

Yolk Sac Infection (Omphalitis) in Kombolcha Poultry Farm, Ethiopia

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Abstract: A study was conducted from December 2010 and June 2011 in Kombolcha Poultry Farm, Ethiopia, to determine the prevalence of yolk sac infection and to isolate and identify yolk sac infection-associated bacteria and to determine drug sensitivity pattern of the predominant isolates. A total of 290 dead chicks of White Leghorn and Rhode Island Red breeds of 1 to 7 days of age were necropsied; yolk sac samples from these chicks were cultured and the bacteria were isolated and identified on biochemical tests and then tested for their susceptibility to 8 antimicrobial agents. Overall prevalence of 33.10 % (96/290) was assessed. Statistically significant difference $p < 0.05$ was noted among the different age groups. A total of 170 bacterial isolates were found, of these *Escherichia coli* (51.2%) was the most frequently isolated bacteria followed by *Staphylococcus aureus* (23.5%) and *Proteus mirabilis* (22.9%). Statistically significant association was established between the chick mortality and the bacterial isolates found $p < 0.05$. Yolk sac infection mortality was highly correlated with *E. coli* isolation. Tested bacterial isolates were showed higher susceptibility to Gentamycin, Chloramphenicol and Streptomycin, which is recommended for the treatment of yolk sac infection (YSI). In conclusion, yolk sac infection results in momentous loss to the poultry industry in Ethiopia in terms of mortality in early age and hence poses a great threat to poultry industry. Further strategies needs to be implemented to reduce the loss due to yolk sac infection.

Key words: Bacterial Isolation Rate • Drug Sensitivity • Poultry • Prevalence • Yolk Sac Infection

INTRODUCTION

Ethiopia has great potential for increased modern poultry production, both for local use and for export. However, expansion was constrained by an unsteady supply of hatching eggs, day-old-chicks, premix, or veterinary drugs, diseases, a lack of support services, insufficient data with which to plan improved services and inadequate information on how to improve animal breeding, marketing and processing [1]. Infectious diseases are remaining among the major health constraints which hampering its intended potential [2].

Yolk sac infection is the main infectious cause of chick mortality during the first week of the post-hatching period [3, 4] and is the main cause of chicks mortality accounting for large economic losses to the poultry industry [5]. It can cause mortality rate of about 5-10%; however the condition has also been associated with much higher mortality especially in chicks during first

week of age [6]. Contamination of unhealed navels has been suggested as a cause of yolk sac infection in newly hatched chicks [7].

Different types of bacterial agents are attributed for causation of yolk sac infection/omphalitis in chicks [8]. *Proteus spp.*, *Enterobacter spp.*, *Pseudomonas spp.*, *Klebsiella spp.*, *Staphylococcus spp.*, *Streptococcus spp.*, *Clostridium spp.*, *Bacillus cereus* and *Enterococcus spp.* were some bacteria that have been isolated from yolk sac infections in chicks in different locations all over the world. Nevertheless, *Escherichia coli* (*E. coli*) was frequently observed [5, 9, 10].

In Ethiopia, investigations on poultry diseases in general and yolk sac infections (omphalitis) in particular have received little attention. Till now no significant research has been reported in the country pertaining to yolk sac infections during their first week of life and continued to be the most neglected and devastating diseases of chicken. Therefore, the objectives of this

study were to assess the prevalence of YSI, to isolate and identify yolk sac infection-associated bacteria, to conduct antimicrobial susceptibility tests and to assess the predisposing factors associated with the occurrence of yolk sac infection in Kombolcha poultry farm.

MATERIALS AND METHODS

Study Area: The study was conducted during the period between December 2010 to June 2011 in Kombolcha Poultry Breeding and Multiplication Centre (KPBMC), Kombolcha, south Wollo, north eastern Ethiopia of the Amhara national regional state. It is located 380 km north of Addis Ababa and 500 km west of Bahir Dar at an altitude of 1864 meter above sea level and the centre is situated at 11° 07' N latitude and 39°44' E longitudes. The area has experienced a bimodal rain fall distribution with a three year an annual average of 1038 mm, annual mean temperature of 18°C and relative humidity from 23.9% to 79% [11].

Study Birds: The bird population on which the study was conducted includes layer types of Rhode Island Red (RIR) and White Leghorn (WLH) breeds of starter chickens aged from 1 to 7 days, which were kept for an extension service programme. During the study period a total of 8718 chicks of three batches were hatched and considered as study population.

Study Design and Sample Size: The study design consists of a cross sectional study to determine the prevalence of yolk sac infections in dead chickens of WLH and RIR breeds and to identify the prevalent bacterial species in the study area. The total numbers of birds required for this study was calculated based on the formula given by Thrusfield [12]. A total of 290 dead chicks in the first week of age were necropsied for bacteriological examination.

Data Management and Analysis: The data and laboratory results were first coded and managed into Microsoft Excel and analyzed using Statistical Package for Social Sciences software (SPSS) version 17. Descriptive analysis such as sum and frequency distribution were computed. In addition, Chi-square was employed in order to statistically determine if there is any significant difference between prevalence of infection in the study populations and age groups. Correlation test was also employed to assess the association between the chick mortality and the bacterial isolates. In all the analyses, confidence level was at 95% and $p < 0.05$ was set for significance.

Postmortem (Necropsy) Examination: All the chicks were subjected to necropsy before sampling in order to record any gross lesion on their viscera with special reference to the yolk sac infections. Postmortem examination was done according to the procedure recommended for poultry by Chauhan and Roy [13].

Sample Collection and Transportation: After necropsy, yolk sac samples were collected aseptically using sterile plain swabs and tryptose soy broth in a sterile test tube. The collected swab samples were labeled, packed and transported along with portable coolant (Ice Pac). The collected samples were stored in refrigerator at + 4 °C as mentioned by Quinn *et al.* [14] until submitted to the Kombolcha regional veterinary diagnostic and research laboratory for bacteriological examination.

Bacterial Isolation: All chicks with gross lesions of yolk sac were sampled with sterile cotton swab from the yolk sac. The swab was aseptically cultured on blood agar and MacConkey's agar for first isolation of the causative agent. All inoculated media were incubated at 37 °C and inspected for growth after 16 to 40 hours of incubation. Based on macroscopic and microscopic appearance, the developed colonies were selected from each sample and subcultured on appropriate differential media for further identification.

Bacterial Identification: Identification of the pure isolates was done on the basis of staining, colony morphology, cultural and biochemical character of pure isolates by using standard bacteriological and biochemical procedures as described by Quinn *et al.* [14] and Swayne *et al.* [15].

Antimicrobial Susceptibility Test: The most predominant bacterial isolates associated with yolk sac infection were subjected to *in vitro* antibiotic sensitivity test by single disc diffusion method as described by Bauer *et al.* [16] with 8 different antibiotic discs. A total of 8 antibiotic discs with Streptomycin 10 [micro]g, Erythromycin 15 [micro]g, Chloramphenicol 30[micro]g, Tetracycline 30[micro]g, Penicillin G 10 [micro]g, Ampicillin 10 [micro]g, Gentamicin 10[micro]g and Bactercin 10 [micro]g were used. The antimicrobial agents were categorized into susceptible, intermediate and resistant categories according to National Committee for Clinical Laboratory Standards [17].

RESULTS

Prevalence of Yolk Sac Infection: Out of the 290 sampled dead chicks, yolk sac infection was observed in 96 (33.10 %) dead chicks. Maximum percentage (64.28%) of yolk sac infection was observed in chicks of 5 day old followed by age of 3 day (42.21%), 2 day (34.48%), 6 day (31.71%), 4 day (31.25%), 1 day (20%) and 7 day (17.74%). There was a statistically significant difference $p < 0.05$ in the prevalence of yolk sac infection (YSI) at the different studied days during the first week of life of birds as shown in Table 1.

Bacterial Isolation Rates: Isolation frequency of the bacterial strains obtained in this study is summarized in Table 2. All the yolk sac infected dead chicks were positive for bacterial isolation, of which, fifty seven samples revealed mixed bacterial infection (59.37 %) whereas single bacterial species was isolated from thirty nine samples (40.62 %). Out of the total 170 different bacterial strains isolated belonging to different genera, *E. coli* 87 (51.17 %) was the most predominant isolate followed by *staphylococcus aureus* 40 (23.53%) and *Proteus mirabilis* 39 (22.94 %), whereas *streptococcus species* 2 (1.18 %) and *Bacillus cerus* 2 (1.18 %) were the least in rate of isolation (Table 2).

Table 1: Mortality rates of chicks due to yolk sac infection during the first week of life

Age	No. of birds examined	No. of positive	Prevalence (%)	95% CI	Chi ²
Day 1	45	9	20.00	0.0107-0.038	29.642
Day 2	58	20	34.48	0.2356-0.4733	
Day 3	26	11	42.31	0.2555-0.6105	
Day 4	16	5	31.25	0.1416-0.556	
Day 5	42	27	64.28	0.4917-0.7701	
Day 6	41	13	31.71	0.1957-0.4699	
Day 7	62	11	17.74	0.102-0.2904	
Total	290	96	33.10	0.2794-0.3871	

Moreover, out of the 96 positive cultured chicks, *E.coli* were isolated from 87 chicks (90.63 %), *staphylococcus aureus* were recovered from 40 chicks (41.67 %), *streptococcus species* were isolated from 2 chicks (2.08 %) and *proteus mirabilis* isolated from 39 chicks (40.63%) and *Bacillus cerus* were isolated from 2 chicks (2.08 %). A correlation between the chick mortality and the frequency of isolation of the bacterial species found in this study is presented in Table 2. Yolk sac infection mortality was highly correlated with *E. coli* infection (Pearson's $R = 0.931$, $p = 0.000$) during the first week of life in this study as shown in Table 2.

Table 2: Association between mortality due to yolk sac infection and the different bacterial isolates during the first week of life

Bacterial Isolates	Mortality rates during the first week of life (1-7 days)							Total Percentage of isolates	95% CI	Pearson's R
	Day 1(n = 45)	Day 2(n = 58)	Day 3(n = 26)	Day 4(n = 16)	Day 5(n = 42)	Day 6(n = 41)	Day 7(n = 62)			
<i>Escherichia coli</i>	9 (20 %)	18 (31%)	9 (34.6%)	5(31.3 %)	25 (59.5%)	12(29.3%)	9(14.5%)	87(51.2%)	0.4372-0.5858	0.931
<i>Staph. aureus</i>	2 (4.4%)	10(17.2%)	6 (23.1%)	0 (0%)	13 (30.9%)	7 (17.1%)	2 (3.2%)	40(23.5%)	0.1778-0.3045	0.569
<i>Protius mirabilis</i>	1 (2.2%)	9 (15.5%)	4 (15.4%)	3(18.8%)	11(26.2%)	7(17.1%)	4(6.5%)	39(22.9%)	0.1726-0.2982	0.560
<i>Streptococcus spp.</i>	0 (0 %)	1 (1.7%)	0 (0%)	0 (0%)	1 (2.4%)	0 (0%)	0 (0%)	2 (1.2%)	0.0032-0.0419	0.118
<i>Bacillus cerus</i>	1 (2.2%)	0 (0%)	1 (3.9%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	2 (1.2%)	0.0032-0.0419	0.118
Total	13(7.7%)	38 (22.4%)	20 (11.8%)	8 (4.7%)	50 (29.4%)	26 (15.3%)	15 (8.8%)	170 (100%)		

n = number of dead birds examined

Table 3: Antimicrobial Susceptibility pattern of the most frequently isolated bacteria due to yolk sac infection during the first week of life

Antimicrobial Agent with their code and disc potency	<i>E.coli</i> (n = 87) isolates			<i>S. aureus</i> (n = 40) isolates			<i>Proteus mirabilis</i> (n = 39) isolates		
	Resistant N (%)	Intermediate N (%)	SusceptibleN (%)	Resistant N (%)	Intermediate N (%)	Susceptible N (%)	Resistant N (%)	Intermediate N (%)	SusceptibleN(%)
Tetracycline (TE) 30µg	81 (93.10)	6 (6.9)	0 (0)	37 (92.5)	3 (7.5)	0 (0)	27 (69.2)	12 (30.8)	0 (0)
Streptomycin (S) 10 µg	0(0)	7 (8.05)	80 (91.95)	0 (0)	5 (12.5)	35 (87.5)	0 (0)	39 (100)	
Gentamycin (GN)10 µg	0 (0)	0 (0)	87 (100)	0 (0)	0 (0)	40 (100)	0 (0)	0 (0)	39 (100)
Bactercin (B) 10 µg	87 (100)	0(0)	0 (0)	40 (100)	0 (0)	0 (0)	39 (100)	0 (0)	0 (0)
Erythromycin (E)15 µg	68 (78.16)	19 (21.83)	0 (0)	13 (32.5)	27 (67.5)	0 (0)	9 (23.1)	30 (76.9)	0 (0)
Ampiciline (AMP) 10 µg	59 (67.82)	28 (32.18)	0 (0)	19 (47.5)	21 (52.5)	0 (0)	17 (43.6)	22 (56.4)	0 (0)
Chloramphenicol (C) 30 µg	0 (0)	0 (0)	87 (100)	0 (0)	0 (0)	40 (100)	0 (0)	4 (10.26)	35 (89.74)
Peniciline G (P) 10 µg	77 (88.5)	10 (11.5)	0 (0)	39 (97.5)	1 (2.5)	0 (0)	33 (84.7)	6 (15.3)	0 (0)

Necropsy (Gross Lesion) Findings: The major gross lesions observed in chicks died of yolk sac infection were unabsorbed yolk sac, congestion and discoloration of the yolk (greenish yellow; dark brown to bright yellow), retained caseous yolk sac and edematous yolk (especially in 3-7 days old chicks). The yolk sac infection was usually associated with peritonitis, pericarditis, petechial and ecchymotic hemorrhages on the serosal surface of visceral organs (the intestine).

In vitro Drug Sensitivity Test Result: The results of the *in vitro* drug sensitivity test were summarized in Table 3.

DISCUSSION

In this study, prevalence rate of 33.1% was recorded in dead sampled White Leg horn (WLH) and Rhode Island Red (RIR) breeds during the first week of life. This is the first report in Ethiopia pertaining to YSI during the first week life of chicks. It was observed that there was a statistically significant difference ($p < 0.05$) in the mortality rate of chicks due to YSI at different days of the first week (1-7 days) life of chicks. The mortality peak was observed at day 5. Mortality rate declined thereafter until 7 days post hatch. Similar results were reported by Rosario *et al.* [10] who indicated that the YSI mortality curve lasts 7-10 days, it peaks at 4-5 days and decreases during the following 3 to 5 days. The relatively higher first week mortality of chicks when compared to different parts of the world [6, 18, 19] due to yolk sac infection in the present study can be attributed to the poor hatchery management and hygienic activities adopted in the farm. Nevertheless, this finding was in conformity with the reports of Rahman *et al.* [6] that assessed 31.45% and 28.42 % mortality in chicks due to yolk sac infection. Yolk Sac Infection mortality was highly correlated with *E. coli* isolation (Pearson's $R = 0.931$, $p = 0.000$), which is in conformity with the findings of Rosario *et al.* [10].

In the present study, *E. coli* 87 (51.17 %) was the most predominant isolate followed by *staphylococcus aureus* 40 (23.53%), *Proteus mirabilis* 39 (22.94 %) and other bacteria like *streptococcus species* and *Bacillus species* in lower proportion. Mixed bacterial infections were the predominant cases recorded due to *E. coli* together with any of the three species (*Staphylococcus aureus*, *Proteus mirabilis* and rarely with *Bacillus cerus*). *Escherichia coli* has been previously reported as one of the most frequently isolated organisms involved in the development of yolk sac infection [20, 21].

Involvement of *Staphylococcus species*, *Proteus species*, *Streptococcus* and *bacillus species* has also been reported previously [10, 21, 22].

The gross lesions observed in chicks died of yolk sac infection included unabsorbed/ retained yolk sac and edematous yolk which was also reported by different workers [18, 21-23]. From the *in-vitro* drug sensitivity test result, tested bacterial isolates were showed higher susceptibility to Gentamycin, Chloramphenicol and Streptomycin, which were in agreement with the previous reports [10, 21, 24-26].

In conclusion, the results of the present study in Kombolcha poultry farm entailed that the importance of yolk sac infection (YSI) in causing the high mortality of chicks during their first week life post-hatching and thus posing a great threat to the poultry industry in Ethiopia. However, the disease has received little attention. Also Gentamicin, Chloramphenicol and Streptomycin may be effective in reducing the early mortality of chicks due to yolk sac infection. Moreover, a further study on epidemiological investigation of Yolk sac infection throughout the country, economical impact of YSI, experimental studies on breed susceptibility to Yolk sac infection and on the solutions to prevent and control the disease is therefore, encouraged.

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