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Prevalence of Avian Tuberculosis in Domestic Chickens in Selected Sites of Ethiopia

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Abstract

This study was conducted on 282 domestic chickens of Bahir Dar, Yilimana densa woreda, and Bishoftu by using avian tuberculosis diagnosis procedures such as single intradermal avian tuberculin test, post mortem examination, and Ziehl-Neelsen (Z-N) staining from tissue samples of naturally infected Domestic chickens (*Gallus domesticus*). The overall of this disease current prevalence was determined based on tuberculin skin test results supported by Z-N stain and post mortem lesions and it was 4.26% (12/282) (95% CI: 1.9-6.6), with higher prevalence in semi-intensively reared exotic chickens (5.85%) than backyard reared indigenous local chicken. This indicates the occurrence of this disease has a statistical significance association both with the breed and production system, both having a p-value of 0.03. Twelve strong positive reactor chickens have shown a swelling of greater than 5 mm in diameter 48 h. of post injection with different variety results, some with edematous swelling, and others with firm erythematic nodular swelling. Typical tuberculous lesions were seen mostly on the liver and spleen. From 12 tuberculi reactor slaughtered chickens, a total of seven (58.3%) chickens had gross lesions on different visceral organs, of which three (42.9%) examined chickens have manifested gross lesions on more than one organ. On acid fast staining five (41.67%) with grossly discernible lesions revealed acid-fast rods. For culturing, Lowenstein-Jensen (L-J) media was used as it yields more positive cultures, greater numbers of colonies on positive tubes, and shorter incubation times. None of the samples shown colonial growth till 8 weeks of incubation period and this may be due to the slow growth nature of mycobacterial species (especially when the infection was due to *Mycobacterium avium* subspecies *paratuberculosis* which needs a considerable long time for growth) and/or the current incubation temperature (37°C) may not be optimal as it does not fit the natural hosts internal body temperature and still the culture result is under process to see any growing colony. Therefore, this finding signaling an urgent need for intervention program to control the disease in domestic chickens and prevent zoonotic transmission.

Keywords: Avian tuberculosis; *Mycobacterium avium* complex; Prevalence; Avian tuberculin skin test; Domestic Chicken

Introduction

The total chicken population in Ethiopia were estimated to be 42 million and with regard to breed, 40.63 million (96.61%), 231,478 (0.55%) and 1.19 million (2.84%) of the total chicken were indigenous, hybrid and exotic, respectively [1]. From the total chicken population of Ethiopia, 99% are raised under the traditional backyard/scavenging system of management, while 1% is reared under intensive management system [2]. Recently, the increased demand for chicken meat and egg consumption in the country level resulting an increase in the number of chickens reared under intensive and semi-intensive management system. The traditional backyard poultry management system is characterized by minimal human care, with birds scavenging in the backyard for food, with poor management and nutrition. The impact of domestic chicken in the national economy of developing countries and its role in improving the nutritional status, income, food security and livelihood of many small holders is significant. Several factors have been suggested for high mortality and low production characteristics of domestic chicken. In Ethiopia, poultry diseases are considered the most important factor responsible for reducing both the number and productivity of chickens. Avian tuberculosis is one of the most important diseases that affect domestic and pet birds [3]. Several *Mycobacterium* spp. can be involved in the etiology of avian tuberculosis [4]. Species of Mycobacteria other than *M. bovis, M. leprae* and *M. tuberculosis* are often referred to as "atypical mycobacteria". The most commonly encountered pathogens among the atypical mycobacteria are species of the *M. avium* complex (MAC). *M. avium* complex consists of two species: *M. avium* and *M. intracellulare*; because these species are difficult to differentiate, they are also collectively referred to as *M. avium-intracellulare* (MAI) [5]. There are over 20 recognized serotypes within the *M. avium* complex. According to the current taxonomy, *M. avium* species contains four subspecies; *M. avium* subspecies *avium* (*M. avium avium)* of serotypes 1, 2, 3, and 6, *M. avium hominissuis* of serotypes 4 to 6, 8 to 11 and 21, *M. avium paratuberculosis*, and *M. avium silvaticum*. *M. avium avium* (often simply called *M. avium*) belonging to serotypes 1, 2, 3, and 6 are the principal cause of avian tuberculosis in wild, domestic and captive birds [6,7]. In addition to *M. avium*, other mycobacteria including *M. genavense*, *M. intracellulare, M. scrofulaceum, M. fortuitum, M. tuberculosis,* and *M. bovis* can also cause avian tuberculosis, but the incidences are rare [4,7].

M. avium, the major cause of avian tuberculosis, considered as "atypical mycobacteria", comprises aerobic, non-spore forming, and non-motile rod shaped bacteria that vary in length from 1-3 μm [6]. They are weakly Gram-Positive and stained specifically by acid fast staining method, due to its high levels of lipids in Mycobacterial cell wall. *M. avium* is highly resistant to environmental challenges and can

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survive in soil up to 4 years, and this makes eradication of the organism difficult [7,8].

The most common route of infection for susceptible birds is the alimentary tract [6,7]. The disease is transmitted to the susceptibles bird by ingestion and inhalation of aerosolized infectious organisms. Infected birds, as they shed large amounts of organism into the environment and contaminated water and soil as the Mycobacteria can survive for several times in the environment, are the main source of infection [9]. The bacilli are exuded from ulcerated lesions of the intestine and are voided in droppings. The disease is more prevalent in places with high population density, poor sanitation, and unhygienic conditions [10]. The disease in the affected individual results unthriftiness, atrophy of the breast muscle, decreased egg production, and increased mortality, which culminates into severe economical losses.

Mycobacterium avium complex is the most commonly associated with human disease. MAC is primarily a pulmonary pathogen that affects individuals who are immune compromised (e.g., from AIDS, hairy cell leukemia, immunosuppressive chemotherapy). In humans, *M. avium* is capable of inducing a progressive and disseminated type of disease that is relatively refractory to treatment both in HIV/AIDS patients [11] and in normal hosts [12]. *M. avium* is the isolate in more than 95% of patients with AIDS who develop *M. avium* complex infections (MAC). MAC lung disease occurs rarely in immunocompetent hosts. *M. intracellulare* is responsible for 40% of such infections in immunocompetent patients [5].

Diagnosis of avian tuberculosis in chickens depends on detection of specific immunological response, in live birds [13] or isolation of *M. avium* by culturing tissue samples of killed birds. The most widely used test in live domestic fowl, and the only test for which an international standard for the reagent exists, is the tuberculin test [4]. This test is used to determine whether an individual has been infected in the past with the causative agent of avian tuberculosis or not. The test result is determined based on Office International Des Epizooties (OIE) 2008 guideline and accordingly this, a positive test reaction is any swelling at the injected site, from a small firm nodule approximately 5 mm in diameter to gross oedema extending to the other wattle and down to the neck. No clinical signs will be provoked in uninfected birds [4].

When clinical signs of the disease are seen in a flock or 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 [13]. 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 (L-J), Stonebrink, and Middlebrook agar must be attempted [14,15]. L-J yields more positive cultures and greater numbers of colonies on positive tubes, and incubation times is shorter, should be incubated for at least 8 weeks [4].

Despite the fact that traditionally reared indigenous local chicken account for a greater proportion of chicken population in Ethiopia, some research has been carried out on them and no organized research at all has been conducted yet on semi-intensively reared commercial chicken on the prevalence of avian tuberculosis. In Ethiopia, avian tuberculosis has been reported previously in indigenous local chickens of Adama, Sebeta, and Debre Birhan with a prevalence of 6.3% [16] and recently a prevalence of 4.23% of avian tuberculosis was reported in indigenous local chickens of Shashemene district [17]. However, there is no information about the epidemiology and significance risk factors for avian tuberculosis in north-western part of Ethiopia (particularly around Bahir Dar area) and in commercial poultry farms of Bishoftu. Therefore, the Objectives of this study were:

- To investigate the prevalence of avian tuberculosis using single intradermal avian tuberculin test on domestic chickens in three selected sites of Ethiopia.
- To identify the possible etiological agents responsible for avian tuberculosis in those avian tuberculin reactor chicken.

Materials and Methods

Geographic description of the study area

This study was conducted in three selected sites of Ethiopia, in Amhara Region (around Bahir dar area and in Yilmana densa Woreda) and in Oromia Region (in Bishoftu town). Bahir dar is located in north-western part of Ethiopia, 487 Kms from Addis Ababa. Bahir dar has a latitude of 11°36'N and longitude of 37°23'E, and has an elevation of 1,830 meters above sea level. Total annual rainfall ranges from about 1100-1530 mm/year. The mean monthly temperature of the area is about 19°C, with monthly mean maximum temperature of 27.3°C and monthly mean minimum temperature of 11.5°C [18].

The second district is Yilmana densa Woreda of Amhara Region and it's Center (Adet) has a latitude of 11°17'N and longitude of 37°43'E and has a distance of about 445 Kms from north of Addis Ababa and 42 Kms south of Bahir dar. It has M-25, Tepid moist cool mountains and plateau agro-ecology. Yilmana densa Woreda has an altitude which ranges from 1500 to greater than 3000 m.a.sl. It have only one rainy season, which extends from mid-may to October and all the remaining months (November to mid-may) are categorized as dry season and have estimated average annual minimum and maximum air temperatures of 9.27°C and 25.74°C, and 860.2 mm and 1770.5 mm rainfall, respectively [19].

The third district is Bishoftu which has a latitude of 8°45'N and longitude of 38°59'E, about 47 Kms southeast of Addis Ababa at an altitude of 1850 meters above sea level. The average annual temperature in Bishoftu is 18.7°C with average annual minimum and maximum air temperatures of 14°C and 26°C and have average annual rainfall is 866 mm. The driest month is December with 5 mm. Most precipitation falls in July, with an average of 232 mm [20].

Animal study procedure

Study animal: The poultry management pattern in Bahir Dar and Yilmana densa Woreda from which some part of this study has been conducted were entirely free-ranging traditional backyard production system of the local indigenous chickens. Generally, this traditional poultry production system is characterized by minimal human care, with birds scavenging in the backyard for food, a few handfuls of local grain, and possibly simple night shades, but no veterinary medical attention. Whereas the poultry production system in Bishoftu from which some part of this study has been conducted were semi-intensively reared commercial chickens and all flocks live together in a small, crowded and unhygienic area and fed grains by the owners. A total of 292 chicken from both sex and above the age of 20 weeks were selected for single intradermal avian tuberculin test. In Bishoftu, at least 50 chickens per farm were selected from four commercial layers farms while in backyard indigenous local chickens 25% of the flock were selected depending on the population in each flock. Age of the chicken was determined by using information from the owners.

Study design and sample size determination: To determine the sample size, an expected prevalence of 50% was taken since there is

no previous organized research conducted on the prevalence of avian tuberculosis on commercial chickens and indigenous local chickens of these selected sites. The desired sample size (N) for this study was calculated using the formula given by Thrusfield [21];

$$N = \frac{1.96^2 P_{exp}(1 - P_{exp})}{d^2}$$

Where, N=required sample size, Pexp=expected prevalence, d=desired absolute precision. Using this formula, the estimated sample size was 384 chickens. But due to the poor availability of the reagent (avian PPD), a total of 292 chickens were sampled despite of their sex, age, and health status to avian tuberculosis to find the present prevalence of avian tuberculosis. To come up with this, a cross-sectional study was conducted and then chickens have been selected by using simple random sampling method.

Avian tuberculin skin test: Before undergoing any examination procedures, each chicken were given an identification number (ID No) using ink on wattle/comb and/or legs. A total of 292 apparently healthy chickens were assessed for avian tuberculosis by using single intradermal avian tuberculin test, the most widely used test in domestic fowl. It is recommended that avian tuberculin should contain the equivalent of at least 25,000 IU/mL, giving a dose for practical use of 2500 IU/0.1 mL [4]. In this test, 0.1 mL of avian tuberculin, PPD (AVITUBAL-25000 IU/ml, Czech Republic) extracted from the *M. avium* (strain D.4 ER) were injected into the wattle using 1 mL of insulin needle intradermally on to a randomly selected 52 indigenous local chickens in Bahir Dar town and its surrounding sites, 31 indigenous breed chickens in Yilmana densa Woreda, and 209 exotic chickens in Bishoftu. The study in Bishoftu was conducted on randomly selected Bovans brown chickens from four semi-intensive commercial farms, Farm 1 (n=53), Farm 2 (n=53), Farm 3 (n=52), and Farm 4 (n=51).

The tuberculin skin test is based on a delayed hypersensitivity reaction. This test is used to determine whether an individual has been infected in the past with the causative agent of tuberculosis or not. A previously infected individual would harbor reactive T-cells in their blood. Avian tuberculin skin test results were read after 48 h. of post injection. The test result was determined based on OIE 2008 guideline and accordingly this, a positive test reaction is any swelling at the site, from a small firm nodule approximately 5 mm in diameter to gross oedema extending into the other wattle and down the neck. No clinical signs will be provoked or the test antigen does not provoke any response at the injection site in uninfected birds and the test was considered as negative for avian tuberculosis [4].

Postmortem gross pathological examinations: For postmortem examination, 12 strong avian tuberculin reactor chickens were purchased and then slaughtered in the poultry post mortem class at College of Veterinary Medicine and Agriculture (CVMA) of Addis Ababa university. At necropsy, all internal organs were examined, any observed gross lesions were recorded, tissues from any organs showing pathologic gross tuberculous lesions were collected; however, tissues from liver, spleen, and intestine at different segments were sampled regardless of the presence or absence of lesions since they can be highly affected by the MAC group. Tuberculosis suspected tissue samples that were collected from different organs by using sterile universal bottles each containing normal saline solution (0.9%) were labeled and kept in a deep freeze (-20°C) in the microbiology laboratory at the College of Veterinary Medicine and Agriculture, Bishoftu until being transported to Aklilu Lemma Institute of Pathobiology (ALIPB-AAU) tuberculosis research laboratory, Addis Ababa, for culturing. During transportation, these tissue samples were transported in cold chain using icebox packed with ice packs to keep low temperature.

Mycobacteriologic culture and ziehl-neelsen staining: At ALIPB Tuberculosis laboratory, two slant types of Lowenstein-Jensen (L-J) media were prepared; one was enriched with 1% sodium pyruvate and the other was enriched with glycerol. Then tissue samples were macerated in sterile Petridish to get fine pieces by using sterile scissor and forceps, and then each sample was homogenized using sterile mortar and pestle for 10 min in 5 mL of normal saline solution. Seven mL of homogenate from each sample was transferred to centrifuge tube and decontaminated by adding an equal volume (7 mL) of 4% NaOH by centrifugation at 3,000 rpm at 4°C for 15 min. After centrifugation, the supernatant was discarded, while the sediment was neutralized with 10% HCl with phenol red as an indicator. Neutralization was achieved when the color of the solution changed from purple to yellow. Thereafter, 0.1 mL of suspension (one to three drop) from each sample was spread on to two slant types of Lowenstein-Jensen (L-J) medium. Cultures were then incubated aerobically at 37°C for up to 8 weeks with weekly observation for growth of colonies.

On the meantime of tissue processing for culturing on L-J media, the remaining sediment portion of the homogenated tissue were smeared and stained using the Ziehl- Neelsen staining technique for direct microscopic examination. Smears were heat-fixed, flooded with concentrated carbon fuchsin, heated gently to steaming for 5 minutes. Then the stain was poured off and the smears were washed with tap water and decolorized with 3% HCl in 95% alcohol for 1 min., with slides being washed under running tap water between each step. The smears were then counter stained with methylene blue for 1 min., air-dried and examined under the 100x oil immersion object of a light microscope for the presence of Acid Fast Bacilli (AFB).

Statistical analysis

The data collected were analyzed using STATA version 11.0 for Windows (Stata Corp. College Station, TX, USA). The prevalence of avian tuberculosis is defined as the number of avian tuberculin skin test reactor chickens from the total number of avian tuberculin injected chicken results expressed in percent. The variation of prevalence in relation to different risk factors was analyzed using chi-square (χ^2) statistical test. Both Postmortem examinations and Ziehl-Neelsen staining of samples which was taken from characteristic post mortem lesions were used to support the prevalence rate. District, altitude, sex, age, breed, Body condition, purpose of keeping, and production system dependent prevalence were compared and analyzed by the χ^2 statistical test. In the analyses, the confidence interval was 95% and $p<0.05$ was set for significance.

Results

Avian tuberculin skin test results

From the total of 292 avian tuberculin injected chickens, 282 chickens were followed up for the test result on the second and third day of post injection while the rest 10 chickens were not seen for the test result by different reasons such as death, sell, get away from the nearby house. Of the 282 observed chickens for the test result, 12 chickens were positive, reactors to avian tuberculin skin test, having a swelling greater than 5 mm in diameter with different variety results, some with edematous swelling, the others with firm erythematic nodular swelling and the prevalence was, therefore, 4.26% (95% CI: 1.9-6.6). Individual reactor chicken observation shown that swelling seen starting 24 h. post- PPD injection increase in size on the second and third day of post

injection (as indicated in the Figures 1 and 2). A total of 29 (10.28%) avian tuberculin injected chickens have shown bluish discoloration around the injection site without any visible swelling resulting doubtful interpretation which still needs further investigation (Figure 3).

Assocation of the risk factors with avian tuberculin positivity showed that exotic breed of chicken had higher rate of avian tuberculin positivity than local breed and the difference was statistically significant (χ^2 =4.7076, Pr =0.03). Similarly, chicken managed under intentsive system had higher tuberculin positivity rate than those in backyard traditional system of production and the difference was ststaistically significant (χ^2=4.7076, Pr=0.03) (Table 1). All the other risk factors considered showed no statistically significant difference among the groups compared in each category with respect to their positivity to tuberculin test (Table 2).

Gross pathological findings

From 12 tuberculin positive slaughtered chickens, a total of seven (58.3%) chickens had gross lesions on different visceral organs (liver, intestine, proventriculus and uterus) (Figures 4 and 5), of which three (42.9%) examined chickens have manifested gross lesions on more than one organ. A total of five (41.67%) of 12 avian tuberculin reactor chickens haven't shown any visible lesion on the visceral organs. The typical tuberculous lesions were grayish-yellow to grayish-white, pin-point to irregularly round, and few to innumerable small nodules and pin-point whitish nodules were observed in liver (Figure 4) and uterus showed extensive disminated lesions with nodular lesions. Calcification was not seen in the nodules. Organs such as the spleen and liver were enlarged; especially spleen was enlarged to about twice of the normal size. Adhesion of the intestinal tract to one unit, and uterus with other reproductive organs were encountered. On Ziehl-Neelsen staining from the total of 12 slaughtered chickens, five (41.67%) with grossly discernible lesions revealed acid-fast rods. From seven chickens with grossly discernible lesions, two of them (28.57%) were negative for acid fast staining.

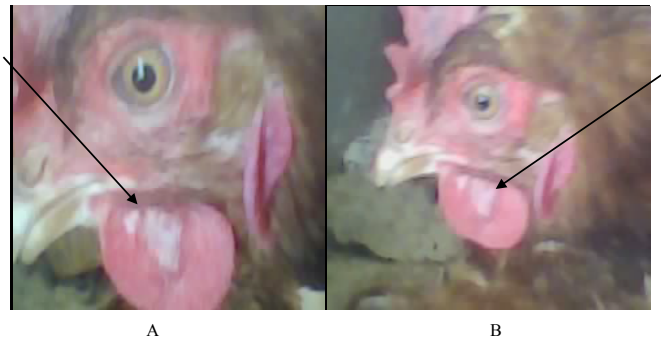

Figure 1: Edematous swelling observed at the left wattle at (A) 48 h and (B) 64 h of post injection of avian tuberculin.

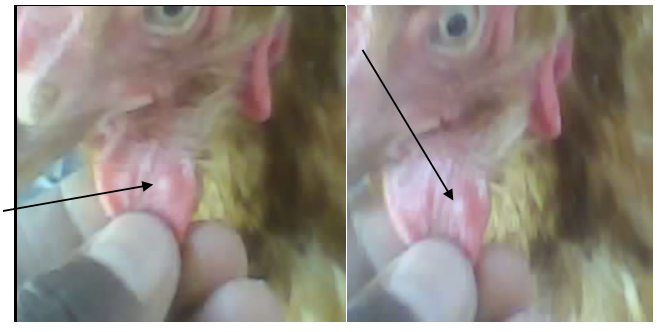

Figure 2: Firm erythematic nodular swelling observed at 48 h of post injection of avian tuberculin.

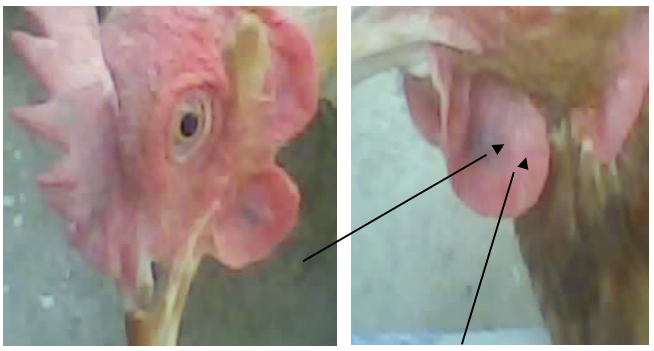

Figure 3: Bluish discoloration after injection of tuberculin shown by the tip of the arrow.

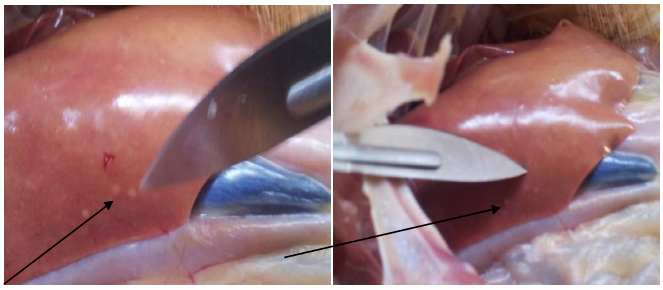

Figure 4: Small whitish nodular tuberculous lesions on the liver of chickens during post mortem examination (indicated by the tip of the arrow).

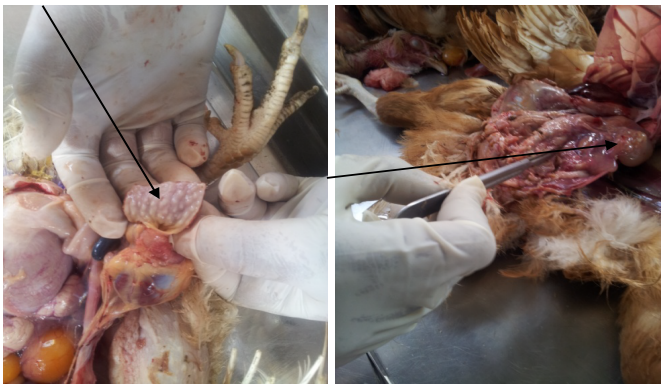

Figure 5: Gross pathological lesions on the (A) proventriculus and (B) uterus.

Mycobacteriologic culture results

From the total of 34 cultured samples (17 on sodium pyruvate enriched L-J slants and the other 17 on glycerol enriched L-J slants) those taken from different organs of 12 slaughtered tuberculin reactor chickens, none of them have shown colonial growth up to 8 weeks of incubation period with weekly observation basis.

Discussion

The present study revealed that the overall prevalence of avian tuberculosis was 4.26% (95% CI: 1.9-6.6) in domestic chickens in

Variables	Categories	No. of examined chickens (%)	No. of tuberculin skin test positive (%)	Chi-square (χ^2)	p-value (Pr)
District	Bahirdar	48(17.0)	0	4.7076	0.095
	Yilmana Densa	29(10.3)	0		
	Debre zeit	205(72.7)	12(5.85)		
Altitude	mid land	253(89.7)	12(4.74)	1.4366	0.231
	high land	29(10.3)	0		
Sex	male	18(6.4)	0	0.8545	0.355
	female	264(93.6)	12(4.54)		
Age	young	89(31.6)	2(2.24)	1.2866	0.257
	adult	193(68.4)	10(5.18)		
Breed	local	77(27.3)	0	4.7076	0.030*
	exotic	205(72.7)	12(5.85)		
Body condition Production Purpose	Poor good layer	167(59.2) 115(40.8) 265(94.0)	12(4.53) 7(4.19) 5(4.34)	0.8040 0.0040	0.370 0.949
	broiler	17(6.0)	0		
Production system	extensive	77(27.3)	0	4.7076	0.030*
	intensive	205(72.7)	12(5.85)		

*Altitude: high altitude >2500 meters & mid altitude >=1500-2500 meter above sea level.

Table 1: Association of risk factors to avian tuberculin skin test results.

Chicken ID NO-	Goss pathological lesions observed at different organs				Z-N stain result
	Liver	Spleen	GIT	Others	
B_1	✓ Y	✓ Y	Y(Pro)	n	Positive
B_2	✓ Y	✓ Y	n	n	Negative
B_3	✓ Y	n	n	n	Positive
B_4	n	n	n	n	Negative
MU_1	✓ Y	✓ Y	n	n	Positive
MU_2	n	n	Y(SI)	Y(UA)	Positive
MU_3	n	Y(Sp)	Y(Pro)	n	Positive
ME_1	n	n	n	n	Negative
ME_2	n	n	n	n	Negative
ME_3	n	n	n	n	Negative
ME_4	n	n	Y(AIT)	n	Negative
ME_5	n	n	n	n	Negative

Key of the table: Y=Small pin-point nodular lesions or other pathological lesions, n=no lesions observed by visualization, Pro=proventriculus, SI=Small Intestine, UA=Uterus Adhered, Sp=Splenomegaly, AIT=Adhesion of Intestinal Tract.

Table 2: Post-mortem findings and acid fast staining results.

selected sites of Ethiopia. This study pointed out the presence of avian tuberculosis in semi-intensive reared exotic chickens in bishoftu (5.85%) for the first time by using single intradermal avian tuberculin test. The study also shown that the disease is not much important in indigenous local chickens at Bahir dar area and Yilimana densa woreda that are kept in backyard reared scavenging production system even if the sampled size were small. With respect to the overall prevalence of this disease occurrence, it was agreed with the previous study in other part of central Ethiopia [16] (6.3%); [17] (4.23%) and comparable with the findings of greater prevalence in the mid-lands than high-lands.

The study indicated that semi-intensively reared chickens are constantly exposed to overcrowding, high contact rate with them selves and to the small space of the floor within unhygienic housing system and that may serve as sources of infection. Overcrowding within a flock brings in stress, which in turn could affect the nature and number of lesions occurring [22]. So in this study, the main risk factors for avian tuberculosis occurrence and transmission are breed and production system. This indicates the occurrence of this disease has a statistical significance association with both the breed and production system, both having a p-value of 0.03. Bovans brown chickens that reared in semi-intensive production system have a higher prevalence of the disease than indigenous local chickens which is reared in backyard system. In semi-intensive commercial chicken production system large number of chickens were kept in one small house as a result they share the same floor (mostly ground soil) for standing and constant movement, and use the same feed and water trough, and share the same atmosphere. That's the risk of exposure of other healthy chickens from one infected chicken is greater in semi-intensive production system than extensive ones.

Sex, purpose of keeping, and age variation on the occurrence of avian tuberculosis was recoded; but all of them had not a statistical significant association. High prevalence of the diseases was shown in Bovans brown adult female chickens that are kept for layer purpose than any other classes. Female chickens are allowed to live longer time than their male counterparts (broiler purpose) because of keeping them for need of egg production and this gives a better chance for bacilli to establish infection over a long period of time and to be shed in the external environment from which other chickens can easily get the bacilli. Thus, older chickens by contaminated the environment could act as a source of infection to other members in the flock especially in unhygienic semi-intensive production system where there is greater number of chickens per unit area than that of extensive production system.

Moreover, the study also attempted to further isolate the possible etiologic agents of avian tuberculosis using culture, postmortem examinations, and Ziehl-Neelsen staining in addition to the prevalence finding by single intradermal avian tuberculin skin test. The variation of swelling with regard to its size and/or time of occurrence after injection of avian tuberculin is due to the variation of individual chickens specific immune response ability to the injected PPD. In the present study granulomatous lesions were found on the liver, spleen, gastrointestinal tract and also in the uterus. Some of the chickens that were positive for avian tuberculin skin test during ante mortem examination don't show any observable lesion at time of post mortem examination and this may be due to the false positivity of the tuberculin skin test or missing of the lesions because it may not still well develop. Chickens that have negative avian tuberculin skin test do not necessarily mean it's free of tuberculosis infection because the test may have false negativity. Because of the false positivity or negativity of the test, the prevalence of this disease may be recorded as little below or above than the exact figure of prevalence within the population.

The reason we obtained no colonial growth up to eight weeks of incubation time may due to culturing still requires longer duration of incubation time as *Mycobacterium* growths slowly and/or the current incubation temperature(37°C) may not be optimal and may cause retardation of growth since it does not much the hosts(chickens) internal body temperature at which the *M. avium* complex growths bestly. This does not necessarily mean cultured sample results are negative on L-J media as mycobacterial colony growth may not shown up to 16 weeks, especially when the infection was due to *M. avium* subspecies *paratuberculosis* which needs a considerable longer time for growth [4].

Conclusions and Recommendations

This study showed an overall avian tuberculosis prevalence of 4.26% in domestic chickens in three selected sites of Ethiopia using avian tuberculin skin test, the most widely used test in domestic fowl [4] with a higher prevalence in semi-intensive reared commercial exotic chickens (5.85%) in bishoftu (the major sources of chickens meat, egg for Addis ababa) for the first time. Some studies [11,12] indicated that there is a zoonotic transmission of *M. avium* complex to human populations, signaling an urgent need for intervention program to control this disease. Based on the above conclusion, the following recommendations were forwarded:

- The sensitivity and specificity of avian tuberculin skin test on local indigenous chickens of Ethiopia should be investigated to validate and verify its use for large scale diagnosis of avian tuberculosis in the country.
- It is suggested that government and policy makers should work together with poultry farm owners and Veterinarians to design methods for the control of avian tuberculosis in semi-intensive commercial farms.
- During chicken flock replacement, the original sites should be thoroughly disinfected and repopulated chickens should not contact with other farm chickens.
- As treatment of infected chicken is not recommended because of the high cost and prolonged time of treatment, control and prevention of the disease before entry is the best option suggested to saving economic losses due to this disease and to prevent Zoonotic transmission.
- Cultural taboos like giving of raw liver of chicken before cooking (immediately after slaughtering) to children for feeding purpose should be prevented.

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