Prevalence of Potentially Zoonotic Intestinal Protozoa, Cryptosporidium and Giardia Species in Pigs in Tigray Region, Northern Ethiopia

Herani Brhanie, Tadesse Dejenie and Zewdneh Tomass

Department of Biology, College of Natural and Computational Science, Mekelle University, Ethiopia.

Abstract: The objective of this study was to determine the prevalence of potentially zoonotic intestinal protozoa, Cryptosporidium and Giardia species (spp), in extensively managed pigs in Mekelle and Southern zone of Tigray, Northern Ethiopia. Fecal samples were collected from the rectum of 400 pigs with strict sanitation during June - September, 2012 and examined through modified Ziehl-Neelsen staining technique and formalin-ether concentration method for oocysts of Cryptosporidium and cysts of Giardia, respectively. Out of 400 pigs examined, 14 (3.5%) and 29 (7.3%) were infected by Cryptosporidium and Giardia species, respectively. Male pigs were more infected with Giardia than females (P=0.033). Moreover, age of pigs had significant effect on the prevalence of both Giardia (P= 0.037) and Cryptosporidium (P= 0.038) in pigs examined. On the other hand, site from where pigs were sampled had effect on the prevalence of Giardia (P=0.009) but not on the prevalence Cryptosporidium spp (P=0.158). Prevalence of Giardia and Cryptosporidium species in extensively managed pigs is a good source of evidence for classifying pigs as one of the potential reservoir hosts of the pathogens with possibility of zoonotic outbreaks. Further investigations on molecular characterization are mandatory for identification of species of the pathogens.

Key words: Cryptosporidium Species Giardia Species Zoonotic Intestinal Protozoa Pig Mekelle Southern Tigray Ethiopia

INTRODUCTION

Cryptosporidium and Giardia spp can infect wide range of animals and responsible for diarrheal diseases in human and animals [1]. Among other animals extensively managed pigs are considered to be important reservoir hosts for zoonotic intestinal protozoa such as Cryptosporidium and Giardia species [2].

Free ranging or extensive pig husbandry is common in rural and urban areas of developing countries [3]. Moreover, feral swine are highly mobile disease reservoirs and of increasing concern due to their potential role in the spread of zoonotic and livestock pathogens such as avian influenza, brucellosis, classical swine fever, foot and mouth disease, cryptosporidiosis and trichinosis [4]. However, very few studies have reported cryptosporidiosis and giardiasis in pigs and hence, the actual and potential contribution pigs for the zoonotic transmission of the diseases is poorly understood [5, 6].

In Ethiopia, there is little practice of swine production, however population of pigs has shown slight increment during the years 1980-2000, which estimated to be 19,000 [7]. Recently small scale pig production is increasing in Ethiopia. However, the production system practiced is mainly extensive in which pigs are left to scavenge and roam on household or municipal garbage for feed. Such pig management is considered to be among the risk factors for infection of pigs with range of gastrointestinal parasites with obvious potential for zoonoses [8, 9]. On the other hand, the prevalence of potentially zoonotic protozoa such as Giardia and Cryptosporidium species in pigs is not well studied in Ethiopia [8-10].

Corresponding Author: Tadesse Dejenie, Department of Biology, College of Natural and Computational Science, Mekelle University, Ethiopia.
Therefore, the objective of this study was to assess the prevalence of potentially zoonotic intestinal protozoa, *Cryptosporidium* and *Giardia* species in extensively managed pigs in Mekelle and Southern Zone of Tigray, Northern Ethiopia, for generating baseline information for control and prevention of potential zoonoses of these parasites.

**MATERIALS AND METHODS**

**Study Area:** The study was carried out in Mekelle (776km far from Addis Ababa, capital city of Ethiopia) and urban areas of Southern Zone of Tigray Region, Northern, Ethiopia. The Southern zone of the region contains major towns including Alamata, Maychew, Adigudom, Mekoni and Koram. The zone lies between longitude 39°5'-39°8' and latitude 12°3'- 13°7' and the altitudinal range of the zone is from 1178masl to 3114masl. The mean annual rainfall of the zone is 628.8mm, reaching its peak from June to September while annual temperature is between 8°C and 30°C with small annual variations [11].

**Study Design and Animals:** This cross sectional survey was conducted during June -September, 2012. Prior to collection of fecal samples, “Tabiyas” (smallest administrative units in the region) were visited for permission and information about the presence of households owning pigs in the area.

After obtaining oral consent from owners, pigs were registered with descriptions including locality, sex and age. According to information from the owners pigs were categorized into three age groups: < 6 months, 6- 12 months and > 12 months.

**Sampling Design:** There was lack of data on the prevalence of *Cryptosporidium* and *Giardia* species among pigs in the study areas, to serve as a basis for the estimation of the sample size. Therefore, sample size was determined by using the statistical formula of sample size calculation $n = p (1-p) z^2/d^2$, where, $n =$ required sample size, $z =$confidence level at 95% which is standard value of 1.96, $p =$ estimated prevalence of intestinal parasite and $d =$ marginal error at 5%, standard value of 0.05 [12]. To minimize sampling error during sample collection 16 pigs were added then the calculated sample size was 400 pigs.

**Fecal Sample Collection and Examination:** Approximately 5 g of fresh fecal samples were collected directly from the rectum of each pig (n= 400) with strict sanitation and preserved in vials containing 10% formalin [24]. The samples were transported to Parasitological and Microbiology laboratory, Mekelle University, Ethiopia and stored in refrigerator at -4°C until processing and examination

**Modified Ziehl Neelsen Staining Technique:** Thin fecal smear was prepared from each of the sample, air-dried, fixed with absolute methanol and stained with carbolfuchsin (primary stain) for 30 minutes. The smear was then washed with tap water followed be decolourization with 1% acid-alcohol for two minutes. The smear was again washed with tap water followed by counter staining with 1% methylene blue for 2 more minutes and rinsed in tap water and air-dried. The smear was then covered with immersion oil and examined under a microscope with 100 objective lense for detection of oocysts of *Cryptosporidium* species [13].

**Formalin-Ether Concentration:** Using an applicator stick, about 1 g of preserved each fecal sample was placed in a clean 15 ml conical centrifuge tube containing 7 ml of 10% formalin. The sample was mixed thoroughly with applicator stick. The resulting suspension was filtered through a sieve into a beaker and the filtrate was poured back into same tube. After adding 3 ml of diethyl ether to the mixture it was hand shaken and centrifuged at 2000 rpm for 3 minutes. After centrifugation, the supernatant was carefully discarded and the residue was smeared on microscope slides, covered with cover glass and examined systematically using the 40 objective lens. The entire field of vision under the cover slip was for cysts of *Giardia* species [13].

**Statistical Analysis:** Statistical analysis was performed by SPSS software. Chi-squared test was used to estimate if age, sex and locality of pigs had significant effect on prevalence of *Cryptosporidium* and *Giardia* species. P value less than 0.05 were considered to be statistically significant.

**RESULTS**

Out of 400 pigs examined 14 (3.5%) and 29 (7.3%) were found to be infected with *Cryptosporidium* and *Giardia* species, respectively.

The prevalence of *Giardia* spp was higher in male pigs than in females (p=0.033), however, there was no significant difference in the prevalence of *Cryptosporidium* infection between sexes (p=0.174) (Table 1).
Table 1: Prevalence of Cryptosporidium and Giardia species in extensively managed pigs according to sex in the study areas.

<table>
<thead>
<tr>
<th>Sex</th>
<th>Cryptosporidium</th>
<th>Giardia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>9 (4.8%)</td>
<td>19 (10.2%)</td>
</tr>
<tr>
<td>Female</td>
<td>5 (2.3%)</td>
<td>10 (4.7%)</td>
</tr>
</tbody>
</table>

Table 2: Prevalence of Cryptosporidium and Giardia species among different age groups of pigs in the study areas

<table>
<thead>
<tr>
<th>Age Group</th>
<th>Cryptosporidium Species</th>
<th>Giardia Species</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 6 months (n = 233)</td>
<td>5 (2.14%)</td>
<td>17 (7.3%)</td>
</tr>
<tr>
<td>6-12 months (n = 140)</td>
<td>6 (4.3%)</td>
<td>7 (5%)</td>
</tr>
<tr>
<td>&gt;12 months (n = 26)</td>
<td>3 (11.5%)</td>
<td>5 (19.2%)</td>
</tr>
<tr>
<td>Total</td>
<td>14 (3.5%)</td>
<td>29 (7.3%)</td>
</tr>
</tbody>
</table>

Table 3: Prevalence of Cryptosporidium and Giardia species according to study locality

<table>
<thead>
<tr>
<th>Study Site</th>
<th># Pigs</th>
<th>Cryptosporidium</th>
<th>Giardia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mekelle</td>
<td>84</td>
<td>5 (5.9%)</td>
<td>2 (2.4%)</td>
</tr>
<tr>
<td>Adigudom</td>
<td>9</td>
<td>1 (11.1%)</td>
<td>3 (33.3%)</td>
</tr>
<tr>
<td>Maychew</td>
<td>143</td>
<td>7 (4.9%)</td>
<td>14 (9.8%)</td>
</tr>
<tr>
<td>Alamata</td>
<td>131</td>
<td>1 (0.8%)</td>
<td>9 (6.9%)</td>
</tr>
<tr>
<td>Korem</td>
<td>23</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Mehoni</td>
<td>10</td>
<td>0 (0%)</td>
<td>1 (10%)</td>
</tr>
<tr>
<td>Total</td>
<td>400</td>
<td>14 (3.5%)</td>
<td>29 (7.3%)</td>
</tr>
</tbody>
</table>

The highest prevalence of Cryptosporidium species was 3(11.5%) in pigs aged greater than 12 month followed by those aged 6-12 months that is 6(4.3%) Moreover, higher prevalence of Giardia species was found to be 3(19.2%) in pigs aged beyond 12 months. The prevalence of Giardia spp (P = 0.037) and Cryptosporidium spp (P= 0.038) among the different age groups were statically significant (Table 2).

Locality had no effect on the prevalence of Cryptosporidium species (P=0.158). However, locality had effect on the prevalence of Giardia species with higest record in pigs from Adigudom 3(33.3%) (P=0.009) (Table 3).

**DISCUSSION**

The observed overall prevalence rate of cryptosporidium and Giardia spp in this study was found to be relatively higher than studies done in Holetta, Ethiopia [8]. The overall prevalence rate of Cryptosporidium spp in the present study was comparable with the report by Tomass et al. [9] in the same region. However, the overall prevalence observed in our survey was lower than the reported prevalence from Western Uganda, Lusaka, Zambia and China [14, 15]. The lower prevalence of the parasites in this report might be due to the classical methods used for oocyst and cyst detection contrary to the highly sensitive molecular methods [16].

The prevalence of Cryptosporidium spp infection in pigs in the current study did not show difference among different sexes this is in agreement with a report from Holetta, Ethiopia [8]. Moreover, studies from other countries reported similar prevalence rates in both sexes [17, 15]. This lack of association between Cryptosporidium infection and sex might be due to the fact that both sexes are equally exposed to the pathogens due to their indiscriminate behavior of roaming on garbage. In the present study, the prevalence of Cryptosporidium spp infection was higher in the age group greater than twelve months old. This was in agreement with the work of Olson et al. [18]. Besides, Yin et al.[15], from China reported that infection due to Cryptosporidium is age dependent. Serologic surveys in animals also support our findings that is, much higher prevalence of cryptosporidiosis in adults than in fattile [19]. This speculation can further be substantiated by the fact that older pigs are highly mobile and with greater chance of encountering with contaminated feed than the younger ones. The reason for the lower prevalence of cryptosporidiosis in young pigs might be due to the fact that these age groups are either lactating or supported by special feed at their pens and not released to the field for free ranging or scavenging. In this study the prevalence of Cryptosporidium spp was not significantly associated with site/locality suggesting that the possibility of getting infection with Cryptosporidium is similar for all sites. This is in agreement with a report from Spain [20]. But contrary observation was also reported from two cities of china where the prevalence of Cryptosporidium in Shanghai 14.3% was lower than in the Shaoxing 25% [21]. The reason for equal exposure of all the study sites of pigs to Cryptosporidium spp in the present study may be due to similar feeding on household and municipal garbage in the study areas.

In the present study male pigs were more infected than females by Giardia spp. This is in agreement with Atwill et al. [22]. On the contrary[14], reported similar prevalence of Giardia spp in both male and female pigs in Lusaka, Zambia. The higher prevalence of Giardia spp observed in male pigs might be due to the fact that pregnant and lactating female (sows) might be kept at home and hence have less exposure to garbage scavenging than male pigs.
In the present study, the prevalence of *Giardia* spp was highest in the pigs aged greater than 12 months. On the contrary [9] reported majority of gastrointestinal infection occurring in pigs with ages ranging from 5-12 months in extensively managing pigs in the same region. On the other hand, *Giardia* spp infections appear to be quite common in pigs and have been found in all age groups from nursing piglets to boars and sows [16]. The present finding was generally in agreement with the reports of Atwill *et al.* [22]. Contrary to this, similar age distribution of infection has been observed in studies in Western Australia; *Giardia* was detected in 17% of fatteners and 20% of sows [16]. However, Hove, Lind and Mukaratirwa [23] reported that domestic pigs reared under different management systems in Zimbabwe had higher rate and intensity of shedding of *Giardia* spp younger ages. The lower prevalence observed in younger age group pigs may be that the mother has a prolonged breast feeding of their calf that may reduce their scavenging dependency on garbage than older pigs and also older pigs can easily acquire diseases from other livestock.

In this study the Prevalence of *Giardia* spp infection has shown a significant variation among study sites. The reason may be due to the response of pigs to *Giardia* spp in different habitat and cultural difference among the communities in the study sites, which might be associated with different sanitary condition practice by pig owners, developmental status of the site and management system. Moreover, developed areas have more sanitation practices and give more attentions for their pigs and supplies additional feed to reduce their scavenging dependency on garbage, which might minimize the chance of getting *Giardia* spp cysts.

Our findings reported that infection due to *Cryptosporidium* spp was associated only with age of pig, but not to the study area and sex. On the other hand, infections due to *Giardia* spp were associated with sex, age and study area. One of the justifications for such difference is the difference in transmission mechanism of the two species, *Cryptosporidium* and *Giardia* and may be also the difference in the immunity response of pigs to the pathogens. On the other hand, from our finding one can deduce that the response of pigs to the infections due to *Cryptosporidium* and *Giardia* spp was different.

**CONCLUSION**

This study added to the knowledge of the epidemiology *Cryptosporidium* and *Giardia* species in extensively managing pigs in Mekelle and urban areas of southern zone of Tigray, Northern Ethiopia. In general, the results of the present survey is a good source of evidence that indicate extensively managed pigs are among the major potential reservoirs for the outbreaks of zoonotic protozoan parasites, *Cryptosporidium* and *Giardia* in the study area. The information may be used in design and application of control strategies. Further investigations on molecular characterization are mandatory for identification of these parasites species.

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**REFERENCES**