

Isolation and Characterization Of Pathogenic Bacteria In Chickens Causing Morbidity and Mortality

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Abstract: A study was conducted between November 2009 and April 2010 in Hawassa town of Sidama zone of Southern Nations, Nationalities and Peoples Regional State (SNNP) to isolate and characterize pathogenic bacteria causing morbidity and mortality in chickens. Bacterial culturing and Gram staining were used for identification of bacteria. Further characterization was done using biochemical tests. A total of 50 chickens including 35 sick and 15 recently dead were used. A total of 250 samples were aseptically collected from lungs, spleens, intestine, livers and heart blood of the chickens. Ten genera of potentially pathogenic bacteria were isolated from 36% (18/50) chickens. The identified genera/species of bacteria were: Enterococcus, *E. coli*, motile Salmonella, *S. aureus*, Pseudomonas, Bacillus, Pasteurella, Yersinia, Edwardsiella and Enterobacter. Enterococcus was the most prevalent bacteria 14% (7/50) followed by *E. coli* with five isolates and Salmonella with three isolates. Pseudomonas, *S. aureus* and *Bacillus* were all isolated from two chickens each and Pasteurella, Yersinia, Edwardsiella and Enterobacter from one chicken each. Most isolates were recovered from lung (13), followed by heart blood (9) and liver (6). Mixed bacterial infections were detected in six chickens while single bacterial pathogens were isolated from twelve chickens. In conclusion, the study revealed that bacterial pathogens are highly involved in morbidity and mortality of chickens in the study area challenging the poultry industry calling for remedial interventions.

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Key words: Bacterial pathogens, Chicken, Hawassa, Mortality, Morbidity

1. Introduction

Ethiopia is among the leading countries by livestock population. Currently Ethiopia has over 49.3 million cattle, 46.9 million small ruminants, 42.1 million poultry and around 2.3 million camels (CSA, 2008). This number ranked the countries livestock population 1st in Africa and 9th from the world. This number is by no means sufficient especially when it is seen in relation to the population of the country and the low productivity of indigenous animals (Aklilu, 2007).

The structure of the poultry industry has changed dramatically over the past 50 year's world wide. There is a world wide movement of poultry production and breeds. However, avian disease problems have no respect for international borders (Hirsh and Zee, 1999). Though it could be done with minimum effort, there are many problems which don't let the sector grow, like poor husbandry system and less attention by the government can be mentioned. But the main constraint is the high mortality rate of poultry because of disease. Mortality due to disease is estimated to be 20% (Alamargot, 1987; Dessie and Jobre, 2004). Especially bacterial diseases continue to cause significant economic losses in the poultry industry. They are of more sporadic occurrence and limited distribution. Their potential public health

significance can be important when and where they occur (Barnes, 1994). The consumer is becoming increasingly sensitive to animal welfare and public health and food safety issues, eg. *Salmonella enteritidis* infection in human associated with table eggs (Calnek *et al.*, 1994).

Diagnostic facilities, both private and government, are available in major poultry producing areas of the world, yet disease takes a heavy toll from all types of poultry enterprises (Adams, 1994). The poultry scientists and veterinary professionals must make every effort to provide the consumer with safe and wholesome poultry products at reasonable cost and remain heavy loss from disease (Zander and Mallinson, 1994). The objective of this study was to isolate and characterize pathogenic bacteria causing morbidity and mortality in chickens.

2. Materials and Methods

2.1 Study area: The study was conducted in Southern Nations Nationalities and Peoples Regional State (SNNPRS) in Sidama Zone, Hawassa town. The town is located in the Great Rift valley, 270 km south of Addis Ababa. The population of the city is estimated about 162,179. Geographically the town lies between 7°3' N 38° 28' E latitude and 7.05° N 38. 467°

E longitude. The annual rain fall of the area is 800-1000 mm and the mean temperature ranges from 20.1°C – 25°C. The town's elevation is 1,708 m above sea level.

There are no large commercial poultry units in Hawassa, there are small scale farms operated by NGOs and private owners mainly for the purpose of table egg production. These farms use exotic breeds of chicken on deep litter floor. The largest poultry farm on the town in the Awassa poultry Multiplication and Breeding center (APMBC), which hold about 26,000 chickens and is run by the region's bureau of agriculture. Nevertheless many households in the town and its vicinity keep local chicken in backyards with extensive management (Scavenging) system.

Table 1. Summary of chickens used in the study: SNNP, Hawassa, 2010

Variables	Number
Source	
<i>ACA</i>	7
<i>APF</i>	23
<i>Backyard</i>	20
Breed	
<i>Local</i>	20
<i>RIR</i>	30
Age	
<i>Young</i>	24
<i>Adult</i>	26
Management	
<i>Extensive</i>	20
<i>Intensive</i>	30
Status	
<i>Sick</i>	35
<i>Dead</i>	15

NB. ACA: Hawassa College of Agriculture; APF: Hawassa Poultry Farm; RIR: Rhode Island Red

2.2 Study animals: A total of 50 sick and dead chickens were used for the study. The study chickens included 35 sick and 15 recently dead birds. Study chickens were obtained from Awassa Poultry Multiplication and Breeding center (APMBC) and Awassa College of agriculture (ACA) free of charge. Sick local birds were bought from the local market for the study. The chickens from the intensive poultry farms were all Rhode Island Red (RIR) kept mainly by the farms for table egg production (ACA) and distribution (APMBC). All sick chickens obtained from the market were of local type and raised under extensive management. All the intensive farms from which sample chickens were collected raise the birds on deep litter. Pullets in APMBC receive vaccines against Newcastle disease and IBD and a prophylactic treatment for coccidian. Additionally the parent stock receives vaccines for fowl typhoid and fowl pox. All

chickens kept at ACA are vaccinated against Newcastle disease and IBD. The details of chickens used in the study are summarized in.

2.3 Study design: The study was a cross sectional observational study conducted between November 2009 and April 2010 at Hawassa. The study involved 50 sick and dead chickens of various age and both sexes obtained from intensive farms and backyard system.

2.4 Sample Collection Procedures

2.4.1 Collection of chickens: Sick and dead chicken those died in less than 12 hours were brought to the microbiology laboratory of veterinary medicine department of Hawassa University for bacteriological examination.

2.4.2 Sample organ collection: Sick birds were first physically examination for any signs like respiratory distress, changes in faeces, signs of ocular and nasal discharge and other visible abnormalities according to Tully *et al.*, (2003). Then this signs were recorded. Sick birds were killed by holding both wings over the back with the hand and the head with other in such a way as to bend the head sharply vertically at the same time it was pulled firmly and quickly forward in a steady manner which breaks the neck and spinal cord instantly. Then the bird was laid on its back and each leg in turn drawn outward away from the abdomen on each side. Both legs were then grasped firmly in the area of the femur and bent forward, downward and outward until the heads of both femurs are broken free of the acetabular attachment so both legs laid flat on the table. The skin was cut between the two previous incisions at a point mid way between keel and vent. The cut edge was then forcibly reflected forward until the entire ventral aspect of the body was exposed. After opening the body, using a sterile forceps, scissors and scalpel blade, samples of liver, lung, spleen, heart blood and part of cecum were taken turn by turn. Between each sampling the equipments were sterilized using 70% ethyl alcohol and Bunsen burner flame. Then the samples were placed on a sterile Petri dish separately and the surfaces of the organs were seared with hot scalpel blade and at the seared area sterilized cotton swabs were introduced through the cut to collect sample for inoculation. Collected samples were then streaked on blood agar (sheep or ox blood) and incubated aerobically at 37°C for 24 hours. Using the above method totally 50 liver, 50 lung, 50 spleen, 50 heart blood and 50 cecum samples were examined during the study.

2.5 Isolation, Identification and Characterization Of Bacteria

2.5.1 Primary identification: Bacterial identification was done according to Quinn *et al.*,

(2004). Growth characteristics of bacteria on blood agar were noted and if there was no growth incubated for additional 24 hours. Then colonies with different growth characteristics were sub cultured on blood and MacKonkey agar. On blood agar; presence and absence of bacterial growth, morphological characteristics of the bacterial colony like size and shape, texture (Rough, Muroid, Smooth, etc). Presence of haemolysis, type of haemolysis (Beta, alpha) and smell. On MacKonkey agar; presence and absence of bacterial growth, colony character (Size, color) and presence and absence of lactose fermentation were noted and recorded. The isolates were Gram stained to see their gram characteristics and morphology. Motility, catalase and oxidase tests were performed and for gram positive cocci bacteria coagulase test was done. The pure isolates were streaked on brain heart infusion (BHI) agar slants and incubated at 37°C for 24 hours and kept in a refrigerator for further characterization.

2.5.2 Secondary biochemical testes: For Gram negative bacteria carbohydrate fermentation test was performed. For gram negative rod shaped bacteria grown on MacKonkey agar Indole, Methyl Red, Voges-Proskauer, Citrate utilization tests – IMVIC and lysine decarboxylation tests were done. The sample was inoculated on blood agar and incubated for 24 hours at 37°C. Then, colonies were characterized phenotypically and Gram staining was conducted to differentiate the bacteria. For further characterization the samples were sub-cultured on MacKonkey agar and BHI and incubated for 24 hours at 37°C. Primary identification was done using biochemical tests such as catalase test, oxidase test, motility test and coagulase test. Whereas, secondary identification was done by carbohydrate fermentation test, IMVIC and Lysine decarboxylation test.

2.6 Data analysis: Microsoft Excel was used for data organization. Percentages, tables and graphs were used to summarize the findings.

Results

Of the total chickens examined 36% were found infected with one or more potentially pathogenic bacteria. A total of ten genera of potentially pathogenic bacteria were identified including: *Enterococcus*, *E. coli*, motile *Salmonella*, *S. aureus*, *Pseudomonas*, *Bacillus*, *Pasteurella*, *Yersinia*, *Edwardsiella* and *Enterobacter*. The organs examined for the presence of the bacteria were liver, lung, spleen, heart blood and cecum.

Enterococcus was the most prevalent bacteria identified in terms of number of infected chickens being found in 14% (7/50) chickens involving lungs (7), heart blood (4) and liver (2). The next most prevalent bacteria were *E. coli* which was isolated

from 10% (5/50) chickens in liver (2), lung (2) and heart blood (2). The above two genera constitute 24% of the prevalence. The other bacteria isolated were motile *Salmonella* (6%) isolated from spleen (1), lungs (1) and heart blood (1). *Staphylococcus aureus* (4%) was recovered from liver (1) and spleen (1). *Pseudomonas* (4%) was identified from liver (1) and heart blood (1), while *Bacillus* (4%) was isolated from lung (1) and intestine (1). *Pasteurella*, *Yersinia*, *Edwardsiella* and *Enterobacter* were isolated from heart blood, lung, intestine and lung, respectively (Table 2).

Table 2. Animal level prevalence of bacterial pathogens isolated from sick and dead chickens: SNNP, Hawassa, 2010.

Bacterial species/genus	Number infected	prevalence
<i>E. coli</i>	5	10%
<i>Enterococcus</i>	7	14%
<i>Salmonella</i>	3	6%
<i>S. aureus</i>	2	4%
<i>Pseudomonas</i>	2	4%
<i>Bacillus</i>	2	4%
<i>Pasteurella</i>	1	2%
<i>Yersinia</i>	1	2%
<i>Edwardsiella</i>	1	2%
<i>Enterobacter</i>	1	2%
Unidentified	1	2%

A total of 32 isolates were recovered from 250 samples from 35 sick and 15 dead birds. The largest numbers of the isolates were from lungs (13) and the next most infected organ/tissue was heart blood (9), followed by liver (6), spleen (2) and intestine (2). Five genera/species of bacteria were isolated from lung and heart blood, 4 from liver and 2 each from spleen and intestine (Table 3).

Different genera of bacteria were found concurrently in some chickens probably causing mixed infection. *Escherichia coli* and *Pseudomonas* from a liver of sick chicken, *Enterococcus* from lung and heart blood and *Edwardsiella* in the intestine from another sick chicken, *Enterococcus* from lung and *Salmonella* from heart blood of a dead chicken, *Enterococcus* from lung and liver and *Bacillus* from intestine in another dead chicken, *Salmonella* from spleen and *E. coli* from heart blood of other dead chicken and *Yersinia*, *Salmonella* and *Bacillus* all from lung was found in a sick chicken. Generally mixed infection occurred in about 12% (6) of the chickens. The summary of mixed infection is expressed in the table below (Table 4).

Some bacteria were found in a given chicken in different organs in a disseminated manner. *Escherichia coli* was found in liver and heart blood of

one chicken. *Enterococcus* was recovered from lung, liver and heart blood of a chicken, in lung and heart

blood in other 3 chickens and in lung and liver of one chicken (Table 5).

Table 3. Bacterial pathogens isolated from study chickens by organ: SNNP, Hawassa, 2010.

Organ	Bacterial species/genus	Frequency	Mixed infection	Frequency
Liver	<i>E. coli</i>	2	<i>E. coli, Pseudomonas</i>	1
	<i>Pseudomonas</i>	1		
	<i>Enterococcus</i>	2		
	<i>S. aureus</i>	1		
Spleen	<i>Salmonella</i>	1		
	<i>S. aureus</i>	1		
	Unidentified	1		
Lung	<i>E. coli</i>	2	<i>Yersinia, Salmonella, Bacillus</i>	1
	<i>Enterobacter</i>	1		
	<i>Enterococcus</i>	7		
	<i>Yersinia</i>	1		
	<i>Salmonella</i>	1		
	<i>Bacillus</i>	1		
Heart blood	<i>E. coli</i>	2		
	<i>Enterococcus</i>	4		
	<i>Pseudomonas</i>	1		
	<i>Pasteurella</i>	1		
	<i>Salmonella</i>	1		
Intestine	<i>Bacillus</i>	1		
	<i>Edwardsiella</i>	1		

Table 4. Multiple bacterial infections of sick and dead chickens: SNNP, Hawassa, 2010

Pathogens Involved	Organs	Status
<i>E. coli</i> and <i>Pseudomonas</i>	Liver	live
<i>Enterococcus</i>	Lung, Heart Blood	live
<i>Edwardsiella</i>	Intestine	
<i>Enterococcus</i>	Lung	dead
<i>Salmonella</i>	Heart blood	
<i>Enterococcus</i>	Lung, liver	dead
<i>Bacillus</i>	Intestine	
<i>Salmonella</i>	Spleen	dead
<i>E. coli</i>	Heart blood	
<i>Yersinia</i>	Lung	live
<i>Salmonella</i>	Lung	
<i>Bacillus</i>	Lung	

Table 5. Pathogenic bacteria isolated from more than one organ in sick and dead chickens: SNNP, Hawassa, 2010

Bacterial species	Organs involved	Number of chicken	Status
<i>E. coli</i>	Liver, Heart blood	1	live
<i>Enterococcus</i>	Lung, liver, Heart blood	1	live
<i>Enterococcus</i>	Lung, Heart blood	3	live
<i>Enterococcus</i>	Lung, Liver	1	dead

Discussion

We found that of the total 50 chickens (35 sick and 15 dead chickens) 18 (36%) of them were infected with potentially pathogenic bacteria. Hossain *et al.* (2006) reported bacterial diseases as major problem in poultry industry in Bangladesh,

responsible for 39.8% mortality and morbidity. A study conducted in intensively managed chickens in Hawassa town revealed bacterial agents were involved in 54% of the mortalities and morbidity (Wondimu, 2009). The proportion of sick and dead chickens with potentially pathogenic bacteria was lower in the

present study compared that of Wondimu (2009). This might be due to change in the epidemiology of diseases with time. Some study chickens were showing signs of viral infections during the present study.

In this study the most prevalent bacteria was *Enterococcus* (14%), which is a normal flora of the intestine, however, known to cause septicemia in immunosuppressed chickens (Quinn *et al.*, 2004). Streptococcosis (*Enterococcus*) in avian species is worldwide in distribution occurring as both acute septicemia and chronic infections with mortality ranging from 0.5% to 50% (Wages, 1994). The bacterial agent was identified in chickens taken from the same farm consecutively; this may indicate that there was a mild outbreak in the farm in a septicemic manner.

The other frequently identified bacterium in this study was *E. coli* (10%). The results from cecum were excluded because *E. coli* is among the normal flora of intestine. *Escherichia coli* infections are responsible for significant economic losses to the poultry industry (Gross, 1994). When an imbalance occurs in bacterial flora of the intestinal tract, *E. coli* may grow and cause an outbreak of colibacillosis. Many investigators doubt that *E. coli* is a primary pathogen; others are convinced that certain serotypes are primary pathogens and agree that *E. coli* frequently can be isolated from a variety of well defined syndromes in poultry especially in young ages (Charlton *et al.*, 2006). Similar to the findings of the present study previous reports indicated that *E. coli* was involved in 10% of sick and dead chicken (Wondimu, 2009). Colibacillosis was also implicated in 8.4% of mortalities and morbidities in chickens in Bangladesh (Rahman and Samed, 2003).

Salmonellae were recovered from 6% (3) of the study chickens. A similar observation (4%) was recorded by Wondimu (2009) in the same study area. However our result is lower compared to a report that identified *Salmonellae* as a cause of mortality in 23.11% of chickens (Islam *et al.*, 2006). It was also lower compared to 11% (Hossain, 2003) and 21% (Akter *et al.*, 2007) prevalence of the bacteria in sick and dead chickens. Salmonellosis in poultry causes heavy economic loss through mortality and reduced production (Khan *et al.*, 2006). Domestic poultry constitutes the largest single reservoir of *Salmonellae* existing in nature. Among all animal species the *Salmonellae* are most frequently reported from poultry. The relatively host-specific and non-motile members of the genus, *S. pullorum* and *S. galinarum* cause pullorum disease and fowl typhoid respectively. Motile *Salmonellae*, the types isolated in the present study, are generally blamed to cause the paratyphoid infection (Snoeyenbos and Williams, 1994).

Staphylococcus aureus was one of the bacterium found with the prevalence of 4%. Staphylococcal septicemia affecting laying birds and causing acute death seems to be prevalent in hot weather (Skeels, 1994). Other study on *staphylococcus* shows that 46% isolated bacteria from dead chickens were staphylococcus (Haider *et al.*, 2004). Wondimu (2009) reported that the bacterium was found in 20% of the dead and sick chickens.

Pseudomonas can cause localized or systemic disease in young and growing poultry, invade fertile eggs causing death of embryos. *P. aeruginosa* is the most common cause of infection (Barnes, 1994). This organism was found to infect 2 of the chickens under study (4%). In another study on this area shows that it was found to involve in 2% bacterial causes mortality and morbidity in chicken (Wondimu, 2009).

Pasteurellosis is a general term used to designate a group of diseases caused by *Pasteurellae* bacteria. *Pasteurella* is among the several opportunistic bacteria. The virulent species is *P. multocida* which is capable of causing up to 68% mortality in susceptible flocks (Rhoades and Rimler, 1994). The prevalence of these bacteria in this study was 4%. This result was not different from a 2% report by Wondimu (2009) in Hawassa and 3.3% elsewhere (Haider *et al.*, 2004). However it was slightly lower compared to 6.5% reported from Bangladesh (Rahman and Samed, 2003).

In the current study about 12% of the chickens were affected by concurrent bacterial infections. Rahman and Samed (2003) recorded concurrent or mixed infections of around 23% in sick and dead chickens. Mixed infections have been recognized as a frequent problem in commercial poultry and may be caused by a combination of different microorganisms (Rahman and Samed, 2003).

Conclusions

We found that *Enterococcus* was the most prevalent potentially pathogenic bacteria followed by *E. coli* and *Salmonella*. *Bacillus*, *Pseudomonas*, *S. aureus*, *Yersina*, *Enterobacter* and *Edwardsiella* were also involved. The identification of motile *Salmonella* in this study could show its public health significance for its zoonotic nature beyond the loss in the poultry industry. In conclusion, the study revealed that bacterial pathogens are highly involved in morbidity and mortality of chickens in the study area challenging the poultry industry calling for remedial interventions.

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