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Salmonella Gallinarum-Pullorum Isolation from Sick and Dead Chickens in Hawassa, Southern Ethiopia

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Abstract: This bacteriological study was conducted with the objective to assess the importance of fowl typhoid and pullorum disease in morbidity and mortality of chickens in and around Hawassa, southern Ethiopia. The study involved bacteriological examination of 115 cloacal swab samples collected from sick chickens (cloacal swab only group [CSG]) and examination of tissue samples collected from additional 42 (22 sick and 20 dead) chickens (tissue sample group [TSG]), for isolation and identification of *Salmonella* enterica serovar Gallinarum (biotypes Gallinarum and Pullorum). The study chickens were obtained from 2 intensive farms in Hawassa and 2 public veterinary clinics in Hawassa and Shashemene towns. The samples collected from the TSG were liver, spleen and ceca (each n = 42), ovary (28) or testis (14) and cloacal swab (38). Standard bacteriological procedures were used to isolate and identify *Salmonella*. All the 115 cloacal swab samples collected from CSG were negative for *S.* Gallinarum-Pullorum, while 11 (26.2%) of the 42 birds which had their tissues examined (TSG) were found infected with the bacteria. At organ level, 8 (19.0%) spleen, 5 (11.9%) liver, 4 (13.8%) ovary and 2 (4.8%) ceca samples cultured positive to *S.* Gallinarum-Pullorum, while none of the testis and cloacal swab samples were positive. The entire chickens positive for *S.* Gallinarum-Pullorum in this study were from one intensive farm, Hawassa Poultry Multiplication and Breeding Center (HPMBC). The study demonstrated that fowl typhoid/pullorum disease is among the important causes of mortality and morbidity in poultry in the study area. Strict biosecurity measures in breeding and multiplication farms are recommended considering their epidemiological significance.

Key words: Fowl Typhoid · Gallinarum · Pullorum Disease · Salmonella · Ethiopia

INTRODUCTION

Demand for livestock products, including poultry, is driven by economic growth, rising per capita income and urbanization [1], in addition to the demand associated with population growth. As a result of expected population and economic growth, it is projected that there will be a net deficit of 223 thousand tons in red meat supply in Ethiopia by 2028 and investment in poultry is suggested as the key in offsetting the expected deficit. To that end, it is planned to transform the traditional backyard poultry production in the country to market-oriented improved family poultry production with semi scavenging crossbred chickens and to expand specialized broiler and layer poultry production [2].

These poultry development endeavors, however, are likely to be challenged by prevailing diseases and feed shortage, among others. Diseases have already been

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very important constraints of poultry production in Ethiopia and affect both village and intensive poultry production [3-7].

Among diseases fowl typhoid and pullorum disease have been reported to occur and cause significant damage to the poultry industry in Ethiopia [5, 7, 8]. Fowl typhoid in chickens, caused by *Salmonella enterica* serovar Gallinarum biotype Gallinarum [9], is a disease of mature fowl that results in either acute enteritis with greenish diarrhea or a chronic disease of the genital tract that reduces egg production. Pullorum disease or bacillary white diarrhea, on the other hand, is caused by the biovar Pullorum and causes a high mortality (50% to 100%) among embryos and then chicks, as well as weakness and white diarrhea [10].

The biotypes are non-motile and highly host adapted avian pathogens [11, 12]. They are widely distributed throughout the world but they have been eradicated from the commercial poultry in many developed countries by a serological testing and slaughter policy for pullorum disease [12]. Chickens are the natural hosts for both biotypes [13].

Pullorum disease and fowl typhoid are economically important diseases, without their effective control through organized national regulatory programs; the profitable production of poultry would be impossible [14]. However, knowledge of the epidemiology and importance of diseases is essential to devise suitable control and prevention strategies. Therefore, the present study was conducted to establish the possible role of fowl typhoid and pullorum disease in mortality and morbidity of chickens in and around Hawassa.

MATERIALS AND METHODS

Study Area: The study was conducted between November 2011 and April 2012 on sick and dead chickens obtained from two farms in Hawassa and public veterinary clinics in Hawassa and Shashemene towns, southern Ethiopia. Hawassa is the capital city of Southern Nations, Nationalities and Peoples Regional State (SNNPRS) located about 275 km south of Addis Ababa. It is geographically positioned at 7°5' latitude N and 38°29' longitude E at an altitude of 1708 m a.s.l. The mean annual rainfall and temperature of the area vary from 800 to 1000 mm and 20.1- 25°C, respectively. Shashemene is a nearby town located at about 25 km from Hawassa. Sick and dead chickens were obtained from Hawassa Poultry Multiplication and Breeding Center (HPMBC) and poultry farm of Hawassa College of Agriculture (HCA) and public veterinary clinics in Hawassa and Shashemene towns. The HPMBC is one of poultry breeding, multiplication and distribution centers in the country, which are engaged in importation of improved commercial chickens from developed countries, rear, multiply and distribute chicks of mostly up to 3 month old to end users [15]. Poultry farm of HCA is a commercial farm which raises layers. Both farms raise chickens on deep litter floor.

Table 1: Description of chickens used in the study

		Sample	
Variable	Category	Cloacal swab only	Tissue
Health status	Sick	115	22
	Dead	0	20
Breed	Local	99	2
	Exotic	16	40
Source	HPMBC	1	35
	HCA	0	3
	Hawassa clinic	41	3
	Shashemene clinic	73	1
Sex	Male	40	14
	Female	75	28
Age	Young (≤5 m)	19	39
	Adult (>5m)	96	3
Management	Intensive	1	38
	Extensive	114	4
Over all		115	42

HPMBC= Hawassa Poultry Multiplication and Breeding Center
HCA= Hawassa College of Agriculture poultry farm

Study Animals: The study involved a total of 157 sick and dead chickens. Only cloacal swab samples were collected from 115 sick birds (cloacal sample only group [CSG]), while tissue samples: liver, spleen and cecum (each n = 42) and ovary (28) or testis (14) and cloacal swabs (38) were collected from the remaining 42 sick or dead chickens (tissue sample group [TSG]). Table 1 summarizes the characteristics of chickens used in the study. The 115 cloacal swab samples from CSG were collected from sick chickens presented to Hawassa and Shashemene veterinary clinics to be diagnosed and treated. Virtually all of the birds obtained from the clinics were from extensive backyard system. The 42 chickens, of TSG were obtained from Hawassa Poultry Multiplication and Breeding Center (HPMBC), Hawassa College of Agriculture (HCA) poultry farm and Hawassa and Shashemene veterinary clinics. The chickens from the intensive poultry farms were exotic chickens of either the layer or dual purpose types raised on deep litter. Almost all sick and dead chickens obtained from veterinary clinics were of local type raised under extensive backyard management.

Study Method: The study involved bacteriological investigation of samples collected from sick and dead chickens for *S. enterica* serovar Gallinarum (biotypes Gallinarum and Pullorum) in an attempt to assess the importance of the bacteria in morbidity and mortality of chickens in the study area. Therefore, cloacal swab samples were collected from sick chickens, while postmortem tissue samples were collected from dead and sacrificed ones.

Sample Collection and Processing: Cloacal samples were collected by gently inserting and rotating sterile cotton tipped swabs moistened with buffered peptone water (BPW) (AES, Combourg, France) into the cloaca of the birds. The swab samples were placed in screw-capped sterile test tubes and transported to the Microbiology Laboratory of School of Veterinary Medicine of Hawassa University for analysis.

For aseptic collection of tissue samples, sick birds and bodies of freshly dead chickens (died within 12 h) were transported to the Laboratory. Sick birds were humanely killed by quick cervical dislocation before opening. Liver, spleen, ovary/testis and cecal samples were collected aseptically. The samples were placed on separate sterile Petri dishes. The surfaces of the samples (liver, spleen, ovary/testis) were sterilized by immersing the tissues for a brief moment in boiling water. The tissues were then cut into small pieces on sterile plates using sterile scalpel blades. One gram of the minced tissue was placed in 9 ml of BPW, vortex mixed and incubated at 37°C for 16 h.

Isolation and Identification: Isolation and identification of *Salmonella* was carried out using standard techniques [16, 17]. The culture in BPW after 16 h of incubation was vortex mixed and 0.1 ml was transferred into 10 ml Rappaport Vassiliadis (RV) medium (HiMedia, Mumbai, India) and was incubated for 24 h at 42°C. A loopful of inoculum from RV broth culture was streaked onto xylose lysine desoxycholate (XLD) (Oxoid, Basingstoke, England) agar plates. The inoculated XLD agar plates were incubated at 37°C for up to 72 h being observed every 24 h for growth of suspect *Salmonella* colonies (Fig. 1). Presumptive *Salmonella* colonies (red colonies, with or without black center with change of the color of the medium to pink) were carefully inoculated to triple sugar iron agar (TSI) (HiMedia, Mumbai, India) and lysine iron agar (LIA) (Oxoid, Basingstoke, England) slants.

Fig. 1: Small colonies of *Salmonella* Gallinarum-Pullorum without black center (no H_2S production) isolated in pure-culture from liver on XLD agar (after 72 h incubation)

The inoculated TSI and LIA media were incubated at 37°C for 24 h and observed for the color of the slant and butt and for gas and H_2S production. Generally *Salmonella* show a red slant and yellow butt, with H_2S (blackening) and gas production in TSI. *S.* Gallinarum-Pullorum show similar reactions with low diffusion of H_2S to the medium from the line of inoculation with variable gas production. *Salmonella* typically decarboxylase lysine and produce H_2S in LIA medium. The isolates were then tested for sulfide production, motility and indole formation using Sulphide Indole Motility (SIM) medium (Oxoid, Basingstoke, England), urease using urea agar base (Oxoid, Basingstoke, England) prepared with addition of 40% urea (HiMedia, Mumbai, India) and citrate utilization using Simmons citrate agar (HiMedia, Mumbai, India). *Salmonella* Pullorum and Gallinarum are non-motile, indole, urease and citrate (Simmons citrate medium) negative. Slow growing isolates (taking 48-72 h) with small colonies [10] which were morphologically and biochemically typical of *Salmonella* and non-motile, were considered *S.* Pullorum or Gallinarum as this serovar is relatively fastidious and is the only serovar of *Salmonella* which is normally non-motile [16].

Data Analysis: Data were stored and managed in Microsoft Excel spread sheet and descriptive statistics such as percentages were used to summarize and present the data.

Table 2: Occurrence of *S*. Gallinarum-Pullorum in sick and dead chickens for which tissue samples were examined

Variable	Category	No. examined	No. (%) positive
Health status	Sick	22	7 (31.8)
	Dead	20	4 (20.0)
Breed	Local	2	0 (0)
	Exotic	40	11 (27.5)
Source	HPMBC	35	11 (31.4)
	HCA	3	0 (0)
	Hawassa clinic	3	0 (0)
	Shashemene clinic	1	0 (0)
Sex	Male	14	3 (21.4)
	Female	28	8 (28.6)
Age	Young (≤5 m)	39	11 (28.2)
	Adult (>5m)	3	0 (0)
Management	Intensive	38	11 (28.9)
	Extensive	4	0 (0)
Over all		42	11 (26.2)

HPMBC= Hawassa Poultry Multiplication and Breeding Center

HCA= Hawassa College of Agriculture poultry farm

Table 2: Occurrence of *S.* Gallinarum-Pullorum by sample type in sick and dead chickens (n=42)

Sample type	No. examined	No. positive	Proportion (%)
Spleen	42	8	19.0
Liver	42	5	11.9
Ovary	29	4	13.8
Cecum	42	2	4.8
Testis	13	0	0
Cloacal swab	38	0	0
Total	206	19	9.2

RESULTS

Cloacal Swabs from Sick Chicken (CSG): Of the 115 cloacal swab samples underwent bacteriological culture test none were positive for *Salmonella*.

Postmortem Tissue Samples from Dead or Sacrificed Chickens (TSG): *Salmonella* Gallinarum-Pullorum was isolated from 11 (26.2%) of the 42 chickens examined in this group (Table 2). All the positive chickens, however, were from just one farm (HPMBC) which was affected by an outbreak of a disease during the study period. All culture positive chickens were white leghorn, aged between 4 and 20 weeks.

A total of 19 samples, 8 (19.0%) spleen, 5 (11.9%) liver, 4 (13.8%) ovary and 2 (4.8%) ceca, cultured positive for *S*. Gallinarum-Pullorum, while none of cloacal swab and testicular samples were positive (Table 3).

Table 4: Distribution of *S*. Gallinarum-Pullorum in tissue samples

Sample type	Frequency
Spleen	3
Liver	2
Ovary	1
Spleen, Liver	1
Spleen, Ovary	1
Spleen, Cecum	1
Spleen, Liver, Ovary	1
Spleen, Liver, Ovary, Cecum	1
Total	11

Salmonella Gallinarum-Pullorum was isolated from at least 2 tissue samples in 5 chickens. The bacteria were recovered from all the 4 tissue samples examined in 1 bird. Spleen was involved in all chickens in which the bacteria were isolated from more than one sample (Table 4). Eight of the 11 infected birds were females (Table 2) and 4 (50%) out of these 8 infected females had the bacteria in their ovaries.

DISCUSSION

In this study it was possible to confirm the occurrence of *S. enterica* serovar Gallinarum only in one farm which was in an outbreak situation during the study. However, none of the sick/dead chickens from other farms and those obtained from veterinary clinics were positive for the serovar. Aragaw *et al.* [7] reported occurrence of the serovar in chickens in Hawassa including in the only farm found affected in the current study. Similar to our observation Abie *et al.* [5] bacteriologically diagnosed fowl typhoid in an outbreak in a university poultry farm in Jimma, west Ethiopia, where there was up to 100 and 97% morbidity and mortality, respectively. A much earlier report documented isolation of *S.* Gallinarum from 77 diseased poultry obtained from small back-yard farms and a government hatchery in Addis Ababa [8]. Similar studies in Bangladesh also demonstrated the importance of fowl typhoid and pullorum disease in morbidity and mortality of chickens [18-23].

The isolation of *S*. Gallinarum-Pullorum from the tissues of 11 (31.4%) of the sick and dead chickens from a farm which was suffering from a disease outbreak during the study indicates that fowl typhoid/pullorum disease was responsible for significant proportion of the morbidity and mortality in the farm. Isolation of the organism from multiple tissues in significant proportion of the chickens indicates that the animals were suffering from septicemia [12].

The fact that we failed to isolate the organism from cloacal swab samples even in chickens which were having widespread systemic infection (as evidenced by isolation of the organism from multiple tissue samples) supports literature reports which indicated that *S.* Gallinarum and Pullorum are not excreted extensively in the feces [10, 24, 25].

The existence of the diseases in the HPMBC farm, which distributes chickens to farmers throughout the southern region of Ethiopia, is of a great concern as the diseases have a great potential for horizontal and vertical transmission [13, 26]. Moreover, 4 (50%) of the infected 8 female chickens had the bacteria in their ovary which may suggest the localization of the infection in the organ. Colonization of the reproductive tract with the serovars has been documented [27]. Localization in the ovary is associated with vertical transmission through eggs [12, 26]. In fact, as a result of the outbreak the farm cleared out all chickens which were on the premise during the outbreak and thoroughly disinfected houses and equipments before restocking.

The spleen was the most frequent organ culture positive for *S.* Gallinarum-Pullorum. Wigley *et al.* [27] demonstrated that *S.* Pullorum persists in spleen and the reproductive tract in experimentally infected convalescent chickens.

The findings of the present study demonstrated that fowl typhoid/pullorum diseases remain important constraints to poultry production in the study area despite earlier demonstrations of the importance of the diseases in poultry morbidity and mortality. This may show the lack or ineffectiveness of control strategies at farm and industry level in the study area. Strict biosecurity measures in farms and establishment of national regulatory programs are recommended for effective control of the diseases.

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