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Evaluation of Internal and External Biosecurity Measures and Their Association with *Mycoplasma gallisepticum* Infection in Broiler Farms in Khartoum State, Sudan

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ABSTRACT

The present study was conducted to investigate the internal and external biosecurity levels and their association with *Mycoplasma gallisepticum* infection in broiler farms in Khartoum State, Sudan. Sixty-six broiler farms were selected. A questionnaire of 77 questions on internal and external biosecurity measures was administered during each farm visit. A total of 360 blood samples were collected and used for serological examination to detect antibodies against *M. gallisepticum*. The overall score of biosecurity was 0.57, and the average score of internal biosecurity (0.75) was higher (p<0.01) than the average score of external biosecurity (0.51). Poor internal biosecurity practices were observed in 23.4% of broiler farms, whereas most farms (93.6%) recorded poor external biosecurity. A significant (p<0.01) positive correlation was encountered between the external biosecurity and the number of houses (0.62), as well as the number of workers (r_s= 0.54). Both internal and external biosecurity displayed significant positive correlations with the number of birds (r_s= 0.64, 0.35; p< 0.05). Fifty percent of the farms were positive for *M. gallisepticum*. The disease incidence correlated positively with the spread of manure on neighboring farms (r_s= 0.44, p<0.05). A negative correlation was observed between the presence of *Mycoplasma* and the distance between farms (r_s= -0.50, p<0.01) as well as farm enclosure (r_s= -0.44, p<0.05). It is concluded that broiler farms in Khartoum State exhibited low biosecurity measures and a high seroprevalence of *M. gallisepticum* infection. Further efforts are necessary to improve external biosecurity practices.

Keywords: Biosecurity measures, Broiler, Manure, ELISA.

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INTRODUCTION

Biosecurity is defined as the implementation of a set of measures that reduces the risks of the introduction and spread of disease agents, preventing infectious agents from entering or exiting the farm (**Maduka *et al.*, 2016).**

Different studies have shown positive associations between biosecurity and some animal production parameters **(Laanen *et al.*, 2013; Postma *et al.*, 2016)** as well as farm profitability **(Rojo-Gimeno *et al.*, 2016).** Moreover, it has become clear that the high level of farm biosecurity not only reduces the use of antimicrobials **(Laanen *et al.*, 2013; Postma *et al.*, 2016)** but also provides the foundation for biosecurity throughout the entire production chain **(Siekkinen *et al.*, 2015).** Significantly, high levels of biosecurity alleviate the incidence of diseases in flocks or diseases considered **zoonotic (Bojesen *et al.*, 2003; Graham *et al.*, 2008; Newell *et al.*, 2011; Maduka *et al.*, 2016; Awad *et al.*, 2019; Erkan *et al.*, 2020).**

Biosecurity is subdivided into two major categories: external and internal. External biosecurity comprises all measures taken to prevent the introduction of off-farm pathogens. On the other hand, "internal biosecurity" includes all measures that address the spread of pathogens within the farm **(Gelaude *et al.*, 2014; Kim *et al.*, 2015).**

There are still great defects in the application of preventive measures on poultry farms, even with the recognized importance of biosecurity **(Van Steenwinkel *et al.*, 2011).** This necessitates a better evaluation of biosecurity measures. For example, a substantial scoring system has been used in several studies conducted in Europe to quantify the level of farm biosecurity **(Gelaude *et al.*, 2014; Postma *et al.*, 2016; Van Limbergen *et al.*, 2018; Rodrigues *et al.*, 2019).**

Although most of the studies conducted in Sudan have shown poor biosecurity measures in poultry farms, they did not demonstrate the level of biosecurity quantitatively **(Mahmoud *et al.*, 2014; Tabidi *et al.*, 2014; Mustafa *et al.*, 2018a; Mustafa *et al.*, 2018b; Omer *et al.*, 2019).** This is mainly because of their design, either as descriptive studies or based on qualitative surveys, without using quantification tools.

Mycoplasma gallisepticum is the causative agent of chronic respiratory disease in poultry. The disease causes immense economic losses as a result of the low performance of birds and increased medication costs. The infection with *M. gallisepticum* also shows immunosuppressive activities resulting in vaccination failure against virulent disease agents such as the Newcastle Disease virus. Serologic ELISA tests are commonly used for flock monitoring and the diagnosis of *M. gallisepticum* **(Raviv and David Ley, 2013).** It is noteworthy that in Sudan, vaccination against *M. gallisepticum* is commonly applied to breeders but not commercial flocks. However, the maternal-derived antibodies against *M. gallisepticum* in broilers could no longer exist by day 10 of age **(Gharaibeh *et al.*, 2013).** Taken together, strict biosecurity measures are recommended as one of the practical solutions to prevent and reduce the adverse effects of *M. gallisepticum* **(Awad *et al.*, 2019).**

The aim of this study was to quantify the internal and external biosecurity levels on broiler farms in Khartoum State, Sudan. A secondary objective was to conduct a *Mycoplasma* serological survey to assess the correlation between the existence of *M. gallisepticum* in broiler flocks and the biosecurity level of the farms.

MATERIALS AND METHODS

The study was carried out in Khartoum State, Sudan, from December 2015 to January 2019. A list of all broiler farms in the state was collated based on information gathered from different sources, including the Ministry of Agriculture, Animal Wealth and Irrigation (Khartoum State), as well as veterinarians and commercial companies working in poultry production. The list included 66 broiler farms distributed through 5 districts of Khartoum State, namely Khartoum North, Sharg En Nile, Omdurman, Jabel Awlya, and South Khartoum **(Fig. 1).** Only forty-seven farms (71.2%) were involved in the study **(Table 1).**

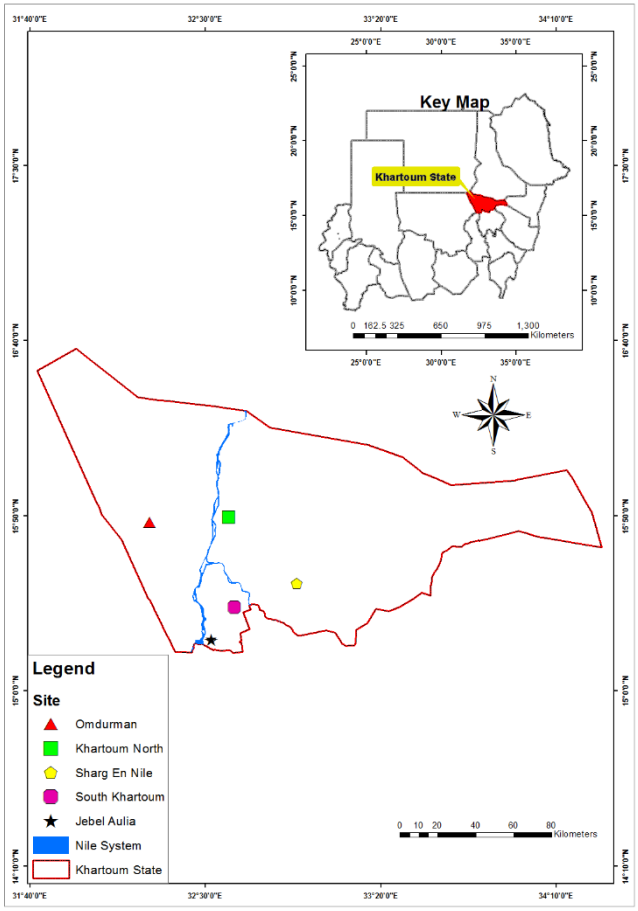

Fig.1: The map of Khartoum State, Sudan displays the five districts of the study: Khartoum North; East Nile; Omdurman; Jabel Awlya; and South Khartoum.

Table 1: Distribution of selected broiler farms in different districts of Khartoum State, Sudan (2016).

District	Broiler farms	
	Total	Selected (%)
Khartoum North	29	25 (86.2)
Sharg En Nile	12	6 (50)
Omdurman	7	7 (100)
Jabel Awlya	13	4 (30.8)
South Khartoum	5	5 (100)
Total	66	47 (71.2)

Study design

A questionnaire (in both Arabic and English) was designed according to the guidelines provided by **Gelaude *et al. (*2014).** General information about the farm **(Table 2),** along with a total of 77 questions on biosecurity measures, were included in the questionnaire. The questions were divided into two major categories, internal and external biosecurity, which in turn comprised several subcategories **(Table 3).** During each farm visit, the questionnaire was administered by a veterinarian using two combined methods: direct observation and an interview with either the farm owner or the supervisor/veterinarian of the farm.

Table 2: General information of broiler farms (n=47) in Khartoum State, Sudan *

General information	Number of farms (%)
Number of houses	
1 - 5	38 (80.9)
6 - 10	6 (12.8)
> 10	3 (6.4)
Number of birds	
1.000 - 10,000	20 (42.6)
11,000 - 30,000	11 (23.4)
> 30,000	16 (34.0)
Production system	
Closed	21 (44.7)
Semi-closed	12 (25.5)
Open	13 (27.7)
Management system	
Cage system	4 (8.5)
Floor	43 (91.5)
Number of workers	
< 5	34 (72.3)
6 - 15	7 (14.9)
16 - 25	3 (6.4)
> 25	3 (6.4)

Serological test for the detection of mycoplasma

A total of 360 blood samples were collected from 24 farms (15 blood samples from one broiler house on each farm) using a simple random sampling technique. Samples were collected from flocks aged 3 weeks or more. About 1 ml of the blood was collected from the wing veins of each bird in a sterile syringe. The serum was then separated and stored until it was used for serological examination. An indirect enzyme-linked immunosorbent assay (ELISA) test was performed to detect the antibodies against *M. gallisepticum* using ELISA test kits (Synbiotics Corporation, USA, item No. 96-6533). The test was applied, and the sample/positive (S/P) ratio was calculated according to the manufacturer's instructions. The samples with S/P ratios <0.50 were deemed negative, whereas those with S/P ratios >0.50 were deemed positive. A farm was considered to be infected with mycoplasma if one or more of the fifteen samples tested positive.

Statistical analysis

Statistical analysis was applied utilizing the Statistical Package for the Social Sciences software (SPSS/PC version 25 for Windows). The collected data were coded and analyzed using descriptive and comparative statistical analysis. The questions were assessed and each subcategory of biosecurity was given a score between 0.00 (absence of biosecurity measure) and 1.00 (full presence of biosecurity measure). The average subtotal scores of internal and external biosecurity [mean (lower and upper 95% confidence interval)] were then calculated, and the overall average of biosecurity [mean (lower and upper 95% confidence interval)] score was determined as well. The scores of all subcategories, along with the subtotal scores of internal and external biosecurity of the broiler farms, were classified as poor (< 0.50), good (0.50–0.59), very good (0.70–0.79), and excellent (> 0.90); the frequency of the broiler farms was calculated accordingly. A student's t-test was used to compare the average scores of internal and external biosecurity. Spearman's Rho Coefficient Correlation Test (r_s) was applied to examine the correlation between internal and external biosecurity, as well as the correlation among all subcategories. The test was also used to determine the correlation between the different subcategories of biosecurity and the incidence of mycoplasma on broiler farms. *A P-value* <0.05 was considered statistically significant.

RESULTS

Table 3 shows the scores of internal and external biosecurity on broiler farms. The average score of biosecurity was 0.57, while 76.19% (10/13) of the subcategories exhibited scores less than 0.70. The average score of internal biosecurity (0.75) was higher (p<0.01) than the average score of external biosecurity (0.51). Poor internal biosecurity practices were observed in 23.4% of broiler farms, whereas almost half of the farms (48.9%) revealed good internal biosecurity with an average score of 0.75. Unlike internal biosecurity, the majority of the farms (93.6%) recorded low scores and poor external biosecurity.

As shown in **Fig.2,** all broiler farms involved in this study displayed poor practices concerning the

supply of materials, infrastructure, and biological vectors. More than 80% of the farms also revealed poor biosecurity measures related to the export of live animals, the removal of manure and dead birds, and the entrance of visitors and personnel.

Table 3: Ranks of internal and external biosecurity scores [Mean (lower and upper 95% confidence interval)] of broiler farms in Khartoum State, Sudan (n=47).

Biosecurity Category	Score (Min - Max)	Frequency of farms (%)	Classification
Internal biosecurity	0.64 (0.62-0.67)	11 (23.4%)	Poor
	0.75 (0.74-76)	23 (48.9)	Good
	0.83 (0.81-0.85)	11 (23.4%)	Very Good
	0.93 (0.76-1.10)	2 (4.3%)	Excellent
External biosecurity	0.49 (0.47-0.52)	44 (93.6%)	Poor
	0.75 (0.67-0.83)	3 (6.4%)	Good
	0.00	-	Very Good
Total scores			
Internal biosecurity	0.75[a] (0.73-0.78)	47 (100%)	Very Good
External biosecurity	0.51[b] (0.48-.054)	47 (100%)	Poor
Overall biosecurity	0.57 (0.55-0.60)	47 (100%)	Poor

Min: Minimum; Max: maximum

Poor: <0.70; Good: 0.70-.79; Very good: 0.80-0.89; Excellent: 0.90-0.99.

[a,b] Different superscripts within scores indicate significant differences at $p<0.01$

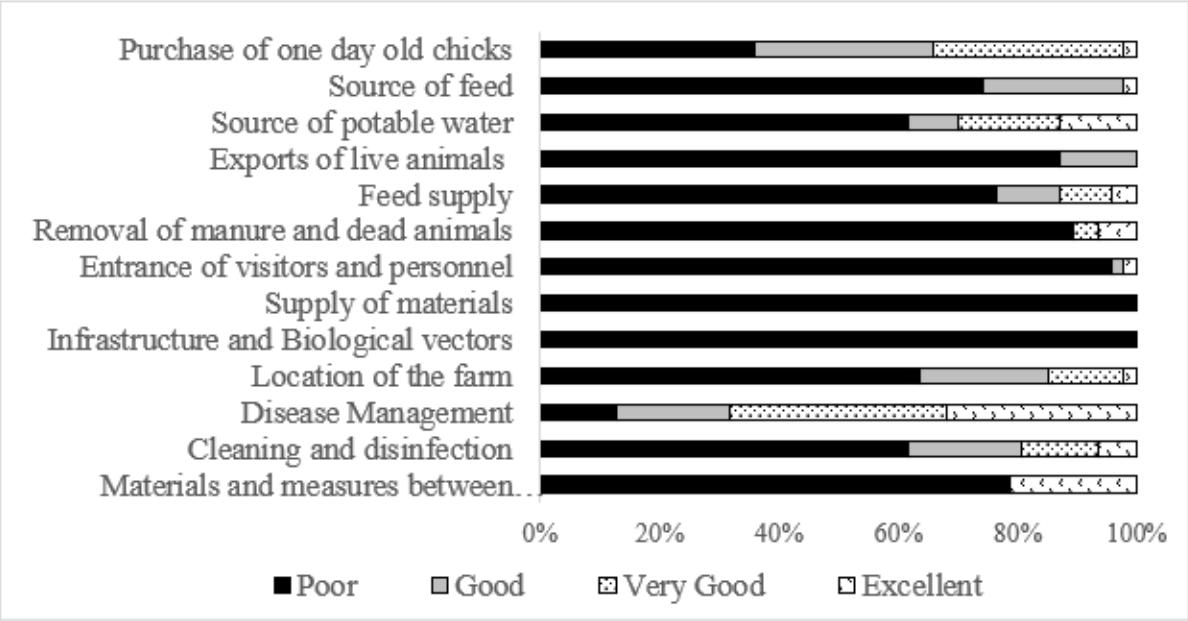

Fig. 2: The level of biosecurity measures in broiler farms in Khartoum State, Sudan. Poor ≤ 0.69; Good 0.7-0.79; Very good 0.80-0.89; Excellent ≥ 0.90.

The subcategory purchase of one-day-old chicks in external biosecurity recorded the highest score (0.7), whereas the supply of materials had the lowest score (0.06). The disease management subcategory in internal biosecurity displayed the highest score (0.84) as compared to all subcategories in both internal and external biosecurity **(Table 4).**

Table 4: External, internal, and overall biosecurity scores [Mean (lower and upper 95% confidence interval)] of broiler farms in Khartoum State, Sudan (n=47).

Biosecurity categories	Biosecurity subcategories	
	Subcategory	**Score**
External biosecurity	Purchase of one-day-old chicks	0.70 (0.66-0.74)
	Source of feed	0.57 (0.52-0.63)
	Source of potable water	0.50 (0.40-0.59)
	Exports of live animals (after the end of production cycle)	0.46 (0.42-0.51)
	Feed supply	0.57 (0.52-.062)
	Removal of manure and dead animals	0.43 (0.36-0.50)
	Entrance of visitors and personnel	0.46 (0.41-0.50)
	Supply of materials	0.06 (0.01-0.11)
	Infrastructure and biological vectors	0.44 (0.40-0.48)
	Location of the farm	0.69 (0.64-0.73)
Internal biosecurity	Disease Management	0.84 (0.81-0.88)
	Cleaning and disinfection	0.68 (0.64-0.72)
	Materials and measures between compartments	0.57 (0.50-0.65)

The external and internal biosecurity showed moderate correlation (R = 0.44) **(Fig. 3).** As shown in **Table 5,** significant positive correlations were also detected between external and internal biosecurity and some of the subcategories of biosecurity. Correlations among the subcategories were also observed. External biosecurity had significant positive correlations with several biosecurity items. The strongest positive correlation (r_s = 0.81) was observed between external biosecurity and the entrance of visitors (p<0.001). A moderate positive correlation (r_s= 0.50) was recorded between the external biosecurity and both the removal of manure and dead animals, as well as materials and measures between compartments (p<0.01). Internal biosecurity revealed a moderate correlation (r_s= 0.49) with cleaning and disinfection (p<0.01). Exports of live animals correlated positively (r_s= 0.42) with materials and measures between compartments. The later has also shown a moderately significant correlation (r_s= 0.51) with the removal of manure and dead birds and with the entrance of visitors and personnel (r_s= 0.41).

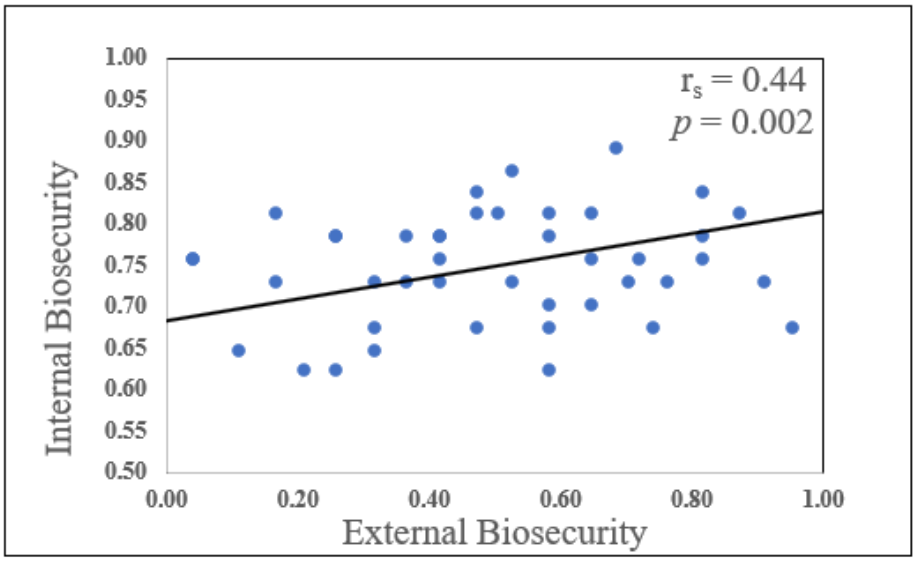

Fig. 3: Correlation between internal and external biosecurity of broiler farms in Khartoum State, Sudan using Spearman's Rho Coefficient correlation test.

Table 5: Correlation between internal/external biosecurity and some items of biosecurity using Spearman's Rho Coefficient correlation test.

	Item	EB[1]	IB[2]	1	2	3	4	5	6	7
1	Purchase of one-day-old chicks	0.11	0.13	--						
2	Exports of live animals	0.42**	0.00	0.15	--					
3	Removal of manure and dead animals	0.50**	0.28	0.09	0.30*	--				
4	Entrance of visitors and personnel	0.81**	0.32*	0.01	0.24	0.64**	--			
5	Location of the farm	0.35*	0.07	0.13	-.06	0.22	0.31*	--		
6	Cleaning and disinfection	0.45**	0.49**	0.30*	0.02	0.26	0.35*	0.08	--	
7	Materials and measures between compartments	0.50**	0.22	0.00	0.42**	0.51**	0.41**	0.12	0.01	--

[1]EB: External biosecurity; [2] IB: Internal biosecurity; *p<0.05; **p<0.001

Table 6 displays the correlation of basic information with internal and external biosecurity. There is a significant (p<0.01) positive correlation between the external biosecurity and the number of houses (0.62), number of workers (r_s= 0.54), and number of birds (0.64). The internal biosecurity showed a significant (p<0.05) positive correlation with the number of birds (r_s= 0.64, 0.35).

Basic information	External biosecurity	Internal biosecurity
District	0.27	0.07
Number of houses	0.62**	0.27
Number of workers	0.54**	0.24
Number of birds	0.64**	0.35*

*p<0.05, **p<0.001

Serological tests for the detection of *Mycoplasma* demonstrated that half of the farms (50 %) were positive for *Mycoplasma* **(Table 7).** The incidence of *Mycoplasma* was positively correlated with the subcategories separated dead birds' storage (r_s = 0.46, p<0.05), and spread of manure on neighboring farms (r_s = 0.44, p<0.05). A negative correlation was observed between the presence of *Mycoplasma* and the distance of the nearest farm (r_s = -0.50, p<0.01) as well as farm enclosure (r_s = -0.44, p<0.05).

Table 7: Seroprevalence of *Mycoplasma gallisepticum* and correlation (using Spearman's Rho Coefficient correlation test) between the infection and some biosecurity measures in broiler farms in Khartoum State, Sudan.

Seroprevalence of *Mycoplasma gallisepticum*		
District	Number of farms	Positive (%)
Khartoum North	13	7 (58.3)
Sharg En Nile	3	0
Omdurman	5	4 (33.3)
Jabel Awlya	1	1 (8.3)
South Khartoum	2	0
Total	24	12 (50)

Correlation between the *Mycoplasma gallisepticum* infection and some biosecurity measures	
Biosecurity measure	Correlation
Separated dead birds' storage	0.46*
Manure from other poultry farms spread on neighboring farmlands	0.44*
Distance where nearest poultry farm is located	-0.51**
Farm enclosing	-0.44*

*p <0.05, **p<0.01.

DISCUSSION

The present investigation covered about seventy percent of broiler farms located in five districts of Khartoum State. This is due to the challenges experienced in accessing all broiler farms. Firstly, some farms were not working because of either a shortage in day-old chick supplies or financial difficulties. Secondly, some farm supervisors or owners refused to participate in the study, possibly to maintain the confidentiality of their production information and flock health status. The obstacles in this study were similar to those faced in an earlier study dealing with poultry farms in Khartoum State **(Sirdar *et al.*, 2012).**

The present study showed a significant difference between internal and external biosecurity scores. There was a low overall score for biosecurity, even though a high score was recorded for internal biosecurity. Similar results on broiler farms were reported by **Gelaude *et al.*, (2014)** in Belgium. Poor external biosecurity practices are therefore responsible for the reduction of the overall scores. One possible explanation for the difference between the external and internal biosecurity scores in this study is that farm supervisors give less attention to external biosecurity measures, most probably due to the increase in cost as compared to internal biosecurity. More efforts are needed to improve such external biosecurity practices on broiler farms. The high score of the subcategory purchase of one-day-old chicks in this study may imply that the breeding companies applied strict biosecurity measures. In addition, the study revealed that most broiler farms used to buy their chicks from one source. This practice is supposed to reduce the risk of the introduction of disease-causing agents as stated by **Hege *et al.*, (2002).**

It has become increasingly clear that human traffic and the unauthorized entrance of visitors are the key factors responsible for the spread of pathogens onto poultry farms **(Thomas *et al.*, 2005; Vieira *et al.*, 2009).** The present study showed poor measures related to the entrance of visitors; the suboptimum measure also had a strong positive correlation with external biosecurity practices. This may indicate that biosecurity hygiene measures for visitors were not properly applied at the entrances of these farms. A similar account has been reported by **Souillard *et al.*, (2024)** in Europe. Thus, effective hygiene locks are required at the main gates of broiler farms.

It is well known that the routine pickup and transport of manure and dead birds from poultry farms can spread pathogenic microorganisms and present a substantial threat **(Blake *et al.*, 1992; Ngogang *et al.*, 2021).** Poultry manure is an affordable organic fertilizer and the main fertilizer used in several African countries **(Ngogang *et al.*, 2021).** The present study showed poor practices in terms of the removal of manure and dead animals. Similar findings have been reported in Nigeria **(Adedayo, 2012; Moreki and Keaikitse, 2013).** The poor results may indicate inefficient methods of manure removal and dead animals. It has been suggested that the dead birds' storage area must be in such a way that the rendering company can collect the mortalities without entering the farm **(McQuiston *et al.*, 2005).**

Mycoplasma spp. is an example of a density-dependent pathogen that can spread via the wind **(Bradburry and Morrow, 2008).** In the present study, serological tests for the detection of *M. gallisepticum* demonstrated that half of the farms (50%) were positive. Similarly, recent studies revealed a high seroprevalence of *M. gallisepticum* among broilers in different African countries **(Mera and Mudasir, 2019; Shiferaw *et al.*, 2022; Dhuha, 2024).** Moreover, the present findings showed a negative correlation between the presence of *M. gallisepticum* infection and the distance between farms. It is well documented that the high density of poultry farms in a certain area is an important factor in the transmission of pathogens that are density-dependent **(Truscott *et al.*, 2007).** Thus, **Kammon *et al.*, (2017)** highlighted the necessity of implementing an appropriate buffer zone between poultry farms.

The current investigation revealed a positive correlation between certain inadequate practices, such as the storage of dead birds, the spread of manure on nearby farms, and the occurrence of mycoplasma infection. This agrees with **Dhuha (2024),** who emphasized the role of inadequate biosecurity measures in increasing infection rates of *M. gallisepticum*. In addition, the present findings may suggest that manure is a potential vector for disease transmission. The negative correlation between *M. gallisepticum* infection and distance between farms and farm enclosures in the present study suggests that these factors may have a protective effect in reducing the spread of infection.

CONCLUSION

The present study revealed low biosecurity measures on broiler farms located in Khartoum State. More efforts are needed to improve farm biosecurity, with more concentration given to external biosecurity practices such as hygiene locks at main gates and the proper removal of manure and dead animals. The present study revealed that the broiler farms had a significant seroprevalence of *M. gallisepticum*, which was positively correlated with the spread of manure on nearby farms, indicating a potential route for disease transmission. The negative correlation between

infection and farm enclosure and the distance between broiler farms suggests that these factors may play a protective role in reducing the spread of infection.

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Author Contributions

This work was carried out in collaboration between both authors. The general supervision over the research, initial drafting of the manuscript, and the edition and reviewing of the final manuscript was performed by Mortada M. O. Elhassan. Material preparation, data collection, and laboratory analysis were performed by Lamyia M. A. Supervision over the serological tests for the detection of mycoplasma, and laboratory analysis was performed by Adil M. A. Salman. *Statistical* analysis was performed by Sanaa A. Enan. The study conception and design and the edition and reviewing of the final manuscript were performed by Elniema A. Mustafa. All authors commented on previous versions of the manuscript. All authors read and approved the final manuscript

Ethical Standards

The manuscript does not contain clinical studies or patient data.

Data Availability

The datasets that support the findings of this study are available upon reasonable request.

Conflict of interest

No competing interests to declare regarding the research tools and data used.

REFERENCES

ADEDAYO, V. 2012. Poultry Waste Management Techniques in Urban Agriculture and its Implications: A Case of Metropolitan Lagos, Nigeria. Asian Journal of Agricultural Science, 4(4): 258-263.

AWAD, N.F.S., ABD EL-HAMID, M.I. HASHEM, Y.M., ERFAN, A.M., ABDELRAHMAN, B.A., and MAHMOUD, H.I., 2019. Impact of single and mixed infections with Escherichia coli and *Mycoplasma gallisepticum* on Newcastle disease virus vaccine performance in broiler chickens: an in vivo perspective. Journal of Applied Microbiology, 127(2): 396–405.

BLAKE, J.P., and DONALD J.O., 1992. Alternatives for the disposable of poultry carcasses. Poultry Science, 71:1130–1135

BOJESEN, A.M, NIELSEN, S.S., and BISGAARD, M., 2003. Prevalence and transmission of haemolytic Gallibacterium species in chicken production systems with different biosecurity levels. Avian Pathology, 32(5):503–510. doi: 10.1080/0307945031000154107

BRADBURRY, J. M., and C. MORROW. 2008. Avian Mycoplasmas. Patisson M., McMullin P. F., Bradburry J. M., and Alexander D. J. (eds). In: Poultry Diseases, 6th ed., (Saunders Elsevier, Beijing, China)

DHUHA K. TAHA, 2024. Detection of antibodies for *Mycoplasma gallisepticum* by ELISA in broiler. Egyptian Journal of Veterinary Sciences, 55 (6):1473 - 1481. doi:10.21608/EJVS.2024.252139.1694

TADA, M. O., SANAA, A. ENAN, EMAN MUSTAFA HAMAD, ADIL, M. A. SALMAN, and ELNIEMA, A. MUSTAFA, 2020. Evaluation of internal and external biosecurity measures in layer farms in Khartoum State, Sudan. World Journal of Pharmacy and Pharmaceutical Sciences, 9 (5): 25-34.

ERKAN CAN, M. 2020. Hygiene conditions and animal-environment relations in farm animals barns. Black Sea Journal of Engineering and Science, 3(2):1–2. doi:10.34248/bsengineering.628540

GELAUDE, P., SCHLEPERS, M., VERLINDEN, M., LAANEN, M., and DEWULF, J., 2014. BiocheckUGent: A quantitative tool to measure biosecurity at broiler farms and the relationship with technical performances and antimicrobial use. Poultry Science, 93(11): 2740–51. doi: 10.3382/ps.2014-04002

GHARAIBEH, S., and MAHMOUD K., 2013. Decay of maternal antibodies in broiler chickens. Poultry Science, 92(9):2333–2336. http://dx.doi.org/10.3382/ps.2013-03249

GRAHAM, J.P., LEIBLER, J.H., PRICE, L.B., OTTE, J.M., PFEIFFER, D.U., TIENSIN, T., and SILBERGELD, E.K., 2008. The animal-human interface and infectious disease in industrial food animal production: Rethinking biosecurity and biocontainment. Public Health Reports, 123(3):282–299.

HEGE, R. ZIMMERMANN, SCHEIDEGGER, W., and STARK, K., 2002. Incidence of reinfections with Mycoplasma hyopneumoniae and Actinobaillus pleuropneumoniae in pig farms located in respiratory-disease-free regions of Switzerland—Identification and quantification of risk factors. Acta Veterinaria Scandinavica, 43:145–156. https://doi.org/10.1186/1751-0147-43-145

AMMON ABDULWAHAB, PAOLO MONICA, MULATTI LORENZETTO, FERRE N., SHARIF, M., ELDAGHAYES, I., and DAYHUM, A., 2017. Biosecurity and geospatial analysis of mycoplasma infections in poultry farms at Al-Jabal Al-Gharbi region of Libya. Open Veterinary Journal, 7(2): 81-85. doi: 10.4314/ovj. v7i2.1

KIM, J.W., KIM, J.H., and KIL, D.Y., 2015. Dietary organic acids for broiler chickens: a review. Revista Colombiana de Ciencias Pecuarias, 28:109-123. DOI: 10.17533/udea.rccp.v28n2a01.

LAANEN, M., PERSOONS, D., RIBBENS, S., DE JONG, E., CALLENS, B., STRUBBE, M., MAES, D., and DEWULF, J., 2013. Relationship between biosecurity and production/antimicrobial treatment characteristics in pig herds. The veterinary journal, 198 (2):508–512. http://dx.doi.org/10.1016/j.tvjl.2013.08.029

MADUKA, C. V., IGBOKWE, I.O., and ATSANDA, N.N., 2016. Appraisal of Chicken Production with Associated Biosecurity Practices in Commercial Poultry Farms Located in Jos, Nigeria. Scientifica, Article ID 1914692, 9 . http://dx.doi.org/10.1155/2016/1914692

MAHMOUD, M.A., ATIF, E.A., and HAYFA, M.I., 2014. Evaluation of biosecurity measures on broiler farms in Khartoum, Sudan. Journal of Veterinary Medicine and Animal Health, 6(5):138–144. https://doi.org/10.5897/JVMAH2014.0276

MCQUISTON, J.H., GARBER, L.P., PORTER-SPALDING, B.A. HAHN, J.W., PIERSON, F.W., WAINWRIGHT, S.H., SENNE, D.A., BRIGNOLE, T.J., AKEY, B.L., and HOLT, T.J., 2005. Evaluation of risk factors for the spread of low pathogenicity H7N2 avian influenza virus among commercial poultry farms. Journal of the American Veterinary Medical Association., 226(5):767-772. doi: 10.2460/javma.2005.226.767

MERA, U.M., and MUDASIR H. B., 2019. Prevalence of *Mycoplasma gallisepticum* (MG) antibodies in chicken in Sokoto, Nigeria. EAS Journal of Veterinary Medical Science, 1(5):58–59. doi: 10.36349/easjvms. 2020. v02i05.003

MOREKI JOHN CASSIUS and KEAIKITSE TETO, 2013. Poultry waste management practices in selected poultry operations around Gaborone, Botswana. International Journal of Current Microbiology and Applied Sciences, 2(7): 240-248.

MUSTAFA E.A., O ELHASSAN, M.M., HAMAD, E.M., A SALMAN, A.M., MANAL, M.E.E., and LAMYIA, M. A., 2018B. Biosecurity measures related to the purchase and transportation of day-old chicks in poultry farms in Khartoum State, Sudan. World Journal of Pharmacy and Pharmaceutical Sciences,7(1):22–31. doi: 10.20959/wjpps201811-12529

MUSTAFA, E.A., MUSTAFA HAMAD, E., ELHASSAN, M., A., SALMAN, A.M., ELSIDDIG, M.M.E., and LAMYIA, M.A., 2018A. Disposal of dead birds and manure in poultry farms under different production and management systems in Khartoum State, Sudan. World Journal of Pharmacy and Pharmaceutical Sciences, 7(11):61–70. doi:10.20959/wjpps201811-12467

NEWELL, D.G., ELVERS, K.T., DOPFER, D., HANSSON, I., JONES, P., JAMES, S., GITTINS, J, STERN, N.J., DAVIES, R., CONNERTON, I., PEARSON, D., SALVAT, G., and ALLEN, V.M., 2011. Biosecurity-based interventions and strategies to reduce Campylobacter spp. on poultry farms. Applied and Environmental Microbiology, 77(24):8605–8614. doi: 10.1128/AEM.01090-10

NGOGANG, M.P., ERNEST, T., KARIUKI, J., MOULIOM MOUICHE, M.M., NGOGANG, J., WADE, A., VAN DER SANDE, M.A.B., 2021. Microbial contamination of chicken litter manure and antimicrobial resistance threat in an urban area setting in Cameroon. *Antibiotics*, *10*, 20. https://doi.org/10.3390/antibiotics10010020

OMER, A., SALIM GIBRIL, and ABDELBASIT, B. HABIB, 2019. Management and Biosecurity Practices on Broiler and Layer Farms in Bahri Locality Khartoum North. Journal of Applied Veterinary Sciences, 4(2): 30.

POSTMA, M., BACKHANS, A., COLLINEAU, L., LOESKEN, S., SJÖLUND, M., and BELLOC, C., 2016. Evaluation of the relationship between the biosecurity status, production parameters, herd characteristics and antimicrobial usage in farrow-to-finish pig production in four EU countries. Porcine Health Management 2:9 pages. doi:10.1186/s40813-016-0028-z

RAVIV, Z.I.V., and DAVID, H. LEY, 2013. *Mycoplasma gallisepticum* infection. In: Diseases of Poultry. Swayne D.E., Glisson J.R., McDougald L. R., Nolan L.K., Suarez D.L., Nair V. (eds). 13th ed., (John Wiley & Sons, Inc. Ames, Iowa, USA)

RODRIGUES DA COSTA, M., GASA, J., CALDERÓN DÍAZ, J.A., POSTMA, M., DEWULF, J., MCCUTCHEON, G., and MANZANILLA, E.G., 2019. Using the Biocheck.UGentTM scoring tool in Irish farrow-to-finish pig farms: Assessing biosecurity and its relation to productive performance. Porcine Health Management, 5(1):1–9. https://doi.org/10.1186/s40813-018-0113-6

ROJO-GIMENO, C., POSTMA, M., DEWULF, J., HOGEVEEN, H., LAUWERS, L., and WAUTERS, E., 2016. Farm-economic analysis of reducing antimicrobial use whilst adopting improved management strategies on farrow-to-finish pig farms. Preventive Veterinary Medicine, 129:74–87.

SHIFERAW J., SHIFARA F., TEFERA M., FEYISA A., and TAMIRU Y., 2022. Seroprevalence and associated risk factors of *Mycoplasma gallisepticum* infection in poultry farms of Hawasa and Bishoftu, central Ethiopia. Veterinary Medicine: Research and Reports, 13:101-107. doi: 10.2147/VMRR.S360669. PMID: 35615601; PMCID: PMC9124814

SIEKKINEN, K.M., HEIKKILÄ, J., TAMMIRANTA, N., and ROSENGREN, H., 2012. Measuring the costs of biosecurity on poultry farms: A case study in broiler production in Finland. Acta Veterinaria Scandinavica, 54:12. http://www.actavetscand.com/content/54/1/12

SIRDAR, M.M., PICARD J, BISSCHOP, S. J, A.R., and GUMMOW B., 2012. A survey of antimicrobial residues in table eggs in Khartoum State, Sudan, 2007 – 2008. Onderstepoort Journal of Veterinary Research, 79(1), Art. #360, 9 pages. https://dx.doi.org/10.4102/ojvr.v79i1.360

SOUILLARD ROZENN, ALLAIN VIRGINIE, CHRISTINE ANNE, NATHALIE ROUSSET, ARTHI AMALRAJ, ANNICK SPAANS, ARTUR ZBIKOWSKI, ALESSANDRA PICCIRILLO, SANDRA SEVILLA-NAVARRO, LÁSZLÓ KOVÁCS, and SOPHIE LE BOUQUIN, 2024. Biosecurity implementation on large-scale poultry farms in Europe: A qualitative interview study with

farmers. Preventive Veterinary Medicine, (224) 106119. https://doi.org/10.1016/j.prevetmed.2024.106119

TABIDI, M.H., MUSTAFA, E.A., and AHMED, A.A., 2014. Data analysis of biosecurity measures for poultry farms registration in Khartoum State, Sudan. International Journal of Current Research; 6 (11): 9714–9718.

THOMAS, M. E., BOUMA, A., EKKER, H. M., FONKEN, A. J. M., STEGEMAN, J. A., and NIELEN, M., 2005. Risk factors for the introduction of high pathogenicity Avian Influenza virus into poultry farms during the epidemic in the Netherlands in 2003. Preventive Veterinary Medicine 10, 69 (1-2):1–11. doi: 10.1016/j.prevetmed.2004.12.001

TRUSCOTT, J., GARSKE, T., CHIS-STER, I. GUITAIN, J., PFEIFFER D. , LUCY SNOW , WILESMITH J , NEIL M FERGUSON N.M., AZRA, C., and GHANI, A.C., 2007. Control of highly pathogenic H5N1 avian influenza outbreak in the GB poultry flock. Proceedings of the Royal Society: Biological Sciences, 274(1623):2287–2295. doi: 10.1098/rspb.2007.0542

VAN LIMBERGEN, T., DEWULF, J., KLINKENBERG, M., DUCATELLE, R., GELAUDE, P., MÉNDEZ, J., HEINOLA, K., PAPASOLOMONTOS, S., SZELESZCZUK, P., and MAES D., 2018. Scoring biosecurity in European conventional broiler production. Poultry Science, 97(1):74–83.

VAN STEENWINKEL, S., RIBBENS, S., DUCHEYNE, E., GOOSSENS, E., and DEWULF, J., 2011. Assessing biosecurity practices, movements and densities of poultry sites across Belgium, resulting in different farm risk-groups for infectious disease introduction and spread. Preventive Veterinary Medicine, 98(4):259–270. http://dx.doi.org/10.1016/j.prevetmed.2010.12.004

VIEIRA, A. R., HOFACRE, C. L., SMITH, J. A., and COLE, D., 2009. Human contacts and potential pathways of disease introduction on Georgia poultry farms. Avian Diseases, 53(1):55-62. doi: 10.1637/8364-051608-Reg.1

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