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Comparison of growth and heterosis of body parameters of two varieties of snail [Arachatina marginata (Swainson1821)] and their crosses reared under tropical conditions

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INTRODUCTION

Breed types, according to Okon et al. (2012a), have a marked effect on performance and production of snails than all other factors considered. Thus, these authors asserted that the performance of any animal is dependent upon the inherent genetic make-up and the environment in which it is raised. In Nigeria, there are different breeds of giant African land snails and they vary in size, colour, adaptability and performance (Amusan and Omidiji, 1998; Okon et al., 2012b). These breeds include Arachatina marginata, Achatina achatina, Achatina fulica, Limicolaria species and Thapsia species. Within a snail breed, there exist varietal differences in foot pigment, length of whorls, aperture size (length and width) and egg clutch size (Okon and Ibom, 2012). Arachatina marginata is the largest known breed of snail in Africa (Omole, 1998; Olawoyin and Ogogo, 2006; Okon et al., 2012b) and has two varieties namely, Arachatina marginata var. ovum and Arachatina marginata var. saturalis. On Arachatina marginata var. ovum, both columella and parietal callus are white, while on Arachatina marginata var. saturalis, both columella and parietal callus are red. Most studies carried out combined these two varieties as one breed and these have not really given the true performance or production of these varieties.

Growth studies of A. marginata var. ovum and A.
Archachatina marginata var. saturalis have been extensively done by Ibom et al. (2011), Ibom and Okon (2012), Okon et al. (2008, 2012a, 2012b, 2014), Imran and Alarape (2012), Isah and Peter (2012), Jayeola et al. (2013), Onuoha (2014), Oyeagu et al. (2015), Fayenuwo et al. (2015), Egbe et al. (2015), and Ejidike and Oladipo (2015). Growth measured in terms of body weight gain (BWG) is the most widely used growth index from birth to maturity (Okon and Ibom, 2012). In snails, the weight at hatch is the first indicator of hatchling growth rate and is useful as a starting point for measuring subsequent growth. Okon and Ibom (2012) also pointed out that the growth rate of snails can be measured as the increase in shell size, this being the obvious phenotypic trait. Similarly, Akinmusi (2002, 2004) opined that snail growth is measured by shell size.

Growth rate values in snails under the tropical condition have been reported by many authors (Ayai et al., 1978; Hodasi, 1984; Udedibia et al., 1986; Oyelade et al., 2013; Okon et al., 2013). The growth rate of snail varies considerably between individuals in each population group, thus Cobbina (1993) opined that snails show differences in growth rates. Ibom (2009) stated that dimensional shell parameters (shell length, shell width and shell thickness) and aperture (shell “mouth”) parameters are indices that can be taken at hatch and used to measure subsequent snail growth. Body weight and body components are the growth parameters used to measure growth in animals and are common measures of size and growth rate.

Heterosis refers to the mean performance of hybrid progeny relative to the mid-parent value (Ibe, 1998). The author added that if the average performance of the hybrid is better than the mid-parent value, heterosis is positive, otherwise it is negative. Positive heterosis is referred to as hybrid vigour. Heterosis, according to Crow (2008) is predicted to be high in small population with gene flow and to increase with population structure, whereas it is reduced by inbreeding. Also, Asumoa (1999) reported that stressful environment may influence the expression of heterosis and hybrid performance may be reduced by out breeding depression. Although, heterosis in these snail varieties does not result from fixation load, its experimental estimation provides relevant information on the trends of heterosis on them, but heterosis is expected to be mainly contributed by mildly deleterious alleles (Clift, 2010) and maximized for moderate to intermediate selection coefficients (Crow, 2008).

Growth traits show moderate heterosis. However, heterosis is not very important for conformation (or body dimension) and composition traits which have high heritabilities and show little or no depression from inbreeding (Ibe, 1998). Integrating snails into target gene pool in an organized breeding project according to Clift (2010) will be beneficial as farmers had used hybrid vigour or heterosis to enhance growth and reproductive performance of farm animals (e.g., cattle, sheep, poultry and pigs).

This paper compared growth traits and heterosis of body and shell parameters of two varieties of Archachatina marginata (var. ovum and var. saturalis) and their crosses under experimental conditions.

MATERIALS AND METHODS

Experimental site

The study was carried out at the Botanical Garden of University of Calabar, Calabar, Nigeria. Calabar (Longitude 8°17' and 10°43'E; Latitude 4°58' and 15°39'N) has annual rainfall and temperature ranges between 1260 to 1280 mm and 25 to 30°C respectively (CRADP, 1992). The botanical garden where the research was carried out provided a mini-environment similar to the natural habitat of snails - with trees (e.g., Cola abuere, Citrus spp., Carica papaya, Musa spp., Gmelina aborea), crops (e.g., cassava and yams) and forages like Centrosoma pubescence and Panicum maximum which provided shades that protected the hutches and snails from direct sunlight and heavy rainfall.

Experimental animals

Three hundred (300) juvenile snails, one hundred (100) each of the three varieties, Archachatina marginata var. ovum (AMO), Archachatina marginata var. saturalis (AMS) and their crosses (AMO x AMS) with mean body weights of 12.40 ± 0.58 g were obtained from an earlier study in the same location for this study. The snails were randomly allotted to the three (3) treatments of the varieties studied. The snails in the three (3) treatments were managed in wooden cells compartments kept under trees shades for the 5 months of study which lasted from May to September, 2015. The cells had dimensions of 40 cm (length), 40 cm (width) and 40 cm (depth). Each treatment was replicated ten (10) times in a completely randomized experimental design (CRD). The juvenile snails were housed in thirty (30) cells with ten (10) snails per cell containing sterilized loamy soil moistened with water for easy management. The snails were placed on mixed-diet of forages (fresh paw-paw leaves) and concentrates (containing 24% CP, 15% Ca and 2650 kcal/kgME). Fresh cool water was supplied ad libitum in shallow troughs during the study. The three (3) varieties of snails were kept under similar environmental and management conditions.

Measurements of body and shell parameters of snails

The body weights (BDW) were taken using Scout Pro® electronic scale with sensitivity of 0.01 g, while dimen-
sional shell parameters were measured using Vernier Caliper. The dimensional shell parameters were shell length (SL), shell width (SW), shell “mouth” length (SML) and shell “mouth” width (SMW). The measurements were as described in Ibom (2009) and El Zaffir et al. (2011).

### Data Analysis

The fixed model used in the experiment is show below as described by Ibom, 2009.

\[ Y_{ij} = \mu + S_i + e_{ij} \]

Where: \( Y_{ij} \) = Single observation, \( \mu \) = Common mean, \( S_i \) = Fixed effect of snail varieties (\( i = 1 - 3 \)) and \( e_{ij} \) = Random error associated with the measurement of each observation

Heterosis was estimated using the formular below (Ibe, 1998):

\[
\% \text{ Heterosis} = \frac{(H_i - P)}{P} \times 100
\]

Where \( H_i \) = Average performance of offspring and \( P \) = Average performance of parents

Data were subjected to analysis of variance (ANOVA) for Completely Randomised Design (CRD) experiment using Statistical Analysis System (SAS, 2006). Significantly different means were separated using Duncan’s New Multiple Range Test (Duncan, 1995) as outlined by Obi (2002).

### RESULTS AND DISCUSSION

Growth performance traits of the three *Archachatina marginata* varieties (AMO, AMS and AMO x AMS) are presented in Table 1. The initial weight recorded no significant (P>0.05) variety effect, while significant variety effect (P<0.05) existed on final body weight (FBW) and average weight gain (AWG). The AMO recorded the highest FBW, followed by AMO x AMS, while AMS recorded the least value. The results of FBW obtained fell within the range mean final body weight values from 16.43 ± 0.6 to 24.57 ± 3.5 g reported by Ademolu et al. (2004) for giant African land snails (*Archachatina marginata*) fed different nitrogen sources, but higher than Egbu (2015) range from 12.87 to 14.89 g for hatchlings of *Archachatina marginata* (A. M.). Opara (2010) reported lower mean FBW values of 16.21 g, 14.07 g and 17.75 g for F1-hybrids of three (3) giant African land snail ecotypes. The differences in the mean FBW values reported by the different authors might be due to the body weight, age, variety and size of the parent stock used, the feed type as well as management system adopted. The better performance observed for final body weight could be due to the combination of forage and concentrate as reported that snail's performance is better on mixed-feeding regime.

The AWG showed significant variety (P<0.05) effect, confirming heterosis or hybrid vigour for this growth trait. The AWG obtained for this study is quite higher than the range of 0.08 ± 0.02 to 0.11 ± 0.01 g reported by Egbu et al. (2015) for hatchlings of *Archachatina marginata* snails fed varying levels of palm kernel cake. The reason being that Egbu et al. (2015) worked with hatchlings of *Archachatina* species, while this study was on juveniles of the three varieties of *A. marginata*.

There were no significant (P>0.05) variety effects on DWG, TFI, DFI, and FCR. The TFI consumed in this study were similar to the mean total fed intake of 17.66 g, 17.98 g and 17.43 g reported by Omole et al. (2007) for *Archachatina marginata* snails raised on different stocking rates. The DFI obtained in Table 1 were similar to the mean DFI intake values of 0.14 ± 0.16 g, 0.13 ± 0.16 g, 0.15 ± 0.16 g and 0.18 ± 0.16 g reported by Oni et al. (2012) for A. M. snails fed vary protein and energy levels. Okonkwo et al. (2000) reported higher average DFI values of 1.41 ± 0.07 g, 1.52 ± 0.07 g, 1.97 ± 0.07 g, 1.41 ± 0.07 g...

### Table 1. Growth Performance Traits of *Archachatina marginata* varieties (var. ovum and var. saturalis) and their crosses.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Varieties</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T1 (AMO)</td>
</tr>
<tr>
<td>Initial body weight (g)</td>
<td>12.40 ± 0.59</td>
</tr>
<tr>
<td>Final body weight (g)</td>
<td>20.03 ± 0.94b</td>
</tr>
<tr>
<td>Average weight gain (g)</td>
<td>7.63 ± 1.11b</td>
</tr>
<tr>
<td>Daily weight gain (g)</td>
<td>0.05 ± 0.01</td>
</tr>
<tr>
<td>Total feed intake (g)</td>
<td>17.02 ± 1.68</td>
</tr>
<tr>
<td>Average Daily feed intake (g)</td>
<td>0.14 ± 0.01</td>
</tr>
<tr>
<td>Feed conversion ratio (FCR)</td>
<td>0.46 ± 0.07</td>
</tr>
</tbody>
</table>

\(^{ab}\)Means with different superscripts along the row differ significantly at P<0.05. AMO, *Archachatina marginata* var. ovum; AMS, *Archachatina marginata* var. saturalis.
Table 2. Shell Parameters of *Archachatina marginata* varieties (var. ovum and var. saturalis) and their crosses.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>T1 (AMO)</th>
<th>T2 (AMS)</th>
<th>T3 (AMO x AMS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial shell length(cm)</td>
<td>3.85 ± 0.08</td>
<td>3.36 ± 0.09</td>
<td>3.82 ± 0.08</td>
</tr>
<tr>
<td>Final shell length(cm)</td>
<td>4.47 ± 0.16</td>
<td>4.48 ± 0.12</td>
<td>4.60 ± 1.11</td>
</tr>
<tr>
<td>Shell length increment(cm)</td>
<td>0.92 ± 1.14b</td>
<td>1.12 ± 1.13a</td>
<td>0.88 ± 0.09b</td>
</tr>
<tr>
<td>Initial shell width(cm)</td>
<td>2.85 ± 0.07ab</td>
<td>2.41 ± 0.06b</td>
<td>2.61 ± 0.05a</td>
</tr>
<tr>
<td>Final shell width(cm)</td>
<td>3.45 ± 0.08a</td>
<td>3.22 ± 0.07b</td>
<td>3.48 ± 0.07a</td>
</tr>
<tr>
<td>Shell width increment(cm)</td>
<td>0.87 ± 0.11</td>
<td>0.81 ± 0.09</td>
<td>0.87 ± 0.06</td>
</tr>
<tr>
<td>Initial shell “mouth” length(cm)</td>
<td>2.62 ± 0.05</td>
<td>2.34 ± 0.05b</td>
<td>2.59 ± 0.04a</td>
</tr>
<tr>
<td>Final shell “mouth” length(cm)</td>
<td>3.34 ± 0.08</td>
<td>3.24 ± 0.08</td>
<td>3.27 ± 0.05</td>
</tr>
<tr>
<td>Shell “mouth” length increment(cm)</td>
<td>0.72 ± 0.07</td>
<td>0.07 ± 0.09</td>
<td>0.68 ± 0.05</td>
</tr>
<tr>
<td>Initial shell “mouth” width(cm)</td>
<td>1.56 ± 0.06a</td>
<td>1.39 ± 0.03ab</td>
<td>1.49 ± 0.03ab</td>
</tr>
<tr>
<td>Final shell “mouth” width (cm)</td>
<td>2.05 ± 0.03</td>
<td>2.06 ± 0.04</td>
<td>2.03 ± 0.05</td>
</tr>
<tr>
<td>Shell “mouth” width increment(cm)</td>
<td>0.49 ± 0.06</td>
<td>0.57 ± 0.04</td>
<td>0.55 ± 0.03</td>
</tr>
</tbody>
</table>

*abMeans within the same row with different superscripts are significantly different (P<0.05). AMO, *Archachatina marginata* var. ovum, AMS, *Archachatina marginata* var. saturalis.

1.61 ± 0.07g, 1.87 ± 0.07 g and 1.50 ± 0.07g for A. M. snails feed dietary levels of *Moringa oleifera* leaf-meal. The differences in TFI and average DFI reported by these authors could be attributed to feed types, forage materials and breed or genotype effect of A. M. snails used as well as the age, size, variety and weight of parental A. M. snails used in their studies. The FCR which is an indication of efficiency of feed utilization in the snail varied significantly across the varieties (P>0.05). The results of FCR obtained in this study were quite higher than those of 0.23, 0.20, 0.19, 0.24, 0.32 and 0.07 reported by Okonkwo et al. (2000) for A. M. snails fed varying dietary levels of *Moringa oleifera* leaf-meal. The feed was better utilized in the purebred AMS with the least FCR value, than the AMO and the AMO x AMS.

Table 2 shows the shell parameters of *A. marginata* varieties (AMO, AMS, and AMO x AMS) studied. Significant difference (P<0.05) variety effects were observed in the initial shell length (SL), initial shell width (SW) and initial shell “mouth” length (SML). No significant (P>0.05) variety effects were observed in final shell length, final shell “mouth” length and final shell “mouth” width, except in final shell width (P<0.05). Similarly, no significant (P>0.05) variety effects were observed for increment in SW, SML and SMW, except in SL (P<0.05).

The AMO x AMS recorded the highest final SL and final SW than the other varieties, indicating heterotic effects on these two parameters. The final SL value of AMO x AMS in this study was similar to mean shell length of 4.62 ± 0.08 cm reported by Badmos et al. (2011) for *A. marginata* fed a mixed-diet of concentrate and forages. Ejidike (2004) reported similar shell length values of 4.70 ± 0.30 cm and 5.70 ± 0.30 cm for grower *A. marginata* fed different dietary protein levels, whereas Okon et al. (2012b) reported higher mean shell length values of 9.76 cm and 10.44 cm for mature *A. marginata* and *A. fulica* respectively. The differences in mean shell length values reported by these authors could be attributed to the feeding regime used, breed, ectotypes and variety of the snail, their different ages and the prevailing environmental conditions. The final shell width value of the AMO x AMS in this study (Table 2) was similar to the mean shell width values of 3.50 ± 0.20 cm and 3.70 ± 0.30 cm reported by Ejidike (2002) for *A. marginata* snails fed different dietary levels.

The values of heterosis for body and shell parameters of *A. marginata* obtained in this study are shown in Table 3 which showed that all the varieties expressed positive heterotic values for body and shell parameters studied. Significant difference (P<0.05) variety effects were observed on body weight (BDW), shell length (SL) and shell “mouth” length (SML). No significant (P>0.05) variety effects were observed on the shell width (SW) and shell “mouth” width (SMW). The positive heterotic values expressed by body and shell parameters were in line with Nwakpu and Omeje (2005) and Ibom et al. (2014) who reported that the effects of heterosis are most often but not always positive, and depend mainly on choice of parents and selection pressure applied on parental lines (breeds).

The AMO x AMS recorded the highest heterotic values than AMO and AMS varieties for all the traits and parameters studied (Table 3), showing that the crossbred variety has advantage over the two purebred varieties.
The results of heterosis of body weight in this study was similar to heterosis of body weight of 53.73% and 57.75% reported by Ibom et al. (2011) for purebred black skinned and purebred white skinned A. marginata ectotypes respectively, but lower than the heterosis of body weight of 71.71% for the crossbred A. marginata variety. However, the heterosis values in this study for body weight were in contrast with Ibom et al. (2014) who reported lower heterosis values of 38.89% (purebred black) and 37.01% (purebred white) and 53.59% (crossbred) A. marginata for body weight at hatch. The heterotic values of shell parameters (SL, SW, SML and SMW) at five (5) months of this study were low (Table 3), as these shell parameters are known to have high heritability; supporting earlier findings of Cliff (2010) and Ibom et al. (2014) that heterosis tends to be greater for traits that have low heritability (e.g., reproductive traits), and small for traits that are highly heritable (e.g., growth traits).

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**CONCLUSION**

This study revealed that there were significant variety effects (P<0.05) on FBW and AWG, confirming heterotic effects for these growth traits. The crossbred (AMO x AMS) variety recorded the highest AWG, followed by the AMS variety, while AMO variety recorded the least value. There were no significant (P>0.05) variety effect on DWG, TFI, DFI and FCR. The AMO x AMS recorded the highest final SL and final SW than the other two varieties, indicating heterotic effects again on the two shell parameters. Results for all body and shell parameters studied showed low, and positive heterotic values. There were significant variety effects (P<0.05) observed on BDW, SL and SML. The heterotic values obtained for body and shell parameters, though low, tend to support the assertion that growth traits have low heterotic values which may be because they are highly heritable. The crossbred variety recorded the highest heterotic values than the two purebred varieties for all the body and shell parameters studied, indicating that the crossbred variety has heterotic advantage over the purebred white variety and purebred black variety.

**REFERENCES**


Management of Peroneal Nerve Paralysis in a Three Month old Puppy using a Tendon Transplantation Technique

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ABSTRACT: A severe right hind limb paralysis due to injury to the peroneal (fibular) nerve was characterized by knuckling of the paw, over extension of the hock, dragging of the limb and excoriations on the dorsum of the digits. Management of the case therapeutically and conservatively over a period of three weeks was not rewarding. The case was eventually and successfully managed by the relocation of the superficial digital flexor tendon (SDFT) to the long digital extensor tendon (LDET). The surgical procedure was relatively easy. However, the post-operative management and retraining of the puppy to enable it use the flexor tendon as an extensor was difficult and protracted but was successful. The puppy regained normal limb placement and near normal gait about two months postoperatively. It was eventually discharged after three and half months of management.

Key words: Peroneal, Peripheral, Neuropathy, Tendon, Transplantation, Prognosis, Management.

INTRODUCTION

Natural recovery from peripheral neuropathies is usually unlikely even after protracted conservative and/or therapeutic interventions (Hart and Tremblay, 1982; Bennett and Vaughan, 1976a, Leighton, 1982; Kilic et al., 2009). In most of such patients, the affected limb is either amputated or the patient eventually is euthanized except if, the condition is managed surgically in which case, the outcome may be favourable. (Bennett and Vaughan, 1976a; Leighton, 1982).

The sciatic nerve and/or its branches (the tibial and peroneal nerves) may be injured giving rise to varying degrees of peripheral neuropathies (neuropaxia, axonotemesis or neurotemesis) affecting different parts of the hind limb distal to the hock joint in animals and man. Such injuries often result from trauma, surgical procedures around the pelvis, hip joint, proximal and distal femur, and proximal fibular regions, during perineal herniorrhaphy or tumours in the vicinity of the nerve (Borrows and Harvey, 1973; Spruell, 1976; Omamegbe, 1976; Weaver and Omamegbe, 1981; Forterre, et al., 2007). Other common causes are dog fights, inappropriate hind limb dressings, intramuscular injections deep in the vicinity of the nerve in the distal thigh muscles and compressions on the nerve at pressure points especially in unconscious, recumbent and emaciated animals (Bennett, 1976; Kilic et al., 2009).

The classical clinical signs of peroneal nerve paralysis include an inability to extend the stifle or flex the hock joints, loss of muscle mass in the tibial region and folding under of the paw (knuckling) which (paw) soon develops excoriations on its dorsum. The affected limb shows loss of cutaneous sensation on the cranial and lateral aspects of the leg below the hock joint and on the dorsum and dorso-lateral aspects of the digits, loss of the extensor reflex and occasional positive cross extension thrust reflex among others (Bennett and Vaughan, 1976a, b). The diagnosis of peroneal nerve paralysis is usually based on the case history and presenting clinical signs (Forterre et al., 2007). Nerve conduction studies are optional only if the rare possibility of re-innervation of the paralysed muscle/tendon is envisaged (Bennett, 1976).

Generally, peripheral neuropathies have rather very poor prognosis in humans and animals if treated therapeutically and/or conservatively only because the
response to such management is usually very poor. Most affected animals so managed usually have the affected limb(s) amputated or are eventually euthanized out of frustration of both the client and the attending veterinarian (Hart and Tremblay, 1982; Bennett and Vaughan, 1976a). In valuable patients like guide, guard, hunting, sport or indeed intimate pets, these options are often unacceptable to most clients. Therefore, some alternative treatment options with better prognoses are usually required.

It has been shown in humans (Wapner et al., 1993; Bradon and Harris, 2015) and animals (Bennett and Vaughan, 1976a; b; Hart and Tremblay, 1982; Leighton, 1982) that certain peripheral neuropathies associated with locomotor dysfunction can be relieved by the relocation of the tendon of some functional and innervated muscle/tendon to the muscle/tendon which has been denervated as a result of nerve damage. These techniques have been used mainly in the management of radial paralysis in the fore-limbs of animals (Sterner and Mollier, 1960; Hussain and Petit, 1967; Bacher and Potkay, 1976).

For this treatment approach to achieve the desired end, the function(s) of the muscle/tendon to be relocated must be duplicated by another muscle/tendon in its group so that the over-all function(s) of the muscle/tendon group is not adversely affected when one member of the group is relocated. Also, the muscle(s)/tendon(s) paralysed, the specific nerve traumatised and the appropriate tendon to be relocated must be clearly determined. This is because these techniques are best suited for the management of mono-neuropathies (Bennett and Vaughan, 1976a). For optimal post-operative results, these procedures must be executed carefully, aseptically and with minimal trauma to and desiccation of tissues at the surgical site (Bennett and Vaughan, 1976a; Leighton, 1982).

A surgical procedure for the relocation of the superficial digital, long digital or caudalis tibialis flexor tendons (SDFT, LDFT or CTT) to the LDET for the management of peroneal nerve paralysis in the hind limb of the dog was described by Bennett and Vaughan (1976a,b), Hart and Tremblay (1982) and others.

Although muscle/tendon relocation procedures have been credited with very good postoperative outcomes in the treatment of such cases, the retraining or rehabilitation of patients to enable them use the flexor tendon as an extensor is usually rather protracted (Malnati 1981; Wapner et al., 1993). Such retraining or rehabilitation programme must be carried out skilfully with attention to details if they are to serve as useful adjuncts to the primary surgical therapy (Bennett and Vaughan 1976a, b; Hart and Tremblay, 1982).

CASE REPORT

On the 8th of May, 2015, a three-month old, male Caucasian puppy weighing 5.0 kilogram was referred to the Veterinary Teaching Hospital (VTH) of the University of Abuja (UA) with the primary history of having been mauled by a much bigger dog three weeks earlier. The attending veterinarian referred it to the VTH because its general health condition had gotten progressively worse while on antibiotic, multivitamin, analgesic and sutured and dressing of cutaneous wounds over a period of three weeks during which period, the puppy had become dehydrated, progressively weak, anorexic, constipated and unkempt. Neither the puppy nor the other dog had been vaccinated against rabies but there was no report of any change of attitude or dog fight among the dogs in the household-kennel after the reported incident.

At presentation, the puppy was weak, recumbent and anorexic. It groaned continuously apparently in pain and could not stand or walk even when encouraged to do so. Further history revealed that the puppy and three others of the same litter were kept in a compound with five adults of large breed of dogs and that they were all fed commercial canned dog food (bonju) and left-overs from the house-hold kitchen. Generally, vaccination regimens for the dogs were haphazard and most times were not carried out. Each adult dog was housed individually in a kennel while puppies were left with their bitches until weaned. It was during one of the feeding times that the reported fight occurred.

On clinical examination, all the vital parameters measured were elevated—(rectal temperature-39.9°C, heart rate-140 beats per minute, respiratory rate-80 cycles per minute). The respiration was laboured with respiratory heaves audible at both inspiration and expiration and the heart sounds appeared more prominent on the right thoracic wall than the left but no murmurs or other abnormal cardiac or respiratory sounds were detected. There were several bite-puncture wounds on the head, thoracic and abdominal walls, and the caudo-lateral aspect of the right hind limb around the region of the stifle joint. Several of these showed signs of healing or carried old suture lines. Of particular note was the deep and penetrating bite wound on the lateral aspect of the lower thigh region of the right hind-limb. The puppy had a very heavy tick (Repicephalussanguinius) infestation. It was mildly to moderately dehydrated (about 5 to 7% body weight loss), with pale ocular and oral mucous membranes and a prolonged capillary refill time (>3min). Apart from piercing wounds on the nasal bones, no other osseous injury or locomotor dysfunction was observed at this stage.

A tentative diagnosis of trauma due to a dog-fight, deep piercing wounds, a diaphragmatic hernia, ecto-prasite associated with haemo-parasitisms, dehydration and severe anaemia was made. While admitted at the kennel of the VTH for further observation and treatment, it was noticed that the puppy was constipated and oliguric.

A survey radiographic examination of the thoracic and abdominal cavities/regions and bony structures revealed...
no obvious abnormality. No evidence of a diaphragmatic hernia was seen. A venous (cephalic vein) blood sample was positive for Babesia canis infection (++) and yielded haemogram values as follows: red blood cell (RBC) count - 4x10⁶ cells/ml, white blood cell (WBC) count - 12x10³ cells/ml, Packed cell volume (PCV) - 22.0% and haemoglobin concentration (Hb)- 7grams/ml. The differential white blood cell count showed a neutrophilia (82%) and a relative lymphopenia (14%) - a shift to the left; eosinophilia (3%) and a monocyte count of 1%.

The case was comprehensively managed therapeutically and conservatively and in particular against haemoparasites with two doses of imidocarb di-propionate @ 6.6 mg/kg body weight at two weeks interval, low doses of prednisolone injections @ 1.0 mg/kg body weight for five days, fluid replacement therapy with Ringers solution given intravenously @ 50.0 ml/kg body weight once daily for two consecutive days and a tick bath with dilute solutions of cypermethrin (twice at 10 days interval), vitamin supplementations, doxineurobion tablets, vitamin E injections intramuscularly for one week and anal suppository administration for three days. The puppy's condition improved markedly over the next nine days. Most of the bite wounds were healing actively and the puppy was able to stand and walk slightly. At this stage, it was noticed that the puppy was mildly ataxic, circled and knuckled slightly on the right hind paw. Its appetite, bladder and bowel movements had returned to normal. It was discharged after three weeks of hospitalisation but to be re-presented for re-assessment especially with respect to the locomotor dysfunction a week later.

On re-presentation for review, the puppy had gained some weight (8.9 kg) but knuckled on the right hind foot almost consistently (about 85 to 90% of limb placement). The stifle joint was over-flexed and the hock joint was hyper-extended and the puppy dragged the right hind limb trailing behind as it walked. Weepy excoriations were present on the dorsum of the right foot and the puppy was again heavily infested with ticks. There were no cutaneous sensations to mild – severe painful stimuli on the cranial aspect of the right foot specifically below the hock joint and the lateral digits. Sensations were present on the volar and dorso-medial aspects of the paw. The muscles of the affected limb below the stifle joint were severely atrophic (Figure 1).

On the basis of these findings, a diagnosis of peroneal nerve paralysis due to injury to the peroneal nerve below the point of separation from the tibial nerve at the distal thigh region was made. A radiographic examination of the limb showed a normal tarso-metatarsal joint.

The puppy was further de-ticked with a bath of dilute cypermethrin solution and administered two intramuscular injections of Diaminazene aceturate (Berenil®) at 3.5 mg per kilogram body weight two weeks apart in the presumptive diagnosis of babesiosis as a result of the tick infestation and going by the previous history of the case. Penicillin and streptomycin powders were applied topically to the weepy excoriations on the dorsum of the paw and the limb was then placed on an Elmer sling (Figure 8 bandage- Egger and Wittick, 1990) in an attempt to encourage flexion of the hock and prevent knuckling of the paw. The dressings and Elmer sling were changed at four to five days intervals. After two weeks of the above management, the excoriations on the dorsum of the foot had healed substantially but the locomotor dysfunction was worse than before the management was initiated.

At this point, it was certain that therapeutic and conservative treatment would yield no positive results in the management of the case. It was then decided to manage the case using a tendon re-location technique.

**Pre-Surgical Evaluation**

A pre-surgical assessment/evaluation of the puppy a day before surgery indicated generally a good bodily condition with adequate hydration, a body weight of 13.5.0 kilogram, heart and pulse rates of 128 beats per minute, a respiratory rate of 24 cycles per minute, a rectal temperature of 37.9°C, and pink mucous membranes with a capillary refill time less than 2 seconds. A venous blood
sample yielded haemogram values that were essentially normal viz: total RBC count \(-7.8 \times 10^6\) cells/ml, PCV - 37%, Hb -13 grams/dl, total WBC count \(-9 \times 10^5\) per ml and differential count of neutrophils - 68%, lymphocyte - 27% eosinophil -2% basophils 2% and monocytes 1%. On a risk assessment basis, the puppy was considered a low risk patient.

Aseptic preparation and Anaesthesia

The entire right hind limb was prepared aseptically for a major surgery in a routine manner and the puppy was pre-medicated with a combination of atropine sulphate at 0.02 milligram per kilogram body weight and xylaxine at 2.0 mg/kg body weight intramuscularly. General anaesthesia was induced with ketamine hydrochloride at 15.0 mg/kg body weight intramuscularly and maintained intra-operatively with the intermittent intravenous administration of a 1.25% thiopentone sodium solution at 10.0 mg/kg body weight via an over the needle cannula pre-placed in the cephalic vein.

Surgical Procedure

The puppy was placed in right lateral recumbency and a 5 to 6 cm linear skin incision was made on the medial aspect of the leg from the proximal 1/3 of the tibial region to just distal to the hock joint. The sub-cutaneous fascia was bluntly dissected and revealed the tendons of the gastrocnemius, superficial digital flexor, hallucis longus, the long digital flexor and the caudalis tibialis muscles starting from the caudal aspect of the incision towards the tibia bone. The SDFT was separated from the gastrocnemius tendon (GT) from the mid tibial region to just distal to the hock joint and was transected at this point. The proximal end of the transected SDFT was tagged with two stay sutures of 2/0 nylon. The tendon pulled with the aid of the stay sutures was then passed under the proximal transverse ligament and sutured side-to-side to the long digital extensor tendon LDET with five simple interrupted sutures of 2/0 monofilament nylon on an eye-less needle proximal to the point at which the LDET divides into the individual ligaments of the metatarsal bones and digits. The surgical site was constantly moistened with physiological saline through-out the procedure to avoid desiccation of the tissues at the surgical site. The muscles were not sutured, while the subcutaneous tissues were approximated with sub-cutaneous sutures of 2/0 chromic catgut. The skin incision was closed with horizontal sutures of size 0 nylon suture material.

Post-Operative Management

The recovery from anaesthesia was uneventful. The post-operative management included topical application of penicillin and streptomycin powders to the surgical site, intramuscular administration of penicillin and streptomycin at 10,000 IU and 20 mg/kg body weight respectively for four days, dressing of the surgical site, bandaging and placing the limb in an Elmer sling. Low doses of Oxymorphone (20 µg/kg/12hrs) were also administered to the puppy intramuscularly for three days postoperatively. On removal of the Elmer sling and dressing six days later, the puppy was able to place the digits/paw normally without knuckling but with a dropped (hyper-flexed) hock when it stood (Figure 2).

The GT appeared intact on palpation and there was no depression (dimple) above the tuber calcis when the hock joint was manually over extended. Neurological examination revealed very little or no changes from the pre-surgical findings. The dropped hock complication was managed by placing the limb in a splint that was bent at the hock joint so that the joint assumed a near-normal angulation over a period of four weeks after which the puppy was able to stand and walk with the limb in appropriate positions both at the hock joint and the digits (Figures 3 and 4).

The puppy was eventually discharged to the owners. A follow-up of the case three weeks later showed appreciable clinical improvement as the puppy placed the limb properly and the hock joint was held in normal flexion though slightly abducted.

DISCUSSION

Tendon relocation/transplantation/transfer techniques
Figure 3. The limb with a dropped hock joint placed in a splint pre-formed to conform to the normal flexion of the stifle and extension of the hock joints.

Figure 4. Notice extension of the digits, flexion of the hock and extension of the stifle joints. The limb bore some weight and was close to normal positioning. The musculature over the tibial region had regained some mass.

have been employed to successfully manage a large spectrum of cases of peripheral neuropathies associated with limb dysfunctions in animals (Lesser, 1978; Lesser and Solimanss, 1980; Leighton, 1982). The primary cause of the peroneal nerve paralysis in the case reported appears to be trauma due to a dog fight. The nerve injury would probably have occurred through the bite wound noticed on the lateral distal aspect of the right thigh region when the puppy was first presented at the VTH of UA. It appears that an initial neuropraxia or axonotmesis caused by the dog bite may have been exacerbated by pressure on the nerve as a result of prolonged right lateral recumbency assumed by the patient, faulty application of dressings to the limb or spreading/intensification of inflammatory reaction around the nerve and has progressed to a neurotemesis. The very severe atrophy of the muscles below the stifle of the affected limb seen at the later stage of the case seems to support this view. These have been cited as common causes of peroneal nerve paralysis by others (Bennett and Vaughan, 1976a, b; Hart and Tremblay, 1982). The peroneal nerve appears more prone to injury than the tibial nerve because it is closer to pressure points, is composed of larger folliculi and has less connective tissue between the folliculi than the tibial nerve. These factors render the nerve more likely to be traumatised than the tibial nerve (Bennett, 1976; Bennett and Vaughan, 1976a).

It would appear that cases of peripheral neuropathy in animals may be under reported given the wide range of its causation like intramuscular administration of medications in both pet and farm animals, surgical procedures around the hip and stifle joints, perineal herniorrhaphy, fights among animals and pelvic fractures and their repair. Some of these are part of routine case management in Veterinary Practices. In large animals particularly where highly irritant medications like long acting oxytetracyclines are traditionally administered in regions close to the sciatic, tibial and peroneal nerves, peripheral neuropathies are theoretically expected to be more common than is currently reported. This may be due to poor reporting, inadequate professional attention to large/farm animals generally or poor awareness of the occurrence of this condition in animals. This may account for the very few reports of the condition in large animals (Kilic et al., 2009; Kilic et al., 2014).

The clinical signs of peroneal nerve paralysis reported in this case are similar to those in most recent reports (Bennett and Vaughan, 1976a, b; Leighton, 1982). They became evident as soon as the patient was ambulant. The continuous maintenance of right lateral recumbency in preference to any other position during the initial presentation at the VTH was a fair clinical manifestation of an attempt to protect and hide an injured and painful part of the body from external interference (Hansen, 2003). This may have caused compression to the nerve or exacerbated the inflammatory response attendant to the initial deep bite wounds.

The poor result from therapeutic and conservative managements in the initial stage of this case agrees with the reports of Bennett and Vaughan (1976a, b), Lesser (1978), Leighton (1982), Forttre et al. (2007), Kilic et al. (2009). This poor outcome may be because in cases so managed recovery is dependent on the resolution of the
neural damage which may take a much longer time than most clients may wish to wait. Traumatic injury to the tendon itself such as lacerations or transactions requires healing of the tendon and re-establishment of continuity before any recovery can occur. This process is reputedly slow due to the poor blood supply to tendons and the retraction of the tendon ends following such injuries. Such cases and those due to neural damage have a better chance of recovery following physical re-establishment of continuity of the tendon or relocation to it of a functional tendon (Spinella et al., 2010; Knecht, 1985).

Most long-standing cases of peripheral peroneal neuropathy which had been managed unsuccessfully by conservative or/and therapeutic interventions have eventually been treated successfully by the relocation of one flexor tendon or the other to the tendon of the long LDET (Bennett and Vaughan, 1976a, b; Hart and Tremblay, 1982). In these and in the case reported, this last method of treatment appears superior to therapeutic or/and conservative management because of its simplicity and more for its better overall outcome.

The basic surgical principle involved in this technique is that most functions of muscles or tendons of the appendicular skeleton are performed by two or more muscles or tendons usually of the same group and innervations. Therefore, one of these muscles/tendons can be spared and used for relocation procedures without affecting the overall function(s) of the group of muscles/tendons. In the case reported, the main flexor of the hock and extensor of the digits- the LDET was denervated and paralyzed due to some damage to the peroneal nerve. On the other hand, the extensors of the hock and flexors of the digits- the GT, the SDFT, the HLT, the LDFT and the CTT all innervated by the tibial nerve were still innervated and functional. Of these, the SDFT, the LDFT and the CTT have been relocated to the LDET for the management of peroneal nerve paralysis in dogs while the HLT has been relocated for the treatment of chronically ruptured Achilles tendon in dogs all with acceptable results (Hart and Tremblay, 1972; Bennett and Vaughan, 1976a, b; Wapner et al., 1993). There is no previous report of a dropped hock joint as a post-operative complication sequel to this procedure irrespective of which flexor tendon was relocated. It may have occurred in this case because the splint applied postoperatively was removed too early or because the function of the GT was substantially compromised by the excision from it of the SDFT as the latter is the second most important component of the GT. The result of the management of the dropped hock joint in this case shows that it may be avoided if the external splint is maintained a little bit longer than six days post-surgically or the LDFT, CTT or the HLT which could be spared is used for the relocation rather than the SDFT. There does not appear to be any report of the re-location of the HLT for the management of peroneal nerve paralysis in dogs.

However, it has been used extensively in the repair of GT injury in humans (Mahalan and Dalal, 2009) and may well be used in dogs and other animals for the management of peroneal nerve peripheral neuropathy.

Although, this procedure is credited with a good prognosis, it must be carried out under optimum aseptic conditions, with minimal trauma to the tendons and with the surgical site kept constantly moistened with some physiological fluid to prevent tissue desiccation. Most non-absorbable suture materials seem suitable for the anastomosis of the tendons. In all, only moderate tension should be applied to the simple interrupted sutures irrespective of the non-absorbable suture material used. It would appear that a side to side should be preferred to an end to end anastomises of the tendons as it is easier to carry out, is less time consuming and preserves the integrity of the entire long digital extensor muscle and tendon so that it could return to normal function if subsequently the de-enervated muscle/tendon becomes re-innervated (Bennett and Vaughan, 1976a).

The long period of retraining or rehabilitation appears important for proper recovery as shown in this case and emphasised by others (Malnati, 198; Wapner et al., 1993). Such retraining could be just passive physical therapy like massaging, walking the dog on a leash, guided exercise or swimming (Hulse, 1990). In most cases, although limb placement in terms of extension of the digits returns to near normal a few days after the operation, appropriate hock flexion requires a period of external casting which must be done with care and the hock joint held at the appropriate angulation (Bennett and Vaughan, 1976a; Hart and Tremblay, 1982). Although a side to side anastomosis of SDFT to the entire (un-section) LDET was employed in this case, the anastomosis needed sufficient time to heal and organize properly to be able to withstand the strain and tension required to adequately hold the hock joint, in particular, in an appropriate weight bearing position.

Conclusion

Tendon relocation/transplantation procedures appear to be superior to therapeutic or/and conservative treatments in the management of peripheral mono-neuropathies. However, once an appropriate diagnosis of the condition is made, no time should be wasted on conservative and/or therapeutic management because the latter are un-rewarding in the management of such cases.

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Small Ruminant Lungworms: Parasite detection, identification and prevalence estimation in Three Districts of South Wollo, Ethiopia

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ABSTRACT: The prevalence, species, age, sex and seasonal variation of lungworm infection in small ruminants were studied using Coprosopic and Postmortem examinations in Three districts of South Wollo: Kombolcha, Dessie and Kalu. Faecal samples were collected from 586 sheep and goats of all age groups (below 6 months, 6 months to 2 years, 2 to 4 years and above 4 years) and both sexes. Modified Baermann technique was used for extraction of L₁ larvae from the faeces. Post-mortem examination had also been done on 98 animals in Dessie abattoir during the study period. An overall prevalence of 31.2% and 7.1% were detected by faecal and post-mortem examinations, respectively. Significant difference (p<0.05) was found between areas of different altitude with an infection rate of 47.5%, 35.5% and 28.5% in Dessie, Kombolcha and Kalu respectively. Factors such as age, species of parasite, season and origin of animals showed significant differences (p<0.05) on the prevalence of small ruminant lungworm infection. However, there was no significant difference (p>0.05) observed among sex, species, management system and body condition score of animals. Due to its impact on production, emphasis should be given for the control and prevention of lungworm infection in the areas.

Key words: Kombolcha, Dessie, Kalu, Prevalence, Lungworm, Small Ruminant.

INTRODUCTION

Sheep and goats provide as much as 30% of the meat and milk consumed in sub-Saharan Africa and is found on smallholdings throughout the continent. Production and marketing of sheep and goats and their products are a vital source of income, especially for small holders who do not have access to credit or farm income. Due to the small size, high reproductive capacity and rapid growth rate, small ruminants provide a more flexible short-term investment than cattle. Yet these species have received much less attention from research and development agencies than cattle (ILCA, 1990).

Helminthes parasites of ruminants are ubiquitous, with many tropical and subtropical environments of the world providing nearly perfect conditions for their survival and development. Although, these parasites are widely prevalent, the clinical signs they cause in infected animals can be less obvious than signs of other livestock diseases. Partly for this reason, infections with gastrointestinal and other helminthes parasites are among the most neglected areas of veterinary care in much of the developing world. High prevalence rates of the infection result in poor production and unthriftiness (Hansen and Perry, 1994).

Among many constraints, which limit productivity in livestock populations, parasites are of major importance (Mboera and Kitalyi, 1992). Endo-parasites are important problem for sheep and goat farmers causing gastroenteritis in these animals in Africa and throughout the world (ILCA, 1991).

Dictyocaulidae and/or certain Metastrongylidae are known to exist in East Africa (Ethiopia, Kenya and Tanzania) and South Africa (Torny, 1989). Endo-
parasites, including *Dictyocaulus filaria* are major causes of mortality in the Ethiopian highlands (ILCA, 1993, 1992). About 50% of mortality in sheep in farms of Ethiopian highlands is caused by pneumonia and endoparasites (ILCA, 1990). Control of these parasites is essential for harnessing the potential of small ruminant production. For proper control to be instituted, however, diseases and their dynamics must be known. At our present state of knowledge of parasitic diseases, it is difficult and even dangerous to lay down rigid rules for their control which are applicable to all regions. For this reason a study of epidemiology of each parasitic disease should be limited to small areas (Rodostitis et al., 1994). The incidence of parasitic diseases, including respiratory helminthosis varies greatly from place to place depending on the relative importance of many of the factors. In this regard, very few studies were conducted so far pertaining to respiratory helminths of small ruminants in Wollo, Amhara National Regional State, Ethiopia.

The present study was undertaken to find out the prevalence and species variation on respiratory helminths of small ruminants in different zones of Wollo, in Amhara Regional State, Ethiopia.

**MATERIAL AND METHODS**

**Study Area**

**Topography and climate**

The study was conducted at Kombolcha town, which is found in South Wollo administrative Zone of Amhara Regional State in North Eastern Ethiopia. The study area is located 376 km North of Addis Ababa with 11°08′44″N latitude and 0.39°73′46″E longitude at an altitude of 1840 meter above sea level (msl) (Figure 1). The Kombolcha town experiences a bi-modal rainfall, the short rainy season occurs usually from March to May while the long rainy season occurs from June to August. The minimum and maximum mean annual rainfall in and around Kombolcha ranges from 750 to 900 mm. The average minimum and maximum daily temperature during short and long rains are 23.9°C and 11.7°C respectively and the relative humidity of the area varies from 23.9% to 79%.

**Vegetation**

The nature of vegetation varies from savannah grass land, bushes and dense shrubs to huge trees like Juniper, Acacia, Hagina abyssinica (Kosso), Cordial africana. Vegetation dries off during the dry season in almost all regions except on high land grazing areas and revival of vegetation with the commencement of rain on the low lands.

**Farming system in the area**

The type of farming in the area is mixed crop to livestock production system. Small ruminant production in the area is an integral component of the traditional farming system. The main livestock grazing land available to animals are swampy water-lodged areas, forest margin, hilly tops and mountain sides, stony and infertile lands, and road sides. According to the Agricultural Office report (2010), livestock population in the area comprises of 1,826,900 cattle, 1,401,470 sheep, 980,750 goats, 262,420 donkeys, 3,420 camels and 2,046,710 poultry.

**Study Animals and their Management**

The animals used in this study are owned by subsistence farmers and small-scale private farms. The breeds of sheep and goats in the study area are Menz and Small East-African breeds respectively.

**Management**

Sheep and goats are traditionally kept on extensive management system. These animals are maintained in small household flocks of mixed ages usually less than 10 animals in lowlands but 20 to 50 or more at the extreme of the high land areas.

Sheep and goats are kept close to the village and they are allowed to graze native pastures on grasslands. Supplementary feeding and forage conservation is not practiced, however, after harvest when there is no risk of damage to crops, animals may have access to hay, stubbles and other leftovers of the year's harvest. Watering is only once at the middle of the day from the nearby streams, rivers or shallow wells.

**Study design**

Cross-sectional study design was carried out to determine the prevalence of small ruminants’ lung worm infection in selected three districts of south Wollo.

**Sample size**

The sample size required for this study was determined depending on the expected prevalence of small ruminant lung worm infection and the desired absolute precision according to Thrusfield (2005) by the following formula.

\[ n = \frac{1.96^2 \cdot P_{exp} \cdot (1 - P_{exp})}{d^2} \]

Where: \( n \) = required sample size; \( P_{exp} \) = expected preva-
Figure 1. Map of Amhara Region; the arrow on the right indicates the study areas. (Source: URL http://en.Wikipedia.Org/wiki/ Amhara-Region, 2009).

Inference (50%); d = desired absolute precision (5%).

According to the above formula, the calculated sample size was 384. However, in order to increase the accuracy the number of sampled small ruminants was 586.

Sampling procedure

In this study, the area was classified into three districts (Woredas) which are Dessie, Kombolcha, and Kalu. The study subjects were managed under extensive and semi-intensive production system.

Sample collection

Faecal sample were collected directly from the rectum of selected animals in screw capped glass bottle (universal bottles) and packed in ice box. While collecting faecal sample, the species of the animals, sex, age, management, date of sampling and the area were properly recorded.

Diagnosis

Laboratory diagnosis

In the laboratory, 2.5 g of fresh faeces was weighed from each sample for the extraction of \( L_1 \) larvae using modified Baermann technique. These were enclosed in gauze fixed on to a string rod and submerged in a clean glass tube filled with warm water. The whole apparatus was left for 2 to 4 hours. The larvae then leave the faeces, migrate through the gauze and settle at the bottom of the glass. After siphoning of the supernatant, the sediment was examined under the low power of the microscope.
When positive, a drop of 1% iodine solution was used to immobilize the larvae for identification of species, otherwise it was registered negative for lung worm infection (Fraser, 1991; Urquhart et al., 1994).

**Post mortem examination**

The lungs were palpated for presence of metastrongyloid nodules, which are usually grayish white in color. If present, they are trimmed of and worms extracted from the tissue by gentle compressing a small non calcified nodule or part a large nodule between two glass slides, and then teasing the worms away from the tissue with thumb forceps. To collect all worms at the bottom of the beaker added with that of the previous and transferred to glass beakers containing saline. The air passages were opened starting from the trachea down to the small bronchi with fine blunt pointed scissors to detect parasites; visible worms were then removed from the opened lungs and transferred to glass beakers containing saline. The worms collected were identified and recorded (ILCA, 1991; Fraser, 1991).

**Data analysis**

The results were analyzed in relation to sex, species of animal, age, management, origin, body condition, species of lungworms and season. Animals were categorized in to four age groups. Age group I (< 6 months), age group II (6 months to 2 years), age group III (2 to 4 years) and age group IV (> 4 years). The data obtained were coded for the above factors and entered in to excel. Then Chi-square was used to compare the prevalence of small ruminants’ lungworm infection for possible significance difference. The differences were regarded as significant if p-value is < 0.05 using SPSS.

**RESULT**

**Coproscopic Examination**

Out of all animals examined, 183 were positive for lungworm infection with an overall prevalence of 31.2% (Table 1).

There was significant difference on the prevalence of lungworm infection of small ruminants in different districts of south Wollo ($\chi^2 = 41.324$, $P<0.05$, df = 3). The highest prevalence was recorded in Dessie (47.5%) followed by Kombolcha (35.5%) while the lowest in Dessie abattoir (7.1%) (Table 1).

There was no significant difference ($p>0.05$) on the prevalence of lungworm infection on the basis of species and sex of small ruminants. However, the prevalence was slightly higher in ovine (31.4%) and males (31.8%) as compared to that of caprine (31.0%) and females (31.0%) (Table 1).

There was significant difference ($\chi^2 = 11.127$, df = 3, $p<0.05$) on the prevalence of lungworms among the four age groups of small ruminants. The highest prevalence was in group I (61.7%) and the lowest was in group IV (11.4%) (Table 1).

There was no significant difference ($\chi^2 = 4.136$, df = 2, $p>0.05$) on body condition score of the animals. However, the highest prevalence was noticed in poor (50%) and the lowest prevalence in medium (28.2%) body condition score of the small ruminants. Similarly, there was no significant difference ($\chi^2 = 0.331$, df = 1, $p>0.05$) of lungworm infection under different management system. However, the highest prevalence was found in extensive management system (31.8%) while the lowest prevalence in semi-intensive management system (29.1%).

There was significant difference ($\chi^2 = 5.860$, df = 4, $p<0.05$) on the prevalence of lungworm infection with species of lungworms. The prevalence was decreased when the age of animals increased in the cases of M. capillaries, P. rufescens and DFMC infection, but not in case of D. filaria infection (Table 2).

There was significant difference ($\chi^2 = 17.660$, df = 4, $p<0.05$) on the basis of monthly prevalence of lungworm infections. The result showed that the highest prevalence was recorded in January (40.1%) while the lowest in November (19.8%) (Table 3 and Figure 2).

**Post-mortem Examination**

A total of 98 lungs from small ruminants slaughtered at Dessie abattoir were examined through post-mortem inspection, of which 7 (7.1%) were positive for lungworm infection. The lungworm species encountered during intact lung incisions during the study period was D. filaria with a prevalence of 7.1% (Table 4).

**DISCUSSION**

**Coproscopic Examination**

The current study revealed that the presence of three nematode species parasitizing the respiratory tract of small ruminants with an overall infection rate of 31.2%. These parasites had also been reported in sheep from different climatic areas of the world and from different regions of Ethiopia (Thomson et al., 1988). A similarly high prevalence (50%) was recorded in previous study conducted at Kombolcha and Dessie by Tefera 1993. Studies made in other parts of Ethiopia had also underlined the relative importance of this disease in small ruminants. For instance, Wondewosen (1992) had reported 58% in Assela, Assaye and Alemneh (2015)
Table 1. The prevalence of lungworm infection in small ruminants on the basis of various factors.

<table>
<thead>
<tr>
<th>Factor</th>
<th>N² of Animals</th>
<th>Prevalence (%)</th>
<th>Chi-square ($\chi^2$) and P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Examined</td>
<td>Positive</td>
<td></td>
</tr>
<tr>
<td>Species</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ovine</td>
<td>360</td>
<td>113</td>
<td>31.4</td>
</tr>
<tr>
<td>Caprine</td>
<td>226</td>
<td>70</td>
<td>31.0</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>192</td>
<td>61</td>
<td>31.8</td>
</tr>
<tr>
<td>Female</td>
<td>394</td>
<td>122</td>
<td>31.0</td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 6 Months</td>
<td>175</td>
<td>108</td>
<td>61.7</td>
</tr>
<tr>
<td>6 M - 2 years</td>
<td>204</td>
<td>46</td>
<td>22.5</td>
</tr>
<tr>
<td>2 - 4 years</td>
<td>75</td>
<td>14</td>
<td>18.7</td>
</tr>
<tr>
<td>&gt; 4 years</td>
<td>132</td>
<td>15</td>
<td>11.4</td>
</tr>
<tr>
<td>Body Condition</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Poor</td>
<td>20</td>
<td>10</td>
<td>50.0</td>
</tr>
<tr>
<td>Medium</td>
<td>188</td>
<td>53</td>
<td>28.2</td>
</tr>
<tr>
<td>Good</td>
<td>378</td>
<td>120</td>
<td>31.7</td>
</tr>
<tr>
<td>Management System</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Extensive</td>
<td>459</td>
<td>146</td>
<td>31.8</td>
</tr>
<tr>
<td>Semi-intensive</td>
<td>127</td>
<td>37</td>
<td>29.1</td>
</tr>
<tr>
<td>District</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kombolcha</td>
<td>259</td>
<td>92</td>
<td>35.5</td>
</tr>
<tr>
<td>Kalu</td>
<td>130</td>
<td>37</td>
<td>28.5</td>
</tr>
<tr>
<td>Dessie</td>
<td>99</td>
<td>47</td>
<td>47.5</td>
</tr>
<tr>
<td>Dessie abattoir</td>
<td>98</td>
<td>7</td>
<td>7.1</td>
</tr>
<tr>
<td>Total</td>
<td>586</td>
<td>183</td>
<td>31.2</td>
</tr>
</tbody>
</table>

Table 2. Prevalence of lungworm species among the four age groups of small ruminants.

<table>
<thead>
<tr>
<th>Age</th>
<th>Total animals examined</th>
<th>D. filaria P (%)</th>
<th>M. capillaries P (%)</th>
<th>P. rufescens P (%)</th>
<th>DFMC P (%)</th>
<th>Total P (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 6 M</td>
<td>175</td>
<td>56(32%)</td>
<td>25(14.3%)</td>
<td>2(1.1%)</td>
<td>9(5.1%)</td>
<td>52.6</td>
</tr>
<tr>
<td>6M-2 year</td>
<td>204</td>
<td>25(12.3%)</td>
<td>10(4.9%)</td>
<td>2(0.98%)</td>
<td>1(0.49%)</td>
<td>18.6</td>
</tr>
<tr>
<td>2-4 years</td>
<td>75</td>
<td>46(61.3%)</td>
<td>0(0%)</td>
<td>0(0%)</td>
<td>0(0%)</td>
<td>61.3</td>
</tr>
<tr>
<td>&gt; 4 years</td>
<td>132</td>
<td>6(4.6%)</td>
<td>0(0%)</td>
<td>0(0%)</td>
<td>1(0.76%)</td>
<td>5.3</td>
</tr>
</tbody>
</table>

* $X^2=125.178$, df=12, p=0.000; DFMC = mixed infection with *D. filaria* and *M. capillaries.*

reported 22.7% in and around Bahir Dar and Muluken (2009) had reported a prevalence of 18.16% in Bahir Dar. The current overall prevalence result almost coincides with the previous report of Brook et al. (1986) (27.8%) in Assela. But it was not similar with the findings of Abdukadir (2009) in the same area who reported higher prevalence (42.96 %). This could be due to the fact that the establishment of
Table 3. The seasonal variation of small ruminants’ lungworm infection.

<table>
<thead>
<tr>
<th>Months</th>
<th>Animals examined</th>
<th>Positive</th>
<th>Prevalence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>November</td>
<td>121</td>
<td>24</td>
<td>19.8</td>
</tr>
<tr>
<td>December</td>
<td>102</td>
<td>34</td>
<td>33.3</td>
</tr>
<tr>
<td>January</td>
<td>172</td>
<td>69</td>
<td>40.1</td>
</tr>
<tr>
<td>February</td>
<td>99</td>
<td>35</td>
<td>35.4</td>
</tr>
<tr>
<td>March</td>
<td>92</td>
<td>21</td>
<td>22.8</td>
</tr>
<tr>
<td>Total</td>
<td>586</td>
<td>183</td>
<td>31.2</td>
</tr>
</tbody>
</table>

$X^2$ cal =17.660, $p = 0.001$, df = 4.

Figure 2. Monthly prevalence of small ruminants’ lungworm infection.

Table 4. The prevalence of lungworm species in small ruminants by post-mortem examination.

<table>
<thead>
<tr>
<th>Adult worm burden (n=80)</th>
<th>D. filaria</th>
<th>M. capillaries</th>
<th>P. rufescens</th>
<th>DFMC</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>7</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td>Prevalence</td>
<td>7.1%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>7.1%</td>
</tr>
</tbody>
</table>

open air clinic in rural kebeles, increased in numbers of private veterinary pharmacies, increased farmers awareness to deworm their small ruminants, prevailing environmental change and differences in rain fall, humidity, temperature and altitude of kebele’s included in the study. The current finding however, disagreed with the previous findings of Eyobe (2008) who reported a prevalence of 72.44% in Assela. Such variation in infection rate could be attributed to the variation in altitude, rainfall, humidity and temperature differences in different area of the country (Blood et al., 1989).

The finding of *M. capillaries* with 14.3% prevalence of the total positives in the study area disagreed with the previous reports of Mezgebu (1995) in Addis Ababa (54.9%) and Sissay (1996) in Bahir Dar (39.3%). But this result agreed with the previous reports of Paulos (2000) in Arsi Zone and Mengstom (2008) in Tigray (Atbhi) reported that *D. filaria* was the most prevalent species.

In the present study, the finding of *D. filaria* as the most prevalent when compared to the other species of lungworms in small ruminants might be due to the fact that *D. filaria* has indirect life cycle, took more time to...
reach the infective stage and after ingestion, larva can appear in faces after several weeks (Soulsby, 1982). The probability of infection, transmission and reinfection with a season could much more high when compared with *M. capillaries* and *P. rufescens*. These factors explained that the young (weaning) animals have higher infection rate of *D. filaria* (Mengestom, 2008).

Influence of sex on the prevalence of infection indicated that there was no significant difference (P>0.05) in susceptibility to infection with lungworms. Hence, sex dependant variation was not encountered. This result was not in agreement with the earlier study of Sissay (1996) and Alemu (1999) who reported significant variation in the infection rate of lungworm in male and female, and coincides with Netsanet (1992) in and around D. Birhan and Teffera (Wendewosen, 1992) in and around Dessie and Kombolcha who reported equal susceptibility in both sexes. This might be due to the improper distribution of sample selection between the two sexes (Paulos, 2000) or most of the samples from female sheep and goats were not in preparturient period during the study period (ILCA, 1990; Urquhart et al., 1994). In addition, most males included in the sample were uncastrated and freely mating in the field might also create stress like that of females.

The influences of management system on the prevalence of lungworms indicated that there was no statistically significant difference (p>0.05) between extensive and semi-intensive management systems. This finding was different from Sissay (1996) in and around Bahir Dar, Alemu (1999) in Wollo and Eyobe (2008) in Assela who reported that management had significant difference on susceptibility. The possible reason for the present finding could be due to the fact that grazing in the same pasture and feeding moist pastures for the small ruminants in the area attributed to similarity on susceptibility to lungworm infection between the two management systems.

The influence of body condition on the prevalence of lungworm infection revealed that there was no statistically significant association (p>0.05). The prevalence of lungworm infection on coproscopic examination was found to be higher (50%) in animals of poor body conformation than that of well confirmed ones (31.7%). This finding coincides with Mengestom (2008) who reported that the affected small ruminants, loss of weight cannot be attributed to lungworm infection alone since *Hamonchus contortus* and other GIT helminthes could be encountered. Poor body condition could be associated with failure to deworm animals or due to lack of feed or nutritional management which lead to lack of resistance to infection and contributed for increased prevalence in poorly conditioned animals.

The monthly dynamics of lung worm infection within the study periods showed that prevalence was higher in January when compared to dry season. This finding coincides with the results of unpublished report of Ferwengle (1995). The survival and development of lungworm larvae was favored by low moisture content and high humidity. For instance, infective larva on pastures minimum during the summer months but reaches peak level during the cooler autumn (Ayalew et al., 1973). Such conditions are obtained after long rainy season (September to November) in Wollo and at high altitude areas.

With regard to age, the prevalence of lungworm infection in small ruminants was found to be higher in young’s (weaning) than adults and showed significant difference (p<0.05). This finding was in agreement with Schanzel (1959) who reported that young animals were found to harbor twice as many *D. filaria* than adults. However, disagree with the idea that the intensity of infection by *M. capillaris* was six times higher in adult small ruminants. This was also not in line with Thomson and Orita (1988) who found an increased infection rate in the age of the animals.

In this study, comparing the susceptibility of species of small ruminants, sheep were found slightly more susceptible to lungworm infection than goats. This finding was not in agreement with Assaye and Alemneh (2015) who reported that goats were more susceptible to both initial and challenge infections than that of sheep.

The prevalence of lung worm infection by coproscopic examination was higher in Dessie (47.5%) followed by Kombolcha (35.5%) while the lowest prevalence was recorded in Kalu (28.5%). The result showed significant difference (p<0.05). This result was due to the reasons of altitude and temperature variation in three selected areas.

### Post-mortem Examination

The observation of the intact lung of slaughtered small ruminants in Dessie town revealed that 7.1% overall prevalence while the prevalence of coprological finding was 31.2%. This decreased prevalence in post-mortem examination disagreed with the finding of Eyobe (2008) in Assela reported higher prevalence in post-mortem than coprological examination. But, the present finding agreed with the finding of Paulos (2000) in Arsi chiallo who reported higher prevalence in faecal than post-mortem examination.

One of the probable reasons attributed for such difference in the present finding could be those larvae which reach the lungs of small ruminant was not remain in the parenchyma and not become encysted in fibrous nodules. Because, such nodules eggs many be deposited in air passage (Rodostitis et al., 1994). Therefore, the finding of the present study strongly supports that coproscopic examination had wide value in estimating the burden of lung worm infection in small ruminants. Hence, coproscopic examination requires serious cautions. In addition, most sheep and goats
brought for slaughter were from rural areas, the probability of deworming might be high. The larvae were found 50 to 100 gm only in faces/pellet of patent cases, otherwise, in the rest phase it might not be dispersed throughout the pellet (Urquhart et al., 1994).

**Conclusion**

The study on lung worm infection of small ruminants by faecal and post-mortem examinations in three districts of south Wollo revealed an overall prevalence of 31.2% and 7.1%, respectively. The respiratory nematodes *D. filaria*, *M. capillaries* and *P. rufescens* were identified. This high prevalence of verminous pneumonia as the result of these three species was considered as one of the important nematode infection in small ruminants in the study area. It was found that young (weaning) small ruminants were most affected by *D. filaria* than adults. The prevalence of lung worm infection had no significant association with sex, species of animals, body condition and management in the study area.

**REFERENCES**


Prevalence and risk factors of the Bovine Thelaziasis at Mersa Town, Ethiopia

Mulat Asrat
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ABSTRACT: A cross-sectional study was conducted from April 2014 to June 2015 in the Mersa Town of South Wollo Zone, Amhara Regional State to determine the prevalence of Thelaziasis in cattle and to assess associated risk factors responsible for the occurrence of the disease. A total of 384 cattle of both ages (98 young and 286 Adult) with two sexes (203 males and 181 females) were grossly examined by flushing the conjunctival sacs and lachrymal duct with sterile saline solution. The prevalence of thelaziosis was 18.23% (70 cases). Age, sex, body condition scores and management systems did not show any statistical significant difference (P>0.05) for the prevalence. The prevalence was higher in male (24.12%), young (26.53%), and extensively managed cattle (20.69%) than their comparative female (11.6%), adult (18.38%), and semi-intensively kept animals (13.01%) respectively. Regarding body condition scores, the highest prevalence of bovine Thelaziasis was recorded in poor body condition cattle (30.19%) while the lowest was recorded in cattle with good body condition scores (14.48%). The results of the present study showed that bovine thelaziosis requires special attention considering its impact on cattle production and productivity.

Key words: Cattle, Eye worm, prevalence, Shawora Alfa, Thelaziosis.

INTRODUCTION

Ethiopia has the largest livestock inventories in Africa, including more than 38,749,320 cattle (Fubini, and Ducharme, 2004). Thelazia nematodes are commonly known as eye worms and cause ocular infections in animals. This genus of spirurids represents one of the most specific taxon among nematodes because of its very close relationship with its intermediate and final hosts (Otranto and Traversa 2005).

However, the immature and mature stages occur in the interior chamber of the eye, thereby being exposed to the external environment. Therefore, it could be considered an “ectoparasite”. Sixteen species of this genus have been reported from ruminants. They have been documented in Europe (Italy, France, Switzerland and Germany), Asia (China, Japan, Korea and Taiwan), North America (Canada), South America (Peru) and South Africa. The adult worms live under the eyelids, nictitating membranes and in lacrimal ducts. Transmission depends upon the continuous presence of the vectors hence has a seasonal occurrence according to the seasonality of the intermediate hosts (Yang et al. 2006).

In final hosts, both the larval stages and adults of Thelazia spp. cause clinical signs such as excessive lacrimation, epiphora, conjunctivitis, keratitis and corneal ulcers (Otranto et al., 2001). A definitive diagnosis is made by detection of the parasites in the conjunctiva sac. Examination of lacrimal secretions may reveal eggs or first-stage larvae. Also, morphological differentiation has been done on some Thelazia species using scanning electron microscopy. Molecular characterization and phylogeny of some Thelazia species have been studied by Nadler et al. (2000), Otranto et al. (2001), and Traversa et al. (2005). Due to the localization of the nematode, thelaziosis can be treated topically by direct application of drugs into the eyes. Removal of the adult parasites with fine forceps, using local anaesthesia is also helpful. Patients with an intraocular infestation with T. callipaeda have been successfully treated with Pars plana vitrectomy. Two ml of levamisole injected into the subconjunctival sac was more effective than levamisole.
given orally. Treatment of dog thelaziosis, caused by *T. callipaeda*, using a topical formulation of 10% imidacloprid and 2.5% moxidectin has been studied by Bianciardi and Otranto (2005).

There are different diseases within the Alfa Shawora district that affect cattle which lead to loss of body condition, draft power and production. Among the eye infection, thelaziosis is the common problem within the district (MWARDO, 2006). So to put forward the major effect of Thelazia and to plan the control strategy, studying the problem within the district needs priority. Therefore, the objectives of this study were to determine the prevalence of bovine thelaziosis in Mersa Town, South Wollo Zone, Amhara Regional State and to assess associated risk factors involved for the occurrence of thelaziosis in cattle in the study area.

**MATERIALS AND METHODS**

**Study Area**

The study was conducted in Mersa Town, South Wollo Zone, Amhara Regional State, which is found in North West of Ethiopia and 491 km away from Addis Ababa. The rearing system of livestock population in the study area depends on natural grazing and crop residues and kept in the traditional management system. The physical feature of the Woreda is hilly, sloppy, plain area, rivers and forested. The altitude of the study area ranges from 1500 to 2500 meter above sea level with average annual temperature of 20 to 25°C (MWARDO, 2006).

**Study design and Sampling Methods**

A cross-sectional study was conducted from April 2014 to June 2015. The study animals were selected randomly from animals that were brought to Mersa town veterinary clinic and they were kept under individual households.

**Study Animals**

Cattle were categorized into groups on the basis of age group (young and adult), sex (male and female), breed (local and cross) (Aiello and Mays, 1998) and the body condition score (poor, medium and good) (Ferguson, 2011).

**Sample size determination**

The sample size requires for this study is determined based on sample size determined in random sampling for the infinite population using expected prevalence of 50% and 5% desired absolute precision according to Thursfield (2005).

\[
\text{n} = \frac{1.96^2 \text{pex} (1 - \text{pex})}{d^2}
\]

Where: \(n = \text{required sample size, pex = expected prevalence and } d^2 = \text{desired absolute precision.}\)

Hence as for as the knowledge of the author concerned, there was no study did concerning thelaziosis of cattle in the study area, the sample size was estimated by using 50% expected prevalence with 95% confidence interval at 5% absolute precision (Thursfield, 1995).

**Statistical Analysis**

The data obtained from this survey were entered in Microsoft excel worksheet. Then descriptive statistics was used to analyze the data using statistical package for social sciences (SPSS) software version 20.0. Chi-Square test \((x^2)\) with computed \(p\)-value of less than 0.05 was used to estimate the statistical significance association of bovine thelaziosis rate with age, sex, body condition score, management system and altitude differences.

**RESULTS**

Out of the total 384 cattle examined, 70 (18.23%) were found positive for bovine thelaziosis after a thorough examination.

**Age-based prevalence**

The prevalence of the disease was higher in the age group of younger (26.53%) than adult (18.38%). However, no any significance difference \((x^2=5.113, p=0.203)\) between the age groups (Table 1).

**Sex-based prevalence**

The prevalence of bovine thelaziosis in male cattle (24.12%) was found greater than in females (11.6%). There was also no significant difference \((x^2=2.149, p=0.716)\) between both sex groups (Table 2).

**Body conditions scores based prevalence**

The prevalence of bovine thelaziosis in poor body condition cattle (30.19%) higher than that of medium body condition (17.79%) as well as good body condition (14.88%). There was no statistically significant difference \((x^2=1.867, p=0.445)\) (Table 3).
Table 1. Prevalence of bovine thelaziasis at Mersa Town, Ethiopia according to age.

<table>
<thead>
<tr>
<th>Age</th>
<th>No. of cattle examined</th>
<th>No. of positive cattle</th>
<th>Prevalence (%)</th>
<th>X² value</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Young</td>
<td>98</td>
<td>26</td>
<td>26.53</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adult</td>
<td>286</td>
<td>44</td>
<td>18.38</td>
<td>5.113</td>
<td>0.203</td>
</tr>
<tr>
<td>Total</td>
<td>384</td>
<td>70</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2. Prevalence of bovine thelaziasis at Mersa Town, Ethiopia according to sex.

<table>
<thead>
<tr>
<th>Sex</th>
<th>No. of cattle examined</th>
<th>No. of positive cattle</th>
<th>Prevalence (%)</th>
<th>X² value</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>203</td>
<td>49</td>
<td>24.12</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>181</td>
<td>21</td>
<td>11.6</td>
<td>2.149</td>
<td>0.716</td>
</tr>
<tr>
<td>Total</td>
<td>384</td>
<td>70</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 3. Prevalence of bovine thelaziasis at Mersa Town, Ethiopia according to body condition score.

<table>
<thead>
<tr>
<th>Body condition score</th>
<th>No. of cattle examined</th>
<th>No. of positive cattle</th>
<th>Prevalence (%)</th>
<th>X² value</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poor</td>
<td>53</td>
<td>16</td>
<td>30.19</td>
<td>1.867</td>
<td>0.445</td>
</tr>
<tr>
<td>Medium</td>
<td>163</td>
<td>29</td>
<td>17.79</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Good</td>
<td>168</td>
<td>25</td>
<td>14.88</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>384</td>
<td>70</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 4. Prevalence of bovine thelaziasis at Mersa Town, Ethiopia according to management system.

<table>
<thead>
<tr>
<th>Management system</th>
<th>No. of cattle examined</th>
<th>No. of positives cattle</th>
<th>Prevalence (%)</th>
<th>X² value</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extensive</td>
<td>261</td>
<td>54</td>
<td>20.69</td>
<td>0.158</td>
<td>0.373</td>
</tr>
<tr>
<td>Semi-intensive</td>
<td>123</td>
<td>16</td>
<td>13.01</td>
<td>0.158</td>
<td>0.373</td>
</tr>
<tr>
<td>Total</td>
<td>384</td>
<td>70</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Management system based prevalence**

The prevalence of the disease was found higher in the extensive management system (20.69%) than in semi-intensive management system (13.01%). However, there was no significance difference between two management systems of cattle (X² =0.158, p=0.373) (Table 4).

**DISCUSSION**

The overall prevalence of bovine Thelaziosis was 18.23%. Despite the fact that no cases of bovine thelaziosis have been reported in the study area, this survey suggests that these parasites were relatively extensive. Prevalence of bovine Thelaziosis reported in this study was less than the prevalence of 22% reported in cattle in endemic areas (Tweedle et al., 2005). The current study indicated there was no significant difference between age groups but higher infection rate was found in young 26.53% than in adult cattle 18.38%. However, it contradicts with the work of Van Aken et al. (1996) who reported that no age and sex preference of cattle in cases exposed to thelaziosis in cattle. The increased prevalence of young animals might be due to the fact that young cattle are not allowed to go far with adult animals for grazing and increased a chance of exposure to vectors around the barn which is a suitable habitat for the vector that results in a high fly density and transmission of the disease.

The study result showed that there was no significant difference in prevalence due to a difference in sex. This finding was in line with the results of Gutierres et al. (1980) who opined that both sexes have an equal chance of exposure for thelaziosis. The highest prevalence of thelaziosis in cattle was observed in poor body conditioned (30.19%) cattle followed by medium body conditioned cattle (17.79%) while the lowest prevalence observed in cattle with good body condition (14.88%). This might be associated with the fact that effect of parasites can be influenced by animals on an inadequate diet which can influence the...
level of immunity.

Higher level prevalence of thelaziosis was observed in cattle kept in the extensive management system (20.69%) compared to cattle kept in semi-intensive management system (13.01%). The result in this study was in agreement with the work of Giangaspero et al. (2000) who reported that thelaziosis infections were not found in cattle from herds managed indoors. The reason might be associated with exposure to transmitting vectors as cattle kept indoors are less exposed to face flies.

Conclusion and Recommendations

The result of the current study with prevalence of 18.23% indicated that the disease is prevalent in the study area. The age, sex, body condition score and management system were not found to have significant influence on the prevalence of Thelaziosis in cattle.

The parasite is known by farmers affecting their cattle and they were trying to control it by using traditional practices and modern treatments. Sex, age, group, body condition, and management system of the area are important factors affecting occurrence of thelaziosis in cattle. High level of prevalence observed in male, five years and above old emaciated and extensively kept in low land.

Based on the above conclusion, the following recommendations are forwarded:

i. Awareness creation for the farmers should be given to the transmission and condition when flies are highly invested.

ii. Integrated prevention and control strategies of the disease should be given and.

iii. Further studies should be conducted in the study area of assessing the species of the parasite and vectors as well as their seasonal dynamics and economic impact of the disease.

REFERENCES


Prevalence, isolation and antimicrobial susceptibility of *Gallibacterium anatis* from Local Breed of Female Muscovy Ducks (*Cairina moschata*) in Maiduguri, Northeastern Nigeria

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ABSTRACT: The present study was carried out from the month of March to December, 2016 and aimed to isolate and investigate the prevalence of *Gallibacterium anatis* infection as well as to study the antimicrobial susceptibility pattern of the bacterium from extensively reared local breed of female Muscovy ducks in Maiduguri, Northeastern Nigeria. To accomplish this, a total of 250 samples (100 tracheal swabs, 100 cloacal swabs and 50 part of ovary) were collected from households where Muscovy ducks are reared and female Muscovy ducks from live birds market. Microbiological isolation and biochemical identification of the phenotypic characteristic consistent with *G. anatis* was used for the diagnosis of a positive sample and this revealed 75/250 (30.0%) positive isolates of the bacterium. The *G. anatis* was more frequently isolated from samples collected from house hold Muscovy ducks 51 (20.40%) than those from the live birds market 24 (9.60%) with a significant difference (*P* < 0.0001 at 95% CI; RR = 1.302). However, isolates where more frequently cultivated in samples of the tracheal swab 49 (19.60%) than those swabs collected from the cloaca 24 (9.60%) and ovary 2 (0.80%). *G. anatis* was discovered to be more frequent in the rainy season 51 (20.40%) when compared to the dry season 24 (9.60%) with a significant difference (*P* = 0.0080 at 95% CI; RR = 0.8466). Moreover, isolates revealed positive reactions to test with catalase, oxidase, phosphatase, sucrose and sorbitol, but show negative reactions to indole, urease, coagulate and maltose. The biochemical investigations differentiated the isolated strains into two biovars; haemolytic *Gallibacterium anatis* biovar 3 (4.0%) and a non-haemolytic *Gallibacterium anatis* biovar 72 (96.0%). Antimicrobial susceptibility test revealed multi-drug resistant of the *Gallibacterium anatis* isolated. The in-vitro antibiotic susceptibility testing revealed that isolates were highly susceptible to Cefotaxime, moderately susceptible to Ciprofloxacain, Doxycycline and Florfenicol. In conclusion, *G. anatis* is prevalent in extensively reared local breed of Muscovy ducks in the study area. Therefore, strict biosecurity measures should be practiced at all level of poultry production systems to curb the spread of the organism. Antimicrobial abuse should be avoided by poultry farmers and the guidance of a registered veterinarian should be sought whenever there is need for medications to avoid misuse and drug resistance.

Key words: Prevalence, *Gallibacterium anatis*, Antimicrobial susceptibility pattern, Muscovy ducks, Maiduguri, Northeastern Nigeria.
INTRODUCTION

Poultry production forms an integral part of many rural families in developing countries of the world (Mwale and Masika, 2009; Fentie et al., 2013; Angieryi et al., 2015). Poultry production in Nigeria is largely dependent on the exotic breeds of chickens, and the preponderance of scientific researches, vaccination awareness campaigns, improvement programmes and commercialization of poultry production are largely concentrated on exotic and village chickens while the other indigenous available poultry species such as guinea fowl, duck, turkey and pigeons are utterly neglected and rarely exploited for domestic and commercial purposes (Oguntunji, 2014). Generally, village poultry species reared in developing countries including Nigeria are given less attentions in terms of management system, feeding, housing and veterinary care, which can lead to low production and prevalence of diseases claiming substantial proportion of the flock among others (Oguntunji and Ayorinde, 2015). Dearth of researches on immediate factors that are responsible for declined relevance of duck, the management practices, mortality, constraints to accelerated duck production etc. are detrimental to the anticipated increased production of this waterfowl in Nigeria (Oguntunji and Ayorinde, 2015). Despite the fact that ducks are easily managed under village conditions, particularly if a waterway is nearby, and unlike the chickens, they are considered to be more resistant to some diseases that may cause huge lose and production decline in poultry production (Oluwayelu et al., 2007; Adegunloye and Adejumo, 2014). Nevertheless, ducks may suffer sub-clinical diseases, serve as reservoir of infectious diseases and also play a significant role in the maintenance and transmission of disease to other susceptible poultry species (Henning et al., 2010; Adegunloye and Adejumo, 2014; Cha et al., 2014). However, ducks do suffer from some diseases, mainly those traceable to mismanagement resulting from poor diet, stagnant unhygienic drinking water, mouldy feed, unhygienic bedding or overcrowded and filthy conditions (Kumar et al., 2004; Mbuthia et al., 2008). *Gallibacterium anatis* has been isolated from apparently healthy ducks in some parts of Africa (Sorour et al., 2015). *Gallibacterium anatis* infection is an emerging disease of poultry (Singh et al., 2016). The increasing global concern about *G. anatis* is the incomplete understanding of its growth kinetics, virulence markers, pathogenesis and vaccine(s) to control. *Gallibacterium anatis* (earlier known as *Pasteurella anatis*) is a commensal in upper respiratory tracts and the lower genital tracts of healthy chickens (Mushtin et al., 1980). It has been reported to be associated with bacteremia, oophoritis, follicle degeneration, salpingitis, peritonitis, hepatitis, enteritis and respiratory tract diseases in chickens (Aarestrup et al., 2004; Jordan et al., 2005; Kristensen et al., 2011). *Gallibacterium anatis* mostly affects intensively farmed poultry birds causing loss in production with heavy mortality in broiler chicken and drop in egg production in layers with increased mortality (Bojesen et al., 2008). *Gallibacterium anatis* has also been reported to infect turkeys, geese, ducks, pheasants, partridges, budgerigars, peacock, cage birds, wild birds, cattle and pig (Christensen et al., 2003; Rzewuska et al., 2007; Bisgaard et al., 2009; Gregersen et al., 2010). The bacterium has been reported to be associated with fatal bacteremia in immune-compromised human patient (Aubin et al., 2013). Poultry diseases caused by *Gallibacterium anatis* has been reported from all continents (Christensen et al., 2003; Bojesen et al., 2007). Its association with a variety of pathology makes it difficult to be diagnosed even after post-mortem in absence of pathognomonic lesion(s) and the disease is often confused with Fowl Coryza, New Castle disease and Bird Flu (Christensen et al., 2003).

Though the infection of *G. anatis* is treatable with antibiotics, the frequency of treatment failure is an emerging and recurrent problem. Multidrug resistant strains of *G. anatis* (Aarestrup et al., 2004; Bojesen et al., 2011) have shown resistance to sulpha drugs, novobiocin, tylosin, clindamycin, tetracycline and penicillin (Malik et al., 2005; Berge et al., 2006; Hendriksen et al., 2008; Guo et al., 2009; Johnson et al., 2011; Jones et al. 2013). Concerns have been shown for biosecurity measures towards control of disease, handling of pathogen and prevention of spread. *Gallibacterium anatis* has not been reported in local breed of the Muscovy ducks in Maiduguri, Northeastern Nigeria. Therefore, this present study was designed to isolate, determine the prevalence and antimicrobial susceptibility patterns of *Gallibacterium anatis* from local breeds of female Muscovy ducks in Maiduguri, Northeastern Nigeria.

MATERIALS AND METHODS

Study area

This study was conducted in Maiduguri, the capital and largest city of Borno State, Nigeria, located within the Sahel savannah zone of the Northeastern Nigeria. It lies approximately between Latitude 11° 5' and 11.83° N and Longitude 13° 09' and 13.50° E at about 350 m (1161 ft) above sea level with an ambient temperature range of 32 to 45°C (http://www.unimaid.edu.ng/About_Maid.aspx). The climate is hot and dry for a greater part of the year with a rainy season from June to September in the Northern part and May to October in the Southern part with a mean annual rainfall of about 650 mm. The mean relative humidity of Maiduguri ranges from 30 to 50% with the minimum been experienced in the months February
and March when it drops to as low as 10% and reaches maximum in August when it rises to as high as 90% (http://www.unimaid.edu.ng/About_Maid.aspx).

Sample Population

Swab samples from the trachea and cloaca as well as ovary samples were collected from extensively reared local breeds of female Muscovy ducks within Maiduguri metropolis and those brought for sales/dressing at major live birds market in the study area. Information on factors such as age and sex that seem to influence results in prevalence study were not included in this study; this is because of the challenges faced with consent for sampling from the duck owners in the study area. Information concerning type of management system employed in the rearing of ducks; availability of swimming ponds and the level of biosecurity around duck shelter were observed and noted.

Sample Size Determination

The desired sample size for the study was calculated using the equation described by Thrusfield (2005), since the exact prevalence of Gallibacterium anatis in extensively reared local breeds of female Muscovy ducks in the study area was not known; so to maximize the sample size it was assumed that the expected prevalence was 50%, absolute precision was 5% and the confidence interval level was set to be 95% as shown below:

\[ n = \frac{1.962 \times p \times (1-p) \times \exp}{l^2} \]

Where, \( n \) = the required sample size, \( p \) = expected prevalence, \( q = 1 – p \); and \( l \) = absolute precision, that is the largest acceptable differences between the true and the estimated prevalence.

As a result, 250 study populations were selected for the sampling area.

Sample collection

During the periods of sample collections, village poultry farms in which ducks were also reared and live birds markets were visited on alternate days of the study period. Swabs samples were collected from the trachea and cloaca of live female ducks while sample of ovary were inclusively collected from slaughtered local breeds of female Muscovy ducks at the poultry dressing slabs of the selected live birds markets. Samples were collected from five (5) different households rearing large numbers of female Muscovy ducks in a flock and two (2) live birds markets in the study area. Consent for sample collections was sought from the duck farmers/owner or sellers in each sampled farm/live birds market within Maiduguri metropolis for the detection of Gallibacterium anatis infection. Two hundred and fifty (250) samples were collected which comprised of One hundred (100) tracheal swabs, One hundred (100) cloaca swabs and Fifty (50) sample of ovary. At least Ten (10) female ducks were sampled from each ducks farm and Twenty five (25) female ducks from each selected live bird markets of the study areas during the study periods. All samples collected were labeled appropriately and transported to the Department of Veterinary Medicine research laboratory, University of Maiduguri and the Microbiology laboratory, University of Maiduguri Teaching Hospital for processing and culturing.

Bacterial isolation and identification

Tracheal and cloacal swabs as well as sample of ovary were inoculated onto a plate of blood agar base (Oxoid), supplemented with 5% citrated bovine blood and incubated aerobically at 37°C for 24 to 48 hours. The colonies of G. anatis on blood agar appeared smooth and shiny, greyish, semi-transparent, circular slightly raised colonies with an entire margin and a butyrous consistency which is 1 to 2 mm in diameter after 24 hours of incubation at 37°C for both the haemolytic and non-haemolytic strains and only the haemolytic strains colonies were surrounded by a wide β-haemolytic zone (1 to 2 mm) after 24 hours incubation adopting the standard protocol described by Christensen et al. (2003) and Bojesen et al. (2008). Such colonies were regarded as suspicious of Gallibacterium, therefore suspected colonies were further sub-cultured on blood agar to obtain pure cultures as described by Neubauer et al. (2009).

Microscopic examination and Biochemical identification

Microscopic examination revealed Gram negative, rod-shaped or pleomorphic, non-motile characteristic of G. anatis as previously described by Christensen et al. (2003). Biochemical identification of G. anatis isolates showed catalase and oxidase positive, indole and urease negative.

Antimicrobial susceptibility testing

Antimicrobials susceptibility testing of G. anatis isolated was performed using disc diffusion test (Oxoid, UK). The antimicrobials used include Cefotaxime, Florfenicol, Norfloxacin, Ciprofloxacin, Gentamycin, Erythromycin, Ampicillin, Amoxicillin, Cephradine, Doxycycline, Oxytetracycline, Sulphamethoxazole + Trimethoprim,
Table 1. Overall prevalence of Gallibacterium anatis isolated from local breeds of female Muscovy ducks in Maiduguri, Northeastern Nigeria.

<table>
<thead>
<tr>
<th>Number of positive samples (CI %)</th>
<th>Number of negative samples (CI %)</th>
<th>Total samples collected</th>
<th>Risk Ratio</th>
<th>Prevalence rate (%)</th>
<th>Prevalence rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>75/250 (30.0)</td>
<td>175/250 (70.0)</td>
<td>250</td>
<td>0.429</td>
<td>30.0</td>
<td></td>
</tr>
</tbody>
</table>

CI%, Cumulative Incidence of infected and uninfected duck sampled; RR, Risk ratio (CI% infected ducks ÷ CI% uninfected ducks).

Table 2. Isolation of Gallibacterium anatis from local breeds of female Muscovy ducks according to sampling location.

<table>
<thead>
<tr>
<th>Study location</th>
<th>Number of positive samples (CI%)</th>
<th>Number of negative samples (CI%)</th>
<th>Total samples collected</th>
<th>Risk Ratio</th>
<th>Prevalence rate (%)</th>
<th>95% CI L – U</th>
<th>P-value</th>
<th>Relative Risk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Live birds market</td>
<td>24 (16.0)</td>
<td>126 (84.0)</td>
<td>150</td>
<td>0.190</td>
<td>9.60</td>
<td>0.8021 – 0.9095</td>
<td>&lt; 0.0001</td>
<td>1.302</td>
</tr>
<tr>
<td>Households</td>
<td>51 (51.0)</td>
<td>49 (49.0)</td>
<td>100</td>
<td>1.041</td>
<td>20.40</td>
<td>0.5810 – 0.7374</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>75 (30.0)</td>
<td>175 (70.0)</td>
<td>250</td>
<td>0.429</td>
<td>30.0</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

RR, Risk ratio (CI% infected ducks ÷ CI% uninfected ducks); L – U, Lower limit and Upper limit 95% Confidence interval; p<0.05 was considered as significant.

Streptomycin, Lincomycin, and Spectinomycin. All isolates were cultured over night on 5% citrated sheep blood agar at 37°C in micro-aerophilic condition. Then, the cultures were suspended in 0.85% NaCl to an optical density equivalent to that of McFarland 0.5 standards. Each isolate was then inoculated onto Mueller Hinton agar medium (Oxoid, UK), then 15 minutes later, the antimicrobial discs were applied. Plates were incubated anaerobically at 37°C for 24 hours and the interpretation was done according to the manufacturer.

Data Analysis

Data generated were entered into Microsoft office Excel spread sheet, Risk Ratios (RR) and 95% CI on the Relative Risk (RR) were calculated using the Fisher's exact test to determine strength and significance of associations between the seasons and infection as well as location of sample collection and infection from sampled ducks. The prevalence of G. anatis among the sampled population was calculated using frequencies and percentages in GraphPad prism® version 5.01 for windows (GraphPad Software, Inc., San Diego, California, USA) computer based program. The observed prevalence and 95% confidence intervals (CI) were evaluated and “p” values equals to or less than 0.05 were regarded significant.

RESULTS

The isolation of Gallibacterium anatis from the samples collected from extensively reared local breed of female Muscovy ducks in Maiduguri were based on the phenotypic morphological characteristic exhibited by the colonies on blood agar plates and their biochemical reactions. Out of the total samples collected, G. anatis was isolated and identified from 75/250 (30.0%) samples which exhibited the entire phenotypic characteristic consistent with those of the bacterium. The cumulative incidence (CI%) of the bacterium in the infected samples was also 75/250 (30.0%) (Table 1).

Considering the isolation of G. anatis from apparently healthy local breed of Muscovy ducks based on the study locations where samples were collected, out of the 150 samples collected in Maiduguri live birds markets, 24 (16.0%) of the samples showed phenotypic characteristics consisted with G. anatis, with a prevalence rate of 9.60%. However, out of the100 samples collected from households/duck farms in Maiduguri, 51 (48.0%) exhibited phenotypic characteristics consisted with those of G. anatis, with a prevalence rate of 20.40%. There was statistical significant difference (P < 0.0001 at 95% confidence interval) between the prevalence rate of G. anatis isolated from samples collected from the live birds markets and those collected from households/duck farms in the study area. The risk of G. anatis infection among the infected ducks sampled from live birds markets and ducks farms was 0.19 and 1.04 times compared to the uninfected ducks respectively (Table 2).

The result of isolation of G. anatis from apparently healthy extensively reared local breed of female Muscovy ducks from the study areas based on the type of samples collected, revealed that the bacteria was more frequently isolated in samples of the tracheal swabs collected from...
the live birds markets 14 (5.60%) and household/duck farms 35 (14.0%) in Maiduguri when compared to isolation of G. anatis from the cloaca swabs collected from live birds markets 8 (3.20%) and duck farms 16 (6.4%). The isolation of G. anatis from the ovaries were the least frequent in the samples collected from the live birds markets in the study area 2 (0.80%) (Table 3).

The results of the distribution of G. anatis isolated from local breeds of female Muscovy ducks according to season of sample collection revealed that, the bacterium is more frequently isolated in samples collected during the rainy season 51 (20.40%) when compared to those collected during the dry season 24 (9.60%) in Maiduguri. There was significant statistical difference ($P = 0.0080$ at 95% confidence interval) between the samples collected during the two seasons. The risk of G. anatis infection among the infected ducks sampled was 0.69 times when compared to the uninfected ducks during the rainy season. However, the risk of G. anatis infection among the infected ducks sampled was 0.24 times when compared to the uninfected ducks during the dry season (Table 4).

The results of the biochemical identification test for G. anatis isolated from extensively reared local breeds of female Muscovy ducks in the present study revealed that the isolated organisms shows positive reactions to test with catalase, oxidase, phosphatase, sucrose and sorbitol, however, demonstrate negative reactions to indole, urease, coagulase and maltose (Table 5).

The result of distribution of G. anatis isolated from extensively reared local breeds of female Muscovy ducks based on their haemolytic and non-haemolytic characteristics on blood agar revealed that the non-haemolytic strain 72 (96.0%) of the bacteria is more frequently isolated than the haemolytic strains 3 (4.0%) amongst the sample collected from the study area. The non-haemolytic strain of G. anatis was more frequently isolated from swabs samples collected from the trachea 46 (61.33%), followed by samples from the cloaca 24 (32.0%) and ovaries 2 (2.67%). While the haemolytic strain of the organism in the present study was only isolated from the cloacal swabs 3 (4.0%) (Table 6).

The in-vitro degree of antimicrobial susceptibility pattern of the isolated G. anatis to 15 different antimicrobials revealed that the isolated bacteria were highly susceptible to Cefotaxime, moderately susceptible to Ciprofloxacin, Doxycycline and Florfenicol, as well as fairly susceptible to Gentamycin and Norfloxacin, but were completely resistant to Erythromycin, Cephradin, Oxytetracycline, Sulpha. + Trimethoprim, Streptomycin, Amoxicillin, Ampicillin, Lincomycin and Spectinomycin (Table 7).

**DISCUSSION**

The research focused on diagnosis of Gallibacterium anatis infection on the phenotypic characteristics of the

### Table 3. Isolation of Gallibacterium anatis from local breeds of female Muscovy ducks according to type of samples collected.

<table>
<thead>
<tr>
<th>Study location</th>
<th>Type of samples</th>
<th>Number of samples collected (y)</th>
<th>Number of positive samples (x) (x/y %)</th>
<th>Prevalence rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Live birds market</td>
<td>Tracheal swabs</td>
<td>50</td>
<td>14 (28.0)</td>
<td>5.60</td>
</tr>
<tr>
<td></td>
<td>Cloacal swabs</td>
<td>50</td>
<td>8 (16.0)</td>
<td>3.20</td>
</tr>
<tr>
<td>Households/farms</td>
<td>Ovary samples</td>
<td>50</td>
<td>2 (4.0)</td>
<td>0.80</td>
</tr>
<tr>
<td></td>
<td>Tracheal swabs</td>
<td>50</td>
<td>35 (70.0)</td>
<td>14.0</td>
</tr>
<tr>
<td></td>
<td>Cloacal swabs</td>
<td>50</td>
<td>16 (32.0)</td>
<td>6.40</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td><strong>250</strong></td>
<td><strong>75 (30.0)</strong></td>
<td><strong>30.0</strong></td>
</tr>
</tbody>
</table>

**Table 4. Distribution of Gallibacterium anatis isolated from local breeds of female Muscovy ducks according to season.**

<table>
<thead>
<tr>
<th>Season of sample collection</th>
<th>Number of samples collected</th>
<th>Number of samples positive (C%)</th>
<th>Number of samples negative (C%)</th>
<th>Risk Ratio</th>
<th>Prevalence rate (%)</th>
<th>95% CI L – U</th>
<th>$P$ - value</th>
<th>Relative Risk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rainy</td>
<td>125</td>
<td>51 (40.80)</td>
<td>74 (59.20)</td>
<td>0.689</td>
<td>20.40</td>
<td>0.6368 – 0.7760</td>
<td>0.0080</td>
<td>0.8466</td>
</tr>
<tr>
<td>Dry</td>
<td>125</td>
<td>24 (19.20)</td>
<td>101 (80.80)</td>
<td>0.238</td>
<td>9.60</td>
<td>0.7697 – 0.8939</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>250</td>
<td>75 (30.0)</td>
<td>175 (70.0)</td>
<td>0.429</td>
<td>30.0</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

RR, Risk ratio (C% infected ducks + C% uninfected ducks); L – U, Lower limit and Upper limit 95% Confidence interval; $p<0.05$ was considered as significant.
isolated organism on blood agar by samples collected from local breed of female Muscovy ducks in the study area. The bacterium has been considered an emerging pathogen of domesticated poultry species, semi-domesticated and wild domiciled birds in developing and developed countries, with no pathognomonic clinical signs (Singh et al., 2016). This is the first report of isolation of *G. anatis* in apparently healthy domesticated

<table>
<thead>
<tr>
<th>Biochemical Test</th>
<th>Number of samples tested (n=75)</th>
<th>Number of sample positive (%) (n=75)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Catalase</td>
<td>75</td>
<td>75</td>
</tr>
<tr>
<td>Indole</td>
<td>75</td>
<td>0</td>
</tr>
<tr>
<td>Urease</td>
<td>75</td>
<td>0</td>
</tr>
<tr>
<td>Oxidase</td>
<td>75</td>
<td>75</td>
</tr>
<tr>
<td>Coagulase</td>
<td>75</td>
<td>0</td>
</tr>
<tr>
<td>Phosphatase</td>
<td>75</td>
<td>75</td>
</tr>
<tr>
<td>Maltose</td>
<td>75</td>
<td>0</td>
</tr>
<tr>
<td>Sucrose</td>
<td>75</td>
<td>75</td>
</tr>
<tr>
<td>Sorbitol</td>
<td>75</td>
<td>75</td>
</tr>
</tbody>
</table>

Table 6. Haemolytic characteristics of isolated serovars of *G. anatis* from female Muscovy ducks on blood agar.

<table>
<thead>
<tr>
<th>Type of Samples collected</th>
<th>Number of Positive samples tested (n = 75)</th>
<th>Type of <em>Gallibacterium</em> biovar isolated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Haemolytic (%)</td>
</tr>
<tr>
<td>Tracheal swabs</td>
<td>49</td>
<td>3 (4.0)</td>
</tr>
<tr>
<td>Cloacal swabs</td>
<td>24</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Ovary samples</td>
<td>2</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Total</td>
<td>75</td>
<td>3 (4.0)</td>
</tr>
</tbody>
</table>

Table 7. Antimicrobial susceptibility of *Gallibacterium anatis* isolated from local breeds of female Muscovy ducks.

<table>
<thead>
<tr>
<th>Antimicrobials</th>
<th>Degree of Antimicrobial susceptibility of isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gentamycin (CN- 10 μg)</td>
<td>+</td>
</tr>
<tr>
<td>Erythromycin (E- 10 μg)</td>
<td>-ve</td>
</tr>
<tr>
<td>Amoxycillin (AML -30 μg)</td>
<td>-ve</td>
</tr>
<tr>
<td>Cefotaxin (CTX- 30μg)</td>
<td>+++</td>
</tr>
<tr>
<td>Florfenicol (FFC- 30μg)</td>
<td>++</td>
</tr>
<tr>
<td>Norfloxacin (NOR- 10 μg)</td>
<td>+</td>
</tr>
<tr>
<td>Ciprofloxacin (CIP - 5 μg)</td>
<td>++</td>
</tr>
<tr>
<td>Oxytetracycline (OT-30μg)</td>
<td>-ve</td>
</tr>
<tr>
<td>Doxycycline (DO- 30μg)</td>
<td>++</td>
</tr>
<tr>
<td>Sulpha.+Trimethoprim (SXT - 25 μg)</td>
<td>-ve</td>
</tr>
<tr>
<td>Streptomycin (S- 30 μg)</td>
<td>-ve</td>
</tr>
<tr>
<td>Lincomycin (MY – 30 μg)</td>
<td>-ve</td>
</tr>
<tr>
<td>Spectinomycin (SH - 100)</td>
<td>-ve</td>
</tr>
<tr>
<td>Ampicillin (AMP - 10 μg )</td>
<td>-ve</td>
</tr>
<tr>
<td>Cephradine (CE - 30 μg)</td>
<td>-ve</td>
</tr>
</tbody>
</table>

+++ , Highly susceptible; ++, Moderately susceptible; +, Fairly susceptible; -ve, Completely resistant.
Plate 1. Extensively reared local breeds of Muscovy ducks scavenging in unhygienic pool of water.

Plate 2. Extensively reared local breeds of male and female Muscovy ducks scavenging on rubbish dump.

local breed of the female Muscovy ducks in the study area. This finding supported the fact that the organism exist among apparently healthy birds, although, the bacterium has previously been isolated from clinically sick ducks in Egypt by Sorour et al., (2015) and Abd El-Hamid et al., (2016). It has also been isolated from apparently healthy domesticated fowl (Gallus domestica) in Nigeria by Addo and Mohan (1985). The disease had been frequently isolated from diseased and apparently healthy layers and cockerels of exotic breeds of chickens as well as a wide range of semi-domestic birds including turkeys, geese, ducks, pheasants, partridges and cattle egrets (Bisgaard, 1993; Christensen et al., 2003; Rzewuska et al., 2007; Bisgaard et al., 2009; Gregersen et al., 2010; Paudel et al., 2014; Sorour et al., 2015). The varying unhygienic environments (Plate 1 and 2) of rearing Muscovy ducks in the study area may possibly expose this species of birds to various infectious bacterial diseases including G. anatis. This observation agrees with the finding of Bojesen et al. (2003) who reported up to 96.0% prevalence rate of G. anatis in free range scavenging domestic fowls and the high prevalent rate has been attributed to poor biosecurity. The occurrence of the bacterium in free range local breed of female Muscovy ducks may pose health threat to chickens and other extensively reared range poultry species since the infection is horizontally transmitted. This agrees with the finding of Singh et al. (2016) who reported that the bacterium is naturally transmitted among poultry species and it is difficult to get rid of the infection on affected poultry farms. The isolation of G. anatis from apparently healthy local breed of female Muscovy ducks in this study supported the findings of Sorour et al. (2015) who reported high prevalence of G. anatis in ducks and Bojesen et al. (2003) who in a similar study isolated the bacterium from apparently healthy chickens. Neubauer et al. (2009) and Sorour et al. (2015) have also reported isolation of Gallibacterium in pure cultures of sample collected from domesticated chickens and ducks with various pathological lesions.

G. anatis infection in this research was found to be more frequently isolated from swab samples collected from free range households ducks compared to swab samples collected from ducks in the live birds markets in the study area. This finding may probably be associated with the scavenging nature of the free range Muscovy ducks which might expose the m to various organisms including G. anatis during scavenging on unhygienic contaminated environment or from horizontal transmission from other infected birds. This finding is in line with those of Bojesen et al. (2003) and Persson and Bojesen (2015) who have also frequently isolated the bacterium from domesticated birds reared under free range unhygienic environment compared to birds reared in an organized farm with modern facilities that maintained adequate biosecurity. From the results of this study, the isolation of G. anatis in samples collected from local breed of female Muscovy ducks in live birds markets may not be surprising, because it was observed that there are no discriminations of health status or screening for diseases among birds before mixing of different poultry species in live birds markets (Plate 3). This habit of live birds’ sellers may facilitate horizontal disease transmissions from infected birds to susceptible uninfected ones. Although, there was a significant
variation of the bacterium in the study area, *G. anatis* was more frequently isolated in samples collected during the raining season compared to samples collected during the dry season. This suggested that free range Muscovy ducks are more predisposed to *G. anatis* infection during the raining season compared to the dry season in the present study. There was significant statistical difference ($P = 0.0080$ at 95% confidence interval) between the prevalent rates of the bacterium in the samples collected during the two seasons. This finding may be associated with the abundance of unhygienic stagnant pool of water usually surrounding households in the rainy season, which may serve as bathing and dabbling pools for extensively reared ducks, such stagnant pool of water may be contaminated with various pathogens including *G. anatis*. This finding is consistent with those of Malik et al. (2005) who have also reported variation in season to be one of the major factors that influence the increased in the susceptibility of domesticated poultry to infection by *G. anatis*. Moreover, several researches have reported significantly higher isolation rate of bacterial diseases in poultry species during the rainy seasons compared to the dry season (Mbuko et al., 2009; Yunus et al., 2009; Zdragas et al., 2012; Balami et al., 2014; Soo-Kyoung et al., 2016).

The finding of this research also revealed that *G. anatis* isolated from local breeds of female Muscovy ducks in the study area shows positive reactions to test with catalase, oxidase, phosphatase, sucrose and sorbitol, however, demonstrate negative reactions to indole, urease, coagulase and maltose. This finding supported those of Christensen et al. (2003) and Bojesen et al. (2007) who have also reported similar reactions of *G. anatis* isolates which indicated that all typical *G. anatis* strains are catalase, oxidase, and phosphatase positive, and they can reduce nitrate. *Gallibacterium* genus can be differentiated from other genera of Pasteurellaceae with catalase, symbiotic growth, hemolysis, urease, indole, acid production from (+) D-xylose, (-) D-mannitol, (-) D-sorbitol, (+) D-mannose, maltose, raffinose and dextrin tests (Christensen et al., 2003; Bojesen et al., 2007).

The identification of *Gallibacterium* organism and their classification into the two basic biovar in the present study relied on the type of phenotypic characteristics exhibited by the inoculated samples on bovine blood agar plates, which at the time of the study was the only detection method available. In previous researches *Gallibacterium* isolates has been differentiated into *Gallibacterium anatis biovar haemolytica* and *Gallibacterium anatis biovar anatis* (Paudel et al., 2013; 2014). The two broad classification or biovars are described within *G. anatis*, as a haemolytic *biovar haemolytica* and a non-haemolytic *biovar anatis* (Kristensen et al., 2010). From the result of this present study the non-haemolytic strain of *Gallibacterium* was more frequently isolated from the infected samples compared to the haemolytic strain. Moreover, the non-

The present study have revealed seasonal prevalence
haemolytic strain of *Gallibacterium* was more frequently isolated from swab samples collected from the trachea followed by the cloaca and ovary in descending order of frequency. This indicated that the non-haemolytic *G. anatis* is the most naturally abundant strain of the bacterium among free range Muscovy ducks in the study area. This finding is consistent with those of Sorour et al. (2015) who have also reported significantly higher prevalence of non-haemolytic *Gallibacterium anatis biovar anatis* (69.2%) in duck compared to haemolytic *Gallibacterium anatis biovar haemolytica* (30.7%).

The *in-vitro* degree of antimicrobial susceptibility pattern of the isolated *G. anatis* to 15 different antimicrobials in the present study revealed that the isolated bacterium were highly susceptible to Cefotaxime, moderately susceptible to Ciprofloxacin, Doxycycline and Florfenicol, as well as fairly susceptible to Gentamycin and Norfloxacin, but were completely resistant to Erythromycin, Cephradin, Oxytetracycline, Sulpha. + Trimethoprim, Streptomycin, Amoxicillin, Ampicillin, Lincomycin and Spectinomycin. This finding supported the antimicrobial susceptibility profile of *G. anatis* isolates from infected ducks and other poultry species which were reported from several investigations (Bojesen et al., 2011; Guo, 2011; Janda, 2011; Jones et al., 2013; El-bestawy, 2014; Sorour et al., 2015; Abd El-Hamid et al., 2016). Chuan-qing et al. (2008) have also reported that all *G. anatis* isolates were highly sensitive to the third generation cephalosporin in antimicrobial resistance testing. Moreover, investigation has also revealed that *G. anatis* isolates were resistant to wide range of antibiotics and were susceptible to very few ones. However, the fact that the organisms remains susceptible to some antimicrobials such as Cefotaxime, Ciprofloxacin, Doxycycline and Florfenicol as demonstrated in the present research makes the organism treatable using chemotherapy, the most appropriate antibiotics with the guidance of a registered Veterinarian.

**Conclusion**

In conclusion, *Gallibacterium anatis* is exist among the free range local breed of female Muscovy ducks reared in the study area, with the non-haemolytic strain occurring more frequent in the trachea compared to isolation from the cloaca and ovary. The occurrence of this organism in swabs samples collected from adult female Muscovy ducks is attributed to natural horizontal mode of transmission of the organism since there was no previous report of the bacterium in the study area. However, there may be possibility of the organism causing mild form of disease in the infected birds without visible clinical signs. The bacterium may occur in both the rainy and dry season, but more frequently in the rainy season which was attributed to abundance of probably contaminated stagnant pool of water in the surroundings in which free range Muscovy ducks swim and dab. Also, the unhygienic environment in which Muscovy ducks scavenge might be considered as the most predisposing factor of the diseases transmission among free range Muscovy ducks. The indiscriminate mixing of several poultry species in live birds local markets might also contribute to the horizontal transmission of the organism. The non-haemolytic strain of the bacterium is more abundant in the sampled ducks and the isolated organism has demonstrated multidrug resistance, but susceptibility to a few ones, this is suggestive that the organism can be treatable with some antimicrobial chemotherapy.

**Recommendations**

The presence of the bacterium should be suspected in atypical bacterial infections of poultry, especially where there is multidrug resistance to treatment with antibiotics. Isolation of the organism should be attempted in poultry diseases associated with uncertain clinical signs. To control disease transmission to susceptible birds, it is recommended that strict biosecurity measures should be maintained in all levels of poultry production systems. Molecular researches involving genotypic characterization of *G. anatis* in several poultry species and other geographical location should be conducted in Northern Nigeria.

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