Sero-prevalence of Fowl Typhoid and Pullorum Disease from Apparently Healthy Chickens in Eastern Ethiopia

Genet Tadele1, Biruhtesfa Asrade2*, Gizachew Bayleyegn1, Mohammed Sanni Ali3
1College of Veterinary Medicine, Haramaya University, Dire Dawa, Ethiopia
2School of Veterinary Medicine, Hawassa University, Ethiopia
3Colleges of Veterinary Medicine and Agriculture, Addis Ababa University, Ethiopia

Abstract
A study was conducted on 393 apparently healthy exotic and local chickens reared at different management systems in eastern Ethiopia in order to determine the sero-prevalence of Salmonella Gallinarum and Salmonella Pullorum. To achieve this objective, blood samples were collected and the expressed sera were used from the selected chickens for antibody detection. The samples were from Haramaya University (309 chickens) and Dire Dawa (84 chickens). Subsequently, serum slide agglutination test was employed. Based on this, the overall prevalence of Salmonella Gallinarum and Salmonella Pullorum was 125/393 (31.8%). The prevalence of each farm was 95 (30.7%) Haramaya University farm, 13 (32.5%) Dire Dawa University farm and 17 (38.6%) local breeds of Dire Dawa. Moreover, the study has recorded higher prevalence in back yard chickens as compared to chickens reared at intensive farms. However, associated risk factors have not been found statistically significant at (P value < 0.05). In conclusion, the present study revealed Salmonella Gallinarum and Salmonella Pullorum were prevalent in both production systems. Therefore, further epidemiological investigations on these pathogens and implementation of control measures are mandatory.

Keywords: Sero-prevalence; Serum slide agglutination; Salmonella Gallinarum; Salmonella Pullorum; Chickens

Introduction
Poultry play an important role in the livelihood of poor rural households in many developing countries including Ethiopia [1]. In Ethiopia, the total poultry population is estimated to be 50 million [2]. Recently commercial flocks have been emerged in urban and peri-urban areas in central parts of the country [1]. However, the country is not benefited from the sector. This is due to different contributing factors, among which are low genetic potential of the indigenous breeds, high prevalent of infectious diseases and traditional feeding practice [3-5].

Previously different poultry diseases have been recorded in exotic and local chicken in Ethiopia; the major diseases include New castle disease, coccidiosis, salmonellosis, chronic respiratory disease and nutritional deficiency [5-8]. Fowl Typhoid and Pullorum Disease are among the most important diseases of poultry. These conditions are caused by Salmonella Gallinarum and Salmonella Pullorum respectively [5]. Both diseases are a serious concern in growing and adult poultry and show similar clinical manifestation. The controls of these diseases are complicated by vertical transmission. Hens can become sub clinically infected carriers, and pass the infection to their embryos in the egg [5-7]. Fowl Typhoid and Pullorum Disease can be diagnosed by culturing on standard media from affected birds (brilliant green, XLD agars, selenite F of tetrathionate broth for enrichment). Detection of the organism and differentiation of the biovars can be done by serologic tests. These tests including serum agglutination test are important to detect infected flocks and estimate the prevalence of infection within a flock [8]. Although Fowl Typhoid and Pullorum Disease are known to cause marked economic loss, up to now few studies have been done in Ethiopia [9]. Presently, there is few sero-prevalence reports exist regarding Salmonella Gallinarum and Salmonella Pullorum [10,11]. In connection to this, further investigation of both diseases using sound serological tests will have significant contribution to show the status in the poultry production sector. Therefore, the objectives of this study are to determine sero-prevalence of Fowl Typhoid and Pullorum Disease and to assess the effect of associated risk factors.

Materials and Methods

Study area
The study was carried out in poultry farms of Haramaya and Dire Dawa Universities. Haramaya University is located in the Eastern Ethiopia, approximately 510 Km from Addis Ababa. The elevation is 2000 meter above sea level and the area receives an average annual rainfall 900mm. Dire Dawa University is located at 515 km from Addis Ababa, at an elevation of 950 – 1250 meter above sea level. The Average annual rainfall varies from 404 mm in the dry “Kola” zone to 900mm in the mid land “Weinadega” zone. The temperature ranges from 28.1°C to 34.6°C [12,13].

Study population
The target population was apparently healthy white leg horn and fiam (Egyptian breed) breed chickens in poultry farms of Haramaya and Dire Dawa Universities including local and exotic breed from Dire Dawa. A total of 353 commercial chickens and 40 chickens of local poultry comprising different age group, management system, and breed and production level were included in this study.

Study design
The sample size was determined using win ecommerce 2 which is improved epidemiological data soft ware for veterinary medicine [14].
Sample size for intensive production was determined using cluster sample formula [11]. Since no research work has been done before in the study areas, 50% prevalence and 95% confidence interval were considered.

**Study methodology**

Approximately 2-3ml of blood sample was collected from the wing vein and the blood in the plane vacutainer tube was put in slant position at room temperature. After 12 hrs of clotting, the sera were subjected to centrifugation at 1000 rpm for 10 minutes [15]. Finally, clarified sera were stored in deep freeze until it was tested. Serum slide agglutination test was performed following the test procedure described by Quinn [16]. All harvested sera and the reagent were brought to room temperature (22 ± 5°C) before use. 30 µl of crystal violet stained Salmonella Gallinarum antigens and equal volume of the serum were added on sterile slide. Thereafter, the two contents were mixed gently by rocking and rotating it for about 2-3 minutes. And then, presence or absence of agglutination reactions was checked. Any level of agglutination is considered as positive.

**Data analysis**

Data were classified, filtered and coded using MS Excel 5. SPSS version 17 was deployed to generate statistical associations. The descriptive statistics were used to determine the prevalence of *Salmonella Gallinarum* in both local and commercial chickens Pearson chi-square was used to evaluate the statistical significance of *Salmonella Gallinarum* and associated risk factors. A probability value <0.05 was considered statistically significant.

**Result**

The overall prevalence of Fowl Typhoid and Pullorum Disease in the chicken of the study area was 31.9% (125/393). Based on the study, chickens from Haramaya were with a prevalence of 30.7% (95/309), while the prevalence in Dire Dawa was 32.5% and 38.6% for the commercial and the local breeds, respectively (Table 1). According to the breeds classification Egyptian fayoumis are less affected with prevalence of 25% while local chickens from Dire Dawa relatively highly affected 38.64% when compared with other chickens (Table 1). Based on this study all age group were similarly affected by the disease (Table 2). Furthermore, the association of potential risk factors for sero-positivity is presented in Table 2.

**Discussion**

Poultry has been the major reservoir of the Salmonellosis. There are epidemiological studies published on prevalence of Fowl Typhoid and Pullorum Disease in Ethiopia [3,11]. However, the sero prevalence of these diseases based on serum agglutination test has not been studied in eastern ethiopia. In present study the serum agglutination response to *Salmonella Gallinarum* and *Salmonella Pullorum* in apparently healthy chickens is relatively high when compare to the previous report in poultry in other countries.

The possible causes of the high prevalence of this study are the facts that both diseases are complicated by vertical and horizontal transmission occur via the respiratory and oral routes. Hens might become sub clinically infected carriers and pass the infections to their embryos in the egg. The other important facts are chickens are natural hosts and highly adapted for these pathogens. In addition, *Salmonella Gallinarum* and *Salmonella Pullorum* survive in a favorable environment from months up to several years and this characteristic contributes persistent infection in these hosts. Previous studies on the basis of bacteriological and culturing have confirmed the above facts [5,7,17,18]. A Sero prevalence study from Nigeria has reported

<table>
<thead>
<tr>
<th>Origin</th>
<th>Number of chickens examined</th>
<th>SAT</th>
<th>Prevalence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>HU(Exotic-intensive)</td>
<td>309</td>
<td>95</td>
<td>214</td>
</tr>
<tr>
<td>DU(Exotic-intensive)</td>
<td>40</td>
<td>13</td>
<td>27</td>
</tr>
<tr>
<td>Dire Dawa(Local-back yard)</td>
<td>44</td>
<td>17</td>
<td>27</td>
</tr>
<tr>
<td>Total</td>
<td>393</td>
<td>125</td>
<td>268</td>
</tr>
</tbody>
</table>

HU: Haramaya University; DU: Dire Dawa University, SAT= Serum agglutination test.

**Table 2:** Association between different potential risk factor and sero-positivity.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Total Examined</th>
<th>No of positive</th>
<th>Prevalence (%)</th>
<th>χ2</th>
<th>P- Value</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td></td>
<td>No of positive</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;70 weeks</td>
<td>200</td>
<td>61</td>
<td>30.50</td>
<td>0.279</td>
<td>0.597</td>
<td>0.584-1.363</td>
</tr>
<tr>
<td>&lt; 70 weeks</td>
<td>193</td>
<td>64</td>
<td>33.16</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Breed</td>
<td></td>
<td>No of positive</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WLH</td>
<td>309</td>
<td>98</td>
<td>31.72</td>
<td>1.789</td>
<td>0.409</td>
<td>0.416-1.211</td>
</tr>
<tr>
<td>Fayoumis</td>
<td>40</td>
<td>10</td>
<td>25.00</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Local</td>
<td>44</td>
<td>17</td>
<td>38.64</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td>No of positive</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>218</td>
<td>69</td>
<td>31.65</td>
<td>0.002</td>
<td>0.963</td>
<td>0.659-1.847</td>
</tr>
<tr>
<td>Female</td>
<td>175</td>
<td>56</td>
<td>32</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Production level</td>
<td></td>
<td>No of positive</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>55-80 eggs/year</td>
<td>349</td>
<td>108</td>
<td>31.00</td>
<td>0.983</td>
<td>0.321</td>
<td>0.377-1.379</td>
</tr>
<tr>
<td>280 eggs/year</td>
<td>44</td>
<td>17</td>
<td>38.64</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

WLH: White leg horn; χ2: Chi-square.
similar prevalence (37.9%) from two different management systems [19]. Moreover, research reports conducted on chicken carcasses have also revealed high prevalence and both diseases are long-established worldwide [7,20–22]. However, in Africa, moderate prevalence (16%) of these diseases has been published by Mbuko [23] as compare to the present study.

Previously, there are few reports on Fowl Typhoid and Pullorum Disease in Ethiopia, in particular, regarding the sero prevalence. These preceding works have indicated that the diseases were highly prevalent and economically important diseases in chickens in central and northern part of the country [3,10,11,24]. However, the high prevalence rate of the present study was inconsistent with the previous survey on salmonella contamination in chickens’ carcass and giblets [25] with moderate prevalence rate of 21.1%. The difference could be due to the methods that are applied and also the tests used. Here it is good to remember that the possibility of false positive and false negative results on slide agglutination (SAT). Serological tests are normally designed to detect a limited range of Salmonella serovars or serogroups. It is well recognised that some animals with a positive serological response may no longer be infected with Salmonella organisms. Chickens that are actively excreting salmonellae may be serologically negative in the early stages of disease and some individual infected chickens never seroconvert. Poultry that are serologically positive 6 may have ceased to excrete salmonellae although circulating immunoglobulin concentrations may remain high. Other chickens on the farm may still be infected. Serologically negative poultry may result from a recent infection causing excretion before immunoglobulin production is maximal, or infection with less invasive serovars. Animals that have been infected recently would eventually be detected serologically by an appropriate monitoring programme throughout the life of the flock but there are often cost limitations to the application of effective monitoring programmes [26].

In conclusion, the present study revealed the overall sero-prevalence of Fowl Typhoid and Pullorum Diseases were prevalent in both production systems. Therefore, further epidemiological investigations; proper management practice, application of prevention and control measures are recommended.

References
13. DDAEPA (2011) Dire Dawa Administration Program of Adaptation to Climate Change (DDAPEA), Dire Dawa, Ethiopia.