

## Seroprevalencia de la infección por *Salmonella pullorum* en pollos locales (criollos) y exóticos comerciales en áreas de Mekelle, norte de Ethiopia

Seroprevalence of *Salmonella pullorum* infection in local and exotic commercial chicken from Mekelle areas, northern Ethiopia

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### RESUMEN

Se realizó un estudio regional cruzado con el objetivo de determinar la seroprevalencia de enfermedad producida por la *Salmonella pullorum* y algunos factores de riesgo asociados dentro y alrededor de la región de Mekelle, región de Tigray, al Norte de Ethiopia. Un total de 770 muestras de sueros de pollos fueron examinado utilizando un test aglutinación (SAT) para detectar anticuerpos post infección con *Salmonella pullorum*, en centro avícola de multiplicación de Mekelle y en los contornos. El paquete estadístico SPSS versión 17 se utilizó para la determinación de la prevalencia y la seroprevalencia global de la enfermedad resultó ser un 32.1 %. La mayor prevalencia de la infección por *S. pullorum* fue observado en razas locales (37.5 %) mayor que en los exóticos (27.2 %). La diferencia fue estadísticamente significativa ( $p < 0.05$ ). De modo semejante la en la seroprevalencia de la edad agrupa a menores de 6 meses, de 6 meses de, 10 meses y más de 10 meses se registró una prevalencia de 16.1 %, 35.1 % y 34.6 % respectivamente resultando estadísticamente significativo entre los grupos de edades diferentes. 31.7 % y 33 % fue la prevalencia de machos y hembras, respectivamente. Los resultados también indicaron que el 29.7 % de los pollos bajo sistema intensivo y el 37.2 % de los pollos criados en patios, resultaron ser serorreacores. Se infiere que es necesario

promover un profundo estudio para determinar los factores de riesgo asociados a la enfermedad.

**Palabras claves:** Seroprevalencia, pollos, enfermedad pullorum, sistema de granja, test de aglutinación, Norte de Ethiopia.

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## ABSTRACT

A cross sectional study was conducted to study the seroprevalence of pullorum disease in and around Mekelle Tigray region which is located north of Ethiopia with the aim of determining the seroprevalence and its associated risk factors for the occurrence of the disease in the different intensive and backyard chickens of the study sites. In this study a total of 770 chicken sera were examined using slide agglutination test with a principle of detecting antibody following infection of the poultries with *Salmonella pullorum* in the poultry multiplication centre of Mekelle and backyard chickens of the surrounding areas. The over all seroprevalence of the disease was found to be 32.8%. In addition sero surveillance result of the between different sexes, breeds, farming systems and age was also determined using slide agglutination tests and the prevalence in the local and exotic breeds was 39.3% and 29.2% respectively the result showed it was statistically significant ( $p < 0.05$ ) in the different breeds due to different management systems. Similarly the seroprevalence in age groups less than 6 month, 6-10month and greater than 10 months was also recorded with the prevalence of 5.1%, 35.1% and 34.6% respectively and this showed there was statistical significant among the different age groups as the disease is more common in the layers than young chickens but the prevalence in the different sexes was not statistical significant ( $p > 0.05$ ) as indicated in the result which was 31.7% and 33% in male and female chickens respectively which might be due to the fact that the disease is vertically transmitted via eggs which can infect both male and female equally. Finally seroprevalence was determined between the different farming systems such as intensive and backyard chickens and the result was 29.7% and 37.2% respectively in both production systems which was also statistically significant ( $p < 0.05$ ). For prevention of the spread of the disease from one farm to another farm and among the different production systems proper management such as feeding, watering, disinfection, vaccination, treatment using antibiotics and separation of infected and healthy chickens and proper disposal of dead poultries are essential.

**Key words:** Age, breed, chickens, farming systems, seroprevalence, sex, slide agglutination tests.

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## 1. INTRODUCTION

Poultry is now by far the largest livestock group in the world with a population estimated to be approximately 14,000 million. Around, 80% of the world poultry population is found in traditional scavenging systems, where the indigenous domestic fowl (*Gallus domesticus*) is the predominant species in the rural poultry sector (FAO, 2000). The free range poultry production system has also been designated as the “low in put – low out put” system. Diseases are also easily contracted under free range conditions due to scavenging habits. With an unconfined type of management, disease control is very difficult to carry out and is therefore rarely practiced by the owners (Pandey, 1992). (SAERT, 1996).

Poultry occupies a very crucial part of our economy for being affordable, easily manageable and fast growing compared with other species of animals that provides people with animal protein. The total poultry population of Ethiopia is estimated as 56.5 million, which represents 60% of the total chicken population in East Africa. From the total population of chicken in Ethiopia, 99% are raised under the traditional backyard system of management, while 1% is under intensive management system (Tadelle *et al.*, 2003; Ashenafi and Eshetu, 2004). It is quite evident that poultry farms are flourishing today but in the past mostly extensive type of production was predominating because the major part of poultry production was occupied by individual farmers and consequently, the outcome as a whole was below expectation and limited. Among the factors that played an important role in this regard are poor husbandry practices, low productive breed of the birds and various viral and bacterial avian diseases. Newcastle disease, Marek’s disease, Infectious bursal disease, Fowl typhoid, Pullorum disease and Fowl cholera are the most economically important poultry diseases (Tadesse *et al.*, 2005).

The Ethiopian indigenous chickens are non descriptive breeds closely related to the Jungle fowl. They vary in color, comb type, body conformation and weight. They are characterized by slow growth, late maturity and low production performance. In Ethiopia, the importation of exotic breeds goes back to the early 1950s. According to Alamargot (1987), about 99% of the Ethiopian poultry population consists of indigenous chickens, while the remaining 1% consists of imported exotic breeds of chickens during the 1970s and 1980s. There has been an increase in the number of exotic breeds of chickens and at present it is estimated that these make up about 2.18% of the national poultry population (CACC, 2005). Unfortunately, however, the contribution of exotic poultry to the Ethiopian economy is significantly lower than that of other African countries (Alemu and Tadelle, 1997).

The major uses and benefits of poultry & eggs in rural societies of Ethiopia are summarized as follows: eggs for hatching (51.8%), sale (22.6%) and home consumption (20.2%), and production of poultry for sale (26.6%), sacrifice (healing ceremonies) (25%), replacement (20.3%) and home consumption (19.5%) (Tadelle and Ogle, 1996). However, the productivity of poultry in Ethiopia is very low. This low production potential may be due to high incidence of diseases, lack of improved poultry breeds, poor feeding and management conditions. Among the infectious diseases, poultry salmonellosis is one of the main problems of economic concern for direct and indirect losses to all phases of the poultry industry from production to marketing (Alemu, 1995).

All species of birds are susceptible to *Salmonella* infection. However, the outcome of infection depends on a variety of factors, including age, host species susceptibility, and bacterial virulence. Although pullorum disease affects birds at any age, mortality rates are higher in young animals. Animals that survive may become carriers, may not meet expected animal production parameters and may produce contaminated eggs. The history of the disease and the development of industrial poultry breeding are mingled; artificial incubation of eggs was highly influenced by the occurrence of the disease, because it led to high mortality and culling rates among chicks. As eggs of different origins were incubated together, the agent of pullorum disease was transferred to other birds of commercial interest. Ability to survive infection increases with age and mortality is greatest in newly hatched chicks. Differences in susceptibility of breeds to *S. pullorum* have been reported (Shivaprasad, 1997).

Pullorum disease (Bacillary white diarrhea, BWD) is mainly an egg transmitted disease but also horizontally by contact in the hatcheries and by placement of chicks on contaminated litter (caused by *Salmonella pullorum* which is a Gram negative, non-motile, non-sporogenic, and facultative anaerobic rod bacterium adapted to poultry) that spreads during incubation or just after hatching. White diarrhea can be seen from 3 days to several weeks of age. The chicks refuse to eat, keep their heads tucked in and their wings hanging down. They huddle together and produce a peeping sound. Mortality in the acute form ranges from 20 to 80 percent and in the chronic form is around 5 percent. In the chronic form the signs are marked swelling of the hock joints, poor feather development, lack of appetite and depression (Berchieri *et al.*, 2001).

In many developing nations, *S. pullorum* infections of poultry are common and pullorum disease remains among the principal disease threats to poultry producers (Nabbut, 1993).

Although the principal current economic significance of *S.pullorum* in developed nations is the cost of testing programs, reminders of the potential for catastrophic losses have been provided by the occasional appearance of pullorum disease in commercial flocks (Salem *et al.*, 1992).

Only scattered data are available on their prevalence in any farm level. High prevalence has been reported from Thailand and Nigeria, while only low prevalence around 5-10% has been reported from other parts of Asia and Africa (Aini, 1990).

Liver and heart lesions should be differentiated from infections due to other Salmonellae and from campylobacteriosis, colibacillosis and omphalitis. Nervous lesions should be distinguished from nervous signs observed in Newcastle disease. Respiratory tract lesions should be differentiated from aspergillosis and joint lesions with synovitis and bursitis caused by other bacteria or viruses. Omphalitis caused by coliform infection may resemble pullorum disease in newly hatched chicks (Proux *et al.*, 2002). Antibacterial treatment helps to reduce mortality but treated chicken remain carriers. Furazolidone 0.022% in feed is effective (DACA, 2006).

Poultry salmonellosis causes great economic losses, but no data are available on the possible economic losses they incur, however, scattered documents revealed that it causes high mortality rates which can reach up to 100%, decrease in production (eggs and chicks), condemnation of affected carcass and viscera at abattoirs and cost of medication both in humans and animals. Direct health costs (e.g., hospitalization, consulting a physician, and laboratory testing) as well as the costs of lost labor (e.g., loss of production per day away from work) in relation to a case of salmonellosis were evaluated as part of a multidisciplinary task force (Calnek *et al.*, 1997). Serological tests are best applied as a flock test as results for individual birds will vary according to the stage of infection. It is therefore necessary to take sufficient individual samples to determine infection in the flock. The number of samples will depend up on the expected prevalence and level of confidence desired and the tests that are most readily applied include rapid whole blood agglutination test, rapid serum agglutination test, tube agglutination and micro agglutination tests (Barrow, 1992).

The main objectives of this study are to:

- Estimate the prevalence of *S.pullorum* in chickens of commercial and back yards in and around Mekelle.
- Recommend relevant control strategies pertinent to the prevailing local situation in the study area.

## 2. MATERIALS AND METHODS

### 2.1. Study Area

The study was conducted in and around Mekelle. Mekelle is located at 39° 29'E and 13° 30'N. It has an altitude of 2000m.a.s.l and is situated 783 km North of Addis Ababa. The mean annual rainfall of the study area is 628.8mm with bimodal rainfall of short rainy season occurring from March to May and long rainy season from June to August followed by the dry season from middle of September to February. The minimum and maximum temperature is 11.8°C and 29.9°C, respectively (BoPED, 1998).

### 2.2. Study Animals

The target populations were both the commercial (exotic breeds) and local chickens from Mekelle poultry multiplication centre and its surrounding

### 2.3. Study Design and Sample Size

A cross-sectional epidemiological study was conducted at Mekelle poultry multiplication center and Tabias (areas) that are found in and around Mekelle.

For sample size determination 95% Confidence interval, an estimated prevalence of 50% and desirable error limit of 5% was used and the formula stated by Thrusfield (1995) was used to determine the sample which was indicated in the formula below:

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

Where, N is required sample size,  
 $P_{exp}$  is expected prevalence and  
d is desired absolute precision

Hence the sample size was calculated to be at least 384, but 770 chickens were sampled so as to increase the precision of the study.

To determine the seroprevalence in randomly selected apparently healthy poultry in two different farming systems: in intensive (Mekelle poultry multiplication center) and in backyard farming systems (from Ellala, Ayder, Debri, Quiha, Hadinet and Adihaqui and Adigudem). During the study chickens were grouped in to three age groups: Group 1 (<6 months of age), Group 2 (6-10 months of age) and Group 3 (>10 months of age). Grouping

of ages was according the Mekelle poultry multiplication center in which chickens were kept in blocks in such a manner that the age is grouped and according to the owner's information in the different areas of the backyard poultry small holders.

## 2.4. Sampling and Data Collection

### 2.4.1. Sample collection and preparation

A total of 770 blood samples were collected from the wing vein of chickens where no salmonella vaccine was given previously. Approximately 3-4 ml of blood samples were collected aseptically using the disposable plastic syringe and plain vacutainer tube and kept for 3-4 hours at room temperature, following clotting, the serum was harvested using sterile cryovials, labeled individually and stored in a deep freeze at -20°C until tested using proper tests of crystal violet stained *salmonella pullorum* antigen.

### 2.4.2. Slide Agglutination test (SAT)

The principle of the agglutination test is based on the presence of corpuscular antigen (such as bacteria), which is complex, by specific antibodies forming an antigen-antibody network. This results in visible clumping of the antigen. By gravity, these clumps are deposited on the bottom of micro titer cup clearing the formerly turbid supernatant (Almaz, 2006).

The SAT test was performed according to the procedure described by OIE (2000) with crystal violet stained standard *Salmonella pullorum* antigen. *S. pullorum* antigens of 30 µl and chicken sera of 30 µl were placed side by side with a micropipette on ceramic tiles (plate) and mixed thoroughly by stirring with tooth pick followed by rocking. The results were observed within two minutes. In positive cases agglutination or precipitation reactions were observed where as in case of negative there is no agglutination reaction. Other data's like sex and breed was recorded by observation; age and farming system was classified based on owner's information.

## 2.5. Data Analysis

Microsoft Excel spread sheet was used for data entry and these data were analyzed using descriptive statistics, using (SPSS) statistical package for windows version 15. The total prevalence was calculated by dividing the number of *S. pullorum* seropositive animals by the total number of animals examined and multiplied by hundred (Thrusfield, 1995). The chi-square test was applied to determine existence of any association between prevalence of *S. pullorum* and the risk factors such as age, sex, breed and farming

systems. Meanwhile Univariate logistic regression was applied to measure the strength of the association. The logistic model was checked for goodness-of-fit using the Hosmer and Lemeshow test. The logistic-regression model was fitted with seroprevalence of *S.pullorum* (positive/negative) as the out come.  $P<0.05$  was considered statistically significant in all cases.

### 3. RESULTS

The overall seroprevalence of *S. pullorum* was recorded as 32.8% (Table 1).

**Table 1.** Overall seroprevalence of *Salmonella pullorum*

Total no. of samples tested	Number of positive cases	Prevalence (%)
770	253	32.8

The prevalence on the basis of age group which was grouped as group 1 (<6 months of age), group 2 (6-10 months of age) and group 3 (>10 months of age) were found to be 5.1%, 35.1% and 34.6 %, respectively (Table 2) and there was statistical significant difference ( $P<0.05$ ) among age groups as the disease is chronic in nature which mostly affects adult chickens.

**Table 2.** Prevalence of *S. pullorum* by age

Variables	Category	Total (N)	+ve (%)	OR	P-value	95% CI for OR	
						Lower	Upper
Age	<6 months	80	13 (5.1)	3.00	0.004		
	6-10 months	225	79 (35.1)	1.31		0.16	0.58
	>10 months	465	161 (34.6)	9		7	8
						0.48	1.05
						8	7

N refers to total number of chickens; OR: odds ratio; CI: confidence interval; +ve: positive

The prevalence in the two sexes was also recorded with higher prevalence in female 33% chickens than male having the prevalence of 31.7% (Table 3) and this shows there was no significant difference ( $P>0.05$ ) between the two sexes.

**Table 3.** Prevalence of *S. pullorum* by sex

Variables	Category	Total (N)	+ve (%)	OR	P-value	95% CI for OR	
						Lower	Upper
Sex	Male	63	20 (31.7)	-	0.845	-	-
	Female	707	233 (33)	-		0.51	1.22
						1	3

N refers to total number of chickens; OR: odds ratio; CI: confidence interval; +ve: positive

Seroprevalence on the basis of breed was also recorded with higher prevalence in local breeds as compared with exotic breeds having the seroprevalence of 39.3% and 29.2%, respectively (Table 4) and this showed that there was significant difference (P <0.05).

**Table 4.** Prevalence of *S. pullorum* by breed

Variables	Category	Total (N)	+ve (%)	OR	P-value	95% CI for OR	
						Lower	Upper
Breed	Exotic	490	143 (29.2)	-	0.004	0.66	1.64
	Local	280	110(39.3)	0.61		-	-
						1	1

N refers to total number of chickens; OR: odds ratio; CI: confidence interval; +ve: positive

The prevalence on the basis of farming (management) system which was classified as intensive and backyard farming systems were recorded to be 29.7% and 37.2%, respectively (Table 5) and the rate showed significance difference (P <0.05).

**Table 5.** Prevalence of *S. pullorum* by farming (management) system

Variables	Category	Total (N)	+ve (%)	OR	P-value	95% CI for OR	
						Lower	Upper
Farming system	Intensive	445	132 (29.7)	-	0.027	-	-
	Backyard	325	121 (37.2)	0.58		0.38	0.97
						7	2

N refers to total number of chickens; OR: odds ratio; CI: confidence interval; +ve: positive

#### 4. DISCUSSION

In the current study, the prevalence of *S. pullorum* was recorded as 32.8% which is higher than the study conducted by Melese (1991) and Assefa (1992) having the prevalence of 10.44%, 28.25% and 19.71% in Shola, Denbi, and around Addis Ababa respectively and lower than Ashenafi *et al* (2003) and Yang *et al* (1996) having the prevalence of 64.2% (39.02%) in Central and Eastern Ethiopia. The variation of seroprevalence might be speculating to be geographical variations or differences of management systems. The significance of all these diseases, however, remains to be investigated. In addition, it should be noted here that a general trend for these studies is that they have only looked for antibodies against selected diseases.

Similarly prevalence in different sexes was recorded as 31.7% and 33% in male and female, respectively and the rate showed no significance difference between sexes ( $P > 0.05$ ). This may be due to the fact that transmission in pullorum disease occurs mainly by vertical route through infective egg laid by carrier hen (trans-ovarian), but also horizontally by contact in the hatcheries and by placement of chicks on contaminated litter. Many of the infected chicks hatch and then transmit the organism laterally and infect incubators, hatchers, check boxes, contaminated houses and equipment and other birds in the brooder area via the digestive and respiratory system. Dissemination of the subclinical carriers to many purchasers results in wide dissemination of the etiological agents. Transmission may also occur within a flock as a result of cannibalism of infected birds, egg eating, and through wounds on the skin. Feces from infected birds are also a source of bacteria for non-infected birds (Johnson *et al.*, 1992). Therefore all means of transmission were not affected by sex differences. Hence there is no discrimination that both sexes to be affected.

Age specific rates were calculated for the three age groups. The proportion of seropositive in the age group 1 (<6 months of age), group 2 (6-10 months of age) and group 3 (>10 months of age) were 5.1%, 35.1% and 34.6%, respectively. Melese (1991) recorded highest proportion of positive in the age group of greater than one year of old (22.375%) and no seropositive was detected in the age group of less than one year old. This finding corresponded with reports of Sikder *et al* (2005) and Truong and Tieuquang (2003). The present study was higher than the previous study this might be due to the difference between the management systems practiced.

The strength of the association (Odds Ratio) was also calculated and was found 3.616 and 1.139 in age group 2 and 3, respectively in relation to the

age group 1. Therefore it was found that seroprevalence increased with age of birds and there was high significance difference ( $P < 0.05$ ). This is because *S. pullorum* antibody found more in adult poultry is that young chicks will die shortly after hatching and a clinical sign in pullorum disease usually seen in chicks younger than 3 weeks old (Calnek *et al.*, 1997) that it is difficult to get the antibody on these chickens unless survive and becoming carriers.

The proportion of seropositive in local and exotic breeds was 39.3% and 29.2%, respectively and the result showed significance difference ( $P < 0.05$ ). It was also calculated that to what extent is the degree of seroprevalence rate (OR) varies with breed difference and was found to be 0.611 in exotic breed in relation to the local breeds. The present finding (33.1%) in intensive (commercial) farms was higher than the seroprevalence (23.46%) recorded by Sikder *et al* (2005). The difference with Sikder *et al* (2005) was corresponded with the findings of Jha *et al* (1995) and Robinson *et al* (2000), who recorded seroprevalence rate higher in commercial chickens. This might be due to the fact that whenever exotic breeds are brought to the tropics, they become easily susceptible to the disease and hence there might be high mortality whereas the local chickens are adapted to the disease prevailing in the tropics and become as a carrier for the disease. That is why the prevalence of the disease was higher in local chickens.

Out of 445 apparently healthy chickens screened from intensive farming system (Mekelle poultry multiplication center and other areas), 132 (29.7%) were found to be seropositive. The percentage of infection, 37.2% (121) of the 325 chickens screened were found to be carriers from backyard poultry farms. The extent of the infection rate (OR) was 0.587 in back yard in relation to the intensive farming system. There was significant difference in seroprevalence between the two management systems ( $P < 0.05$ ). Assefa (1992) reported 22.6% from the small scale poultry farms and the percentage of infection varied between 10.41% and 40.0% from the backyard poultry farms. The low prevalence in the intensive might be because in the commercial poultry farms there is routine vaccination programme, good ventilation, proper spacing of poultry houses and again there is no mixing of breeds (species). But in backyard farming systems such activities may not be performed and traditionally they used some drugs which might not be the appropriate on its dosage and its quality. Then after the chickens develop resistance against the diseases and this makes them to be continuing their life as carriers. This might be the cause to have high prevalence rate.

## 5. CONCLUSION AND RECOMMENDATIONS

Pullorum disease caused by *Salmonella pullorum*, is severe septicemia disease of domestic and wild fowl and remains an important disease for the poultry industry due to high morbidity and mortality. In Ethiopia, even though there were frequent complaints by the state and private poultry farms due to the effect of the disease which causes high morbidity, mortality, loss of production and high treatment cost. Poor husbandry practices, low productive potential of local breed, and various bacterial and viral avian diseases have made the outcome obtained from poultry in Ethiopia below expectation of which pullorum is one of the most economically important bacterial poultry diseases.

Based on the above findings the following points are recommended

- ❖ There have been economic losses due to pullorum disease in the intensive and extensive poultry production systems in Ethiopia as a result improvement of the production and managerial conditions are essential.
- ❖ Continuous assessment to determine the prevalence of the disease are essential as the test are screening tests which needs confirmatory tests to have clear data about the status of the disease.
- ❖ Studies must be done to identify the strains which would help in producing more effective vaccines.
- ❖ Reduction of contact between wild birds and domestic bird's in order to reduce the risk of transmission of infection from wild birds to domestic fowl.
- ❖ Disinfection of the poultry premises and shoe and vehicle tyre are essential to reduce the wide spread of the disease among different poultry houses of the same or different farms.
- ❖ Proper treatment and vaccination of birds using appropriate antibiotics and vaccines are mandatory.
- ❖ Avoid mixing of birds having different age groups and also infected and healthy birds.
- ❖ Proper ventilation and disinfection of poultry houses.
- ❖ Proper disposal of dead birds and debeaking are important.

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