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Research article

Epidemiological investigations of Salmonella and Escherichia coli associated morbidity and mortality in layer chickens in Hawassa city, Southern Ethiopia

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ABSTRACT

Background: This study was conducted with the objectives of estimating the morbidity and mortality rates in layer chickens, identifying the risk factors associated with morbidity and mortality, and identifying the major bacterial pathogens affecting small-scale commercial layers in Hawassa. A longitudinal observational study design was employed from November 2019 to March 2020. The chickens on selected farms were checked for morbidity and mortality twice a week. During each visit, clinical examination of sick birds and pathological investigation of dead birds were conducted. Cloaca samples were collected for isolation and identification of *Salmonella* spp. and *E. coli*. A Cox proportional hazard model was used to quantify the effects of various risk factors on the morbidity and mortality rates observed.

Results: Of the 8976 chickens followed, 106 developed clinical disease, giving a morbidity of 1.18% (95% CI: 0.97, 1.43). The overall morbidity rate was 2.37 (95% CI: 1.94, 2.87) per 1000 chicken months. A total of 101 of the chickens under study were found dead, yielding a mortality of 1.13% (95% CI: 0.92, 1.37) and a mortality rate of 2.26 (95% CI: 1.84, 2.75). Multivariable Cox regression analysis showed that farm hygiene, the experience of farm manager, housing condition, housing systems, the availability of veterinary services and age of chicken were important risk factors for morbidity and mortality. Out of 58 cloacal samples collected from sick chickens, 7 (12.07%; 95% CI: 4.99, 23.29) yielded positive results for *Salmonella* spp., while 25 (43.10%; 95% CI: 30.16, 56.77) yielded positive results for *E. coli*. Out of swabs collected from 8 randomly selected sick chickens after necropsy, 3 (37.5%) were found to be positive for *Salmonella* spp. Four (50%) of them were positive for *E. coli*. Swabs were collected and cultured from 15 dead chickens, and of these, 2 (13.33%) and 7 (46.67%) were found to be positive for *Salmonella* spp. and *E. coli*, respectively. Farm hygiene, age of chickens, housing conditions and frequency antibiotics use were important risk factors for colibacillosis and salmonellosis.

Conclusions: Although the incidence of chicken morbidity and mortality was relatively low in the present study, important risk factors have been identified in the poultry farms of Hawassa City, southern Ethiopia. Therefore, comprehensive poultry farm management practices are needed to mitigate risk factors for morbidity and mortality as well as colibacillosis and salmonellosis. Identification of the serotypes of *Salmonella* spp. and *E. coli* should be carried out.

1. Introduction

Poultry production is an integral part of urban agriculture in Ethiopia. It is a viable income generation activity to support and improve the welfare of the producers and the community. Poultry products are a cheap source of protein that is vital for maintaining the health of children, mothers, and needy people. It seeks to create employment opportunities for the youth and women so that they cater for themselves and, to some extent, the household needs. Hawassa city is one of the densely populated cities in Ethiopia. There are a large number of needy mothers,

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children, and others who need sufficient protein. In addition, the city is one of the destinations for tourists. Basically, there is a huge demand for animal protein in Hawassa. Poultry production, thus, plays an important role in improving the national economy, household sustainability, and food and nutrition security in developing countries [1, 2]. Therefore, poultry production contributes to the reduction of the supply-demand gap in animal proteins.

Taking this into account, several government-led programs have attempted to improve chicken production as a strategy to reduce poverty [3]. Elsewhere in the country, especially in central Ethiopia, there is a gradual increase in commercial and small-scale chicken enterprises [4]. In contrast, in Hawassa, which is the capital of the Sidama Region, as well as a business and tourism center in the southern half of the country, commercial chicken production is a more recent enterprise. Several small-scale farms have been established and have started to produce and supply table eggs and meat to various supermarkets, kiosks, and roadside restaurants in Hawassa and beyond.

Chicken production in Hawassa is, however, affected by several technical and nontechnical factors. Among the known problems that negatively affect the productivity of chickens are the occurrences of infectious and noninfectious diseases [5]. Infectious diseases reduce egg production and retard growth (meat production), thereby significantly impairing the economics of the poultry industry [1, 6]. Optimum poultry production, therefore, requires an understanding of the epidemiology of infectious diseases, such as the incidence of morbidity and mortality, which are useful for quantifying the financial losses incurred. The magnitude of morbidity and mortality in small-scale poultry farms in Hawassa has not been studied. The differential diagnosis of the pathogens responsible for morbidity and mortality has also not been established. This study was carried out with the objectives of (1) estimating the morbidity and mortality rates in layer chickens, (2) identifying the risk factors associated with morbidity and mortality, and (3) identifying the major bacterial pathogens affecting small-scale commercial layers in Hawassa.

2. Results

2.1. Morbidity and mortality rates in laying chickens in Hawassa city

Of the 8976 chickens followed for five months (November 2019 to March 2020), 106 developed clinical diseases. That is, the morbidity was 1.18% (95% CI: 0.97, 1.43). The study chickens developed the clinical disease within a total of 44,666 chicken months at risk given an overall morbidity rate of 2.37 (95% CI: 1.94, 2.87) per 1000 (Table 1). The morbidity rate was highest in Farm 1 and lowest in Farm 3. During the period under investigation, 101 of the chickens under study were found dead, yielding a mortality of 1.13% (95% CI: 0.92, 1.37). The overall chicken months elapsed for mortality was 44,674, giving a mortality rate of 2.26 (95% CI: 1.84, 2.75). Similar to the morbidity rate, the highest mortality rate was observed in chickens reared on Farm 1, whereas the lowest was observed on Farm 3 (Table 2). The case fatality observed in this study was 95.28% (95% CI: 89.33, 98.45).

Table 1. The morbidity rates observed in layer chickens at different farms in Hawassa city, Ethiopia.

Farm	Chicken at risk	No. of cases	Chicken months	Morbidity rate per 1000
Farm 1	798	21	3940	5.32 (95% CI: 3.29, 8.12)
Farm 2	300	4	1492	2.68 (95% CI: 0.73, 6.85)
Farm 3	1000	6	4983	1.20 (95% CI: 0.44, 2.62)
Farm 4	6878	75	34,251	2.19 (95% CI: 1.72, 2.74)
Total	**8976**	**106**	**44,666**	**2.37 (95% CI: 0.97, 1.43)**

Table 2. Mortality rates in layer chickens reared on different farms in Hawssa city, southern Ethiopia.

Farm	Chicken at risk	Deaths	Chicken months	Incidence rate per 1000
Farm 1	798	51	3868	13.19 (95% CI: 9.83, 7.29)
Farm 2	300	3	1496	2.00 (95% CI: 0.41, 5.85)
Farm 3	1000	0	5000	0 .00 (95% CI: 0.00, 5.97)
Farm 4	6878	47	34,310	1.37 (95% CI: 1.01, 1.82)
Total	**8976**	**101**	**44,674**	**2.26 (95% CI: 1.84, 2.75)**

2.2. Factors associated with morbidity in layer chickens in Hawassa city

The results of multivariable Cox regression analysis indicated that farm hygiene, the experience of the farm manager, housing condition, and age of chicken were significantly associated with morbidity (Table 3). Keeping layer chickens on farms with good hygiene significantly reduced morbidity (HR = 0.005, P < 0.001) compared to keeping them on farms with poor hygiene. In farms that were managed by more experienced managers, the hazard of morbidity was lower by nearly 44% (HR = 0.435, P = 0.002) compared to those farms managed by less experienced managers (Table 3).

The experience of the farm managers, breed of chicken, availability of veterinary service, regular vaccination programs, type of farm ownership, housing system, and age of chicken were significantly associated with mortality. The results of multivariable Cox regression analysis showed that the relative hazard of mortality was significantly lower in chickens managed by more experienced farm managers than in chickens managed by less experienced farm managers. The relative hazard of mortality was significantly lower in Bovans brown than in Sasso. Interestingly, the hazard of mortality was nearly 22 times higher in farms that did not have adequate veterinary services than in those farms having adequate veterinary services. The occurrence of mortality was significantly higher in private farms (HR = 2.508) than in government-owned farms in chickens reared on deep litter (HR = 4.102) than in those reared in cage systems (Table 4).

2.3. Results of clinical and necropsy examination

The prominent clinical findings observed in chickens suspected of infection with *E. coli* include depression, reduced appetite, weight loss, diarrhea with pasting of feathers around the vent, sitting on hocks, arching back, drooping of the head, neck, and wings, and reduced egg production. The common signs recorded in chickens affected by salmonellosis comprise depression, pale comb, ruffled feathers, chalky white diarrhea stained with green bile, vent soiled with feces, sulfur (yellow)-colored diarrhea, and reduced egg production. The results of a necropsy revealed a congested trachea, swollen and discolored liver, congested spleen, hemorrhagic lung, presence of yolk material in the peritoneal cavity, hemorrhagic enteritis and distended intestinal loops with fluid,

Table 3. Results of multivariable Cox regression analysis revealing the risk variables significantly associated with the morbidity of layer chickens in Hawassa city.

Variable	Category	HR	P value	95% CI
Farm hygiene	Good	0.005	0.000	0.003, 0.009
	Poor			
Farm experience	≥3 yrs.	0.435	0.002	0.254, 0.746
	<3 yrs.			
Housing condition	Good	0.190	0.000	0.112, 0.325
	Poor			
Age category	4–5 month	0.155	0.000	0.080, 0.302
	>5 month			

HR = hazard ratio; CI = confidence interval.

Table 4. Results of multivariable Cox regression analysis revealing risk factors significantly associated with mortality of layer chickens.

Variable	Category	HR	P value	95% CI
Farm experience	≥3 yrs.	0.007	0.000	0.003, 0.012
	<3 yrs.			
Breed	Bovans brown	0.279	0.000	0.161, 0.483
	Sasso			
Availability of veterinary services	In adequate	21.667	0.000	5.635, 83.306
	Adequate			
Regular vaccination	Yes	0.016	0.000	0.004, 0 .085
	No			
Farm ownership type	Private	2.508	0.002	1.403, 4.482
	Government			
Housing system	deep litter	4.102	0.000	2.300, 7.315
	cage system			
Age category	4–5 month	0.269	0.010	0.099, 0.732
	>5 month			

hemorrhagic oviduct, congested ovarian follicles, congested and swollen kidney and salpingitis in the case of colibacillosis. In the case of salmonellosis, the major gross lesions observed were swollen and fragile liver with a distinctive copper-bronze sheen on its surface, congested and swollen spleen, irregularly shaped ova with prominently thick stalks, caseous exudate in the oviduct, and salpingitis.

2.4. Results of the bacteriological investigation

A total of 58 cloacal samples were collected from 58 sick chickens and subjected to bacteriological analysis, of which 7 (12.07%; 95% CI: 4.99, 23.29) gave positive results for *Salmonella* spp. Similarly, 25 (43.10%; 95% CI: 30.16, 56.77) of them yielded positive results for *E. coli*. Swabs were also collected from the organs of 8 randomly selected sick chickens after necropsy, of which 3 (37.50%) were found to be positive for *Salmonella* spp. Four (50.00%) of them were positive for *E. coli*. Swabs were collected and cultured from 15 dead chickens, and of these, 2 (13.33%) and 7 (46.67%) were found to be positive for *Salmonella* spp. and *E. coli*, respectively.

2.5. Risk factors associated with colibacillosis and salmonellosis

Farm hygiene, age of study chicken, and housing condition were significantly associated ($P < 0.05$) with the occurrence of colibacillosis. The hazard of colibacillosis was significantly lower ($HR = 0.006$) in chickens raised on farms with good hygiene than in those reared on farms with poor hygiene, in young chickens ($HR = 0.079$) than in older ones and in chickens reared on well-constructed houses (0.074) than in those reared on poorly constructed houses (Table 5). Farm hygiene, use of antibiotics, and age of chicken were found to be significantly associated with salmonellosis. The hazard of salmonellosis was significantly lower ($HR = 0.011$) in chickens kept on farms with good hygiene than those kept on farms with poor hygiene; in flocks given antibiotics ($HR = 0.044$) regularly than those that did not receive antibiotics (Table 6).

Table 5. Results of Cox regression analysis revealing risk factors for colibacillosis in layer chickens in Hawassa.

Variable	Category	HR	P value	95% CI
Farm hygiene	Good	0.006	0.000	0.003, 0.015
	Poor			
Age of chicken	4–5 month	0.079	0.000	0.027, 0.232
	>5 month			
Housing condition	Good	0.074	0.000	0.035, 0.154
	Poor			

Table 6. Results of Cox regression analysis revealing risk factors for salmonellosis in layer chickens in Hawassa.

Variables	Categories	HR	P value	95% CI
Farm hygiene	Good	0.011	0.000	0.002, 0.046
	Poor			
Use of antibiotics	Yes	0.044	0.000	0.011,0.169
	No			
Age of chicken	4–5 month	0.069	0.003	0.012,0.398
	>5 month			

3. Discussion

3.1. Morbidity and mortality in layer chickens

In Ethiopia, poultry production is an important economic activity providing cash, nutritional and sociocultural services. The Ethiopian government has made poultry production a priority sector for achieving food and nutrition security. The goal is to raise the number of small-scale and commercial poultry farms with greater emphasis on small-scale farms. Poultry enterprises are particularly interesting in highly growing urban centers such as Hawassa. To achieve this goal, the identification of standing problems that can counteract the productivity of chickens is of vital importance. This study provided preliminary results on the morbidity and mortality of layer chickens in Hawassa city. Factors that have the potential to set back the productivity of layers in the area have also been identified. Therefore, veterinary and livestock authorities should take this into account when planning poultry development activities and setting up systems of livestock production and health monitoring.

The prevalence of morbidity and mortality reported in this study was lower than those reported elsewhere in the world, Bangladesh [14], Nigeria [15], Europe [16] and [17], which does not rule out the negative effects it has on the sustainability of the farms. The lower morbidity and mortality observed in the present study could be ascribed to the fact that commercial poultry production is a recent activity in the area where there is limited contact among farms, thereby limiting the spread of the pathogens. In addition, there are only few commercial poultry farms holding less than 20,000 chickens, which contributed to a lowered chance of accumulation of the disease agents in the area. The morbidity and mortality rates observed in this study are, however, comparable to previously published reports from Bangladesh [18, 19], who studied morbidity and mortality rates in the Fayoumi and Rhode Island Red breeds, respectively. This suggests that although the proportion of chickens that succumb to illness and die is low, the speed with which it occurs in the flocks in the study area is relatively high, implying the need to take action before it becomes very high.

3.2. Factors affecting morbidity and mortality

In this study, the age of chickens was significantly associated with morbidity and mortality. It was shown that both morbidity and mortality rates were higher in adult chickens than younger ones. This could be due to the increased susceptibility of adult layers as a result of the effects of egg production, which stresses the chickens and suppresses their immunity [20]. This could also happen due to the increased chance of exposure to disease agents or predisposing factors compared with the newly arrived younger ones. That is, once the chickens start laying eggs, they need close observation and necessary health provision. Consistent with our observation [14, 21, 22, 23], similar effects of age on morbidity and mortality rates have been published elsewhere in the world. However, our results disagree with the reports of [18, 24, 25], who observed higher morbidity and mortality rates in younger chickens than adults. This difference could be due to differences in the level of management and biosecurity measures.

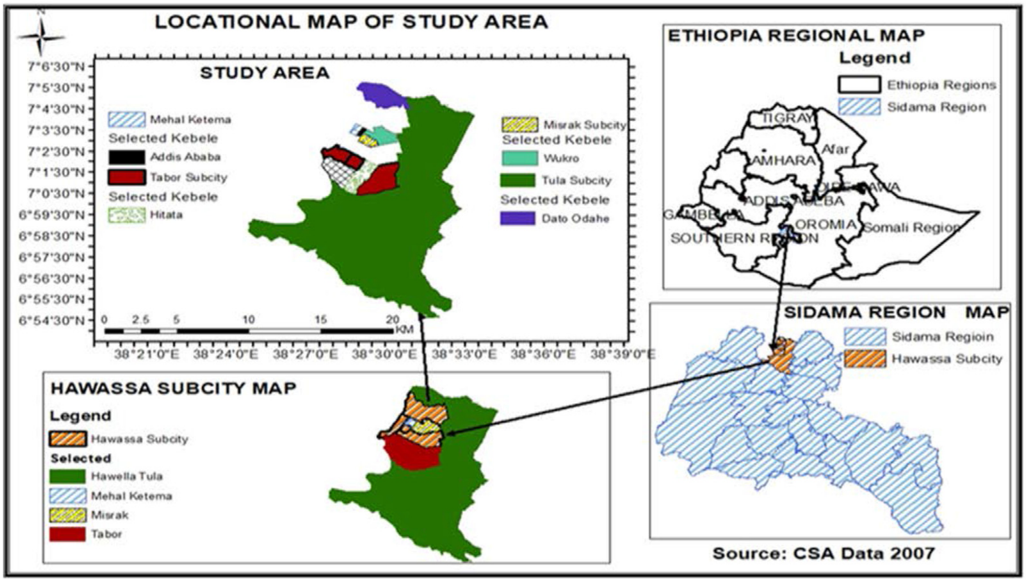

Figure 1. Map of Ethiopia and Hawassa city, Sidama Regional State showing the selected study areas.

Higher morbidity and mortality rates were observed in layer farms with poor hygiene than in those with good hygiene. This underlines the importance of maintaining farm hygiene to sustain the health of chickens. Unhygienic farms are favorable for the survival and multiplication of pathogens, especially bacteria. This is further supported by the fact that the occurrence of both *Salmonella* and *E. coli* infections was significantly associated with poor farm hygiene. The effect of farm hygiene on the occurrence of diseases and mortality in poultry farms as a source of contamination and spread of infectious pathogens from bird to bird and farm to farm has been well documented [21, 26, 27]. The higher morbidity and mortality rates in chickens reared on deep litter systems than those reared on cage systems could also be associated with farm hygiene. In deep litter systems, there is an accumulation of waste materials, especially feces, and contaminated water for long periods of time, especially on farms with poor hygienic practices. This allows the survival and multiplication of bacterial pathogens such as *Salmonella* spp. and *E. coli* since both are environmental pathogens. The chickens can ingest the pathogens contaminating the feed and water while feeding and watering and spreading to others. In contrast, in cage systems, excreta and other wastes drop to the ground, and the probability of chickens acquiring pathogens is lower. This observation is supported by the reports of [28, 29], who observed higher morbidity and mortality in chickens reared on deep litter systems than those kept on cage systems.

The higher morbidity and mortality in chickens kept in poorly maintained buildings could be due to the access of rodents to the poultry house. Rodents can transmit pathogens to chickens and contaminate feed and water. In addition, poorly constructed buildings also stress chickens due to the direct effects of temperature and sun light, which increase the susceptibility of chickens to diseases. This is in agreement with the findings of [25, 30, 31], which showed flock-to-flock spread of infectious agents in chickens reared on poorly constructed houses.

The effects of other factors, such as the availability of adequate veterinary services in the vicinity of the farms, regular administration of vaccination, and the experience of farm managers in reducing the magnitude of morbidity and mortality, are directly associated with the implementation of biosecurity measures. Experienced managers understand the importance of vaccines and veterinary services in the prevention of diseases and implement the necessary measures to keep chickens healthy. This justifies the significantly lower morbidity and mortality in chickens having more than 3 years of farm experience. A similar observation has been published by [32], in which poor veterinary services and poor understanding of biological chicken behavior were associated with increased morbidity and mortality.

The higher mortality reported in the Sasso breed than in Bovans brown in this study reveals the genetic liability between the two breeds to diseases or climatic and other predisposing factors. In agreement with our observation, these studies [14, 16, 19, 20, 21, 29, 33] reported significant differences in mortality among various breeds of layers.

3.3. Occurrence of infection with Salmonella spp. and E. coli

The occurrence of infection with *E. coli* observed in this study is slightly higher than that in previous reports [18, 34] from Bangladesh. In contrast, it is lower than the reports of [23, 35, 36] from Bangladesh. The difference observed in the magnitude of infection with *E. coli* might result from the difference in the study design, isolation techniques used, the difference in sample type and size, the difference in geographical location, breeds of chickens, management system, and difference in the standard of laboratory facilities available. The incidence rate of colibacillosis recorded in this study is also comparable to the reports of [18] from Bangladesh. Apart from its negative effects on the productivity of chickens, the occurrence of *E. coli* bears public health implications. Therefore, veterinary and public health authorities should take this into account and monitor its spread to consumers.

The occurrence of infection with *Salmonella* spp. observed in this study is lower than the results reported previously [18, 23, 34, 35] elsewhere in the world. The incidence rate of infection with *Salmonella* spp. is also in agreement with the reports of [19] in Fayoumi and RIR chickens. The lower prevalence of infection with *Salmonella* spp. could be due to the relatively recent establishment of commercial small-scale poultry farms in Hawassa. Since the environment is an important source of *Salmonella* spp., a sufficient amount of the bacteria has not been accumulated on the firms. However, the low prevalence does not rule out its importance, as *Salmonella* infection causes a prolonged reduction in egg production, and some of the serotypes also cause human infection.

The occurrence of infection with *Salmonella* spp. was significantly lower in chickens reared on farms that used antibiotics than in those that did not use or less frequently used antibiotics. On the one hand, this shows the importance of veterinary services, including the application of antibiotics for treatment and prophylaxis, and implies a lower prevalence of *Salmonella* infection reported in this study since antibiotic administration augments the immunity of chickens and ultimately reduces the incidence of infection. On the other hand, the inadvertent use of antibiotics can lead to the development of resistance and the outbreak of disease that cannot be treated with available antibiotics. The lower prevalence of *Salmonella* spp. in poultry farms that frequently use antibiotics has also been reported previously [27].

4. Conclusions

The present study revealed the occurrence of relatively lower morbidity and mortality in chickens in Hawassa city. Farm hygiene, housing systems, housing conditions, age of chickens, and the experience of farm managers are important factors that affect the morbidity and mortality of chickens in the area. Limited number of farms willing to be involved in the follow-up and non-identification of bacterial serotypes using further laboratory methods was the limitation of this study. The occurrence of infection with *Salmonella* spp. and *E. coli* is identified, suggesting their role in affecting productivity and public health. The veterinary and public health authorities should take this into account. Identification of the serotypes of *Salmonella* spp. and *E. coli* should be carried out.

5. Methods

5.1. The study area

The study was conducted from November 2019 to March 2020 in commercial poultry farms in Hawassa city. Hawassa is the capital of Sidama Regional State, covering an area of 50 square km. It is located between 7°3′N latitude and 38°28′E longitude at an elevation of 1,708 m above sea level. It is located 273 km south of Addis Ababa in the Ethiopian Great Rift Valley. The average monthly minimum and maximum temperatures and mean annual rainfall of the area are 13 °C, 27 °C, and 1,091 mm, respectively. According to the Hawassa city office of Agriculture, there are 13 small-scale and 5 medium-scale registered layer farms holding a total number of 17,914 chickens. Hawassa city has a total of eight subcities and 32 Kebeles (lower administration units) (Figure 1).

5.2. Study population

The study populations were layer chickens reared on poultry farms in Hawassa city. The chickens included in the study were chickens belonging to Bovans brown and Sasso between 4-12 months of age. The farms were owned by the government (Hawassa University Enterprise Poultry Farm) and private farms (all commercial small- and medium-scale farms). Data obtained from the Hawassa city livestock and fishery department revealed that there were 13 small-scale and 5 medium-scale layer farms holding a total of 17,914 chickens. Four farms willed to participate in the follow-up study from each of the four subcities designated Mehal Ketema, Tula, Tabor, and Misrak were selected. To maintain the confidentiality of the data generated from farms, their names were not mentioned. The numbers of chickens recruited for the study were 798 from Farm 1, 300 from Farm 2, 1000 from Farm 3, and 6878 from Farm 4, with a total of 8976.

5.3. Study design

A longitudinal prospective study design was employed for five months from November 2019 to March 2020. The poultry farms included in the study were selected based on willingness to participate in the follow-up study, and all chickens reared on the selected farms were followed for five months. During the study period, chickens reared on the selected farms were monitored for morbidity and mortality twice per week. However, the farm attendants were instructed to record the time at which incidents occurred whether it was signs of disease or death. During each visit, clinical examination of sick birds and pathological investigation of dead birds were conducted. Samples of cloaca were collected for isolation and identification of *Salmonella* spp. and *E. coli* as described below. In addition, a questionnaire survey was administered to farm owners or managers to gather information on the potential risk factors for morbidity and mortality.

5.4. Study techniques used

5.4.1. Questionnaire survey and farm visits

During the study, the attendants or managers of each farm selected for this study were interviewed using a semistructured questionnaire. The information gathered during an interview included biosecurity measures, flock management practices, waste handling activities, general farm history, disease management information, housing conditions, flock number, source of drinking water, and hygiene status of the farms. Emphasis was given to critical management practices and risk factors that might have a potential association with morbidity and mortality. Each farm was regularly visited twice weekly. In addition to the questionnaire, data were recorded on husbandry practices related to the health of the chicken, clinical signs, duration of illness, and associated mortality. In addition, for any mortalities that occurred on other days (other than the days of visits), data enumerators were assigned to record the incidents.

5.4.2. Sample collection and processing

Whenever chickens were affected clinically with diseases suspected to be salmonellosis or colibacillosis, samples were collected for isolation and identification of the causative bacteria following the recommendations of OIE [7]. The samples were labeled individually with specific identification numbers, dates of sampling, and sample type. Fifty-eight (58) cloacal samples were collected from clinically sick chickens using sterile cotton-tipped swabs moistened with buffered peptone water. Each swab sample was placed in separate screw-capped sterile test tubes containing buffered peptone water, kept in an icebox containing ice packs and transported to Hawassa University's Microbiology laboratory. In addition, 23 clinically sick and dead chickens were autopsied in the farms following standard postmortem procedures [8, 9, 10]. At necropsy, each organ and system of the body of each chicken was examined for gross lesions, and the changes observed were recorded. When gross lesions suggesting the occurrence of salmonellosis and/or colibacillosis were encountered, tissue samples were collected from vital organs such as the liver, spleen, ovary, and kidney using sterile forceps and scalpel blade following aseptic conditions. Before sampling, the surface of each organ was decontaminated using a hot sterile spatula. After making an incision on each organ using a sterile scalpel blade, sterile cotton-tipped swabs were inserted. The swabs were kept in sterile test tubes containing buffered peptone water and transported to the laboratory.

5.4.3. Isolation and identification of Salmonella and E. coli

For isolation and identification of *E. coli*, a loop full of inoculum from swab samples pre-enriched in buffered peptone water was streaked onto MacConkey agar and incubated at 37 °C for 24 h. Then, the suspected colonies of *E. coli* were inoculated onto EMB agar. Finally, the pure colonies of *E. coli* with characteristic red color on MAC agar and green metallic shine appearance on EMB agar were further analyzed by biochemical tests such as indole production tests, triple sugar iron (TSI) tests, and citrate utilization tests [11, 12].

For isolation and identification of *Salmonella* spp., the pre-enriched samples from buffered peptone water were transferred onto selective enrichment media (Rappaport Vassiliadis) and incubated at 41.5 °C for 24–48 h. Then, the loop full of the samples was inoculated onto xylose lysine deoxycholate (XLD) and incubated at 37 °C for 48 h. The pure colonies that resemble *Salmonella* spp. with characteristic red color with black center on XLD agar were subjected to biochemical tests such as triple sugar iron (TSI) test, citrate utilization test, and indole production test following the procedures described by [11, 12].

5.5. Data management and analysis

Epidemiological data obtained from the questionnaires, follow-up of the farms and laboratory tests were summarized and analyzed using STATA version 14 (StataCorp, 4905 Lakeway Drive, College Station, Texas 77845, USA). Data were gathered on thirty-two hypothesized explanatory variables to test their association with morbidity and mortality in layer chickens in the study area. The overall cumulative incidence, morbidity, and mortality rates as well as the cause-specific rates (CSR) were calculated using the formula described by Thrusfield [13]. During the analysis, all chickens developed an outcome variable (became sick, died, infected with *E. coli* or *Salmonella* spp.) constitute the numerator, and the time in months that the chickens were at risk was used as a denominator for computation of the morbidity and mortality rates. The rates computed were expressed as the number of cases per 1000 chicken months at risk. Cox's proportional hazard model was used to quantify the effects of various risk factors on the morbidity and mortality rates observed. Initially, the association of individual risk factors with morbidity and mortality was screened by univariable Cox regression. The explanatory variables (factors) significantly associated with morbidity at a 5% significance level in the univariable analysis were recruited for multivariable analysis using multiple Cox regression. In the multivariable Cox regression analysis, the final model was fitted for morbidity by stepwise backward elimination of those variables that had no significant effect ($P > 0.05$).

Declarations

Author contribution statement

Gizachew Hailegebreal: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Kabech Gedeno: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Bereket Molla Tanga; Mishamao Sulayeman: Contributed reagents, materials, analysis tools or data; Wrote the paper.

Teshale Sori: Analyzed and interpreted the data; Wrote the paper.

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Data availability statement

Data will be made available on request.

Declaration of interest's statement

The authors declare no competing interests.

Additional information

No additional information is available for this paper.

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