Environmental and Epidemiological Aspects of *Alphitobius diaperinus* Infestation and Pathogen Shedding: A Biosecurity Threat in Poultry Farms

Naglaa Mhny [1], Samah F Darwish [2], Ahmed R El-Dahshan [1] and Asmaa M Ali [1*]

1. Department of Veterinary Hygiene and Management, Faculty of Veterinary Medicine, Cairo University, Egypt.
2. Biotechnology Research Unit, Animal Reproduction Research Institute, Agriculture Research Center

* Corresponding author: Asmaa M Ali, e-mail address: dr_fulla_2007@cu.edu.eg

1. Abstract

Darkling beetles (***Alphitobius diaperinus)*** are ubiquitous generalist pests of poultry facilities. They are known for their persistence in the environment, retaining several avian pathogens, causing economic losses through structural damage and dissemination of pathogens within poultry flocks. This study aimed to investigate the possible role of litter in beetle survival and distribution and determine if beetles could carry pathogens related to bird health and threaten public health. A total of 510 samples were obtained from 30 different poultry farms, including beetles (n=90), cloacal swabs (n=150), litter (n=90), swabs from feeders (n=90), and drinkers (n=90). Litter physical parameters (temperature, moisture, and pH) were measured. Correlation between litter parameter and beetle population was performed. All samples were examined bacteriologically and molecularly identified for the presence of *Staphylococcus aureus* and *Escherichia coli.* The darkling beetles were counted significantly ($P < 0.05$) in all poultry farms, recording the highest prevalence of 79.5/m^2 in broiler farms. Statistical analysis revealed that litter temperature (from 26 to 31°C) and moisture (up to 40%) have a strong correlation with beetle density, while pH (from 7.5 to 9) has no impact on beetle number. *S. aureus* was isolated from beetles (2.6%), cloacal swabs (1.7%), litter (1.3%), feeder (1.7%), and drinkers (12%). In addition, *E. coli* prevalence was in beetles (78.9%), cloacal swabs (82.7%), litter (84.4%), feeders (35.6%), and drinkers (33.3%). The presence of darkling beetles in poultry farms, along with their ability to shed pathogens into the environment, underscores the importance of these insects in the persistence of infection between flocks, posing a significant health risk to poultry production and humans. Therefore, implementing alternative control methods and an effective integrated pest management program are essential to mitigate the beetle population and lower the risk of pathogen transmission in poultry farms.

Key words: Darkling beetle; beetle population; litter parameter; poultry flock; *S. aureus; E. coli*.

2. Introduction

Alphitobius diaperinus is a worldwide pest commonly found in poultry farms. In the larval stage, it is known as a lesser mealworm, while in the adult stage, it is known as a litter beetle or a darkling beetle [1]. It adapted to the microenvironment of poultry farms, causing significant economic losses

Online ISSN: 2537-1045
Print ISSN: 1110-1423
DOI: 10.21608/vmjg.2025.398306.1046

through damaging farm infrastructure and disease transmission [2].

Darkling beetles commonly colonize under litter, at corners, under feeders, and near cooling pads. They survive within flocks by feeding on litter, spilled feed, bird droppings, and even dead birds [3]. The degree of infestation is linked to suboptimal litter management, high organic load, and physical parameters of litter (defined as moisture content, pH, and temperature), which encourage beetles' survival and reproduction [4].

Darkling beetles are significant vectors for several pathogens infecting poultry flocks, such as viruses [5], fungi [6], and bacteria (including *E. coli, Campylobacter, Staphylococcus* spp, *and Salmonella)* [7]. It plays a significant role in the horizontal spread of pathogens within poultry flocks. The beetles obtain bacteria from a contaminated environment and spread them across flocks, perpetuating the pathogens' presence in the environment [8].

Poultry can be infected directly by ingesting infected beetles or indirectly by contaminated litter, food, and water [9].

On the other hand, poultry consumes infected beetles, which remain undigested due to their keratinized structure, contaminating carcasses during slaughtering. In addition, they threaten human health through the dissemination of zoonotic pathogens, rendering the meat unfit for sale and making it unmarketable [10].

Staphylococcus spp. and *E. coli* are the most common pathogens in poultry farms and threaten the poultry industry. The main virulence factors of *Staphylococcus* spp. are their ability to form biofilm, which enhances bacterial survival, persistence, and shedding [11]. Moreover, *S. aureus* carries antibiotic resistance genes, raising significant public health concerns [12]. In breeder farms, Staphylococci are responsible for skeletal infections associated with locomotor problems [13], leading to a decrease in production and reproduction, inflicting economic damage

Avian pathogenic *Escherichia coli* (APEC) is a major etiological agent responsible for colibacillosis, septicemia, omphalitis, and yolk sac infection, leading to high morbidity and mortality, especially for broiler chicks [14]. It is a high-prevalence and economically impactful bacterial disease that impairs egg production and causes systemic infections, leading to economic losses among layer production farms [15].

Despite the recognized role of beetles as pathogen vectors, there is a notable gap in understanding the prevalence of beetle populations and the degree of infestation among different poultry systems.

This study aimed to evaluate the environmental factors affecting the persistence of these insects and investigate the correlations between darkling beetle populations and litter parameters (moisture, pH, temperature) in poultry farms. Moreover, screening for the most common bacterial diseases shed by beetles contaminates the surrounding poultry environment. Additionally, it evaluates the risk factors (beetles, litter, or other environmental sources) for infection to help apply biosecurity measures to minimize the cross-contamination of these pathogens between poultry flocks.

3. Materials and Methods

3.1. Ethical Approval

The study followed the ethical guidelines for poultry sampling provided by the Institutional Animal Care and Use Committee (IACUC), Faculty of Veterinary Medicine, Cairo University, Egypt. It was approved by the IACUC (VET-CU-IACUC-110520251073).

3.2. Poultry Houses and Sampling

Online ISSN: 2537-1045
Print ISSN: 1110-1423
DOI: 10.21608/vmjg.2025.398306.1046

The study was conducted in thirteen poultry farms with a deep litter system of housing distributed in the governorates of Giza, Fayoum, Menoufia, Beni-Suef, and Alexandria. Table 1 lists the number of samples taken from each governorate.

The farms studied differ in their biosecurity level. Still, they have a closed system with a controlled environment and share the same noticeable characteristics, such as cracks in walls, door gaps, poorly sealed ventilation openings, damaged insulation materials, poorly maintained cooling pads, and wooden doors. The farms are variable in drinking and feeding equipment (nipples or drinkers) and (feeding alley or open feeders).

The samples were collected following complete personal protective equipment and aseptic technique. 510 samples were examined, including 90 darkling beetle samples, 150 cloacal swabs, 90 litter samples, 90 feeders swabs, and 90 drinkers swabs.

Beetles' samples were collected using sterile tweezers from the house corners, under the feeders, near cooling pads, and the window. Afterwards, sterile cups were filled with beetles and labelled. Swabs were taken from birds, feeders, and drinkers using sterile soaked swabs placed in non-selective Buffered Peptone Water (BPW; HIMEDIA).

Litter samples were collected in sterile plastic bags, ensuring representative sampling within the poultry house. The litter was collected using a randomized sampling method. All samples were collected, stored in an ice box, and brought to the lab to be tested.

3.3. Estimation of Beetle Population

Beetle colonization sites were observed under the litter, at corners of the house, under feeders, near cooling pads, and windows. The beetle population was counted in three different sites within a farm.

A wooden frame (10 x 10 cm) was put on the floor surface after exposing the litter, and then direct collection of beetles distributed in this area for further counting in the lab [16].

3.4. Physical Examination of Litter

Litter temperature was measured in situ using digital thermometers. Measurements were taken at a consistent depth (5 cm)

The moisture content of litter was determined gravimetrically by weighing 10 g of fresh litter, then dried at 105°C for 24 hours in a hot air oven until constant weight was achieved [17].
Moisture Content (%) = Fresh Weight−Dry Weight ×100

Litter pH was determined by extracting pore water through a standardized method. A small, representative sample of litter was mixed with distilled water at a ratio of 1:5 (w/v), shaken for 30 minutes, and allowed to settle. The pH of the supernatant was then measured using a calibrated portable pH meter [18].

3.5. Bacteriological Isolation and Identification

The beetles were prepared to assess internal bacterial carriage according to [19]. Briefly, the beetles' surface was washed three times, disinfected using ethanol 95% and 10% H_2O_2, and rinsed thrice with sterile distilled water. In a sterile mortar, the beetles were crushed and added to 9 ml BPW, which was incubated at 37 ˚C for 24 hrs.

All swabs from the birds' cloaca, feeders, and drinkers, which were soaked in 9 ml BPW, were directly incubated at 37 ˚C for 24 hrs [20]. Three grams of litter were added to 27 mL BPW, vortexed, and filtered. One mL of filtrate was added to 9 mL of BPW and incubated at 37°C for 24 hrs.

Online ISSN: 2537-1045
Print ISSN: 1110-1423
DOI: 10.21608/vmjg.2025.398306.1046

After 24 hours of pre-enrichment culturing, 7% sheep blood agar (Oxoid Ltd., Hampshire, UK) and mannitol salt agar (HIMEDIA, India) were used for selective isolation of *Staphylococcus* spp., and EMB agar (Oxoid, Hampshire, UK) was used for *E. coli* isolation. The selective agar media were incubated aerobically at 37 °C for 24 hrs. According to [21]. Suspected colonies of *Staphylococcus* spp. biochemically confirmed using the catalase reaction and the coagulase test. *E. coli* biochemically identified according to [22].

3.6. Bacterial Gene Identification

Chromosomal DNA was extracted from the broth of bacterial isolates by using a quick boiling process method described by [23].

In Polymerase Chain Reaction (PCR) amplification, 5 µl of extracted DNA, 25 µl of 2X (Dream Taq Green PCR Master Mix, Waltham, USA) and 0.5 µl of each primer (table 2) at 20 pmol concentration; nuclease free water was added up to 50 µl.

Multiplex PCR assay when targeting 16S rRNA gene (Staphylococcus genus-specific), nuc (*S. aureus* species-specific).

Amplicons were examined after gel electrophoresis, gel were stained with ethidium bromide and seen with 100 bp DNA ladder (Sigma, Darmstadt, Germany) under UV light. Negative and positive controls were used in each batch of reactions.

3.7. Statistical Analysis

Data was collected, tabulated, and analyzed using SPSS 26.0 (Statistical Package for Social Sciences) and Microsoft Excel.

Descriptive statistics were performed for numerical data as mean±SD (standard deviation), while for categorical data as number and percentage. Inferential analyses were done for quantitative variables using the ANOVA test in cases of more than two independent groups with parametric data. Inferential analyses were done for qualitative data using the chi-square test or the Fisher exact test for independent groups.

The Person correlation coefficient was used to detect the correlation between quantitative data. The level of significance was set at P value <0.05, which is significant; otherwise, it is non-significant. The p-value indicates how likely it is that the results of the study could have happened by chance.

4. Results

4.1 Estimation of Beetle Population

The prevalence of beetles/m^2 across different poultry production farms is represented in fig. (1). The mean number was significantly different between farms, with the highest number in broiler farms (79.5 ± 16.57), followed by breeder farms (64.5 ± 12.12), and the lowest in layer farms (62.7 ± 8.54).

4.2. Relation Between Beetle Population and Measured Litter Parameters

Fig. (2, 3 and 4) illustrate the relationship between litter parameters and beetle populations in different poultry farms. The temperature shows a strong positive correlation (r = 0.692 to 0.887) across all farm types, with highly significant (P < 0.001), indicating a consistently impactful relationship. pH, however, demonstrates very weak and statistically insignificant correlations (r=*-0.237* to 0.231; $P > 0.05$), suggesting little to no effect. Meanwhile, moisture content reveals a robust negative correlation (r= -0.706 to -0.971) with highly significant *P-values* (<0.001), emphasizing a powerful inverse relationship with the variable studied.

Online ISSN: 2537-1045
Print ISSN: 1110-1423
DOI: 10.21608/vmjg.2025.398306.1046

4.3 Prevalence of Bacteria Isolated from Different Sources in Poultry Farms:

4.3.1 Prevalence of Staphylococcus spp. and S. aureus

The results in table (3) present the prevalence of *Staphylococcus* spp**.** from different sources in poultry farms. High prevalence was recorded from darkling beetles (85.6%), litter (84.4%), and cloacal swabs (80.6%). While drinkers and feeders recorded 55.6% and 66.7%, respectively.

Fig. (5) represents the prevalence of *S. aureus* isolated from poultry farms**.** Out of 384 samples positive for *Staphylococcus* spp., the prevalence of *S. aureus* was 12 isolates (3.1%). Significance was found between different sources, as the highest prevalence was observed from drinkers (12%), Cloacal swabs (1.7%), and darkling beetles (2.6%). In comparison, the lowest prevalence was observed in litter (1.3%) and feeder (1.7%).

4.3.2 Prevalence of E. coli in Different Poultry Farms

The results presented in Table 4 revealed the significant difference in *E. Coli* prevalence from different sources in poultry farms. A high prevalence was recorded from litter (84.4%), cloacal swabs (82.7%), and Darkling beetles (78.9%). While drinkers and feeders record low prevalence (33.3% and 35.6%, respectively).

5. Discussion

The poultry house structure, environmental conditions, and hygienic measures can provide an ideal biotope for the darkling beetle to complete its life cycle [27]. Beetle infestation can vary according to the housing system, the duration of bird rearing, the stocking density, the recycling of litter, and the insecticide used.

This epidemiological study found that beetle numbers were significantly different between farms, with the highest mean number in broiler farms (79.5 ± 16.57), followed by breeder farms (64.5 ± 12.12), and the lowest in layer farms (62.7 ± 8.54). This finding agrees with [28], who estimated that there are 34.7 million adult beetles and other stages in a single house of broiler production. Also, Chernaki-Leffer *et al.* [29] studied broiler flocks and observed that the number of insects per trap increased from 5,137 in the first week to approximately 18,494 in the sixth week, and the population increased in successive flocks.

Variation in beetle population recorded in our studied farms may be due to broiler chicken farmers prioritizing chicken growth and productivity while neglecting pest control programs. However, layer and breeder farms implement some control measures without paying attention to the evolving insecticides or adjusting the optimal concentration. As a result, beetles persist in consecutive flocks and become resistant to many insecticides.

Darkling beetles seek a favorable site in a poultry farm for pupation and development. Our survey detected a high density of beetle populations in the corners, near the windows, and under the feeders compared to other farm sites. This may be due to food sources, suitable temperature, and moisture content. Besides, uncleaned surfaces in corners are difficult for workers to access during the cleaning and disinfection. This agrees with [30], who found that spilled feed and neglected corner cleaning increase the population.

Quality, managemental practice, and litter physical parameters also play a significant role in the beetle population. Figs (2, 3, and 4) illustrate the correlation between litter physical parameters

(temperature, pH, and moisture) and the number of beetles in different poultry farms.

According to the litter temperature, we found that the litter temperature between 26°C and 31°C has a strong positive correlation and significant *P-values* (<0.001) with the number of beetles. The same observation was recorded by [4], who experimentally proved that suitable temperatures for adult survival and optimal reproduction of darkling beetles within the spectrum of (25°C to 32 °C) and above 35°C cause a negative impact on survival rate. This is ensured by Bjørge *et al.* [31], who concluded that temperature is a major factor influencing beetle growth and reproduction. In contrast, [29] found that temperature did not significantly affect the number of darkling beetles.

On the other hand, our Statistical correlations between litter moisture and beetle number provided a strong inverse relationship, as when litter moisture increased over 40%, the beetle number significantly decreased.

This can be explained as excessively high moisture content promotes the growth of microorganisms, which can compete with adult beetles for resources or even become pathogens, thereby reducing their survival rate. Fungus-killed insects, such as *Beauveria bassiana*, which grows naturally in soils and is pathogenic to the species of darkling beetles [32]. In contrast,[4] found that darkling beetle development and survival across its life cycle are mostly influenced by temperature rather than moisture content, since the species can adapt to a range of humidity conditions, even relatively dry ones.

Regarding litter pH, we found no significant correlations between measured pH (ranging from 7.5 to 9) and the number of beetles. This finding agrees with [33], who found that different pH values don't affect the beetle population. In an experimental trial by [34], they found that increasing pH (≥12) by adding hydrated lime to poultry litter increased larval and adult darkling beetle mortality.

Understanding these factors is an important tool for eradicating beetles and applying a successful integrated pest management (IPM) program. Otherwise, they become resilient pests with a vectorial capacity to harbor and disseminate pathogens through poultry flocks.

The present investigation pointed out that bacterial pathogens can significantly impact poultry production, such as *Staphylococcus* spp. and *E. coli*. Table (3) discusses the prevalence of *Staphylococcus* spp. isolated from beetles, poultry, and other environmental sources in the poultry house. *Staphylococcus* spp. High prevalence was recorded from darkling beetles (85.6%), litter (84.4%), cloacal swabs (80.6%), drinkers (55.6%), and feeders 66.7%. The prevalence is nearly as isolated by [35], who recorded *Staphylococcus spp.* 30% from poultry, litter 25%, drinkers 20%, and feeders 20%. Also, [30] and [36] detected *Staphylococcus spp.* from beetles.

The isolated *Staphylococcus* spp. from beetles were positively identified as *S. aureus* with 2.6% prevalence; from litter 1.3%, cloacal swabs 1.7%, while drinkers 12% and feeders 1.7%. In contrast, [30] and [36] negatively identified *S. aureus* from beetles. While a similar result was obtained by [36] and [37], who isolated *S. aureus* within 5.1% and 1.6% from cloacal swabs, respectively. A high prevalence of *S. aureus* 21.3% *was* recorded by [39]. From litter samples [40], isolate *S. aureus* with 14.29%.

E. coli is always present in birds' gastrointestinal tracts and spreads widely in droppings, contaminating water and the environment [41], resulting in significant health problems and economic

Online ISSN: 2537-1045
Print ISSN: 1110-1423
DOI: 10.21608/vmjg.2025.398306.1046

losses [42]. Table 4 shows the prevalence of *E. Coli* isolated from different sources in poultry farms. The high prevalence of *E. Coli* 84.4% within litter, followed by cloacal swabs 82.7%, Darkling beetles (78.9%), and a low prevalence was detected from drinkers (33.3%) and feeders (35.6%).

Many researchers agree with our results that [30] isolated *E. coli* from darkling beetles with 89% prevalence, and [43] reported that darkling beetles were able to carry and shed *E. coli* for 12 days. Meanwhile, [44] recorded a 25 % low *E. coli* prevalence. From poultry flocks [45] and [46], isolated *E. coli* with 80% and 73% respectively. [47] isolated *E. coli* from feeders with 33.33%.[48] found *E. coli* with a prevalence of 44.1% in litter.

Variations in prevalence explain the level of farm hygiene, the number of samples, intermittent shedding of bacteria in droppings, and easy transmission and spread of pathogens via direct and indirect contact within poultry, beetles, litter, and other environmental samples. Besides, the *S. aureus* and *E. coli* have the potential to form biofilm on different surfaces, and carry biocide resistance genes (BRG) [49], making them persistent in the poultry environment.

6. Conclusion

Darkling beetles are prevalent pests in poultry production systems, with the highest infestation levels observed in broiler farms. Their distribution within facilities is uneven, with higher densities commonly found in corners, beneath feeders, and near cooling pads. Litter conditions significantly influence their colonization patterns; beetle development and survival are favored by temperatures between 26–31°C and low humidity levels (≤40%), while litter pH (7.5–9) appears to have minimal impact.

Importantly, these beetles can act as vectors for *Staphylococcus* spp. and *Escherichia coli*, alongside other environmental sources, potentially influencing pathogen dynamics and serving as a reservoir of infection within poultry environments. Strengthening biosecurity measures and adopting alternative control methods are crucial to reducing beetle populations and mitigating the risk of infection in poultry flocks. Incorporating these findings into integrated pest management (IPM) programs is critical for improving poultry health and maintaining farm biosecurity.

Conflict of interest: Nothing to declare

7. References

1. Mariod A A, Mirghani M E S, Hussein I. *Alphitobius diaperinus* the Lesser Mealworm and the Litter Beetle. Unconventional oilseeds and oil sources. Elsevier, Amsterdam, the Netherlands. 2017; 327-330.

2. Renault D, Colinet H. Differences in the susceptibility to commercial insecticides among populations of the lesser mealworm (*Alphitobius diaperinus)* collected from poultry houses in France. Insects. 2021; 12(4): 309.

3. Sparagano O, Di Domenico D, Venturelli C, Papadopoulos E, Smallegange R C, & Giangaspero A. Arthropod pests in the poultry industry. In Pests and vector-borne diseases in the livestock industry. Wageningen Academic. Chapter 2. 2018:17-53.

4. Zafeiriadis S, Baliota G V, Athanassiou C G. The effect of temperature and moisture content on population growth of *Alphitobius diaperinus* (Panzer) (Coleoptera:

Tenebrionidae). Agronomy. 2023; 13 (10): 2535.

5. Ou S C, Giambrone J J, Macklin K S. Detection of infectious laryngotracheitis virus from darkling beetles and their immature stage (lesser mealworms) by quantitative polymerase chain reaction and virus isolation. Journal of Applied Poultry Research. 2012; (1):33–38.

6. da Silva Soares CE, Weber A, Scussel V M. Stereo and scanning electron microscopy characteristics of poultry breeding beetle (*Alphitobius diaperinus*), a filamentous toxigenic fungi carrier. Emirates Journal of Food and Agriculture. 2018; 30 (2): 150-156.

7. Donoso A, Paredes N, Retamal P. Detection of antimicrobial-resistant Salmonella enterica strains in larval and adult forms of lesser mealworm (*Alphitobius diaperinus*) from industrial poultry farms. Frontiers in Veterinary Science. 2020; (7): 577848.

8. Crippen T L, Sheffield C L, Beier R C, Nisbet D J. The horizontal transfer of *Salmonella* between the lesser mealworm (*Alphitobius diaperinus*) and poultry manure. Zoonoses Public Health 2018; (65): e23–e33.

9. Buyukyavuz A, Northcutt JK, Dawson PL. Incidence of bacterial pathogens in flying insects collected near poultry farms. Journal of Applied Poultry Research. 2024; 33 (4): 100462.

10. Polat, B, Cengiz A, Koc S, Kahraman Kokten, S, Gultekin Z N, Caliskan C, et al. Efficacy of Steinernema feltiae nematode against lesser mealworm (*Alphitobius diaperinus*) populations from poultry farms in Türkiye. Veterinary Sciences. 2024; 11 (11): 567.

11. Mack D, Rohde H, Harris L G, Davies A P, Horstkotte M A, & Knobloch J M. Biofilm formation in medical device-related infection. The International Journal of Artificial Organs. .2006; 29(4):343-359.

12. Saharan V V, Verma P, Singh A P. *Escherichia coli*, *Salmonella spp*., and *Staphylococcus aureus* susceptibility to antimicrobials of human and veterinary importance in poultry sector of India. Journal of food safety. 2020; 40 (1): e12742.

13. Szafraniec G M, Szeleszczuk P, Dolka B. Review on skeletal disorders caused by *Staphylococcus spp*. in poultry. Veterinary Quarterly. 2022; 42(1): 21-40.

14. Islam M S, Nayeem M M H, Sobur M A, Ievy S, Islam M A, Rahman S, et al. Virulence determinants and multidrug resistance of *Escherichia coli* isolated from migratory birds. Antibiotics. 2021; 10 (2): 190.

15. Joseph J, Zhang L, Adhikari P, Evans J D, Ramachandran R. Avian pathogenic *Escherichia coli* (APEC) in broiler breeders: an overview. Pathogens. 2023; 12(11): 1280.

16. Stark J D, Banks J E. Population-level effects of pesticides and other toxicants on arthropods. Annual review of entomology. 2003; 48(1): 505-519.

17. Dumas M D, Polson S W, Ritter D, Ravel J, Morgan R, Wommack K E, et al. Impacts of poultry house

environment on poultry litter bacterial community composition. PLoS One. 2011; 6(9): e24785.

18. Cools N, De Vos B. Forest soil: characterization, sampling, physical, and chemical analyses. Developments in environmental science. 2013; (12): 267-300.

19. Crippen T L, Sheffield C. External surface disinfection of the lesser mealworm (Coleoptera: Tenebrionidae). Journal of Medical Entomology. 2006; 43 (5): 916-923.

20. Soliman E S, Reddy P G, Sobieh M A A, Busby H, Rowe S E. Epidemiological surveillance on environmental contaminants in poultry farms. Int. J. Poult. Sci. 2009; 8 (2): 151-155.

21. Quinn P J, Markey B K, Leonard F. C., Hartigan, P., Fanning, S., & Fitzpatrick, E. Veterinary microbiology and microbial disease. 2011. John Wiley & Sons.

22. Feng P, Weagant S D, Grant M A, Burkhardt W, Shellfish M, & Water B. BAM: Enumeration of *Escherichia coli* and the Coliform Bacteria. Bacteriological analytical manual. 2002; 13(9): 1-13.

23. Darwish S F, Asfour H A. Investigation of biofilm-forming ability in Staphylococci causing bovine mastitis using phenotypic and genotypic assays. The Scientific World Journal. 2013;1, 378492.

24. Monday S R, Bohach G A. Use of multiplex PCR to detect classical and newly described pyogenic toxin genes in staphylococcal isolates. Journal of Clinical Microbiology. 1999; 37(10): 3411-3414.

25. Brakstad O G, Aasbakk K, Maeland J A. Detection of *Staphylococcus aureus* by polymerase chain reaction amplification of the nuc gene. Journal of clinical microbiology. 1992; 30 (7): 1654-1660.

26. Hu Q, Han X, Zhou X, Ding C, Zhu Y, Yu S. OmpA is a virulence factor of Riemerella anatipestifer. Veterinary microbiology. 2011; 150 (3-4): 278-283.

27. Agabou A, Alloui N. Spatio-temporal variability of broiler house litter infestation with *Alphitobius diaperinus* during three successive breeding cycles in the North-East of Algeria. International Journal of Current Research. 2012; 4 (8): 140-142.

28. Singh N. Chemical ecology, population dynamics, and insecticide susceptibility of lesser mealworm *Alphitobius diaperinus* (Panzer) (Coleoptera: Tenebrionidae). Ph.D. dissertation, University of Arkansas, Fayetteville. 2011

29. Chernaki-Leffer A M, Almeida L M, Sosa-Gómez D R., Anjos A, Vogado K M. Populational fluctuation and spatial distribution of *Alphitobius diaperinus* (Panzer) (Coleoptera; Tenebrionidae) in a poultry house, Cascavel, Parana state, Brazil. Brazilian Journal of Biology. 2007; (67): 209-213.

30. Tamburro M, Sammarco M L, Trematerra P, Colacci M, Ripabelli G. Alphitobius diaperinus Panzer (Insecta, Coleoptera) in a single house of a broiler production facility as a potential source of pathogenic bacteria for broilers and humans. Letters in

Online ISSN: 2537-1045
Print ISSN: 1110-1423
DOI: 10.21608/vmjg.2025.398306.1046

Applied Microbiology. 2022; 74 (6): 883-892.

31. Bjørge J D, Overgaard J, Malte H, Gianotten N, Heckmann L H. Role of temperature on growth and metabolic rate in the tenebrionid beetles Alphitobius diaperinus and Tenebrio molitor. Journal of Insect Physiology. 2018; 107: 89-96.

32. Kucharska K, Zajdel B, Pezowicz E, Jarmul-Pietraszczyk J. Compatybility of entomopathogenic nematodes with active substances of popular biocidal products, in controlling of the lesser mealworm beetle *Alphitobius diaperinus* (Panzer, 1797). Annals of Warsaw University of Life Sciences-SGGW. Animal Science. 2018; 57 (3).

33. Abreu V M N, Paiva D P D, Abreu P G D, Coldebella A. Evaluation of litter material and ventilation systems in poultry production: III. Litter reuse, darkling beetle populations and intestinal parasites. Revista Brasileira de Zootecnia. 2011; 40:1372-1380.

34. Watson D W, Denning S S, Zurek L, Stringham SM, Elliott J. Effects of lime hydrate on the growth and development of darkling beetle, Alphitobius diaperinus. International Journal of Poultry Science. 2003; 2 (2): 91-96.

35. Khalefa H S, Abdel-Kader F, Kadry M. Public Health Importance of Biofilm-Producing *Staphylococcus* species in Poultry Farms, Nearby Wild Bird Residents, and Farm Workers in Egypt. Egyptian Journal of Veterinary Sciences. 2025; 1-10.

36. Antonuccio G I, Sauka D H. Isolation and identification of culturable gut microbiota in the larval stage of lesser mealworm (*Alphitobius diaperinus*). In Biology and Life Sciences Forum. 2023;31 (1): 12.

37. Radwan I A, Abed A H, Abd Allah M M, Abd El-Latif M A A. Bacterial pathogens associated with cellulitis in chickens. Journal of Veterinary Medicine Research. 2018;(25):68 – 79.

38. Alfifi A, Christensen J P, Hounmanou Y M G, Sandberg M, Dalsgaard A. Characterization of *Escherichia coli* and other bacteria isolated from condemned broilers at a Danish abattoir. Frontiers in Microbiology. 2022; 13: 1020586.

39. Abou-Khadra S H, El-Azzouny, M M, Tawakol M M, & Nabil N M. Antimicrobial efficacy of quaternary ammonium compounds (QACs) against multidrug-resistant bacterial species causing cellulitis in broiler chicken. Journal of Advanced Veterinary Research. 2024; 14 (5): 874-880.

40. Abunna F, Adugna B, Tufa T B, Ayana D, Gutema F D, Waktole H, et al. Detection and antimicrobial resistance of *Staphylococcus* species from chicken, chicken litter, and humans in Addis Ababa, Ethiopia. Veterinary Medicine International. 2022; (1): 9084334.

41. Lutful Kabir S M. Avian colibacillosis and salmonellosis: a closer look at epidemiology, pathogenesis, diagnosis, control, and public health concerns. International journal of environmental research and public health. 2010; 7 (1): 89-114.

42. Nolan L K, Barnes H J, Vaillancourt J P, Abdul-Aziz T, Logue

Online ISSN: 2537-1045
Print ISSN: 1110-1423
DOI: 10.21608/vmjg.2025.398306.1046

C M. Colibacillosis. Diseases of poultry. 2013; 751-805.

43. Agabou A, Alloui N. Importance of *Alphitobius diaperinus* (Panzer) as a reservoir for pathogenic bacteria in Algerian broiler houses. Veterinary World. 2010; 3 (2): 71.

44. Mokhtari P, Jalalizand A, Mahmoudi E, Ghalamkari G. Tracking and Identifying Enterobacteriaceae Contamination in Darkling Beetles (Tenebrionidae) as One of the Reservoirs of Bacteria Persistence Poultry Farms. Journal of Veterinary Research. 2023; 78 (2).

45. Eid S, Merfan Ahmed. Characterization of *E. coli* associated with high mortality in poultry flocks. Assiut Veterinary Medical Journal. 2013; 59 (139): 51-61.

46. Dawadi P, Bista S, Bista S. Prevalence of colistin-resistant *Escherichia coli* from poultry in South Asian developing countries. Veterinary Medicine International. 2021; (1): 6398838.

47. Hossain M J, Islam M S, Sobur M A, Zaman S B, Nahar A, Rahman M, & Rahman, M T. Exploring Poultry Farm Environment for Antibiotic Resistant *Escherichia coli, Salmonella spp., and Staphylococcus spp.* Having Public Health Significance. Journal of the Bangladesh Agricultural University. 2020; 18 (3): 615–622.

48. Moffo F, Mouiche M M M, Djomgang H K, Tombe P, Wade A, Kochivi F L, et al. Poultry litter contamination by *Escherichia coli* resistant to critically important antimicrobials for human and animal use and risk for public health in Cameroon. Antibiotics. 2021; 10 (4): 402.

49. Chieffi D, Fanelli F, Fusco V. Antimicrobial and biocide resistance in *Staphylococcus aureus*: genomic features, decontamination strategies, and the role of *S. aureus* complex-related species, with a focus on ready-to-eat food and food-contact surfaces. Frontiers in Food Science and Technology. 2023: (3): 1165871.

Table (1): Number of Samples in the studied area

Governorates	Number of farms			
	Layer	Breeder	Broiler	**Total Samples**
Giza	7	-	5	204
Fayoum	1	-	3	68
Menoufia	-	4	-	68
Alexandria	-	6	2	136
Beni-Suef	2	-	-	34
Total farms	10	10	10	30 farms 510 samples

Table (2): Primers used in PCR

Gene target	**Name**	**Sequence 5'- 3'**	**Annealing Temp** (°C)	**Size** (bp)	**References**
G. *Staphylococcus*	16SrRNA f	GTA GGT GGC AAG CGTTAT CC	55	228	[24]
	16SrRNA r	CGC ACA TCA GCG TCA G			
S. aureus	nuc 1	GCGATTGATGGT GATACGGTT	55	279	[25]
	nuc 2	AGCCAAGCCTTGACGAACTAAAGC			
E. coli	phoA f	CGA TTC TGG AAA TGG CAA AAG	62	720	[26]
	phoA r	CGT GAT CAG CGG TGA CTA TGAC			

Table (3): Prevalence of *Staphylococcus* spp. from different sources in different poultry farms

Sources	Sample type	Breeders		Layer		Broilers		Total Prevalence (%)
		Positive/n	Prevalence (%)	Positive/n	Prevalence (%)	Positive/n	Prevalence (%)	
Insect	Darkling Beetles	24/30	80	26/30	86.7	27/30	90	77/90 85.6
Environmental sample	Litter	27/30	90	21/30	70	28/30	93.3	76/90 84.4
	Feeders	20/30	66.7	19/30	63.3	21/30	70	60/90 66.7
	Drinkers	18/30	60	15/30	50	17/30	56.7	50/90 55.6
Poultry	Cloacal swabs	41/50	82	38/50	76	42/50	84	121/150 80.6
Total		130/170	76.5	119/170	70	135/170	79.4	384/510 75.3
p-value		0.036*		0.025*		0.001*		<0.001*

*Indicate significance *at P<0.05.*

Table (4): Prevalence of *Staphylococcus* spp. from different sources in different poultry farms

Sources	Sample type	Breeders		Layer		Broilers		Total Prevalence (%)
		Positive/n	Prevalence (%)	Positive/n	Prevalence (%)	Positive/n	Prevalence (%)	
Insect	Darkling Beetles	20/30	66.7	27/30	90	24/30	80	71/90 78.9
Environmental sample	Litter	21/30	70	28/30	93.3	27/30	90	76/90 84.4
	Feeders	9/30	30	5/30	16.6	18/30	60	32/90 35.6
	Drinkers	9/30	30	9/30	30	12/30	40	30/90 33.3
Poultry	Cloacal swabs	28/50	56	48/50	96	48/50	96	124/150 82.7
Total		87/170	51.2	117/170	68.9	129/170	75.9	333/510 65.3
p-value		0.001*		<0.001*		<0.001*		<0.001*

*Indicate significance *at P<0.05.*

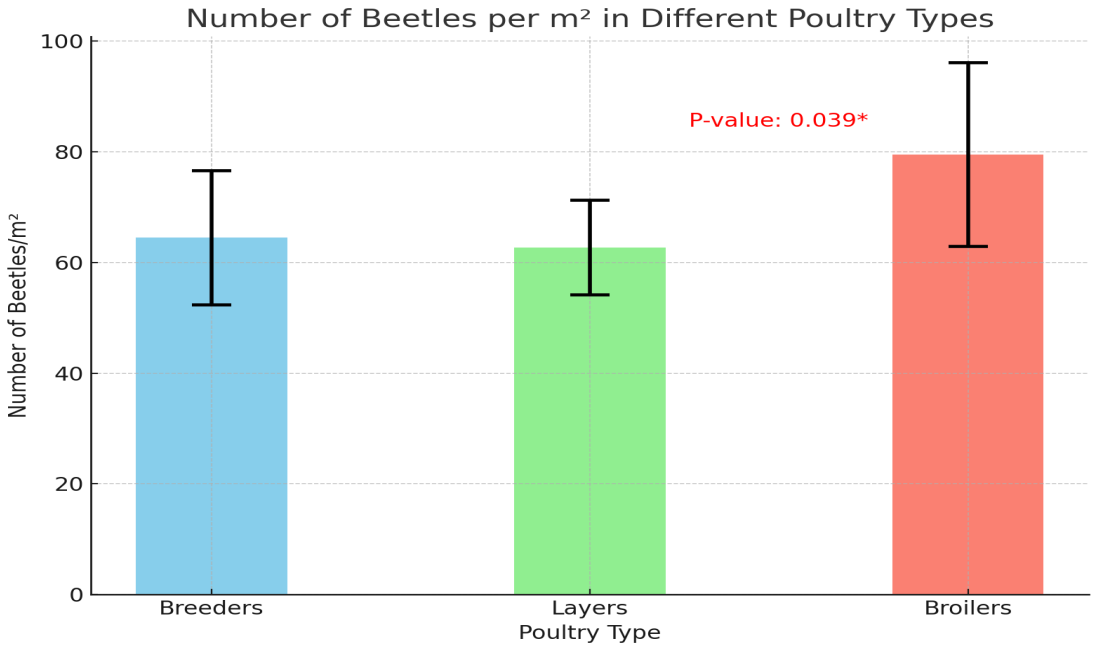


Fig. 1: The prevalence of beetles/m^2 across different poultry production farms

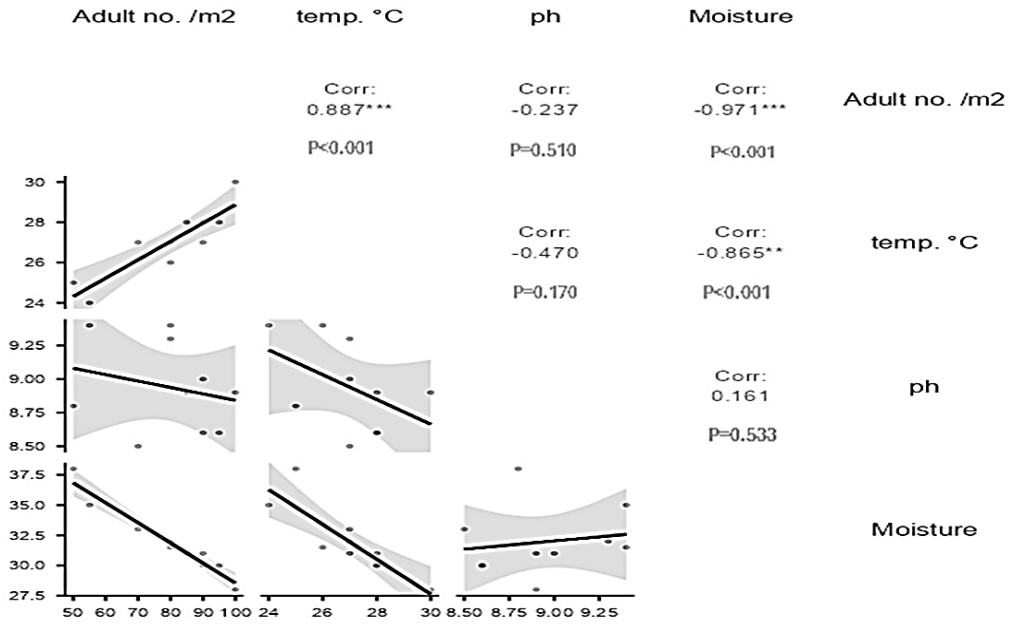
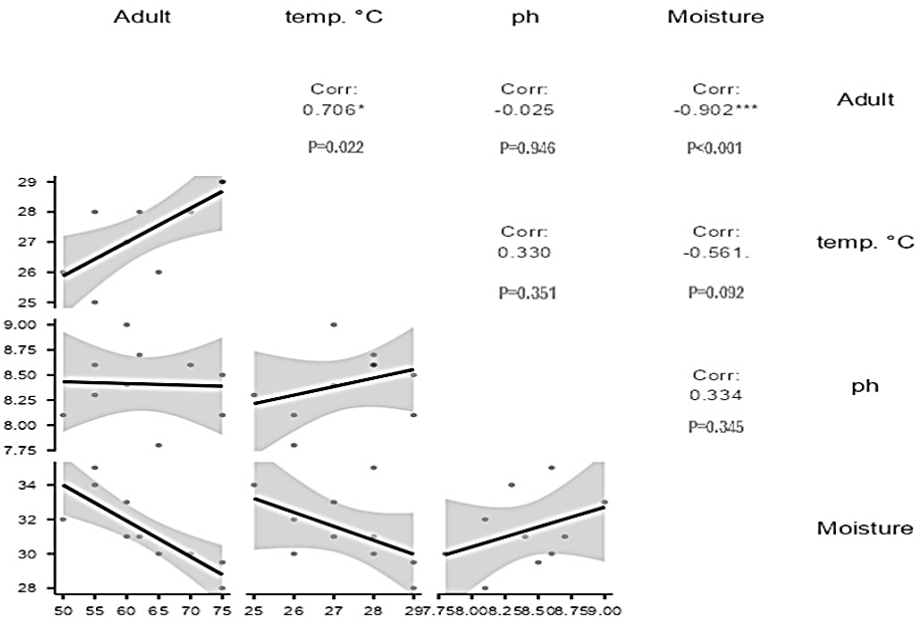


Fig. 2: Relation between litter parameters and population of beetles among the Broiler farms.



Fig

farms.

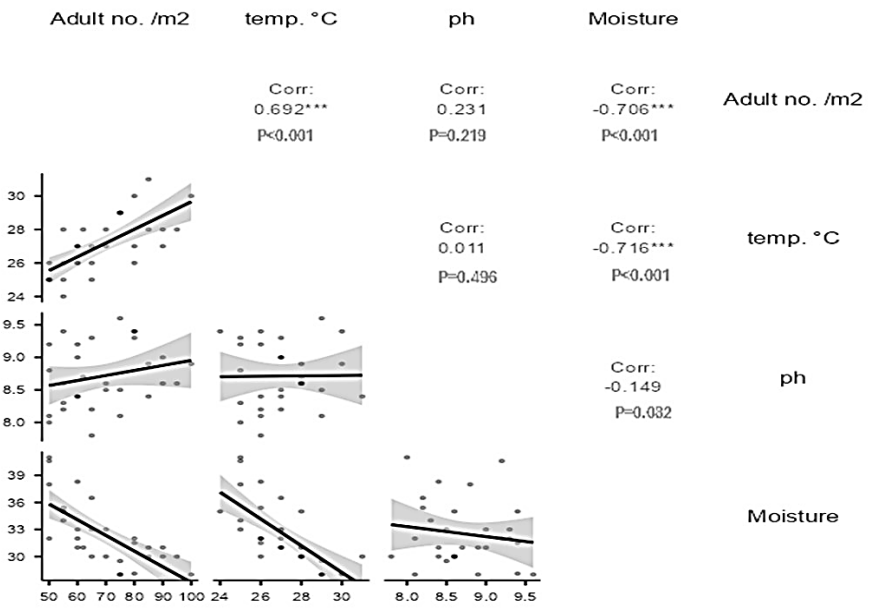


Fig 4: Relation between litter parameters and population of beetles among the breeder farms.

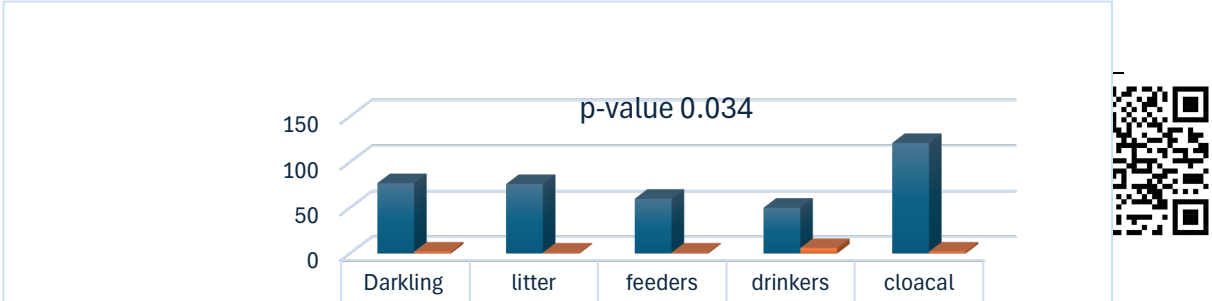


Fig. 5: Prevalence of *Staphylococcus* spp. and *S. aureus* from different sources in poultry farms.

Online ISSN: 2537-1045
Print ISSN: 1110-1423
DOI: 10.21608/vmjg.2025.398306.1046