Full Length Research Paper

Gastrointestinal helminths of semi-domesticated helmeted guinea fowl (*Numida meleagris*) under different management systems in Arua district, Uganda

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Multi-stage and purposive sampling designs were undertaken to identify the smallest unit for extraction of samples to determine the prevalence of gastrointestinal helminths of the guinea fowls in the free-range management system (FRMS) and semi-scavenging management system (SSMS) in Arua district. Gastrointestinal tracts (GIT) were extracted from 120 guinea fowls, 60 from each management system. All the 60(100%) guinea fowls from FRMS harboured helminths, whereas only 49(81.7%) from SSMS were infected. The number of helminths *Hymenolepis carioca* ($X^2=17$, $p<0.001$), *Heterakis gallinarum* ($X^2=7.60$, $p<0.01$) and *Subulura brumpti* ($X^2=4.82$, $p<0.05$) were significantly higher in FRMS than in the SSMS. The prevalence of all species was higher in the FRMS compared to SSMS except for *Hartertia gallinarum* (3.3%) which was the same in both systems. Ten helminth species, namely; *Hymenolepis carioca*, *Ascaridia galli*, *Heterakis gallinarum*, *Dispharynx spiralis*, *Raillietina tetragona*, *Subulura brumpti*, *Prosthogonimus* spp., *Hartertia gallinarum*, *Strongyloides avium* and *Raillietina echinobothrida* were identified. The mean worm burden of *Hymenolepis carioca* (FRMS, 140±21.7; SSMS, 63.4±14.7), *Ascaridia galli* (FRMS, 7.3±3.5; SSMS, 0.03±0.0) and *Subulura brumpti* (FRMS, 12.7±2.8; SSMS, 4.3±2.1) were significantly higher in FRMS than SSMS. It is important to separate guinea fowls from other poultry as well as improve hygiene measures in both management systems in order to realise a healthy flock.

Keywords: Helminths, helmeted guinea fowl, management systems, Uganda.

INTRODUCTION

Guinea fowl production is one area with a potential to alleviate poverty if it is successfully pursued and incorporated into Uganda’s poultry sector, taking into consideration the various advantages it has over other poultry. The guinea fowl provides high-quality meat, which has been classified as game meat and described by many as halfway between free-range chicken and pheasant, and commands high premium rates (Hastings, 1985; Hayes, 1999). A well-fed guinea fowl at 20 weeks will weigh about 1.6-1.8 Kg and will have more meat to bone ratio compared to conventional poultry (Hastings,

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1985). Their eggs have a more york-white ratio and are a delicacy with good flavour (Hastings, 1985). Each guinea fowl is reported to lay between 180-200 eggs under intensive management system, 80-100 eggs under the semi-scavenge management system (SSMS) and not less than 40 eggs under the free-range management system (FRMS) in a single laying season (Hastings, 1985; Hayes, 1999; Saina, 2005). Unlike chickens, guinea fowls do little damage to vegetables and can be used in biological control of pests like ticks, mites and flies (Ferguson, 1999). There is an increasing demand for the production of guinea fowls in Uganda, with many enterprises found mainly in Arua, Yumbe, Mukono and Rakai districts. Despite the high demand, guinea fowls like other poultry are faced with morbidities and mortalities due to mismanagement, poor feeding and diseases (Haziev and Khan, 1991; Saina 2005). The commonest infections affecting guinea fowls include endoparasites, particularly helminths, which cause a lot of production losses (Okaema, 1988; Haziev and Khan, 1991; Hayes, 1999).

Birds raised by FRMS are more exposed to many infections with various parasites (Soulsby, 1983; Kabatange and Katule, 1989), especially helminths. In many FRMS and SSMS, guinea fowl parasitic infestations are often neglected because many think they are less prone to parasites compared chickens. Much as this may be true, poorly managed productions systems always expose guinea fowls to a variety of helminths and other endoparasites. Guinea fowls are highly susceptible to helmint infection and may be severely affected in many instances if control measures are not put in place (Haziev and Khan, 1991). Guinea fowls are often kept in households with other poultry, probably exposing them to varieties of poultry helminths. Gastrointestinal (GIT) helminths common in poultry include but not limited to those of the genus Hymenolepis, Raillietina, Ascaridia, Heterakis and Capillaria (Băcescu et al., 2011; Shukla and Priti, 2013). There is no information on helminths of guinea fowls in Uganda to compare with, but it is known that some helminths of chicken and other poultry affect them. Infection with helmint varies from one management system to another. Studies on the differences of endoparasite prevalence in free-range and intensive poultry have been done (Zetterman et al., 2005; Ibrahim et al., 2006). Studies on variations in helmint burdens between free-range and captive guinea fowls were previously described in Brazil and have been associated with cross infections (Zetterman et al., 2005). This is partially attributed to high infection densities where the wild birds or free-living ones are associated with exposure to a more varied parasitic fauna in the wild than the captive ones (Zetterman et al., 2005). Other factors like feeding ecology, habitat, population and immunity can also dictate differences in helmint parasite prevalence and burdens (Garvon et al., 2011).

Studies have demonstrated that helmint infections cause a lot of economic losses in poultry and captive bird production. Heavy helmint infestations which are often common in scavenging birds can have an impact on their health and growth (Permin et al., 1997). Many investigations concerning helmint and other endoparasites in sub-Saharan Africa have been done mainly in domestic chicken (Muhairwa, 2007) and not guinea fowls. Research on the management and health of guinea fowls has mainly been done in Southern Africa, Europe and North America (Cooper and Hilgarth, 1989; Cooper et al., 1996; Hayes, 1999).

The growing interest of guinea fowl farming in Uganda and the general lack of documented information on the diseases that affect them necessitates research in this area. This study was therefore designed to investigate the prevalence of gastrointestinal helminths in the FRMS and SSMS, ultimately producing information on a variety of helminths affecting guinea fowls in Uganda.

MATERIALS AND METHODS

Study Area

This study was carried out in Arua district, West Nile, 520km from Kampala City covering a total area of 5419.6 Km$^2$, composed of 6 counties (Maracha, Tivu, Koboko, Aivu, Terego, Vura and two urban councils (Koboko town council &Arua municipality, and lying between latitude 2°30' N and 3°50' N, longitude 30°30' E, 31°30' in the North West part of Uganda. It is bordered by the Republic of Sudan in the Northwest, Yumbe in the North East, DRC in the West and Nebbi in the South and Gulu in the East.

Study design and data collection:

Sample size determination (Total number of birds)

The required overall guinea fowl sample size was calculated according to Martin et al. (1987) and Thrusfield (2007) using the formula $n = \frac{4PQ}{L^2}$.

Where:

- $P =$ prevalence/ expected prevalence in the flock/population
- $Q =$ 1-P and
- $L =$ specifies the desired limit of error of prevalence/ required precision, i.e. the largest acceptable difference between true and estimated prevalence.
- $n =$ total number of samples to collect.

The exact prevalence of helmint infection in that particular area was not known so to maximize the sample size, it was assumed that expected prevalence was 50%, precision (desired error limit) was 13% and the confidence level was 95%; i.e. the prevalence can be calculated to be within 13% of the true prevalence 95% of the time. The number of birds in the population has little
influence on the required sample size except when is greater than 0.1XN, where N indicates the population size (Martin et al., 1987).

The required sample size \( n = \frac{4 \times 0.5 \times 0.5}{0.13^2} = 59.2 \)

Therefore, for each management system identified, 60 guinea fowl were examined.

Sampling strategy

A multi-stage and purposive sampling design (Martin et al., 1987; Thrusfield, 2007) was undertaken. Arua district was selected conveniently based on the reports that it had most of the guinea fowls in the region. From the Veterinarian in charge of the area, information on general guinea fowl distribution was got, establishing the counties that had the majority of guinea fowls in the district. Koboko, Aivu and Maracha counties were selected. Koboko was then picked because it had most guinea fowls and was originally the source for all the other counties. Since the county was large, further sampling was done according to Local Council (LC) Zones. A list frame of all LC zones was made and only those with guinea flock sizes greater than 10 (purpose sampling) were taken. Thirty farmers were identified to provide the study specimen.

Humane guinea fowl slaughter and extraction of gastrointestinal tract (GIT)

The birds were slaughtered humanely and GIT extracted as described by Arnall and Keymer (1975), Gordon and Jordan (1982). The left hand held the legs or base of the wings together over the back, whilst the right hand grasped the head with the palm against the forefinger and thumb. The head was bent vertically upward by the thumb under the beak, whilst at the same time, the head was pulled firmly and steadily forward, hence stretching the neck, dislocating the skull from the neck and breaking the cord. The entire GIT was extracted from each bird and examined for helminths. Following postmortem extraction of the GIT, the guinea fowl carcasses in each village were distributed to the individuals who helped with the work as part of an incentive. The slaughter of the birds occurred every evening prior to the day of traveling from Arua district to the Veterinary parasitology laboratory at Makerere University.

Extraction of GIT Helminths and their processing

Each intestine was put on a tray and spread out to expose the esophagus, crop, proventriculus, duodenum, jejunum, ileum, caecum, rectum, and cloaca. Each section was opened longitudinally and the contents carefully put onto a petri dish (Polystyrene disposable, 60×15mm, Carolina® lab supplies and equipment, USA). The intestinal wall was slowly scraped with specula to collect helminths embedded in the muscular layer. Water was added to the petri dish and helminths were sorted under a stereomicroscope. After sorting helminths were transferred to falcon tubes (Falcon® Polypropylene, Supplier, Discovery Labware, USA) containing 70% of ethanol.

Morphological (Microscopic) identification of the organisms at genus and species level

To determine the genus/species of each helminth, external and internal features were observed (Kauffman, 1996; Soulsby, 1982). Preserved parasites were poured onto Petri dish and Nematodes, Cestodes, and Trematodes were separated according to their morphology under a light microscope (Olympus® light Microscope, USA). Helminths were placed on glass slides and 2-4 drops of lactophenol (25g phenol crystals+25mls lactic acid +50mls of water) were added to clear and make the worms transparent in order to see the identifying features. After about six minutes, the mounted helminths were observed under light microscope x10 objective and identified accordingly.

After 12 hours, the helminths were removed from alcohol, stained with canine for 20 minutes and discolored in 1% acid alcohol for one minute. To be able to clearly identify large helminths like Ascaridia, parasites were moved into a series of alcohol concentrations as follows; 70% - 80% - 85% - 90% -95% -100% for two hours in each percentage and one hour for smaller helminths like Heterakis spp. The helminths were then cleared in creosote for 12 hours to make them transparent in order to see the identifying features of the parasite. Canada balsam mountant was poured onto the glass slide to cover the parasite after which a cover slip was put over the parasite and examined under a light microscope using the x10 objective.

STATISTICAL ANALYSIS

Data was analysed using IBM SPSS version 22. Numerical variables were summarised using mean and standard error of the mean (SEM). Univariate analysis to compare the prevalence of the helminths across management systems was done using cross-tabulation with a Chi-square test. Variables with a p-value of ≤ 0.05 were taken to be significant.

RESULTS

Morphological (Microscopic) identification of the organisms at genus and species level

Six different nematodes (Ascaridia galli, Heterakis gallinarum, Synhimantus (Dispharynx) spiralis, Subulura...
brumpti, Strongyloides avium, Harteria gallinarum), three cestodes (Raillietina tetragna, Raillietina echinobothrida, Hymenolepis carioca) and one trematode (Prosthogonimus spp.) were isolated in this study (Table 1).

Comparing of adult helminth prevalence in FRMS and SSMS

Using Chi-square ($\chi^2$) test to compare the association in the two systems of management; Hymenolepis carioca ($\chi^2=17$, $p<0.001$), Heterakis gallinarum ($\chi^2=7.60$, $p<0.01$) and Subulura brumpti ($\chi^2=4.82$, $p<0.05$) parasite numbers were significantly higher in FRMS than in the SSMS. Meanwhile Ascaridia galli ($\chi^2=2.88$, $p>0.05$), Dispharynx spiralis ($\chi^2=0.07$, $p>0.05$), Strongyloides avium ($\chi^2=0.00$, $p>0.05$), Harteria gallinarum ($\chi^2=0.00$, $p>0.05$), Raillietina tetragna ($\chi^2=0.00$, $p>0.05$), Raillietina echinobothrida ($\chi^2=0.00$, $p>0.05$) and Prosthogonimus spp. ($\chi^2=0.90$, $p>0.05$) were non significant (Table 1).

<table>
<thead>
<tr>
<th>Helminths</th>
<th>FRMS (n=60)</th>
<th>SSMS (n=60)</th>
<th>Level of Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\chi^2$</td>
<td>$p$-value</td>
<td></td>
</tr>
<tr>
<td>Ascaridia agalli</td>
<td>27</td>
<td>18</td>
<td>2.88</td>
</tr>
<tr>
<td>Heterakis gallinarum</td>
<td>17</td>
<td>10</td>
<td>7.6</td>
</tr>
<tr>
<td>Dispharynx spiralis</td>
<td>9</td>
<td>8</td>
<td>0.07</td>
</tr>
<tr>
<td>Subulura brumpti</td>
<td>7</td>
<td>1</td>
<td>4.82</td>
</tr>
<tr>
<td>Strongyloides avium</td>
<td>2</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Harteria gallinarum</td>
<td>8</td>
<td>6</td>
<td>0.03</td>
</tr>
<tr>
<td>Raillietina tetragna</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Raillietina echinobothrida</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Hymenolepis carioca</td>
<td>54</td>
<td>34</td>
<td>17</td>
</tr>
<tr>
<td>Prosthogonimus spp.</td>
<td>7</td>
<td>4</td>
<td>0.9</td>
</tr>
</tbody>
</table>

* $p<0.05$; ** $p<0.01$; *** $p<0.001$ (significant at these levels)
ns: Non significant

Helminth prevalence and mean (SEM)

The prevalence of all species was higher in the FRMS compared to the SSMS except for Harteria gallinarum which was the same (3.3%). The commonest helminth was Hymenolepis carioca (FRMS, 90%; SSMS, 56.7%). This was followed by Ascaridia galli (FRMS, 45%; SSMS, 30%) and then Heterakis gallinarum (FRMS, 28.3%; SSMS, 16.7 %). Prosthogonimus spp. that could not be identified to species level was processed, preserved and stored.

The mean worm burden of Hymenolepis carioca (FRMS, 140±21.7; SSMS, 63.4±14.7), Ascaridia galli (FRMS, 7.3±3.5; SSMS, 0.03±0.0) and Subulura brumpti (FRMS, 12.7±2.8; SSMS, 4.3±2.1) were significantly higher in FRMS than SSMS. The difference in mean numbers for the other helminth in FRMS and SSMS was not significant (Table 2). Although the helminth egg prevalence was mostly higher in FRMS, it did not fully represent the level of infection (Table 2).

DISCUSSION

The largest numbers of guinea fowls in Uganda are concentrated in the West Nile region, particularly Arua district, but little is known about the prevalence and burden of parasites that affect them. This is the first study in Uganda looking at guinea fowl helminths, a group of parasites that are known to cause disease and production loss in animals. The study reports the presence of nematodes (Ascaridia galli, Heterakis gallinarum, Synhimantus (Dispharynx) spiralis, Subulura brumpti, Strongyloides vium, Harteria gallinarum), cestodes (Raillietina tetragna, Raillietina echinobothrida, Hymenolepis carioca) and a trematode (prosthogonimus spp.). Many of these helminth species have been reported in domestic chicken and guinea fowls elsewhere in the world (Muhairwa et al., 2007; Garvon et al., 2011; Băcescu et al., 2011; Ferdushy et al., 2014; Nalubamba et al., 2015). In Uganda, the helminth Ascaridia spp., Heterakis spp., Syngamus trachea, Capillaria spp., Strongyloides avium, Gongylonema ingluvicola, Raillietina spp., Postharmostomum commutatum and Hymenolepis carioca have been associated with broilers and indigenous chicken (Senyonga, 1982; Kabatange and Katula, 1989). The trematode Prosthogonimus spp. identified in the guinea fowls was different from that often identified in the guinea fowls.
Table 2. Prevalence (%) and mean worm count in two guinea fowl management systems.

<table>
<thead>
<tr>
<th>Worm type</th>
<th>FRMS (n=60)</th>
<th>SSMS (n=60)</th>
<th></th>
<th></th>
<th></th>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Worm prev (%)</td>
<td>Egg prev (%)</td>
<td>Worm count</td>
<td>Mean±SE</td>
<td>Worm prev (%)</td>
<td>Egg prev (%)</td>
<td>Worm count</td>
</tr>
<tr>
<td>Nematodes</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Ascaridia galli</td>
<td>45</td>
<td>16.7</td>
<td>764</td>
<td>12.7±2.8</td>
<td>30</td>
<td>5</td>
<td>262</td>
</tr>
<tr>
<td>Heterakis gallinarum</td>
<td>*28</td>
<td>8.3</td>
<td>157</td>
<td>2.6±0.1</td>
<td>16.7</td>
<td>8.3</td>
<td>121</td>
</tr>
<tr>
<td>Dispharynx spiralis</td>
<td>15</td>
<td>6.7</td>
<td>22</td>
<td>0.4±0.2</td>
<td>13.3</td>
<td>3.3</td>
<td>31</td>
</tr>
<tr>
<td>Subulura brumpti</td>
<td>12</td>
<td>-</td>
<td>437</td>
<td>7.3±3.5</td>
<td>1.7</td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td>Strongyloides avium</td>
<td>1.7</td>
<td>-</td>
<td>1</td>
<td>0.02±0.0</td>
<td>0</td>
<td>-</td>
<td>0</td>
</tr>
<tr>
<td>Hartertia gallinarum</td>
<td>3</td>
<td>-</td>
<td>13</td>
<td>2.2±0.2</td>
<td>-</td>
<td>-</td>
<td>5</td>
</tr>
<tr>
<td>Cestodes</td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>Raillietina tetragona</td>
<td>13</td>
<td>3.3</td>
<td>311</td>
<td>5.2±0.3</td>
<td>10</td>
<td>-</td>
<td>139</td>
</tr>
<tr>
<td>R. echinobothrida</td>
<td>1.7</td>
<td>-</td>
<td>60</td>
<td>1±0.1</td>
<td>0</td>
<td>-</td>
<td>51</td>
</tr>
<tr>
<td>Hymenolepis carioca</td>
<td>* 90.0</td>
<td>11.7</td>
<td>8402</td>
<td>140±21.7</td>
<td>56.7</td>
<td>3.3</td>
<td>3805</td>
</tr>
<tr>
<td>Trematodes</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prosthogonimus pp.</td>
<td>11.7</td>
<td>5</td>
<td>53</td>
<td>0.9±0.5</td>
<td>6.7</td>
<td>-</td>
<td>30</td>
</tr>
</tbody>
</table>

prev = prevalence
n is the number of birds examined
SE is the standard error of mean
*Significant difference (prevalence) compared to the other system
** Significant difference (mean worm count) compared to the other system
isolated from other poultry, necessitating further studies to differentiate it. Helminth prevalence was significantly higher in FRMS compared to SSMS. High helminth prevalence in guinea fowls have been documented elsewhere in the world but investigators have often reported different species from the ones found in this study. This high helminth prevalence is highly consistent with reports from research done on guinea fowls from tropical areas in South and West Africa (Ayeni, 1973; Crowe, 1977; Ayeni, 1983; Ferdushy et al., 2014; Nalubamba et al., 2015) as well as on other poultry from Central, East and West Africa (Ssenyonga, 1982; Msanga and Tungaraza, 1985; Mpoame and Agbede, 1995). The life cycle of A. galli and H. gallinarum is directly from faeces contaminated environment (Permin, 1997). This explains the success rate of the parasites in FRMS and SSMS. The life cycle of Hymenolepis carioca is indirect with infection occurring by eating up intermediate hosts like beetles and leeches (Soulsby, 1982) which are usually delicacies for guinea fowls. Ascaridia galli eggs often act as vectors of salmonella (Chadfield et al., 1997). Heavy Ascaridia infections may affect the transmission of salmonella within the flock. Similarly, it has been shown that Heterakis gallinarum may play an important role in the transmission of Histomonas meleagridis.

The differences in the prevalence and means (SEM) of helminth in the two systems could also be due to differences management. The prevalences of Raillietina tetragona, Synhimanus (Dispharynx) spiralis, Subulura brumpti, Hartertia gallinarum, Prosthogonimus spp. were low and their differences in the two management systems were not very significant. Strongyloides avium and Raillietina echinobothrida were only isolated in FRMS guinea fowls. Free-range guinea fowls had all the ten species of helminth while semi-scavenging guinea fowls had only eight species. This could be attributed to the fact that free-ranging guinea fowls spent more time scavenging and so were more predisposed to faeces contaminated environments and various intermediate hosts. However, in both management systems, the birds were predisposed to ingestion of free-living stages of helminth in the faeces contaminated environment and intermediate hosts.

The findings of this study suggest that the risk of guinea fowls becoming infected by helminths is higher on FRMS, however, results show high helminth prevalence in both systems. This indicates poor husbandry practices among the rural farmers, which is attributed to lack of sufficient knowledge on the guinea fowl management and breeding. It is, therefore, imperative to provide for best husbandry techniques coupled with regular handling and clinical examination in order to contribute to better health and welfare of the domesticated guinea fowls with the intent of providing for sustainable development in communities as well as bio-diversity conservation.

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