The effect of biosecurity measures application on the level of diseases risk in different poultry farms

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Research Article

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Abstract

The development and implementation of a biosecurity plan in a poultry production enterprise, require a documented approach and scoring systems to rank biosecurity protocols and their implementation. Hence, this study was carried out to monitor some biosecurity measures applied in different poultry farms and its effect on the presence of some pathogenic bacteria, such as *E. coli*, Pasteurella and Campylobacter in different poultry farm's production. The obtained results showed that the highest incidence of isolated pathogenic bacteria was in the duck farms. The incidence of *E.coli* spp was (61%), Pasterulla spp was (18.52%) and Campylobacter spp was (43.5%) in duck farms. There was a negative relationship between the application of biosecurity measures and the isolation of pathogenic bacteria in poultry farm's production. The EaeA and Stx1 virulence genes were detected from some serotypes of *E.coli*; while the virulence genes pfhA and hgbB were isolated from some *P. multocida* serotypes. Also, the cdtB and cdtC virulence genes were recovered from Campylobacter serotypes. Finally, the biosecurity measures are essential for the success of poultry production. Indeed, biosecurity measures reduce the risk of introducing pathogens during the poultry production cycle; In addition, it reduces financial losses through decreased mortality rates and treatment costs.

Introduction

Poultry is one of the most progressive animal enterprise and one of the major and fastest producers of meat worldwide. In addition to that, poultry has been a significant contributor to the country’s agriculture sector. Poultry products are the most essential sources of protein for all population and it considers the main important effectiveness for economy and income of some countries (Attia et al. 2022). The prosperity of poultry production is mostly related to good management and hygiene of the farm (Díaz Carrasco et al. 2022).

The biosecurity and hygienic level of the farms are critical points in poultry industry and should be a part of any poultry production system. Adequate biosecurity measures can improve overall flock health, decrease the cost of treatment, reduce losses and improve farm profitability, prevent disease from entering a facility and manage a disease once it is on the premises (Goualie et al. 2020).

The Food and Agriculture Organization (FAO) classifies poultry production system into four sectors based on their levels of biosecurity and strongly recommends the strict application of biosecurity measures as the most effective way to prevent and control the spread of infectious diseases (FAO 2020). The biosecurity level could be mapped to identify areas at high risk for the spread of disease. This would be valuable in case of epidemic disease outbreaks and makes targeted surveillance strategies more achievable (Cuc et al. 2020).

The general biosecurity scoring system includes groups of questions that are divided into several subcategories for external and internal biosecurity. The answer to every question results in a score. This score can range from zero, indicating a total absence of the described biosecurity measures, to 100, indicating a full application of the described measures. The final overall biosecurity sore was the sum of the external and internal biosecurity scores which subdivided into different subcategory scores (Dewulf et al. 2018).

Colibacillosis disease caused by Avian pathogenic *Escherichia coli (APEC)* has an important economic impact on poultry production worldwide as it is one of the most common causes of mortality up to 30% and high first week mortality in commercial layer and breeder chickens (Lozica et al. 2022). *Escherichia coli* represents a part of the normal microflora of the intestinal tract of poultry (Borgatta et al. 2012).

Pasteurella mainly inhabits in the upper respiratory tracts of domestic and wild birds such as chicken, duck which can cause fowl cholera. Currently, the infection of *P. multocida* is still one of the most serious threats to the poultry industry that causes several diseases and economic loses in poultry and wild birds (Xiao et al. 2021). Five capsular serogroups are recognized among the avian strains of *P. multocida*, namely: A, B, D, E and F which affecting multi host species (Smith et al. 2021).

Campylobacter is generally isolated from the poultry caecum. The poultry is the common animal food source of Campylobacter infection for human causing many food-borne outbreaks (Tedersoo et al. 2022). There are three Campylobacter species (*C. jejuni*, *C. coli* and *C. lari*) that related to the poultry digestive system and foodborne infections (Ugarte-Ruiz et al. 2018). Most Campylobacter transmission to poultry occurs from the environment as well as through horizontal transmission between flock mates, but once Campylobacter colonizes a poultry flock, the spread is fast, making an eradication approach impossible (Plishka et al. 2022).

Finally, A well-designed and planned biosecurity approach at farm level have been established as a fundamental method to counteract colonization of flocks (Georgiev et al. 2017). The present study aimed to monitor some biosecurity measures applied in different poultry farms and its effect on the presence of some pathogenic bacteria in different poultry farms production.

Materials And Methods

Poultry farms

The present study was carried out on twelve poultry farms (three broiler chicken, three-layer chicken, three breeder chicken and three duck farms). All farms located at Qalubia Governorate, Egypt. The selection of the farms was based on their geographical location, variation in farm hygiene, housing system and the type of production (broilers, layers and breeders).

Hygienic scoring of poultry farms

General biosecurity scoring system which applied in each farm were evaluated depending on internal and external parameters such as traffic control, surrounding of the farm, outer and inner farm building status, hygiene of feed, hygiene of water, litter hygiene, visitors control and workers control list in Table

(1). The final total score of farm biosecurity was the sum of the different subcategory scores according to (Dewulf et al. 2018).

$$\text{Calculation} = \frac{\text{Sum scores of total applied biosecurity measures}}{\text{Total full application of biosecurity measures (score 2)}} \times 100$$

Sampling

A total of 2160 environmental and bird samples were collected from twelve poultry farms (n = 180 from each farm) along three visits per each farm and five samples were collected per each visit from feed storage, feeder, water source, drinker, storage litter, pen litter, droppings and air dust as well as swabs were taken from walls, birds' cloaca, worker's hand and wheals of vehicles (n = 15 of each from each farm). The collected sample were approved with Ethical Approval Number **(BUFVTM 18-03-22).**

Samples preparation and enrichment

The collected samples and swabs were preserved in a dry insulated ice box supplied with gel bags to maintain the samples characters and retard any biological changes then transferred to the laboratory within 3 hrs. Taking 1g of feed, litter and droppings samples then were separately grounded in a sterile manual blender that cleaned and disinfected in between samples to prevent cross-contamination. After that, it mixed with sterile buffered peptone water. Before bacterial isolation, the prepared samples with buffered peptone water (BPW) were incubated aerobically at the temperature of 37°C for 24 hours according to (levy et al. 2020).

Isolation and identification of some pathogenic bacteria

Isolation of E. coli

The enriched samples were streaked on Eosin Methylene Blue agar (EMB) plates and incubated aerobically at 37°C overnight. Single metallic sheen colonies on the EMB agar plates were considered as indicative of *E. coli* then the typical colony was confirmed by morphological study by Gram staining according to (levy et al. 2020).

Isolation of Pasteurella spp

The prepared samples were inoculated separately into Blood agar (BA) containing 5% sheep blood. The cultured media were incubated at 37°C for 24 hrs. The appearance of characteristic colony based on the colony characteristics, subsequent selective subculture was done to obtain pure culture. The isolated pure culture was subjected for Gram staining and Leishman's staining for morphological identification of the bacteria according to (Panna et al. 2015).

Isolation of Campylobacter spp

The previous enriched swabs and samples were streaked directly onto plates of Campylobacter Blood Free selective agar (Oxoid) which called Charcoal Cefoperozone Deoxycholate agar (CCDA). Streaked plates were incubated at 42°C for 48 hrs under microaerophilic (10% CO_2 and 5% O_2) condition which was achieved by placing the plates in anaerobic jar together with a gas pack then confirmed the suspected colony by Gram staining (El Baaboua et al. 2022).

Biochemical identification.

Biochemical identification of E. coli

Separate colony subculture on EMB and incubated aerobically at 37°C for 16hrs then take fresh colony for performance of TSI (Triple Sugar Iron) test (positive), Simmon citrate test (negative), Methyl red test (positive), Voges–Proskauer test (negative) and Indole test (positive) according to (levy et al. 2020).

Biochemical identification of Pasteurella spp

The suspected isolated colony was identified by Methyl red test (negative), Nitrate reduction test (negative), Indole production test (variable), Catalase test (positive), Oxidase test (positive) and Sugar fermentation test (positive) according to (Panna et al. 2015).

Biochemical identification of Campylobacter species

The suspected isolated colonies were identified biochemically using Catalase test (positive), Oxidase test (positive), TSI test (negative), Lead acetate test (positive), Growth in the presence of 1% glycine (positive expect *C. lanienae*), Nitrate reduction test (positive) and Hippurate hydrolysis test (negative expect *C. jejuni*) according to (Hedges and Colles 2022).

Serological identification

Serological identification of E. coli

The serologically identification of *E.coli* was identified by using antisera sets **(**DENKA SEIKEN Co., Japan**)** for diagnosis of the Enteropathogenic types according to (Sobur et al. 2019).

Serological identification of Pasteurella spp

The serologically methods were used to identify the Pasteurella are the commercial latex agglutination Bionor Mono-kits that were used for rapid serological identification of *P. multocida* type. Also, the commercial *Pasteurella multocida* ELISA test kits (Glory Science Co., Ltd, China Manufacturers, China) were used for rapid serological identification of such pathogen according to (Mahrous et al. 2022).

Serological identification of Campylobacter species

The Latex Agglutination Kits namely dry spot Campylobacter (Oxoid, Basingstoke, Hampshire, England) were used for rapid serological identification of Campylobacter species according to (Oyarzabal et al. 2007). The recommended technique was applied depending upon the manufacture directions.

Polymerase chain reaction identification (PCR).

Molecular detection of virulence genes of some E. coli serotypes

By using conventional polymerase chain reactions (cPCRs) for detection of two virulence genes (E. *coli* stx1 and *E. coli* eaeA) **.** Extraction of DNA according to QIAamp DNA mini kit instructions (Catalogue no.51304). Preparation of PCR Master Mix according to Emerald Amp GT PCR master mix (Takara)Code No. RR310A kit with total volume of 25 µl per reaction. The following Oligonucleotide primers of Metabion, Germany source were used. For *E. coli* stx1 F, 5' ACACTGGATGATCTCAGTGG '3, R 5' CTGAATCCCCCTCCATTATG '3 with amplified product 614 bp (Dipineto et al. 2006) while of *E. coli* eaeA F 5' ATG CTT AGT GCT GGT TTA GG '3, R 5' GCC TTC ATC ATT TCG CTT TC '3 with 248 bp according to (Bisi-Johnson et al. 2011). Cycling conditions of the primers during cPCR during primary denaturation at 94˚C / 5 min, 94˚C / 30 sec during secondary denaturation, 58˚C / 40 sec during annealing of first gene and 51˚C / 30 sec for the second, 72˚C / 45 sec for extension of first gene and 72˚C / 30 sec for second. Number of cycles are 35 and the final extension of first gene at 72˚C / 10 min and at 72˚C / 7 min for second. DNA Molecular weight marker Gene ruler 100 bp DNA ladder (cat. no. SM0243) supplied from Fermentas. Number of bands: 10 Size range: 100–1000 bp.. Agarose gel electrophoreses. The steps were carried out according to (Khafagy et al. 2018) with modification and the gel was photographed by a gel documentation system and the data was analyzed through computer software.

Molecular detection of virulence genes of some Pasteurella multocida strains

By using multiplex PCR and genomic DNA extraction according to (Panna et al. 2015). Using Gene JET Genomic DNA Purification Kit (Fermentas). DNA amplification: Amplification for pfhA, hgbB and toxA genes of *P. multocida* by multiplex PCR. The amplification was performed on a Thermal Cycler (Master cycler, Eppendorf, Hamburg, Germany). Accurately, by using 20 µL mixture, containing 0.5 µL of each primer (10 picomol), 1.5 µL of 2.5 mM $MgCl_2$, 0.5 µL of 10 mM dNTPs, 2U of Taq DNA polymerase, 2.5 µL of 10x PCR buffer and sterile distilled water. The following primers were used pfhA (F) 5' AGCTGATCAAGTGGTGAAC '3, (R) 5' TGGTACATTGGTGAATGCTG '3 with Product size 275 pb, hgbB (F)5'ACCGCGTTGGAATTATGATTG'3, (R) 5'ATTGAGTACGGCTTGACA'3 with size 499 bp, toxA (F) 5'CTTAGATGAGCGACAAGGT'3, toxA R(5'GGAATGCCACACCTCT'3 with 846 bp according to (Cucco et al. 2017).The cycling conditions for PCR included an initial denaturation of DNA at 95°C for 5 min followed by 25 cycles each of 45 sec denaturation at 94°C, 30 sec annealing at 55°C and 1 min extension at 72°C, followed by a final extension of 5 min at 72°C and held at 4°C. Thus, 10 µL of PCR products were separated by electrophoresis (100 volts for 1 hour) in a 1% agarose gel, stained with 0.5 µg/mL ethidium bromide per ml for 15 min, visualized under an ultraviolet transilluminator and photographed. A 100 bp plus DNA Ladder (Qiagen, Germany, GmbH) was used to determine the fragment sizes according to (Gharibi et al. 2017).

Molecular detection of virulence genes of some Campylobacter strains

Using multiplex Polymerase chain reaction (PCRs) and genomic DNA extraction using Gene JET Genomic DNA Purification Kit (Fermentas). Amplification of virulence genes of *C. jejuni* by using 40 µl of PCR mixture. All reactions contained appropriate concentrations of 3 primer sets, 0.2 mM each of dATP, dCTP, dGTP and dTTP, 1 × Ex *Taq* DNA polymerase buffer and 1.0 U of Ex *Taq* DNA polymerase in a 40 µl reaction volume. The following pair of primers (Pharmacia Biotech) of cdtB were used (F) 5' ATCTTTTAACCTTGCTTTTGC '3 (R) 5' GCAAGCATTAAAATCGCAGC '3 with product size (bp) 714 while of cdtC (F) 5' TTTAGCCTTTGCAACTCCTA '3 (R) 5' AAGGGGTAGCAGCTGTTAA'3 (Asakura et al. 2008). The PCR cycling protocol was applied as following; An initial denaturation at 94°C for 60 secs, followed by 35 cycles of denaturation at 94°C for 30 secs, annealing at 55°C for 30 sec and extension at 72°C for 30 sec. Finally, 5 µl of each amplicon was electrophoresed in 2% agrose gel) stained with ethidium bromide and visualized on UV transilluminator. A 100 bp DNA ladder was used as a marker for PCR products according to (Carvalho et al. 2013).

Statistical analysis

The statistical analysis was carried out by using two-way ANOVA (analysis of variance) using SPSS, ver. 25 (IBM Corp. Released 2013). Multiple comparisons were carried out applying Duncun test. The significance level was set at < 0.05.

Results

The score of biosecurity was the highest in the breeder chicken when compared with other different chicken and duck production. The biosecurity score was different in each type of production. It was the highest in the farm C of broiler chicken (67.5%), layer chicken (75%) and duck (52.5%). While, it was the highest in the farm B of breeder chicken (90%) in Fig. 1.

The prevalence of *E.coli* isolated from different poultry farm was the highest in the duck farms (61.11%) followed by (56.85%) layer chicken, (45.9%) breeder chicken and (42.4%) in the broiler chicken farm. There are highly significant difference of *E.coli* prevalence between different poultry farms production. The *E.coli* was highest isolated from droppings samples that were 97.78%, 100%, 91.11% and 97.78% in broiler chicken, layer chicken, breeder chicken and duck farms, respectively. In contrast, it was the lowest in the feed storage at 6.67%, 2.22%, 6.67% and 26.67% in broiler chicken, layer chicken, breeder chicken and duck farms, respectively in Table (2).

In addition to, the results in Table 3 that showed the highly significant difference of isolated Pasteurella between different poultry farms production. The prevalence of suspected Pasteurella spp were the highest in duck's farms (18.5%) followed by breeder chicken (12.59%), broiler chicken (9.075%) and layer chicken (7.22%). The isolated Pasteurella spp were the highest in droppings samples which were 26.67%, 24.45%, 46.67% and 55.56% in broiler chicken, layer

chicken, breeder chicken and duck farms, respectively. In contrast, it couldn't isolate from feed storage, water source, storage litter, dust, wheal swabs and wall swabs in all examined poultry farms in Table (3).

Furthermore, the highest prevalence of isolated Campylobacter was 43.52% in duck followed by 43.5% in layer chicken, 33.33% in broiler chicken and 25.37% in breeder chicken in Table 4. There is highly significant difference of isolated Campylobacter spp. between different production of poultry farms. The Campylobacter prevalence was the highest isolated in the droppings samples at 97.78%, 88.89%, 80% and 95.56% in broiler chicken, layer chicken, breeder chicken and duck, respectively as in Table (4).

The prevailed serotypes of *E. coli* were *O114: K90, O112, O153*, at prevalence 16.67%, 13.89% and 13.89%, respectively for broiler chicken. While, in the layer chicken they were *O26: K60, O128 : K67* and *O91 : K* at 22.22%, 19.44% and 13.89%, respectively. Furthermore, in the breeder chicken *E. coli* serotypes were *O44: K74, O124: K72* and *O86 : K61* at 22.22%, 13.89% and 13.89%, respectively. Finally, in the duck they were *O128: K67*, *O119: K69, O157: K-, ,O124 : K72*, at prevalence 16.67%, 13.89%, 11.1% and 11.1%, respectively in Table (5).

The most isolated serotype of Pasteurella was *P. multocida* A:1 at prevalence 58.33%, 50% and 47.2% in duck, breeder chicken and broiler chicken, respectively. On other hand, *P. multocida* A:3 was the highest serotype in the layer chicken at 47.2% in Table (6).

The results in Table (7) showed that the most prevailing Campylobacter serotype was *C. Jejuni* at prevalence 44.44%,41.67%, 38.89% and 33.33% in layer chicken, breeder chicken, duck and broiler chicken, respectively.

The molecular detection of *E. coli* virulence genes (Stx1 and EaeA) confirmed presence of Stx1 gene in*O164* and *O26* serotypes while EaeA gene present in *O114, O164, O119, O26* and *O124* serotypes, respectively in Table (8) and illustrated in Fig. 2. The virulence genes of some *Pasteurella multocida* serotypes were pfhA, hgbB and toxA genes in *Pasteurella multocida* A:1 serotype, pfhA and hgbB genes in *Pasteurella multocida* A: 3 serotype and pfhA gene in *Pasteurella multocida* A:12 serotype in Table 8 and cleared in Fig. 3. The Occurrence of virulence genes in *C. jejuni* strains was 100% for cdtB and 58.3% for cdtC in Table (8) & Fig. 4.

Discussion

Poultry include a number of domesticated avian species which encompasses the chicken (meat production – “broiler” or reared for laying eggs – “layer”) and ducks birds (Al-Nasser et al. 2020). In general, the application of the biosecurity measures is essential for the success of poultry production. Indeed, biosecurity measures reduce the risk of introducing of pathogens during the poultry production cycle (Goualie et al. 2020). In addition to, it reduces financial losses through decrease mortality rate and treatment costs (Soro et al. 2020).

Development and implementation of a biosecurity plan in a livestock enterprise require a documented approach and scoring systems have been developed to rank biosecurity protocols and their implementation (Van Steenwinkel et al. 2011).

Our results showed that the farm C of broiler chicken, layer chicken and duck farms as well as farm B of breeder chicken were recorded the highest scores per each type of production due to the application of internal and external biosecurity measures, while broiler chicken A, layer chicken B, breeder chicken C and duck A had the lowest biosecurity score due to presence of many defects in internal and external biosecurity measures linking with high prevalence of some pathogenic bacteria. The biosecurity is an important part of any avian health management program reduce interactions between poultry and infectious agents (Morishita and Derksen 2021). The farms with high biosecurity score had low percentage of suspected isolated pathogenic bacteria. In contrast, low score biosecurity farms were related to high risk of bacterial and viral poultry diseases (Greening et al. 2020) such as *Escherichia coli*, *Campylobacter, Clostridial and* Salmonella bacterial disease (Bernd et al. 2022), More ever, Newcastle, HPAI, IBV, MPV, MS, and MG viral diseases (Yoo et al. 2022).

Our results recorded the highest prevalence of *E. coli* was 61.11% in the duck as a result of the absence of some internal and external biosecurity measures. On the other hand, the lowest *E. coli* prevalence was 42.4% in broiler chicken followed by breeder chicken (45.9%) (Musiev et al. 2020). This is due to the highest application of internal and external biosecurity measures. In addition to, the age factor theory that plays role in the *E. coli* prevalence in breeder chicken farms (Awad et al. 2016).

The highest incidences of *E. coli* were found in droppings samples, pen litter and cloacal samples this is could be due to the habitat of E. *coli* in the normal microflora of poultry the intestinal tract furthermore, the droppings contaminate the pen litter (Borgatta et al. 2012). On the other hand, the lowest percentages of it was recorded in the water source this might due to the chlorination treatment of water (Jonsson et al. 2012) as well as in the feed samples collected from feed storage this is due to heat treatment during pelleting process in addition the effect of organic acid (Steghöfer et al. 2021).

Our results mentioned that the most popular *E. coli* serotypes in broiler chicken were *O114*, *O153*, *O112*, *O44* and *O164*. While, *O26*, *O128*, *O91*, *O44* and *O152* were most popular prevailed serotypes in layer chicken. In addition to that, in the breeder chicken were *O44*, *O124*, *O86* and *O26*. Also, in the duck farms were *O114*, *O119*, *O157* and *O124*. Finally, the most commonly isolated O groups in poultry farms were *O44, O114, O91, O26, O127, O164, O86, O157, O55 and O128* (El-Jakee et al. 2012; El-Sayed et al. 2015).

The EaeA virulence gene was isolated from *O114, O164, O119, O26* and *O124* serotypes of *E.coli*. On the other hand, the Stx1 virulence gene was recorded in *O164* and *O26 E. coli* serotypes (Hossain et al. 2021).

Our result showed that the highest prevalence of Pasteurella was found in duck's farms followed by breeder chicken, broilers chicken while the lowest in layer chicken this is might be due to the management of farms, vaccination and nutrition status of the poultry (Panna et al. 2015).

The most Pasteuella spp was isolated from bird samples (cloacal and droppings) as well as water from drinkers, feed from feeders and pen litter this is due to the contamination of feed, water and pen litter from nasal discharge and droppings of infected birds (Musiev et al. 2020). In contrast, there were complete absence of Pasteurella in dust, storage litter, water source, wheel, wall and feed storage (Nasrin et al. 2007)

The most popular serologically isolated Pasteuella spp was *P. multocida*. The *P. multocida* serotypes were A:1, A:3 and A:12 isolated from different poultry farms production. The *P. multocida* serotypes A:1 and A:3 are widely recognized as the causative agent of the most fowl cholera outbreaks in poultry flocks (Mohamed et al. 2012).

The virulence genes pfhA were isolated from A:1, A:3 and A:12 *P.multocida* serotypes, in addition to hgbB virulence genes were recorded in A:1 and A:3 serotypes of *P.multocida* while, the toxA virulence genes were founded in A:1 serotype of *P.multocida* (Abd-Elsadek et al. 2021).

The incidence of Campylobacter spp was the highest in duck's farms in contrast, the lowest incidence was in breeder chicken. The Application of some biosecurity measures were affecting on the flock to be positive with Campylobacter and there was a negative relationship between application of biosecurity measures and the prevalence of Campylobacter (Newell et al. 2011 ; Schweitzer et al. 2021)

The highest prevalence of Campylobacter were isolated from droppings, cloaca as it is a normal inhabitant in poultry intestine (Di Marcantonio et al. 2022; Wayou et al. 2022) followed by pen litter due to droppings contamination as it is a manure born pathogen (Sahin et al. 2015) then, in feeder and drinker due to bird droppings contamination (Mota-Gutierrez et al. 2022). In contrast, lowest prevalence was recorded in water source this might due to chlorination treatment of water (Jonsson et al. 2012), feed storage and fresh litter as a result of the low water activity in the dry feed and litter which prevent Campylobacter survival (Newell et al. 2011).

Our result showed that the most serotypes of Campylobacter were *C. Jejuni* and *C. coli* and they are a common finding in the poultry farms (Wayou et al. 2022). While, *C. Lari, C. Upsaliens and C. Lanienae* were less frequent or absent in some farms. Also, the poultry was a reservoir of other Campylobacter species including *C. lari, C. upsaliensis* and *C. concisus* (Kaakoush et al. 2014).

The Campylobacter virulence genes cdtB and cdtC were isolated from Campylobacter serotypes. The cdtB was one of the most important Campylobacter isolates virulence genes (Karikari et al. 2021).

Conclusion

Finally, the biosecurity measures are critical control points in poultry farms; It is essential for the success of poultry production. Indeed, biosecurity measures reduce the risk of introducing pathogens during the poultry production cycle; In addition to the reduction of financial losses through decrease possibility of diseases spreading, mortality rate and treatment costs. Investment in biosecurity and hygiene are the best way for success stories of poultry production.

Declarations

Authors contributions HAA and HEKE designed the concept for this research and scientific paper. HEKE and EMH has sampled, analyzed the data and interpreted the result. All authors participated in manuscript's draft and revision. All authors have read and approved the final manuscript.

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Consent to participate and publish All authors have approved to participate and publish this scientific work.

Data availability All data generalized or analyzed during this study are included in this article.

Conflicts of Interest The authors declare no conflict of interest.

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Tables

Table (1): Biosecurity scoring system of different poultry production farms.

Items			Score (0)	Score (1)	Score (2)
Outside the farm:	1-Distance from other farms	Broiler	< 0.6 km	0.6 to < 1 km	≥ 1km
		Layer chicken	<1km	1-2 km	> 2km
		Breeder	< 1km	1 km till < 5km	5 km
	2-Distance from road	Broiler	< 100 m	100-300 m	> 300 m
		Layer chicken	<200 m	200-400 m	>400 m
		Breeder	< 300 m	300-500 m	> 500 m
	3-Fence		Absent	•	Present
	4-Wheel dip		Absent Or not used	Disinfectant not regularly change	Disinfectant regularly change
	5- Foot bath		Absent Or not used	Disinfectant not regularly change	Disinfectant regularly change
Outer farm construction			Bad hygiene	Fair	Good
Inner farm construction:			Bad hygiene	Fair	Good
Pollution sources			Present	Moderate	No pollution
Type and state of ventilation system			Un sufficient	Fair	Sufficient
Chick source			Not Trustable	Moderate	Trustable
Birds type and age			Different types or ages in same Pen	Different ages or types indifferent pens	The same type and age
Stocking density of broiler chicken at end of cycle	Open system		≥ 22 kg/m^2	19-22 kg/m^2	≤ 18 kg/m^2
	Closed system		≥ 40 kg /m^2	31-39 kg/m^2	≤ 30 kg/m^2
Stocking density of broiler ducks at end of cycle	Open system		≥ 17 kg/m^2	13-16 Kg/m^2	≤ 12 Kg/m^2
	Closed system		≥ 25 kg/m^2	16-24 kg /m^2	≤15 kg/m^2
Stocking density of layer chicken at deep litter system	Open System	Rearing	≥ 15 birds / m^2	10-15 birds / m^2	< 10 birds / m^2
		Production	≥ 8 birds / m^2	5-7birds / m^2	< 5 birds / m^2
	Closed system	Rearing	≥ 20 birds / m^2	15-20 birds / m^2	<15 birds / m^2
		Production	≥ 10 birds / m^2	8-10 birds / m^2	< 8 birds / m^2
Stocking density of layer chicken at battery system	Open system	Rearing	≥ 15 birds / m^2	10-14 birds / m^2	< 10 birds / m^2
		Production	≥ 25 birds / m^2	20-24 birds / m^2	< 20 birds / m^2
	Closed system	Rearing	≥ 20 birds / m^2	15-19 birds / m^2	< 15 birds / m^2
		Production	≥ 30 birds / m^2	25-29 birds / m^2	< 25birds / m^2
Stocking density of broiler breeders chicken	Open system	Rearing	≥ 15 birds /m^2	10-14 birds /m^2	< 10 birds /m^2
		Production	> 8 birds /m^2	4-8 birds /m^2	< 4 birds /m^2
	Closed system	Rearing	> 20 birds /m^2	14-20 birds /m^2	≤ 13 birds /m^2
		Production	> 10 birds /m^2	6-10 birds /m^2	< 6 birds /m^2
Stocking density of breeder ducks	Open system	Rearing	≥ 7 birds /m^2	6 birds /m^2	≤ 5 birds /m^2
		Production	> 4 birds /m^2	3 birds /m^2	≤ 2 birds /m^2
	Closed system	Rearing	> 10 birds /m^2	8-10 birds /m^2	≤ 7 birds /m^2

Production	> 8 birds /m^2	5-8 birds /m^2	≤ 4 birds /m^2
Control of visitors	Bad control	Fair	Good
Water hygiene	Bad hygiene	Fair	Good
Feed hygiene	Bad hygiene	Fair	Good
Litter hygiene	Bad hygiene	Fair	Good
Control of pets and pests	Bad control	Fair	Good
Sick bird's quarantine	No	-	Yes
Hygiene of workers	Bad hygiene	Fair	Good
knowledge about biosecurity	No	Fair	Good

< means less than, > means more than, ≥ means equal or more than and ≤ means equal or less than

Table (2): The prevalence of *E. coli* isolated from different poultry farms.

Parameters	Broiler chicken			Total	Layer chicken			Total	Breed chicken			Total
	A	B	C		A	B	C		A	B	C	
Wall swabs	60.00 ±18.56 ^{Ca}	53.33 ±11.55^{cB}	40.00 ±26.67^{deC}	51.11 ±5.88 c	80.00 ±11.55^{abB}	100.00 ±0.00^{aA}	73.33 ±13.33^{bcdB}	84.44 ±13.74 bc	40.00 ±11.55 dB	53.33 ±8.82 bcA	60.00 ±6.67 bA	51.11 ±5.88 c
Air dust	53.33 ±20.28 ^{Ca}	40.00 ±23.09^{dB}	40.00 ±17.64^{deB}	44.44 ±4.44 c	53.33 ±6.67^{cB}	73.33 ±17.64^{bcdA}	53.33 ±6.67^{deB}	60 ±6.67 c	40.00 ±11.55 dA	46.67 ±8.82 cA	46.67 ±17.64 bA	44.45 ±2.22 cd
Feed storage	20.00 ±6.67 ^{Fa}	0±0^{gB}	0±0^{iB}	6.67 ±6.67 gh	6.67 ±6.67^{efA}	0±0^{fA}	0±0^{gA}	2.22 ±2.22 ^{f}	13.33 ±13.13 eA	0.00^{eB}	6.67 ±3.33 cdAB	6.67 ±3.85 d
Feed feeder	40.00 ±16.67^{Da}	33.33 ±20.00^{deB}	26.67 ±13.33^{fgC}	33.33 ±3.85 de	80.00 ±17.64^{abB}	86.67 ±6.67^{abA}	73.33 ±13.33^{bcdB}	66.67 ±13.1 c	40.00 ±6.67 dB	46.67 ±6.67 cB	60.00 ±11.55 bA	48.89 ±5.88 c
Water source	0.00^{Ga}	0.00^{gA}	0±0^{iA}	0±0 h	0±0^{Fb}	40.00 ±20.00^{eA}	0±0^{gB}	13.33 ±8.82 ef	33.33 ±6.67 dA	0.00^{eC}	20.00 ±11.55 cB	17.78 ±9.69 d
Drinker	40.00 ±25.17^{Db}	46.67 ±17.64^{cdA}	33.33 ±33.33^{efC}	40 ±3.85 cd	66.67 ±17.64^{bcA}	53.33 ±26.67^{deA}	20.00 ±20.00^{fgC}	46.67 ±12.91 cd	46.67 ±6.67 cdB	20.00 ±5.77 dC	60.00 ±11.55 bA	42.22 ±11.76 c
Storage litter	33.33 ±16.67^{deA}	26.67 ±20.00^{efB}	20.00 ±13.33^{ghC}	26.67 ±3.85 ef	33.33 ±17.64^{dC}	80.00 ±0.00^{abcA}	66.67 ±17.64^{cdB}	60 ±10 ^{c}	60.00 ±13.33 bcAB	66.67 ±5.77 bA	53.33 ±6.67 bB	60 ±3.85 bc
Pen litter	80.00 ±18.56^{Ba}	80.00 ±6.67^{bA}	60.00 ±30.55^{cB}	73.33 ±6.67 b	100.00 ±0.00^{aA}	93.33 ±6.67^{abA}	93.33 ±6.67^{abA}	95.56 ±2.94 a	86.67 ±13.33 aA	93.33 ±10.00 aA	86.67 ±13.33 aA	88.89 ±2.22 a
Bird cloacal swab	93.33 ±3.33^{Aa}	73.33 ±6.67^{bC}	80.00 ±0.00^{bB}	82.22 ±5.88 ab	80.00 ±11.55^{abB}	93.33 ±6.67^{abA}	86.67 ±6.67^{abcAB}	86.67 ±4.71 ab	73.33 ±13.33 abB	66.67 ±8.82 bB	86.67 ±6.67 aA	75.56 ±5.88 b
Bird droppings sample	100.0 ±16.670^{Aa}	100.00 ±0.00^{aA}	93.33 ±33.33^{aB}	97.78 ±2.22 a	100.00 ±0.00^{aA}	100.00 ±0.00^{aA}	100.00 ±0.00^{aA}	100±0 a	86.67 ±6.67 aB	86.67 ±6.67 aB	100.00 ±0.00 aA	91.11 ±4.44 a
Hand swab	20.00 ±3.33^{Fa}	20.00 ±13.33^{fA}	13.33 ± 6.67^{hB}	17.78 ±2.22 d	26.67 ±26.67^{deC}	46.67 ±24.04^{eA}	13.33 ±13.33^{gC}	28.89 ±12.07 de	6.67 ±6.67 eB	6.67 ±8.82 deB	20.00 ±11.55 cA	11.11 ±4.44 fg
Wheel swab	26.67 ±20.82 ^{Ec}	33.33 ±33.33^{deB}	46.67 ±29.06^{dA}	35.56 ±5.88 de	26.67 ±17.64^{deC}	86.67 ±30.55^{abA}	40.00 ±23.09^{efB}	51.1 ±13.1 cd	40.00 ±17.64 dA	0±0^{eB}	0±0 ^{dB}	13.33 ±13.33 d
Total	47.22^{A}	42.22^{AB}	37.78^{B}	42.4	54.44^{B}	70.33^{A}	51.67^{B}	56.85	47.22^{A}	40.56^{A}	50.00^{A}	45.9

a, b & c: There is no significant difference (P>0.05) between any two means, within the same column have the same superscript letter.

A, B & C: There is no significant difference (P>0.05) between any two means for the same attribute, within the same row have the same superscript let

Table (3): The prevalence of Pasteurella spp isolated from different poultry farms.

Parameters	Broiler chicken			Total	Layer chicken			Total	Breeder chicken			Total	Du
	A	B	C		A	B	C		A	B	C		A
Wall swabs	0 ± 0^{Ca}	0 ± 0^{cA}	0 ± 0^{Ca}	0 ± 0^{d}	0 ± 0^{cA}	0 ± 0^{eA}	0 ± 0^{bA}	0 ± 0^{d}	0.00^{dA}	0 ± 0^{bA}	0 ± 0^{dA}	0 ± 0^{c}	0±
Air dust	0 ± 0^{Ca}	0 ± 0^{cA}	0 ± 0^{Ca}	0 ± 0^{d}	0 ± 0^{cA}	0 ± 0^{eA}	0 ± 0^{bA}	0 ± 0^{d}	0 ± 0^{dA}	0 ± 0^{bA}	0 ± 0^{dA}	0 ± 0^{c}	0±
Feed storage	0 ± 0^{Ca}	0 ± 0^{cA}	0 ± 0^{cA}	0 ± 0^{d}	0 ± 0^{cA}	0 ± 0^{eA}	0 ± 0^{bA}	0 ± 0^{d}	0 ± 0^{dA}	0 ± 0^{bA}	0 ± 0^{dA}	0 ± 0^{c}	0±
Feed feeder	13.33 ±6.67 bcA	13.33 ±3.33 bA	0 ± 0^{Cb}	8.89 ±4.44^{cd}	6.67 ±6.67^{bcB}	26.67± 17.64^{bcA}	0 ± 0^{bC}	11.11 ±6.76^{bcd}	13.33 ±13.33^{cdB}	6.67 ±6.67 abC	33.33 ±24.04 cA	17.78 ±8.01^{bc}	26 ±6
Water source	0 ± 0^{Ca}	0 ± 0^{cA}	0 ± 0^{Ca}	0 ± 0^{d}	0 ± 0^{cA}	0 ± 0^{eA}	0 ± 0^{bA}	0 ± 0^{d}	0 ± 0^{dA}	0 ± 0^{bA}	0 ± 0^{dA}	0 ± 0^{c}	0±
Drinker	26.67 ±11.55 bA	0.00^{cC}	13.33 ±6.67 Bb	13.33 ±7.7^{c}	26.67 ±17.64^{aA}	26.67± 13.33^{bcA}	0 ± 0^{bB}	17.78 ±7.78^{ab}	40.00 ±5.77 ^{abA}	13.33 ±13.33 abC	33.33 ±24.04 cB	28.89 ±8.01^{b}	40 ±1
Storage litter	0 ± 0^{Ca}	0 ± 0^{cA}	0 ± 0^{Ca}	0 ± 0^{d}	0 ± 0^{cA}	0 ± 0^{eA}	0 ± 0^{bA}	0 ± 0^{d}	0 ± 0^{dA}	0 ± 0^{bA}	0 ± 0^{dA}	0 ± 0^{c}	0±
Pen litter	60.00 ±14.24 aA	46.67 ±5.77 aB	26.67 ±5.27 Ac	44.45 ±9.69^{ac}	0 ± 0^{cB}	53.33±16.38^{bA}	0 ± 0^{bB}	17.78 ±7.47^{bcd}	46.67 ±14.53 ^{aA}	0 ± 0^{bC}	40.00 ±11.55 bcB	28.89 ±14.57^{b}	46 ±6
Bird cloacal swab	13.33 ±5.77 bcA	13.33 ±3.33 bcA	0 ± 0^{Cb}	8.89 ±4.44^{c}	13.33 ±13.33^{bB}	20.00±11.55^{cdA}	0±0 bC	11.11 ±5.77^{bc}	26.67 ±18.56 bcB	13.33 ±13.33 abC	46.67 ±6.67 bA	28.89 ±9.69^{b}	33 ±6
Bird droppings sample	33.33 ±9.72 bA	26.67 ±3.33 abB	20.00 ±5.27 abC	26.67 ±3.85^{b}	13.33 ±6.67^{bB}	60.00± 11.55^{aA}	0±0 bC	24.45 ±9.49^{a}	53.33 ±13.33^{aB}	20.00 ±20.00 aC	66.67 ±13.33 aA	46.67 ±13.88^{a}	53 ±3
Hand swab	20.00 ±7.99 bcA	0 ± 0^{cB}	0 ± 0^{Cb}	6.67 ±6.67^{cd}	0 ± 0^{cB}	13.33±13.33^{dB}	0 ± 0^{bB}	4.44 ±4.44^{cd}	0 ± 0^{dA}	0 ± 0^{bA}	0 ± 0^{dA}	0 ± 0^{c}	6.6 ±6
Wheel swab	0 ± 0^{Ca}	0 ± 0^{cA}	0 ± 0^{Ca}	0 ± 0^{d}	0 ± 0^{cA}	0 ± 0^{eA}	0 ± 0^{bA}	0 ± 0^{d}	0 ± 0^{dA}	0 ± 0^{bA}	0 ± 0^{dA}	0 ± 0^{c}	0±
Total	13.89^{A}	8.33^{B}	5^{B}	9.075	5^{B}	16.67^{A}	0^{C}	7.22	15.00^{A}	4.44^{B}	18.33^{A}	12.59	17

a, b & c: There is no significant difference (P>0.05) between any two means, within the same column have the same superscript letter.

A, B & C: There is no significant difference (P>0.05) between any two means for the same attribute, within the same row have the same superscript letter.

Table (4): The prevalence of Campylobacter spp isolated from different poultry farms.

Parameters	Broiler chicken			Total	Layer chicken		
	A	B	C		A	B	C
Wall swabs	13.33 ±3.33 ^{efA}	20.00 ±6.67 ^{dA}	0±0^{dB}	11.11 ±5.88^{ef}	13.33 ±13.33^{deA}	20.00 ±20.00^{defA}	0±0d^{B}
Air dust	0±0^{Fa}	0±0^{eA}	0±0^{dA}	0±0^{f}	26.67 ±17.64^{dA}	33.33 ±13.33^{dA}	0±0^{dB}
Feed storage	33.33 ±6.67^{dA}	20.00 ±13.33 ^{dB}	0±0^{dC}	17.78 ±9.69^{e}	13.33 ±6.67^{deA}	6.67 ±6.67^{fgA}	13.33 ±13.33^{cA}
Feed feeder	26.67 ±6.67 ^{deB}	40.00 ±6.67^{cA}	33.33 ±11.55^{cAB}	33.33 ±3.85^{d}	66.67 ±17.64^{cB}	80.00 ±11.55^{abA}	20.00 ±20.00^{cC}
Water source	13.33 ±6.67 ^{efA}	0±0^{eB}	0±0^{dB}	4.44 ±4.44^{ef}	0±0^{eA}	0±0^{gA}	0±0^{dA}
Drinker	60.00 ±12.02 ^{cB}	73.33 ±29.06 ^{bA}	40.0 ±11.550 ^{cC}	57.78 ±9.69^{c}	66.67 ±6.67^{cA}	60.00 ±23.09^{cA}	0±0^{dB}
Storage litter	0±0^{Fb}	20.00 ±13.33 ^{dA}	0±0^{dB}	6.67 ±6.67^{ef}	6.67 ±6.67^{eA}	0±0^{gA}	0±0^{dA}
Pen litter	80.00 ±6.67 ^{Ba}	66.67 ±11.55 ^{bB}	73.33 ±6.67 ^{bAB}	73.33 ±3.85^{b}	100.00 ±0.00^{aA}	66.67 ±13.33^{bcB}	46.67 ±17.64^{bC}
Bird cloacal swab	86.67 ±3.33 ^{abAB}	93.33 ±6.67 ^{aA}	80.00 ±6.67 ^{abB}	86.67 ±3.85^{ab}	80.00 ±0.00^{bcA}	80.00 ±11.55^{aA}	80.00 ±11.55^{aA}
Bird droppings sample	100.00 ±0.00 ^{Aa}	100.00 ±0.00 ^{aA}	93.33 ±6.67 ^{aA}	97.78 ±2.22^{a}	86.67 ±13.33^{aA}	93.33 ±6.67^{aA}	86.67 ±13.33^{aA}
	Hand swab	26.67 ±3.33 ^{deA}	0±0^{eC}	13.33 ±6.67 ^{dB}	13.33 ±7.7^{e}	13.33 ±13.33^{deA}	26.67 ±13.33^{fgA}	13.33 ±13.33
	Wheel swab	0±0^{Fa}	0±0^{eA}	0±0^{dA}	0±0^{f}	0±0^{eA}	0±0^{gA}	0±0d^{A}
	Total	36.67^{A}	36.11^{A}	27.78^{B}	33.52	39.44^{A}	38.89^{A}	21.67^{A}

a, b & c: There is no significant difference (P>0.05) between any two means, within the same column have the same superscript letter.

A, B & C: There is no significant difference (P>0.05) between any two means for the same attribute, within the same row have the same superscript letter.

Table (5): Different *Ecoli* serotypes isolated from samples were collected from different poultry farm.

.		Broiler chicken					Layer chicken					Breeder chicken					Duck				
Ecoli strains monovalent	Polyvalent	A	B	C	No.	%	A	B	C	No	%	A	B	C	No	%	A	B	C	No	%
O55	Poly 2	+	+	-	3	8.3	-	-	-	-	-	-	-	+	1	2.78	+	-	+	3	8.3
O164	Poly 2	+	+	+	4	11.1	+	+	-	3	8.3	-	-	-	-	-	-	-	-	-	-
O112	Poly 7	+	+	+	5	13.89	-	-	-	-	-	+	+	+	3	8.3	+	-	-	1	2.78
O153	Poly 5	+	+	+	5	13.89	+	-	-	1	2.78	-	-	-	-	-	+	-	+	3	8.3
O152	Poly 8	+	+	+	3	8.3	+	+	-	4	11.1	-	-	-	-	-	-	-	-	-	-
O119 : K69	2	-	+	-	1	2.78	-	-	-	-	-	+	-	-	2	5.55	+	+	+	5	13.89
O157: K	3	-	-	-	-	-	-	+	-	1	2.78	-	-	-	-	-	+	+	-	4	11.1
O114 : K90	1	+	+	+	6	16.67	+	+	-	3	8.3	+	+	-	3	8.3	+	+	+	6	16.67
O26 : K60	1	+	-	-	2	5.55	+	+	+	8	22.22	+	+	+	4	11.1	-	-	-	-	-
O127 : K63	2	-	-	+	1	2.78	-	-	-	-	-	+	+	+	3	8.3	+	+	+	3	8.3
O128 : K67	2	+	-	-	1	2.78	+	+	+	7	19.44	-	-	-	-	-	+	+	-	3	8.3
O44 : K74	1	+	+	+	4	11.1	+	-	+	4	11.1	+	+	+	8	22.22	-	-	-	-	-
O124 : K72	3	-	-	-	-	-	-	-	-	-	-	+	+	+	5	13.89	+	+	-	4	11.1
O86 : K61	2	-	-	-	-	-	-	-	-	-	-	+	+	+	5	13.89	-	+	-	2	5.55
O91 : K	2	-	-	-	-	-	+	+	+	5	13.89	-	-	-	-	-	+	-	+	2	5.55
Total					36					36					36					36	

Table (6): Different Pasteurella serotypes isolated from samples were collected from different poultry farms.

		Broiler chicken					Layer chicken					Breeder chicken					Duck				
Pasteurella strains	Serotype	A	B	C	No.	%	A	B	C	No	%	A	B	C	No	%	A	B	C	No	%
Pasteurella multocida	A:1	+	+	+	17	47.2	+	+	-	12	33.33	+	+	+	18	50	+	+	+	21	58.33
Pasteurella multocida	A:3	+	+	+	12	33.33	+	+	+	17	47.2	+	+	+	15	41.67	+	+	+	11	30.55
Pasteurella multocida	A:12	+	+	-	3	8.33	+	+	-	5	13.89	-	-	+	3	8.33	-	+	+	4	11.1
Pasteurella multocida	Untypable	+	+	+	4	11.1	-	+	-	2	5.55	-	-	-	-		-	-	-	-	-
Total					36					36					36					36	

Table (7): Different Campylobacter serotypes isolated from samples were collected from different poultry farms

	Broiler chicken					Layer chicken					Breeder chicken					Duck				
Campylobacter strains	A	B	C	No.	%	A	B	C	No	%	A	B	C	No	%	A	B	C	No	%
Campylobacter Jejuni	+	+	+	12	33.33	+	+	+	16	44.44	+	+	+	15	41.67	+	+	+	14	38.89
Campylobacter coli	+	+	+	9	25	+	+	+	10	27.78	+	+	+	12	33.33	+	+	+	9	25
Campylobacter Lari	+	+	+	7	19.44	+	+	-	5	13.89	+	-	+	6	16.67	+	+	+	6	16.67`
Campylobacter Upsaliens	+	+	-	6	16.67	+	-	-	2	5.55	+	-	+	3	8.3	+	+	+	4	11.1
Campylobacter Lanienae	-	+	-	2	5.55	+	+	-	3	8.3	-	-	-	-	-	+	+	+	3	8.3
Total				36					36					36					36	

Table (8): The virulence genes of some pathogenic bacteria isolated from different poultry farms .

Pathogenic Bacteria	Serotypes	*E.coli* virulence genes		Pasteurella virulence genes			Campylobacter virulence genes	
		Stx1	EaeA	PfhA	hgbB	toxA	cdtB	cdtC
E.coli	*O44*	-	-					
	O114	-	+					
	O164	+	+					
	O112	-	-					
	O55	-	-					
	O157	-	-					
	O119	-	+					
	O26	+	+					
	O124	-	+					
	O86	-	-					
Pasteurella	*A: 1*			+	+	+		
	A: 3			+	+	-		
	A: 12			+	-	-		
Campylobacter	*C. jejuni* (C_1-C_{12})						+	+
%		20%	50%	100%	66.67%	33.33%	100%	58.3%

Figures

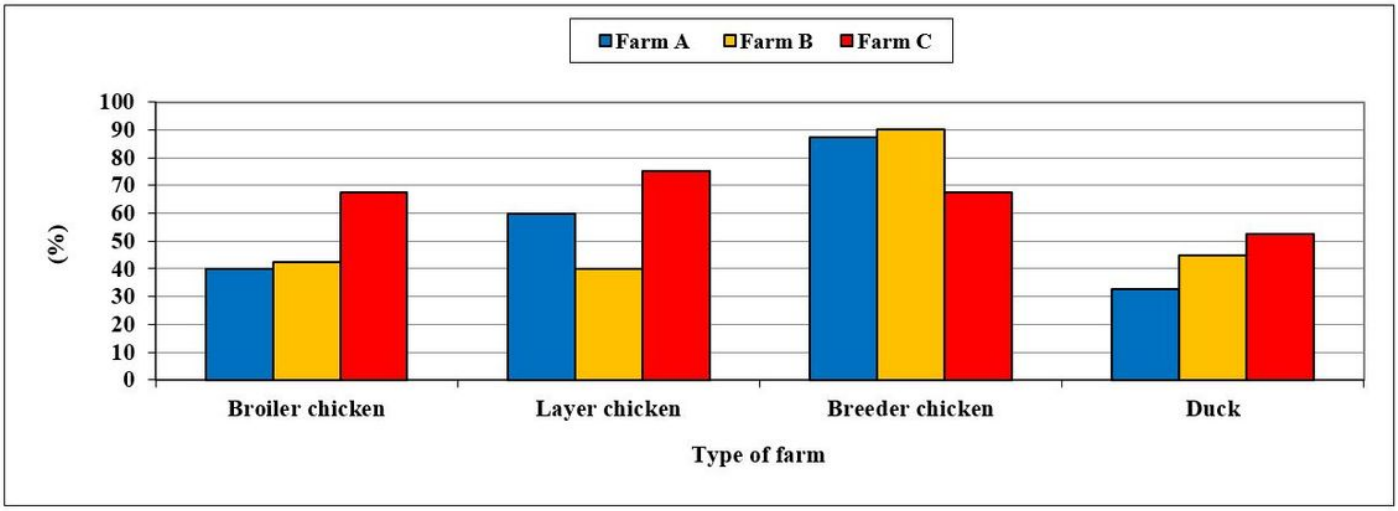

Figure 1

The biosecurity scoring percentage of different poultry farms.

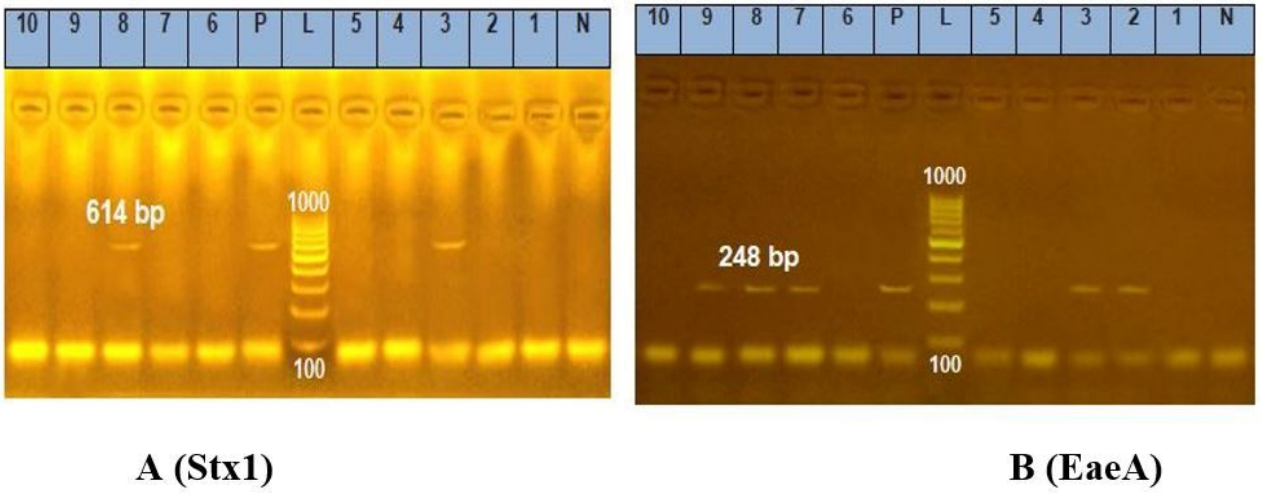

Figure 2

Characterization virulence genes of *some E. coli serotypes* on agarose gel electrophoresis of conventional PCR.

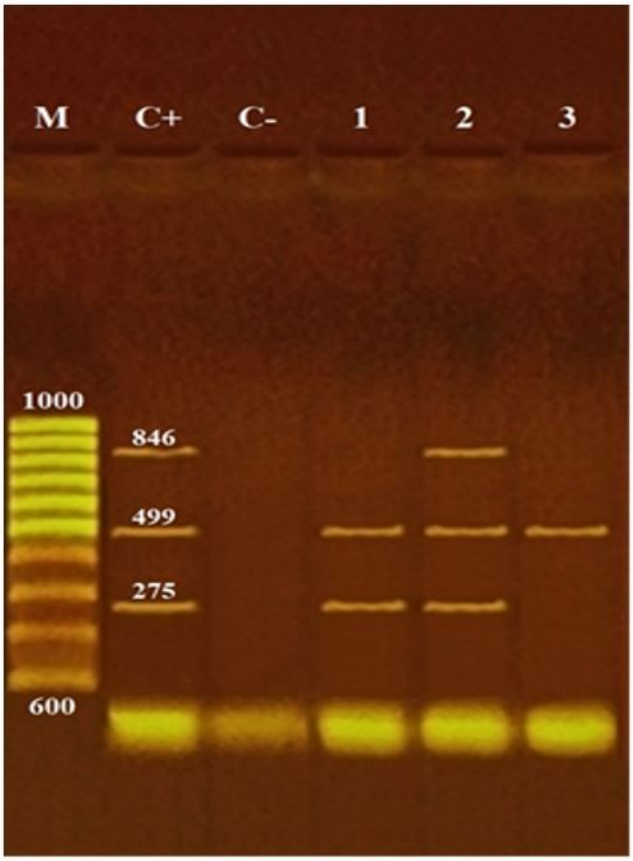

Figure 3

Characterization virulence genes of *Pasteurella multocida* [pfhA (275 bp), hgbB (499 bp) and toxA (846 bp)] on agarose gel electrophoresis of multiplex PCR

Lane M: 100 bp ladder as molecular size DNA marker.

Lane C+: Control positive *P. multocida* for pfhA, hgbB and toxA genes.

Lane C-: Control negative.

Lane 2 : Positive strain (A:1) for pfhA, hgbB and toxA genes.

Lane 1: Positive strains (A:3) for pfhA and hgbB genes.

Lane 3: Positive strain (A:12) for pfhA gene.

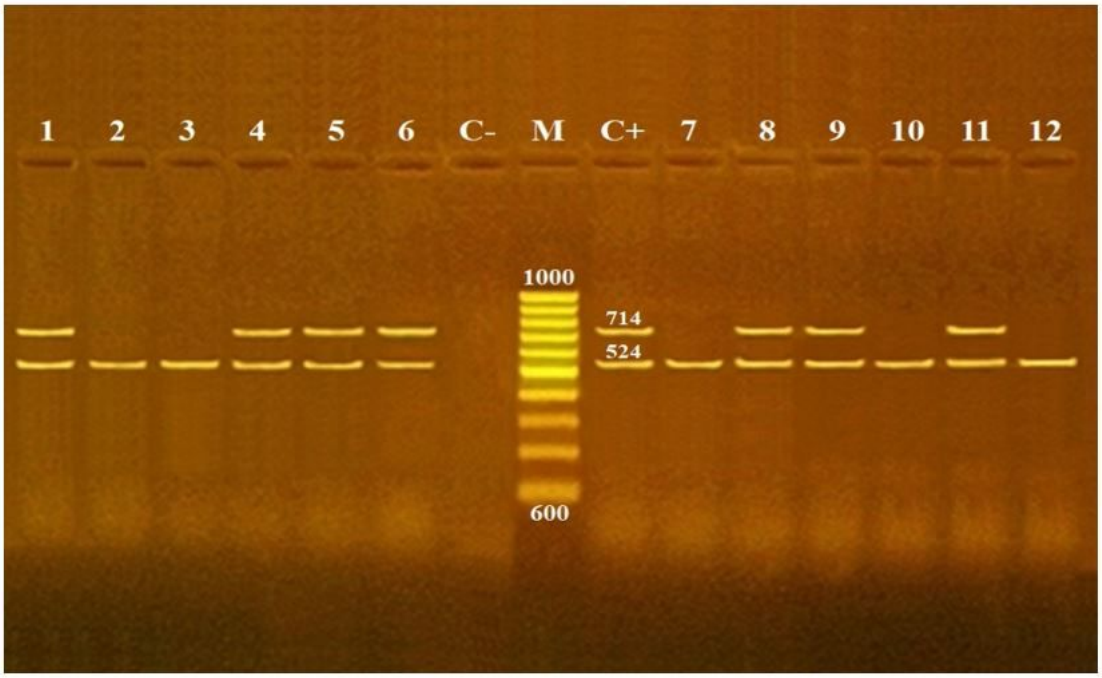

Figure 4

Characterization virulence genes of *Campylobacter jejuni* [cdtB (714 bp) and cdtC (524 bp)] on agarose gel electrophoresis of multiplex PCR for cytological distending toxins

Lane M: 100 bp ladder as molecular size DNA marker.

Lane C+: Control positive *C. jejuni* for cdtA, cdtB and cdtC gene

Lane C-: Control negative

Lanes 1, 4, 5, 6, 8, 9 & 11: Positive *C. jejuni* for cdtB & cdtC genes.

Lanes 2, 3, 7, 10 & 12: Positive *C. jejuni* for cdtB gene.