oocysts recovered from Farin Gada (115) and the Gada Biu (75) areas were all from cattle manure only. The frequency of occurrence of the oocysts in Yanshanu area was higher for the three categories of manure screened, the cattle manure had the highest frequency of 240, followed by the manure from the goats 90, and the least frequency of oocysts in manure was the Yanshanu area with the sheep manure that recorded 80. In the case of Abattoir, the cattle manure still recorded highest frequency of 220 oocysts but the sheep manure recorded slightly higher frequency (65) than in the case of the goats 45 (Figure 1).

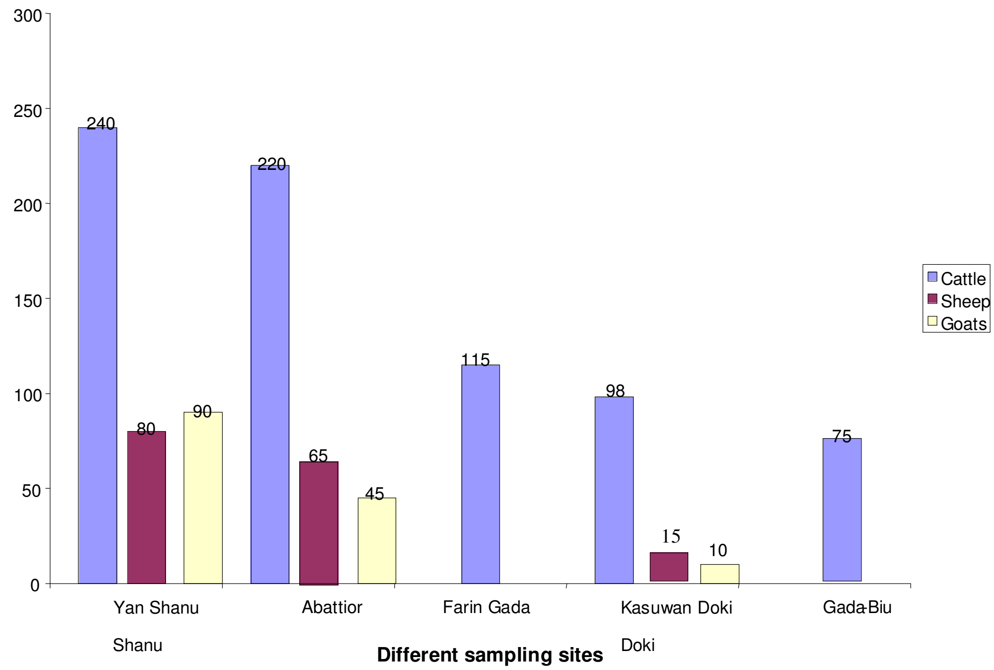

Figure1: The frequency of occurrence of *Cryptosporidium s*pecies in Soil and Manure Samples

Mean Occurrence of C*ryptosporidium* Oocysts in soil according to Sites of Collection.

Figure 1 reveals the mean occurrence of *Cryptosporidium* in soil in the different sampling sites. The result shows that there were differences in the mean occurrence of the oocyst in the different sampling sites. The soil sample from Yanshanu had the highest mean (19.3) followed by samples from Abattoir with a mean of 13.6, Kasuwa Doki had a mean of 4.8, prevalence of 4.3 was noted in the Faringada area. The least mean 2.1 was noted with the samples collected from Gada Biu.

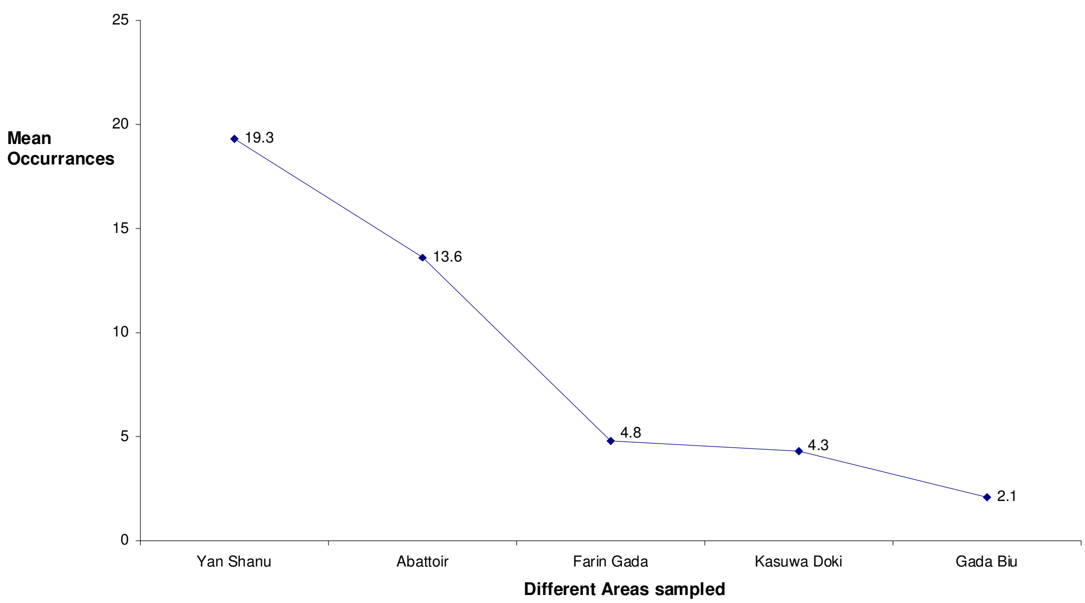

Figure 2:The Mean Occurrence of *Cryposporidium* Oocysts in soil at the different collection sites

The Occurrence of *Cryptosporidium* Oocysts in Soil according to Season

Figure 2 shows contamination rate in soil which indicates that infection rate decreases along the months; the infection was found to be highest during the dry season and less during the early and late rainy season, the mean occurrence is seen to start decreasing shortly after the rains.

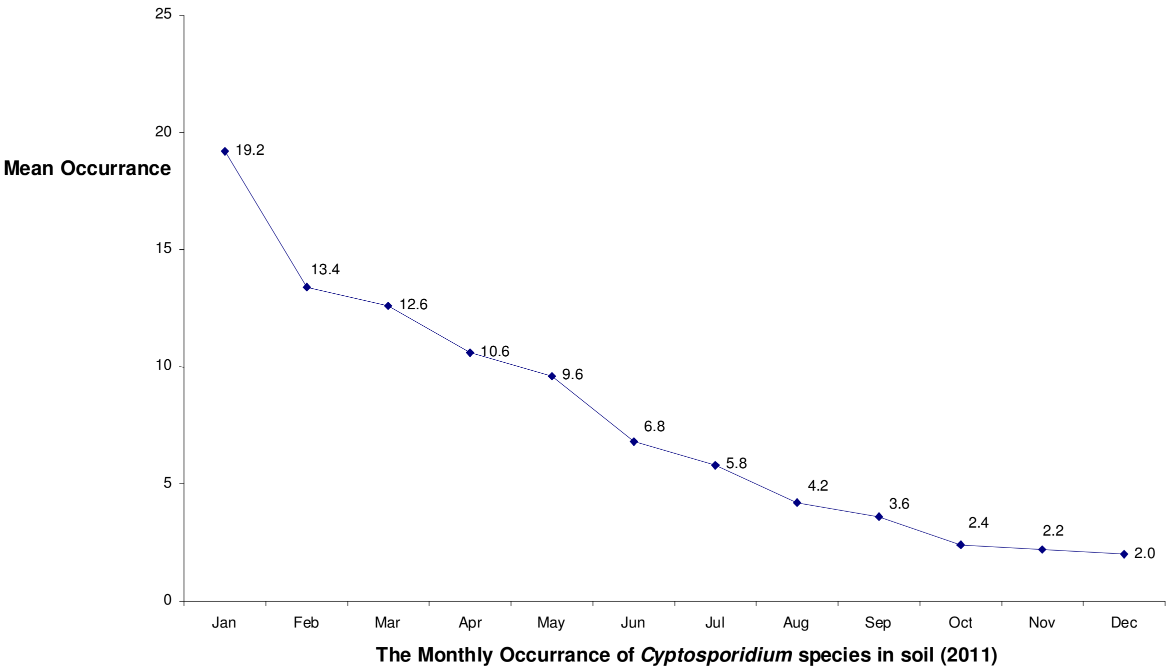

Figure 3: The Mean Occurrence of *Cryptosporidium* oocysts according to months in soil

The Frequency of Occurrence of Oocysts in Manure and Soil Samples according to Sites of Collection

The presence of *Cryptosporidium* sp was higher in cattle manure than sheep and goats at all collection sites. The highest frequency was at yanshanu (240) followed by abattoir (220) and the least at Gada-Biu. In regards to soil samples, the highest mean occurrence was at Yanshanu (19.3) followed by abattoir (13.6) and the least was at Gada-Biu (2.1). This observation could only imply the presence of infected animals in those areas and the arbitrary use of both contaminated animal and human excreta as supplement to chemical fertilizer which will directly contaminate the farmers and the food crops (Robertson et al., 1992, Ewakochzynska and Daniel 1999, Atwill et al. 2006). Children (0 to 10years) and young adults (11-20years) are at higher risk of infection with *Cryptosporidium* sp because of their active participation in out door activities.

Although the occurrence of *Cryptosporidium species* was high in manure at all collection sites, its presence in the soil and in these sites is an indication of endemicity of Cryptosporidiosis, sipping/ leaking into the soils of oocysts from manure heaps around the soil, high environmental pollution due to indiscriminate defecation and disposal of both human and animal excreta in the areas under study can also result in contamination of the areas (Fayer and Ellis, 1993; Garber *et al*., 1994, Xiao et al., 2009 and Ewakuczynska et al., 2005). Promiscuous defecation can be a source of pollution in the environment (Elizabeth and Dwigth, 2003; Atiwill *et al*., 2006, Alves et al., 2003, Enriquez et al., 1997).

In cognisance with our report, Brandford and Schijven, (2002), Bjorkman and Mattsson, (2006) reported 15-31% *Cryptosporidium* oocyst in packed manure in Kentucky and Ohio. In another similar studies of farm animals in Canada, Caroline *et al.,* (2006) reported that 20% of horses re-infected with Giardia and 17% with *Cryptosporidium* from manure. On the contrary, Fayer and Andrew, (1998) in California did not find Giardia or *Cryptosporidium* in manure. Our finding of *Cryptosporidium* is consistent with that by Garber *et al.,* (1994). Neonatal calves are particularly susceptible to infection and can excrete several billion oocysts if they develop cryptosporidiosis (Atiwill et al. 2006, Anderson and Hall, 2006). Recent studies indicate that post weaned and adult, asymptomatic cattle also excrete oocysts (Alves et al., 2006, Braam, 2005, Castro-Hermida et al., 2002a, b & 2006). Although excretion rates (oocysts/gram of faeces) may be lower, the total number of oocysts excreted may still be substantial due to the quantity of faeces produced, oocysts must first be "released" from the manure matrix before they can be transported to surface water or groundwater (Bradford and Schijven , 2002 , Castro -

Hermida et al., 2005). However, in general, manure enhanced the attachment of oocysts to soil particles, indicating that some component of manure facilitated oocyst attachment. Our results therefore indicate that oocyst attachment to soil and its later contamination to humans is substantially affected by the availability of infected manure (Rose, 1997, Gracczyk and Cranfield, 1998, Xiao et al., 2004, Yu et al., 2004).

This study provides evidence that cryptosporidiosis is prevalent in both humans and animals in Jos and environs. The prevalence of the oocysts in soil and manure samples was encountered more during the dry season. Manure management programs to reduce runoff from agricultural sites, testing and repair or elimination of leaky septic tanks, and improved cleaning of waste-water that is released into surface waters will reduce loading of all water-borne pathogens including *Cryptosporidium species*. High prevalence was recorded in the water samples during the rainy season.

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