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Preliminary Study on Avian Tuberculosis and Associated Risks in Domestic Chickens at Shashemene District, Ethiopia

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Abstract
The study was conducted to estimate the prevalence of avian tuberculosis (TB) and assess associated risk factors in Shashemene District. In addition, the zoonotic implication of avian tuberculosis in the District was investigated. In this study, 260 adult domestic chickens of both sexes were tested by tuberculin test on their wattle. Test positive chickens were sacrificed and examined for the presence of tuberculous lesion. Suspicious lesions were cultured for mycobacterial isolation and characterization using multiplex polymerase chain reaction (PCR). On top of these, questionnaire was used to assess public perception and the potential public health risks of the disease. The prevalence of avian TB at Shashemene District was 4.23% (11/260). Gross TB lesions were detected in six of the 11 tuberculin positive chickens. Culture positivity was confirmed in three of the chicken with gross lesions. These isolates were confirmed to be members of the Genus Mycobacterium. Multivariable logistic regression analysis showed that male owners had a better knowledge of avian TB compared to females (adjusted OR=2.01; 95% CI: 0. 35-11.26). Furthermore, human TB exposed owners had better knowledge of avian TB compared to TB unexposed owners (adjusted OR=3.92; 95% CI: 0.98-15.76). The survey indicated that chicken kept in extensive production system and as there exist a close physical contact between the chicken and their owners, there could a possibility of transmission of mycobacteria between chicken and their owners. On top of this, the low perception of the owners about zoonotic TB including avian TB could add up to the transmission.
Keywords: Avian tuberculosis, domestic chicken, *M. avium complex*, prevalence, Shashemene.

Introduction

Ethiopia has large population of chicken, estimated to be 42 million of which local breeds representing 96.6%, hybrid chicken 0.55% and exotic breeds of chicken 2.84% (CSA, 2009). From the total population of chicken in Ethiopia, 99% are raised under the traditional back yard system of management, while 1% is under intensive management system (Tadelle *et al.,* 2003).

Poultry occupies a unique position in terms of its contribution to the provision of high quality protein food to rural smallholder farming families in Africa (Sonaiya *et al.,* 1999) and particularly in Ethiopia (Tadelle and Ogle 2001). There are only few alternative animal protein sources available. There are also few cultural or religious taboos that stand against the consumption of eggs and poultry meat in most countries. Both poultry meat and eggs enrich and contribute to a well-balanced diet to satisfy human needs. The backyard poultry could contribute for better dietary requirement of the family particularly in improving the diet of young children in Ethiopia. To date there are no detailed studies conducted targeting the backyard chicken production patterns, disease prevalence and associated risks to public health. Investigating the farmers understanding on poultry diseases and their potential spill-over to the family while rising and utilizing poultry product, will have considerable relevance in view of determining potential zoonotic risks of the diseases.

Avian tuberculosis (Avian TB) is one of the most important diseases that affect domestic and pet birds. It is a zoonotic disease that also affects human beings. Though several mycobacterial species have been reported to be involved in the aetiology of TB in chicken, the disease is most often caused by *Mycobacterium avium* (*M. avium*) belonging to serotypes 1, 2, 3, and 6 (genotype IS901 and IS1245) and *Mycobacterium genavense (M. genavense)* (Fulton and Thoen, 2003; Dvorska *et al.,* 2007).

The occurrence of avian TB has been reported from various parts of the world and in many species of animals, including domestic poultry (OIE, 2000; Pavlik *et al.,* 2000) pet birds, (Pavlik *et al.,* 2000), wild and zoologic birds (Thoen, 1997; OIE, 2000; Pavlik *et al.,* 2000), swine (OIE, 2000), cattle (Thorel *et al.,* 1997; Pavlik *et al.,* 2000) and humans (Pavlik *et al.,* 2000). In humans, *M. avium* is capable of inducing a progressive and disseminated type disease that is relatively refractory to treatment both in HIV/AIDS patients (Horsburgh, 1996) and in immunocompetent individuals (Iseman, 1989). Infected birds and contaminated water and soil are the main source of infection as the Mycobacteria can survive for several months in the environment (Fulton and Thoen, 2003; Dhama *et al.,* 2008). The impact of avian TB in domestic chickens has been diminished in many

countries as a result of the introduction of more intensive production systems (Thoen, 1997) that encompass better nutrition, shelter, and hygiene and management practices. However, it remains a problem in extensive (traditional) production systems under which chickens scavenge for survival in unhygienic environments. The prevailing production characteristics like flock size, housing at night, and poor nutrition and management practices reported in our country (Ashenafi, 2000; Tadesse *et al.,* 2004) indicated that chickens are constantly exposed to overcrowding (which may lead to stress), unhygienic external environments, other domestic animals, and free-living birds that may serve as sources of infection. This may predisposes them for various infections, including avian TB (Mwalusanya *et al.,* 2002).

Diagnosis of avian TB in chickens depends on demonstration of *M. avium* complex (MAC) in dead birds or detection of a cellular immune response in live birds (Thoen, 1997; OIE, 2000). In addition, clinical manifestations in birds like emaciation, depression and diarrhoea along with marked atrophy of breast muscle can be used for tentative diagnosis of avian TB. Unlike TB in animals and man, lesions in lungs are rare. Tubercular nodules can be seen in liver, spleen, intestine and bone marrow. Granulomatous lesion without calcification is a prominent feature. If typical lesions of tuberculous are present at necropsy, demonstration of acid-fast bacilli in smears or histopathologic sections made from affected organs is regarded as sufficient for positive diagnosis (OIE, 2000). If acid-fast bacilli are not found, but typical signs or lesions are present in the birds, culture of the organisms on artificial media such as Lowenstein-Jensen (LJ), Stonebrink, and Middlebrook agar must be attempted (Barron *et al.,* 1994). In tropical countries such as Ethiopia, where laboratory facilities such as molecular techniques or chromatography are limited, tuberculin test is the most economical and technically simple ante-mortem test for the diagnosis of avian TB.

In Ethiopia, in spite of the existence large population of chicken and potential future expansion of the poultry industry in the country, infectious diseases such as avian TB which has direct impact on the sector has not been well studied. A single study carried out so far on avian TB in central Ethiopia has indicated that the disease is prevalent in traditionally managed local chicken and reported a prevalence of 6.3% (Tadesse *et al.,* 2004). In addition, avian TB is known to have zoonotic significance for risk groups like HIV patients. Therefore, it is of paramount importance to generate more epidemiological data that helps to design appropriate control strategy. Therefore, the present study was conceived to determine the prevalence of avian TB and associated risk factors in domestic chickens at Shashemene district and to evaluate public perception on the zoonotic risks of avian TB in the area.

Materials and Methods

Study Area

The study was conducted from October 2012 to May 2013; at Shashemene Districts of west Arsi zone of Oromia Regional State. The District is located at 250 km south of Addis Ababa. The area lies within the mid Rift Valley with altitudes ranging from 1700 to 2600 metres above sea level (masl). It receives an annual rainfall of 700-950 mm with bimodal type of rainfall, and has an annual average temperature range of 12-27^{o}C (SDARDVA, 2011). The farmers in the District also practices mixed crop-livestock production system. According to Shashemene Woreda Animal Resource Development and Veterinary Agency (SDARDVA), majority (about 96%) of the chicken are reared under extensive backyard production system consisting of local indigenous chicken kept for home consumption (egg and meat) and income generation (Personal communication). The chicken feely scavenges in the backyard for feed in the vicinity of the household. A complete list of kebeles in the district was obtained from the district head-quarter and 8 kebeles namely: Alelu, Awasho, Bute filicha, Kebele 03, kebele 04, kebele 05, Kebele 06, and Melka Uda, were selected randomly, so as to study the prevalence of Avian TB.

Study Animal and Sample Size

The study animal consists of domestic chickens raised under extensive backyard production system. A total of 260 adult chicken of both sex were randomly selected for the study. The sample size was estimated by considering the previous prevalence (6.3%) reported in Adama (Tadesse *et al.,* 2004) and following the formula by Thrusfield (2005), for simple random sampling with 95% confidence interval and 5% desired precision.

Study Design

A cross-sectional study design was used primarily to estimate the apparent prevalence of avian TB in Shashemene District using single intradermal tuberculin test. The tuberculin test was conducted on the randomly selected chickens. Those chickens with test positive were bought and sacrificed for post-mortem examination and suspected tissues were sampled for further laboratory investigation. Additionally the tissue distribution of tuberculous lesion was determined. Chicken owners were interviewed to evaluate their perception about the disease in question, associated risk factors and also characterize the prevailing production system.

Clinical examination and tuberculin test

Individual chicken was examined clinically for any disease conditions and the findings were recorded. The single intradermal tuberculin test was carried out according to the method described (Fulton and Thoen, 2003). Briefly, 0.1ml of 2500 IU/ml avian PPD (Veterinary Laboratories Agency, Addlestone, Surrey KT15 3NB, U.K.) was injected intradermally on the right wattle and the site of injection was marked with ink. The test result was read and measured for the presence of indurations after 48 hours. A positive reaction is identified as a hot and oedematous swelling at the site of inoculation or by the presence of a small firm nodule of approximately 5 mm or above in diameter which is clearly observed by comparison with the control one (un inoculated) wattle (Tell *et al.,*2001; Dhama *et al.,*2008). Avian TB positive chickens were bought from the farmer, ante-mortem examined, live body weight measured and body condition scored according to Gregory and Robins (1998).

Post-mortem examination

Tuberculin test reactor chickens were slaughtered and thoroughly examined for the presence of tuberculous lesion in the visceral organs including liver, intestine, spleen, lung, bone, kidney skin and walls of body cavity. During post-mortem examination, gross lesions were sliced using separate sterile surgical blades and the lesion were described grossly on characteristic and distribution of the lesion. The suspected lesion were sampled in pair and kept in universal bottle containing phosphate buffered saline solution and stored at -20^{o}C deep freeze until transported to Aklilu Lema Institutes of Pathobiology (ALIPB) for cultural isolation of Mycobacterium.

Mycobacteriological culture

Tissue samples were transported in a cold chain using ice box packed with ice block to ALIPB laboratory. Isolation of mycobacteria from tissues was done in accordance with OIE (2004) protocols. Tissue samples were sectioned into pieces using sterile blades, and then homogenized by pestle and mortar. The homogenate was decontaminated by adding an equal volume of 4% NaOH followed by centrifugation at 3000 rpm for 15 minutes. The supernatant was discarded while the sediment was neutralized by 1% (0.1N) HCl using phenol red as an indicator. Neutralization was achieved when the color of the solution changed from purple to yellow. Thereafter, 0.1ml of suspension from each sample was spread onto a slope of LJ medium. Duplicates of LJ were used; one enriched with sodium pyruvate while the other was enriched with glycerol LJ media.

Cultures were incubated at 37^{o}C in a slant position for one week and in upright position for 16 weeks with weekly observation for mycobacterial growth. Whenever, colonies were seen, sub-culturing and Ziehl-Neelson staining were performed to confirm the presence of acid fast bacilli; further colonies were frozen and killed by incubating at 80^{o}C for 1 h for molecular typing.

Acid fast stain

In this study, Ziehl Neelsen staining was performed on the culture positive colonies to confirm the presence of acid-fast bacilli according to Quinn *et al*., (1994) and WHO, (1998).

Multiplex PCR of the isolated colony

A loop full of the AFB positive colony isolate was heat killed. The PCR targets the sequence of the genus *Mycobacterium,* within the 16S rRNA gene (G1,G2) sequences, within the hyper variable region of 16S rRNA, that is known to be specific to *M. intracellularae* (MYCINT-F) and *M. avium* (MYCAV-R), and the MTB70 gene specific for MTBC (TB-A, TB-1B).

The primers used were MYCGEN-F, 5'-AGA GTT TGA TCC TGG CTC GA 3'; (35 ng/µL), MYCGEN-R, 5'-TGC ACA CAG GCC ACA AGG GA 3', (35 ng/µL); MYCAV-R, 5'-ACC AGA AGA CAT GCG TCT TG 3' (35 ng/µL); MYCINT-F, 5'-CCT TTA GGC GCA TGT CTT TA 3' (75 ng/µL); TB1-F, 5'-GAA CAA TCC GGA GTT GAC AA 3' (20 ng/µL); TB1-R, 5'-AGC ACG CTG TCA ATC ATG TA 3' (20 ng/µL) (Parsons *et al.,* 2002) The reaction mixture in each PCR tube consisted of 5.2 µL H_2O Qiagen, 8 µL Hot Star Taq Master Mix, 0.3 µL of each of the six primers (concentration given above), and 5 µL of DNA templates of samples or controls, making the total volume 20 µL. The reaction was carried out using thermal cycler (Applied Bio-systems, Gene AMP 9700) and reaction mixture was heated for 10 min at 95°C, further 35 cycles of 1 min at 95°C 1 min at 61°C, and 1.5 min at 72°C; and 10 min at 72°C. *M. avium*, *M. intracellularae*, H37Rv and 2122/97 *M. avium* strain were used as positive controls, while H_2O Qiagen was as a negative control. The product was electrophoresed in 2% agarose gel in TAE running buffer 10X. The 100 bp DNA ladder, and orange 6X loading dye were used in gel electrophoresis. DNA bands were visualized by ethidium bromide staining and photographed. All members of the genus *Mycobacterium* produce a band of 1030 bp.

Questionnaire survey

A structured questionnaire survey was performed on all of the owners of the chicken. The survey was intended to

access the public perception about avian tuberculosis and associated risk factors. These individuals were first asked for their willingness to be interviewed and also consulted to sell their chicken if it was found to be positive on tuberculin test. It was also designed to characterize the overall production system in the area. The questionnaire form was first prepared in English and translated to local language for ease of interview.

Data Analysis

Descriptive statistics, chi-square ($\chi 2$), univariate and multivariate logistic regression were performed to analyze the data using Statistical Program of Social Sciences (SPSS) version 16.0 software packages (SPSS Inc, Chicago, IL, USA). For all analysis performed, 95% CI and *P*-value < 0.05 was set for statistical significance of an estimate.

Results

Prevalence of Avian Tuberculosis and Associated Risk Factors

The prevalence of avian TB at Shashemene District was 4.23% (95% CI: 1.7 to 6.6%) on the basis of the single intradermal tuberculin test. The prevalence of the disease varies with different risk factors. The male to female sex ratio of the adult chicken selected for this study was 1:3.48 (which means 22.3% male and 77.7% female. From 11 test positive chicken, 10 were females (90.9%). The diameter of swollen area on the injection site and the characteristics of typical reactions like oedematous swelling, redness and hot up on touching the wattle were observed on positive reactors (Figure 1).

The multivariable logistic regression analysis for the association between prevalence and various risk factors is presented in Table 1. The analysis showed that female chicken were found to be more prone to infection with avian TB as compared to male chicken (adjusted OR=2.93; 95% CI: 0.37-23.55). The association of other risk factors with the prevalence of avian TB was not statistically significant ($p > 0.05$).

Ante-mortem and Post-mortem Findings of the Test Positive Chicken

Ante-mortem examination was conducted on the 11 tuberculin test positive chicken for clinical signs of any disease. All of them did not have clinical sign of sickness. Their live body weight ranges from 0.92-1.45kg in which 8 chickens were poor body condition (Score 0), two chickens with medium body condition (Score 1) and one chicken with good body condition (Score 2). During detail examination of the organs from the 11 tuberculin test positive chicken, gross lesions were observed in liver, lung, spleen and intestine of 6 chickens (54.5 percent detection) and typical tuberculous lesions were seen on intestine (Figure 2). Grossly the lesions were characterized as small; multiple circular nodules with light-yellowish color appear up on dissection with no calcification seen in the nodules. Additionally, cestode parasite infestations were seen on two chickens on lung and liver.

The distribution of lesions in different tissue was scored with their respective frequency of occurrence. From 11 sacrificed and examined chickens, lesions were found at 4 anatomical sites: larger proportions (44.5%) of the lesions were observed in intestine, followed by lung (22.2%) and liver (22.2%) and spleen (11.1%). In some of the chickens that have manifested gross lesions, more than one type of organ was affected.

Isolation and Characterization Mycobacteria from Chicken Tissues

From the suspected TB lesion of six chickens, growth of Mycobacterium was observed on pyruvate enriched three LJ culture media after 3 weeks of incubation. The colony was characterized and found to be smooth, light yellowish and slightly raised colony. The origin of the three isolate was from the liver and intestinal tissue. The culture isolates were further identified by conducting acid-fast stain and multiplex PCR. The three isolates were confirmed to be acid-fast by Ziehl Neelsen staining. The multiplex PCR result showed that the three isolate belongs to the Genus *Mycobacterium* (See figure 3, lane 7-9).

Knowledge, Perception and Practice of the Chicken Owners

The poultry production system in the area was mainly scavenging backyard chicken production system. Chickens were basically kept for hatching, sale and home consumption. The present study revealed that women have considerable close contact with poultry more than their men counterparts. In all the 8 study sites it was found that mostly the women (75%) who own and manage the birds (Table 3). In this study, half of the chicken owners share their home to keep their chicken during the night time of which 57.2% were kept with other domestic animals. Most of the owners (98%) provided irregular grain supplement (Wheat, Maize and Sorghum) and water to their chickens. For those chickens that share the same house with human, the house was cleaned in a daily basis. The houses which were not cleaned regularly were belongs to male owner ($P < 0.05$). Though majority (74%) of the owner treat their chicken in case of sickness, 88% of their treatment was traditional

treatment. All of the chickens had exposure to wild birds particularly to Sparrows (Table 2). The prevalence distribution of avian TB was not significantly varies with host risk factors like chicken sex and chicken body condition (Table 2), however it was found to have significant association with feed supplement practice as wheat supplement significantly increased the prevalence of the disease ($p < 0.05$).

From total of 100 interviewed peoples, 82 of them know avian disease and they described the disease by local name called "Sombe" and mainly characterized the disease with symptoms like diarrhoea, fail to respire and eat, highly communicable and eventually death. Although 42% of the interviewed owners knew about zoonotic disease (Table 4), they mainly gave rabies and anthrax as examples. The perception of peoples about human TB was better as 89.5% of the respondents knew the disease by sign and its mode of transmission; out of which 26% reported the existence of TB patient in their family. Yet 8% and 13% of them heard about zoonotic TB and avian TB respectively, remarkably only 2% of them knows the zoonotic contribution of avian TB (Table 4). As shown in Table 3, the public awareness about avian TB varied with their education status where illiterate peoples did not know avian TB ($p < 0.05$). Multivariable logistic regression analysis for knowledge of the owners on avian TB showed that male owners had a better knowledge of avian TB compared to females (adjusted OR=2.01; 95% CI: 0. 35-11.26). Furthermore, human TB exposed owners had better knowledge of avian TB compared to TB unexposed owners (adjusted OR=3.92; 95% CI: 0.98-15.76).

From those 11 owners whom their chickens were tested positive for TB, only one of them knows about zoonotic TB. Most of the owners whom their chickens were tested positive were found to share their home with the chicken. There is a significant association between human TB exposure rate and perception of the people about the disease ($P < 0.05$). The questionnaire-based assessment for the occurrence of human TB case per interviewed family also showed significant variation with risk factors like house cleaning practice and educational status of the people ($P < 0.05$).

DISCUSSION

In the present study the prevalence of avian TB in Shashemene District was 4.23% (95% CI: 1.7 to 6.6%) and the result was in line with the finding of previous report by Tadesse *et al.* (2004) who reported 6.3% prevalence at Adama Town. This study is the first avian tuberculin skin test based survey on local chicken in Ethiopia. In the present study, the majority of the chickens infected were females. Similar to this finding, sex variation on the occurrence of avian TB was also recoded, with slightly higher occurrence in female adult chickens than male chicken (Tadesse *et al.,* 2004). The reason could be due to the fact that female chickens are allowed to live longer than their male counterparts as they are needed for long term for egg production. This gives the bacilli a better chance to establish over a long period of time and to be shed in the external environment. Thus, female older hens could act as a source of infection to other members in the flock. In this study, there was a significant association of avian TB prevalence with feeding practice (wheat supplement) which may be due to the increase of contact between chicken and wild birds while they compete for the feed dispersed outdoor as a supplement which attract the wild birds and increase faeco-oral transmission of the disease. Additionally the hands, feet, and clothing of attendants may play an important role in disease transmission (Dhama *et al.,* 2008).

The clinical examination and ante-mortem inspection of the test positive chickens revealed that most of them were having poor to medium body condition and lighter live weight at slaughter. Similarly Miguel (2012) described that one of the most commonly observed feature due to TB in bird is poor body condition with bird appearing thin and emaciated, especially in backyard domestic poultry. Concomitant to tuberculin test, post-mortem examination of gross lesions have been commonly used for diagnosis of avian TB (Tell *et al.,* 2001; Fulton and Thoen, 2003; OIE, 2010). The post-mortem detection rate of tuberculous lesion from the tuberculin test positive chicken was 54.6%. The gross lesions were not detected on the rest of the chicken (45.4%) and this may be associated with the possibility of missing the lesions of recent infection and existence of non-visible lesion which are common in mycobacterial infections (Gallagher, 1998).

The fact that the naturally infected chickens showed gross lesions mainly (44.5%) in the small intestine in this study suggests that the probable route of infection were oral. In some of the chickens that have manifested gross lesions however, different organ were affected including lung, liver, spleen and intestine. The varied distribution of lesions that were encountered in such naturally infected chickens may be due to simultaneous exposure by different route of infection. The overall characteristics of gross lesions and its distribution in different organs which were found in this study were in line with the previous findings (Tadesse *et al.,* 2004). The variation in size and number of lesions recorded in adult chickens could also be caused by successive episodes of reinfection from previously established lesions (Thoen, 1997). The cestode parasite infestation seen in this study was also in line with the previous study conducted in central Ethiopia, which showed a significant relationship of cestode

coinfection with *M. avium* (Tadesse *et al.,* 2003).

In mycobacteriological culture examination, three isolate identified showed characteristics of Mycobacterium as confirmed by colony morphology and acid-fast staining of the bacili. Typically species like *M. avium* produces "smooth" colonies which are virulent for chickens while variants with smooth domed or rough colonies are avirulent (Fulton and Thoen, 2003; Dhama *et al*., 2007). The three out of six (50%) recovery of culture growth for Mycobacterium could be the non-optimal condition of the culture for *M. avium* complex growth which assumed to be the major isolates causing pathology in chicken while this study used the available LJ media for culture of the isolates (Mamo *et al*., 2011). It could also be due to the absence of viable mycobacteria in necrotized TB lesion in which completely necrotic/calcified lesions, tubercle bacilli are dead and therefore no growth will be obtained up on culture on LJ media (Gracy, 1986; Quinn *et al*., 1994). As this study was a preliminary study in our laboratory to isolate mycobacteria from the chicken tissue, getting optimum culturing and isolation condition and other up-stream procedures like accuracy of collecting representative specimen also determine the degree of getting culture positive.

The multiplex PCR result indicated that all the culture and AFB positive isolates were confirmed to be within genus *Mycobacterium* showing signal for genus marker. However, further species differentiation showed no band signal on the gel. This could be related to the M*ycobacterium* isolated from the chicken might be different species of mycobacterium. In addition, the possibility of presence of primer competition in multiplex PCR method might also contribute for the lack of band signal for species differentiation. The overall study showed the presence of virulent avian TB causing *Mycobacterium* circulating in the local chicken raised in extensive production system in the Shashemene district, which warrants further investigation.

The questionnaire survey revealed that women had considerable close contact with poultry more than men counterparts. This is associated with the fact that the management of backyard chickens can easily be combined with other women's activities because of the proximity of the chickens to homesteads (Bradley, 1992). Chicken products are also among the few agricultural products directly accessible to women in rural areas (Kitalyi, 1998). In this study, specific poultry houses were rare. Most of the chicken owners share their home with chicken and other domestic animals. This finding agrees with the study of Tadesse *et al.* (2003). Such large association of chicken with other domestic animals and human beings is stressful for the chicken and also create conducive environment for transmission of mycobacterial infection from chicken to other species including human and vice versa.

Although most of the owners give irregular grain supplements to their chickens, they are still free to scavenge. Hence, there is a strong probability that these scavenging chickens may acquire the infection from the soil in a contaminated environment with the droppings of other infected chickens and other wild birds (Tell *et al.,* 2001). The wild birds such as sparrows, crows, and pigeons have been identified in this study to have contact with the chicken and these wild birds could be a potential source of infection. Previous studies in other countries have reported the role of these wild birds in spread of *M. avium* to poultry flocks (Dhama *et al.,* 2008).

In this study, there was a statistically significant association ($\chi 2 = 6.12$, P=0.013) between owner's sex and poultry house cleaning practice in which female owners clean better than male counterpart, this could be due to the difference in the gender emphasis in house cleaning and poultry raising habit. Moreover, the flock size, housing at night, poor nutrition, poor health care and management practices reported by the survey are in agreement with reports from Tanzania (Mwalusanya *et al.,* 2002) and Ethiopia (Ashenafi, 2000). Generally, the current chicken production patterns might create potential favourable conditions for survival, infection and transmission of endemic poultry diseases including avian TB which has a potential zoonotic risk.

Based on the interview result, most farmers described an avian disease with the local name called "Sombe" which is also called "Fengle" in most parts of the country to be the cause of major mortality and economic loss in the area. Similarly previous research have reported the so called "Fengel" as the most important cause of economic loss in poultry production in Ethiopia (Nasser *et al*., 2000). Although 42% of the interviewed owners know about zoonotic disease, they mainly give example rabies and anthrax and only 8% of them aware about zoonotic TB. Recently similar result was reported by Tesfaye *et al.* (2013) in which 13% knew about zoonotic TB. And yet in our study though 13% of them heard about avian TB, only 2% of them know its zoonotic contribution. As the awareness about the transmission of the disease is low, the unrevealed zoonotic partake of avian TB particularly for the risk groups is conceivable. In contrary, the perception of peoples about human TB is better as 82.5% of the respondents know the disease by sign and mode of transmission. Such awareness is created up-on hospitalization due to TB infection. Moreover, out of these respondents 26% reported to have a

previous history of TB patient in their family which indicates the existence of human TB infection in the area. Since the current routine human TB diagnosis was not based on culture isolation of the agent and molecular techniques, the underlying Mycobacterium species and the role of *M. avium* in human TB in the area is not yet investigated.

Conclusion

In Ethiopia, there are limited epidemiological information on avian TB in chicken and no information regarding the public health impact of the disease. In the present study, ante-mortem surveillance conducted using avian tuberculin test showed 4.24% apparent prevalence of avian TB. Post-mortem examination revealed varied distribution of tuberculous lesions in liver, lung, spleen and intestine that associated with simultaneous exposure by different route of infection. The underlying cause of avian TB identified were belongs to genus Mycobacterium. On top of that the questionnaire survey also showed the difference in the prevalence of avian tuberculosis with chicken feed supplement practices, wild bird interference and education status of the animal owners. There was also great discrepancy of public awareness about zoonotic diseases including zoonotic TB. Overall, the results from this study indicate the presence of avian TB in local chicken in the society who has low awareness about zoonotic TB reflecting the possibility of unrevealed disease risks. Hence, further epidemiological study, zoonotic impact of *Mycobacterium avium* complex on the risk groups like the HIV/AIDS and immunocompromised individuals should be investigated. Creation of public awareness, validation of tuberculin test for large-scale use and adoption of sound control option(s) is highly recommended.

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Figure 1. Typical tuberculin test reactors. The frontal and lateral view of reaction site on the wattle of the chicken (The right wattle of the chicken get swollen compared to un-injected left wattle).

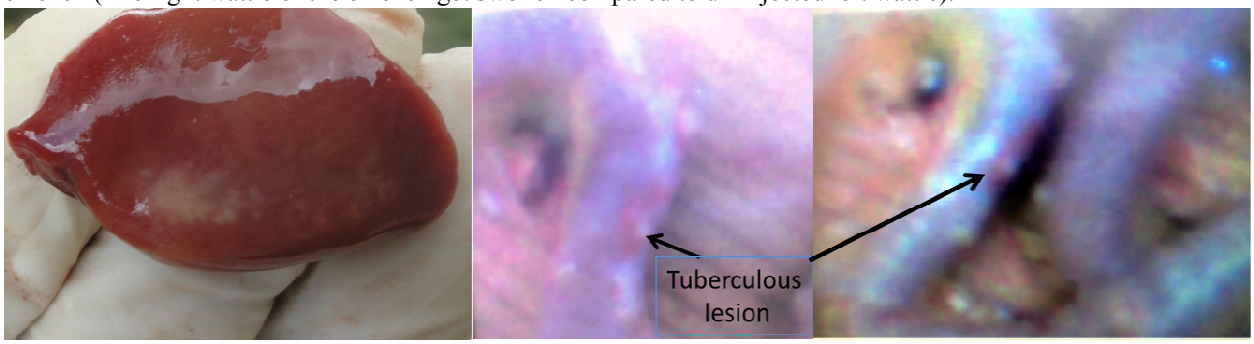

Figure 2. Gross tuberculous lesion on Liver (Left) and Intestine of the chicken (Right). The arrows indicate gross granulomatous lesion.

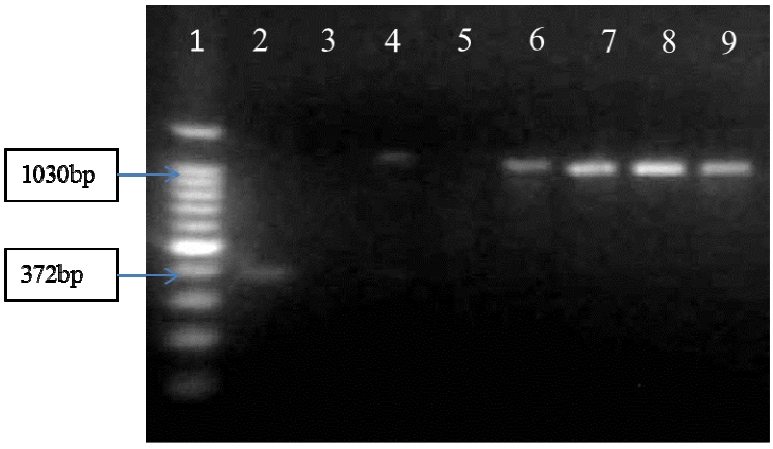

Figure 3. Electrophoretic separation of PCR products obtained by multiplex PCR of genomic DNAs of mycobacteria, isolated from tissue samples of positive reactor chickens. The DNA for each lane is as follows: lane 1, molecular-weight markers representing DNA fragments (Bio-Rad); lane 2, *M. tuberculosis*; lane 3, Blank (Negative Control); lane 4, *M. bovis*; lane 5, *M. intracellulare*,; lane 6, *M. avium* (Positive control); and lane 7-9, Samples.

Table 1. Result of the analysis of logistic regression of the effect of different risk factors on the prevalence of TB in chicken.

Risk factors (Variables)	Categories	Frequency	Positive for TB	Crude OR (95%CI)	Adjusted OR (95% CI)	P-value
Chicken sex	Male	58	1 (1.7%)	1	1	0.043*
	Female	202	10 (5%)	2.96(0.37-23.69)	2.93(0.37-23.55)	
Chicken body condition	Poor	196	8 (4.1%)	1	1	
	Medium	45	2 (4.4%)	1.09(0.22-5.33)	1.05(0.13-5.19)	0.24
	Good	19	1 (5.3%)	1.3(0.15-11.03)	1.17(0.14-10.03)	
Housing system	Separate	51	4 (7.8%)	1	1	
	Shared	49	7 (14.3%)	0.61(0.16-2.24)	1.49(0.14-16.10)	0.09
House cleaning	yes	98	11(11.2%)	1	-	
	No	2	0 (0%)	0.85(0.94-7.67)	-	
Wheat supplement	yes	98	11(11.2%)	-	-	
	No	2	0 (0%)	-	-	
Wild bird access	Yes	100	3(17.6%)	1	1	
	No	0	8 (9.6%)	0.49(0.11-2.11)	0.48(0.08-3.12)	0.11
Have cattle	No	23	3(13.04%)	1	1	
	Yes	77	810.39%)	0.77(0.19-3.19)	3.16(0.21-47.64	0.07
Have sheep	No	64	7(10.94%)	1	1	
	Yes	36	4(11.11%)	1.01(0.28-3.74)	0.67(0.14-3.09)	0.31

* indicate significance ($p < 0.05$)

Table 2. Risks factors associated with avian TB.

Risk factors	Category	Frequency	Test Positive (%)	X^2-value	P-value
Chicken Sex	Male	58	1 (1.7%)	1.15	0.235
	Female	202	10 (5%)		
Chicken body condition	Good	19	1 (5.3%)	0.068	0.968
	Medium	45	2 (4.4%)		
	Poor	196	8 (4.1%)		
Housing System	Separate	51	4 (7.8%)	1.06	0.25
	Shared	49	7(14.3%)		
House Cleaning	Cleaned	98	11 (11.2)	0.252	0.791
	Not cleaned	2	0 (0%)		
Wheat supplement	Supplements	98	11 (11.2)	0.252	0.041*
	No supplements	2	0 (0%)		
Health Care	Treated if Sick	74	7 (9.5%)	0.69	0.308
	Not treated	26	4 (15.4)		
Wild bird interference	Yes	100	11 (11%)	0.924	0.278
	No	0	0 (0%)		

* indicate significance ($p < 0.05$)

Table 3. Logistic regression analysis of knowledge of owners on avian TB

Factors (Variables)	Category	Frequency	Know avian TB	Crude OR (95% CI)	Adjusted OR (95% CI)	p-value
Owner Sex	Male	25	2(8%)	1	1	0.038*
	Female	75	11(14.67)	2.04(0.7-5.9)	2(0.35-11.26)	
Education status	Illiterates	22	0(0%)	-	-	< 0.001*
	Elementary	46	7(14.29%)	-	-	
	High school	27	4(14.81)	-	-	
	Higher edu.	2	2(100%)	-	-	
Know human TB	No	9	4(44.4%)	1	1	0.46
	Yes	78	7(9%)	1.5(0.5-5.01)	0.12(0.03-0.57)	
TB transmission	Not know	13	2(15.38%)	1	1	
	Know	87	11(12.64%)	3.3(0.9-11.4)	0.79(0.15-4.08)	0. 097

* indicate significant (P < 0.05)

Table 4. Factors determining the public understanding about avian TB

Risk factors	Category	Frequency	Know avian TB	χ2-value	P-value
Owner sex	Male	25	2(8%)	0.768	0.391
	Female	75	11(14.67)		
Education status	Illiterate	22	0(0%)	16.822	0.001*
	Elementary	46	7(14.29%)		
	High school	27	4(14.81)		
	Higher Edu.	2	2(100%)		
Know human TB	Yes	9	4(44.4%)	9.19	0.002*
	No	78	7(9%)		
Know TB Transmission	Not know	13	2(15.38%)	0.075	0.784
	Know	87	11(12.64%)		
Zoonotic disease	Not know	58	9(15.52%)	0.773	0.379
	Know	42	4(9.52%)		
Avian TB zoonotic	Not know	98	11(11.2%)	13.68	0.000*
	Know	2	2(100%)		
Human TB	Exposed	74	7(9.46%)	3.154	0.076
	No Exposed	26	6(23.08%)		

* indicate significant (P < 0.05)