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Seroprevalencia del tifus aviar en áreas seleccionadas al este de Shewa, Ethiopia

Seroprevalence of fowl typhoid in selected sites of east shewa, Ethiopia

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RESUMEN

Se realizó el estudio de la seroprevalencia del tifus aviar mediante un test de aglutinación en áreas seleccionadas al este de Shewa específicamente en Debre Zeit distrito de Ziway, en el período comprendido de Noviembre de 2006 a Mayo de 2007.En total se estudiaron 8l4 pollos tipo comercial y locales(criollos) criados en patios. Los resultados ofrecieron una seroprevalencia global de 35.9 % de la enfermedad. Una prevalencia altamente significativa se registró en los pollos de patio o criollos (44.6 %) comparada con el tipo comercial (26.6 %). Respecto a los grupos de edades, la seroprevalencia fue alta en los grupos mayores de 26 semanas de edad con (42 %). La prevalencia resultó similar en ambos grupos mayores de 26 semanas de edad. Los pollos menores de 26 semanas, incluidas las hembras (pollitas) con una prevalencia de (11.6 %).Por lo tanto, este estudio preliminar revela que el tifus aviar es una de las enfermedades ampliamente diseminadas, y ello indica la necesidad de profundos estudios e implementación de medidas de prevención y control.

Palabras claves: edad, pollos, tifus aviar, prevalencia, suero. aglutinación test.

ABSTRACT

Seroprevalence study of fowl typhoid using serum slide agglutination test was conducted from November 2006 to May 2007 in selected sites of East Shewa namely Debre zeit and Ziway District on a total of 814 chickens of commercial and local (backyard) types. The result indicated an overall seroprevalence of 35.9% of the disease. Significantly higher prevalence was recorded in backyard chickens (44.6%) as compared to commercial poultry (26.6%). In addition seroprevalence of the disease was higher in age groups greater than 26 weeks of age with the rate of (42%) and layers with similar prevalence with the age groups greater than 26 weeks of age than those chickens of age less than 26 weeks of age and pullets with the prevalence of (11.6%). Therefore, this preliminary study revealed fowl typhoid was one of the wide spread poultry disease that indicated the need of further study and implementation of preventive and control measures.

Key words: age, chicken, fowl typhoid, prevalence, serum slide agglutination test

INTRODUCTON

Ethiopian economy heavily depends on the earning from agriculture, which take paramount contribution that raising of livestock comprising cattle, donkeys, horses, camels, and chickens etc. Poultry production in Ethiopia is characterized by small scavenging flocks of local chickens kept by individual households and it is concentrated in the temperate zone of the country and recently commercial flocks have emerged in urban and peri-urban areas in central parts of the country (FAO/World Bank, 1993).

As poultry provides relatively cheap animal protein to the human population, efforts to promote this sector of agriculture have received attention in most countries. In Ethiopia, in order to improve the genetic makeup of indigenous breeds, upgrading works have been undertaken since 1950. Modern poultry production (commercial production) began most probably from 1960-1970 (Alemu, 1985 and Habte, 1987).

There are three types of poultry production systems in Ethiopia, namely large scale, commercial and traditional (Backyards) production systems. During the previous years a number of large commercial state poultry farms have started to operate in the rural and urban areas using exotic mainly layer breeds, which yield on average 280 eggs per year. These days, the existing commercial state poultry farms are privately owned and a number

of small-scale poultry farms are mushrooming especially in and around Debre zeit, Addis Ababa and Adama. The small- scale commercial systems deals with broilers with a capacity of 100 to 1000 per farm (Alemu, 1995).

The traditional (backyards) chickens survive on scavenged feed where on average households has 6-10 birds producing 55-80 eggs per year with an average egg weight of 45 grams are kept. Mature body weight ranges from 1.0kg for female chickens to 1.5kg for male. The equipments like drinkers and feeders are usually handmade and traditional in most cases. Besides, extensive service designed to supply farmers with high- grade exotic poultry breeds have been started for the rural farmers as part of the agricultural extension program (Marosi, 1998).

The success of the poultry farming is largely dependent on improved management, proper nutrition and effective disease prevention and control schemes, etc (Mohammed, 1998). Poultry disease was the most important constraints responsible for reducing both the number and productivity. It is apparent that as more exotic breeds are introduced and with the advent of the intensification. New disease and management problems become extremely important both in commercial and backyard poultry production systems (Alamargot, 1987).

Different poultry diseases have been recorded in exotic and local chickens in Ethiopia; the major disease includes Newcastle disease, Coccidiosis, Salmonellosis, Mycoplasmosis and nutritional deficiencies (Dereje, 2002). Fowl typhoid caused by *Salmonella Gallinarum is economically* important disease in poultry particularly of chickens and Turks world wide, in exceptional cases and other birds can also be attacked (Alamargot,1987).

Salmonella Gallinarum belongs to the Kaufmanns, white scheme sero group D, along with *salmonella Enteritids* which was closely related. The organism are gram negative, non motile, sporogenic rods 1.0-2.5μm in length and 0.3-1.5μm in width. They are considered to be non motile under normal condition but inducement of flagella proteins and motility has shown in special motility of the microbes (OIE, 2004).

Usually infection of animals with salmonella may not be clinically apparent. It is subclinical form and the animal harbors salmonella in its intestine and in the lymph nodes intermittently and may excrete the agent in their feces hence, serving as the main reservoir of infection (Bean and Griffin, 1990). The infected bird reactors and carriers are the most important means of spread of the disease. Such birds may infect not only their own but also succeeding generation via their eggs transmissions. It was found that fowl

typhoid is transmitted from artificially infected birds to normal ones by co-habitation (Calnek *et al*., 1991).

Attendants feed dealers, chicken buyers and visitors may also be the source of contamination. Wild birds, animals and flies may be important mechanical spreader of the disease especially if they have been feeding on carcasses of dead birds suscepected of this disease and feeding of offals from packing plant and hatcheries (Dereje, 2002).

Fowl typhoid generally presents as septicemia, where the course of the disease may be acute or chronic. The acute disease is characterized by rapid onset, weakness, diarrhea, respiratory distress, loss of weight and sudden death. Peritonitis, enteritis, and localization of the organisms in the myocardium and the ovary accompany with swelling of the liver, kidney and spleen. Infection with biovar *Salmonella Gallinarum* often induces the development of hemolytic anemia (Assoku *et al*., 1978).

It affects birds of all age mainly those over 3 months (later growing period) are frequently affected. The mortality may be moderate or very high, depending largely on the virulence of the causative agents. The highest mortality occurs in birds of 2-3 weeks of age. In older birds the disease may be mild or in apparent. The course of the *Salmonella Gallinarum* infection varied greatly depending on the breed nutritional, immune status and age of the birds involved and mortality can be 100% in some infected flocks (Shivaprashad *et al*., 1997).

In breeding flocks loss of egg production and hatchability may be the observable clinical signs (Marosi, 1998). The postmortem signs of such infected birds revealed signs of septicemia, enlarge, dark, friable with distinctive copper bronze sheen appearance of liver. The bone marrow is also often dark brown. Although flock history, clinical signs and postmortem finding of fowl typhoid may be highly suggestive of the condition, they are not sufficient to distinguish from other causes of septicemia (Dereje, 2002).

Since both clinical finding and postmortem signs are not confirmatory, it requires further diagnosis by serology and isolation of the organisms to confirm and determine the prevalence of the disease. Serology is used because *Salmonella Gallinarum* is not excreted extensively in the feces unlike any other salmonella serotypes (Jones, 1995). These species are consistently highly invasive following oral route and circulating antibody is readily detectable thus in growing and mature birds shouldn't be treated with antimicrobial drugs for approximately 2-3 weeks previously. Samples may be obtained from live birds, fresh or freshly frozen carcasses, eggs materials, fresh feces, or any contaminated materials from housing,

incubators or transport boxes swabs may be taken from the cloacae of live birds (Jones, 1995).

Samples from visibly abnormal tissue are preferably taken from spleen, liver, gall bladder, kidney, lungs, fecal and environmental samples and in order to identify the agent one has to understand colonial morphology in selective and non- selective medias. *Salmonella Gallinarum* on non- selective media is round, translucent, listening, domed and smooth and 1-2 mm in diameter after 24-48hrs incubation (Calnek *et al*., 1991).

In order to culture and isolate the organism from apparently healthy chickens and those suspected ones a screening test was used as flock test using serum agglutination test. This test is easy to use and has largely eliminated these infection from many countries when use as a flock test. However, the test can yield erratic results, which can be dependent on the quality of the antigen.

There have been minor fragment reports of the existence of *Salmonella Gallinarum* by Marosi, (1998) in Ethiopia, but so far there was no published works done to indicate the magnitude and epidemiology of the disease.The objectives of the study were: To determine seroprevalence of fowl typhoid in commercial and backyard chickens of selected sites of East Shewa.

MATERIALS AND METHODS

Study areas

The study was conducted from November 2006 to May 2007 in Debre zeit and Ziway areas located around 45km and 160kms south east of Addis Ababa, with altitude of about 1850m and 1650m above sea level respectively. The areas experience bimodal rain fall where the long rainy season extends from June to September in both towns with an average rainfall of 1151.1mm and 760mm respectively. The shortest rainy season extends from March to May. The annual average maximum and minimum temperature ranges from 30.7^{o}C to 8.5^{o}C in Debre zeit and 27^{o}C to 12 ^{o}C in Ziway. The highest temperature in both towns attain in May (NMAS, 2003).

Study animals

The target populations were Bovan breed chicken of commercial poultry farms in Debre zeit poultry farm and local (backyard) chickens of Ziway. A total of 395 commercial chickens of Bovans breed and 419 chickens of the backyard poultry comprising different age groups, management systems,

breeds and production level were used. Based on their age the chickens were classified as pullets, which have an age of less than 14 weeks (i.e. 12 weeks-14 weeks) and layers for age greater than 26 weeks of age.

Study design

For sample size determination, win episcope 2.0 which is improved epidemiological data software for veterinary medicine (Thrustfield *et al*., 1995) was used with the following information to be considered for the sample size determination

- Total chicken population of East Shewa of 1.47 million
- Expected prevalence of 50%
- Absolute precision of 5% and level of confidence of 95% were used to calculate the sample size hence the total chicken population to be sampled were 384 from each sites according to the following formula for sample size determination

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

Where n= sample size, p_{exp}= expected prevalence and d= absolute precision.

Hence, to increase the precision of the result the sample size was increased to 814. In this study 814 chicken sera from the two selected sites were taken randomly.

Sampling methodology

The part of the humoral region was plucked and wiped using cotton soaked with alcohol then whole blood was collected from the brachial vein of chickens. Approximately 2-3 ml of blood samples were collected from the brachial vein using 5ml disposable sterile syringe and the syringe was put in a slant position at room temperature which allows the blood to clot; following clotting the serum was harvested after 3-4 hours using cryovials. The sera were put in a refrigerator at +4 ^{o}C until it was tested and finally for testing the whole samples were taken out of the refrigerator and put at room temperature and test using serum slide agglutination test.

Data analysis

Data were entered in to Microsoft excel spread sheet and coded properly for the analysis using descriptive and logistic regression statistics to determine the prevalence of fowl typhoid in both local and commercial chickens and its associated risk factors. The prevalence was determined by dividing animals serologically positive to total number of animals examined. Chi-square –test (X^2) was used to test the association of age, breed, and production levels. The analysis was made using SPSS version 15 for Microsoft windows where confidence level of 95% and p value of 0.05 were used as level of significance, in all cases.

RESULTS

The overall seroprevalence of fowl typhoid in the study site was 35.9%. Furthermore, it has indicated that there was significant difference of fowl typhoid between age groups where significantly ($p<0.05$) higher prevalence (42.0%) was recorded in older fowl of age greater than 26 weeks than age of less than 26 weeks (11.6%) (table1).

Table 1. Seroprevalence of fowl typhoid by age

Age	SAT		
	No. tested	No. positives	Prevalence (%)
<26 weeks	164	19	11.6
>26 weeks	650	273	42.0
Total	814	292	35.9

Pearson's chi-square= 52.6, df=1, p-value= 0.000

The study also indicated significantly higher prevalence ($p<0.05$) of the disease in back yard production systems of local chickens (44.6%) than intensive commercial production systems of the Bovan breeds (26.6%) (Table 2).

Table 2. Seroprevalence of fowl typhoid by breed

Breed	SAT		
	No. tested	No. positives	Prevalence (%)
Bovans	395	105	26.6
Local	419	187	44.6
Total	814	292	35.9

Pearsons chi-square=28.789, df=1 p- value= 0.000

Similarly, significantly ($p < 0.05$) higher prevalence was recorded in layers (42.0%) than pullets (11.6%).The logistic regression of the factors associated with prevalence of fowl typhoid indicated that age and production levels were highly associated with the disease (tables 3 and 4).

Table 3. Seroprevalence of fowl typhoid by production system

Production level	SAT		
	No. tested	No. positives	Prevalence (%)
Pullets	164	19	11.6
Layers	650	273	42.0
Total	814	292	35.9

Pearsons chi-square=52.7, df= 1, p-value= 0.000

Table 4. Logistic regression results of age, breed and production level

variables Age	Total examined	Odds ratio	p-value	95% CI of OR
	814	5.526316	0.000	3.342083,9.138063
Breed	814	1.359012	0.068	.9779727,1.888511
Production level	814	4.526316	0.000	2.617804, 7.82623

Note: significant if the OR>1 and p<0.05

DISCUSSION

Salmonella were considered to be one of the most economically important diseases of animals and human beings worldwide. Poultry has been the major reservoir of the infection. *Salmonella Gallinarum* which is the cause of fowl typhoid causes severe economic loss in poultry farms as the disease is characterized by high mortality and morbidity (Shivaprashad *et al*., 1997).

In this preliminary study of serology a screening test by slide agglutination was used as *Salmonella Gallinarum* is not extensively excreted via feces unlike any other salmonella serotypes which are more frequently associated with human poisoning.

The study revealed that a total of 292 (35.9%) of the total examined chickens were found to be sero-positive for fowl typhoid in the selected sites. The highest prevalence rate of fowl typhoid in the present study was inconsistent with previous preliminary survey on commercial layers and

pullets in Debre zeit and Addis Ababa poultry farms (16.05%) (Melese, 1992) and with the study conducted by Dereje (2002) on commercial layers and replacement pullets in Debre zeit, which was 1.54% prevalent. This inconsistency could be associated with the difference in management of the poultry where in both cases cited above the study was conducted on commercial poultry farms with good hygiene, nutrition and health management.

The present result was also significantly higher than the overall prevalence study in Bangladesh on intensive management with the prevalence of 26.7%. In Ethiopia, since the magnitude of the prevalence and impact was not studied, there was no any intervention taken which could also contribute to the higher prevalence rate presently recorded. In most of the developed countries such as Europe, North America and Australia the prevalence of fowl typhoid was almost nil as a result of serological testing and slaughter policies applied by them (Whiteman and Bickford, 1989).

Previous researchers have also indicated salmonellosis as one of the major disease problems affecting commercial exotic chickens and local chickens in Ethiopia. The incidence rate recorded were 5% and 20% in 1983-1984 and 1995-99 in Debre-zeit commercial poultry farms and Addis Ababa poultry farms, respectively (Alamargot, 1987). (Marosi,1998) pointed out salmonellosis to be one of the major causes for economic loss in Debre zeit commercial poultry farms responsible for high mortality and drug expenditure.

The occurrence of high seroprevalence rate of fowl typhoid indicates that salmonellosis was one of the major diseases in local and exotic chickens of commercial poultry farms of Ziway and Debre zeit. There was no published report of the disease in Ethiopia, but the present study indicated that the disease was highly prevalent and could paramount cause economic loss though attention was not given to such production systems.

The study also indicated large amount of backyard (local chickens) (44.6%) was affected by fowl typhoid compared to those chickens kept intensively with the prevalence of (26.6%). This may be attributed to the poor sanitary and management practices of the small scale holder farmers where in backyard system birds are kept extensively and rely on scavenging feed and exposed to unsanitary environmental conditions (Alamargot, 1987). The result was consistent with the study conducted on avian salmonellosis which is serious problem in most free ranging birds than in commercial poultry farms (Gordon *et al*., 1982).

The high prevalence of fowl typhoid may take the line share in the overall poultry production systems with high mortality rate and low productivity of backyard chickens in the country compared to the intensive production systems. In extensive production systems of Debre zeit it was noted that there were history of occurrence of salmonellosis, minimal distance and lack of physical separation between different units, poor disposal of dead birds, absence of an all in all out systems, poor insecurity measures and maintenance of different types and multi age groups of birds in farm created favorable conditions for the outbreak and persistence of the disease (Dereje, 2002).

Furthermore, the serum examination among the different age groups and production level indicated that of the 164 pullets (age ranging from 12 weeks to 14 weeks), 19 (11.6%) were found to be sero-positive. The study indicated that significantly ($p<0.05$) high sero-prevalence of the disease was recorded in adult (42.0%) chickens than young (11.6%). This result agrees with the reports of OIE (2004) which stated fowl typhoid is more common in the later growing period compared to young ones and it is the disease of mature flocks. Similar observations had been made in America where significantly higher incidence rate were recorded in older birds than in young (Whitemanand and Bickford, 1989). Similarly in Bangladesh over all prevalence rate of 10.85% at the ages 10^{th} week, 33.27% at the age of 24^{th} week and 37.74% at age of 40^{th} weeks that indicated the prevalence rate of the disease in young was also lower than that of adults were recorded which is similar with the current study (Bouzoubaa *et al.,* 1992).

In addition the prevalence of fowl typhoid in layers were much higher than that of pullets where out of the total of 650 layers 273 (42.0%) were positive for the disease compared to pullets where 19 (11.6%) of the 165 pullets were sero-positive. Hence, this result also showed high significance difference ($p<0.05$) in the prevalence of the disease. This is in agreement with the fact that all ages are susceptible even though most outbreaks occur in growing birds, particularly from 3 months of age approaching to laying (Calnek *et al*., 1991). The difference in pattern of age distribution of fowl typhoid could help to distinguish it from pullorum disease, which frequently occur in young chickens (OIE, 2004).

Likewise, significantly different prevalence of fowl typhoid was observed in this study between local and Bovan (exotic) breeds of chicken. The prevalence was significantly higher in local chickens 187 (44.6%) than in Bovan breeds (26.6%). This finding is in consent with previous reports from America where significantly different resistance to the disease was noted (Whiteman and Bickford, 1989). This could be partly associated with the management system where in most of the cases the exotic breeds of poultry

have genetic differences. Experimental researches also indicated that free range birds are more susceptible

CONCLUSION AND RECOMMENDATIONS

The present study clearly indicated that chickens kept under the backyard system of management were more exposed to fowl typhoid infection. The most important cause of loss in both commercial and local chickens was salmonellosis of which fowl typhoid was the most important one. Most outbreaks were associated with poor management, poor hygiene condition, poor nutrition and lack of effective disease preventive and control measures. Although all ages and those of free ranging chickens were susceptible most outbreaks occur in growing birds. Outbreaks in each chicken are comparatively high and the disease can also transmit to lesser extent via egg from one generation to another generation of chicken. For many years the test of choice had been slide agglutination test for the screening of flocks test for fowl typhoid using serum harvested from the local and exotic chickens as the agent is not excreted via feces extensively unlike any other salmonella serotypes. However, the test can yield erratic results, which can be dependent on the quality of the antigen.

Furthermore the lack of trained man power, especially in the field of poultry disease and nutrition, absence of definitive primary avian diagnosis laboratories and the lack of research works to solve the problem of poultry production in Ethiopia have further exacerbate the prevailing situation.

Based on the above remarks the following points are recommended

- Improvement of management in chickens under backyard production systems is required to obtained the required output
- Flocks tested to have positive reactors for fowl typhoid should be replaced with other flocks free of the disease to decrease the chance of carrier birds from spreading the disease
- Cause of high mortality of chickens in both backyard and commercial chickens should be further studied
- introduction of chickens from sources which are free of the disease should be mandatory
- Regular vaccination of layer chickens is essential to protect the transmission from layers to chickens

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ANNEX

SERUM SLIDE AGGLUTINATION TEST

Plug the feather from ventral surface of the wing

⇩

Wiped out with cotton damped with alcohol

⇩

Take 2-3ml of whole blood using 5ml disposable syringe

⇩

Put the syringe in slant position at room temperature for 3-5 hours

⇩

Harvest the serum using cryovials

⇩

Add 30µl of Salmonella Gallinarum antigen on to microscopic clean slide

⇩

Add 30µl of the serum on to the same microscopic slide

⇩

Mix them properly by gently rocking the slides and wait for about 2-3 minutes until serum and the antigen was completely mixed

Read the results

Results

Positive = Agglutination/ Precipitation was observed due to Ag- Ab reaction

Negative = No agglutination/precipitation was observed

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