

ASSESSMENT OF BIOSECURITY MEASURES IMPLEMENTED ON THE BROILER FARMS IN THE REGION OF BELGRADE CITY

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Abstract

The implementation of biosecurity measures as the first line of defense is an essential prerequisite for preventing the occurrence and spread of infectious agents in flocks. Assessment of biosecurity measures on the farms was carried out during farm visits based on the application of an appropriate questionnaire whereby the farmer answered a number of questions regarding the implemented biosecurity measures. The study was performed via Biocheck.UGent online survey on 16 broiler farms in the region of the city of Belgrade. The capacity of farms was 25,000-100,000 broilers, placed in 2-4 houses, depending on the farm. Farm visits were made by the authors to confirm the correct answers to the online survey. Results showed that external biosecurity scores ranged from 57% to 93%, averaging 83.6%. Internal biosecurity score ranged from 48% to 98%, with an average score of 85.7%. The total score for the biosecurity assessment ranged from 56% to 93%, with an average score of 84.3%. The scores for subcategories varied between the farms. Removing manure and carcasses, a subcategory within the category of external biosecurity, had the lowest mean score (farms 9 and 10 had a score of 12%). Relatively low scores were also obtained for the number of steps of broilers depopulation (farm 9 had a score of 44%) and for the location of the farm (farms 2 and 3 had a score of 44%). Concerning internal biosecurity, the lowest score was obtained

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for the subcategory material and measures that they apply between compartments (farm 10 had a score of 29%). These findings should be useful for decision-makers and flock veterinarians and farmers to set feasible targets and sustainable biosecurity programs to improve biosecurity, the health status of the flock, and farm profitability.

Key Words: assessment, biosecurity, broilers, farms, Belgrade

INTRODUCTION

Poultry production is the fastest-growing branch of animal husbandry on agricultural holdings. In Serbia, poultry production has a long tradition and plays an important economic role. According to the data of the Statistical Office of the Republic of Serbia, from year to year, there is a steady increase in the number of poultry of different categories (Statistical Office of the Republic of Serbia, 2022). However, only a healthy flock brings real economic profit.

Implementing biosecurity measures is critical to maintaining a farm free from diseases (Robertson, 2020). In broiler production, the risk of introducing pathogens causing disease and their downstream dissemination and pathogens of importance for food safety and public health can be reduced with the corrected implementation of biosecurity measures. *Campylobacter*, *Salmonella*, avian pathogenic *Escherichia coli*, and *Gallibacterium* are such pathogens. (Bojesen et al., 2003; Newel et al. 2011; Limbergen et al. 2018; Limbergen et al., 2020). In Serbia in 2021, the incidence of salmonellosis in humans was 9.83 in 100,000 people and the incidence of campylobacteriosis was 6.4 in 100,000 people (Institute of public health of Serbia “Dr Milan Jovanovic Batut”, 2022). Biosecurity is an essential component of preventive measures aimed at preserving public and animal health, as well as the health of plants and the environment, and as such is part of the One Health concept (Renault et al., 2022). The existing animal production systems in Serbia need to identify the lapses in their biosecurity measures so that appropriate steps could be taken and risks minimized (Kureljušić et al., 2023).

Biosecurity implies the sum of management and physical measures that will extenuate the risk of introduction (external biosecurity), development, and spread (internal biosecurity) of diseases between and within farms (Regulation (EU) 2016/429; EU, 2021). Each production system aims to prevent disease outbreaks rather than treat them, and biosecurity can help to decrease antimicrobial use and the development of antimicrobial resistance in veterinary and human medicine (Gelaude et al., 2014). Every broiler farm has implemented a biosecurity program, but it is very important to regularly monitor and evaluate the current biosecurity status (Dewulf et al., 2018). The Biocheck.UGent scoring system is used worldwide and enables quantifying biosecurity at the herd level. With this scoring system, it is possible to monitor broiler farms over time or compare biosecurity levels between farms (Gelaude et al., 2014). Also, it may serve as a device to fortify the diminishment of antimicrobial usage (Raasch et al, 2018).

In the Belgrade city area, there are 20 broiler farms. According to the previous findings, there is a lot of space for improvement in the biosecurity protocols on both, broiler

and breeder farms in Serbia (Maletić et al., 2023). This study included broiler farms that were willing to review the situation regarding implemented biosecurity protocols. The objective of this study was to quantify the biosecurity practices at the flock level and to identify farm-specific strengths and weaknesses with regard to implemented protocols. Each farmer needs to be aware of their farm's status to effectively reduce the risk of disease transmission between flocks and different housing facilities on the premises. As a consequence of that, overall health and welfare would be improved, and profits as well.

MATERIALS AND METHODS

Data were collected from 16 broiler farms (labeled farm 1 to farm 16) in the Belgrade area during 2022. The broiler farms were 20 years old, on average, and most of them are contract growers. The capacity of farms was 25,000 (middle-scale commercial producers, farms labeled from 1 to 11) to 100,000 broilers (large-scale commercial producers, farms labeled from 12 to 16), with birds placed in 2-4 houses, depending on the farm.

Assessment of biosecurity measures on the farms was carried out based on the application of an appropriate questionnaire where farmers on a voluntary basis answered several questions regarding the implemented biosecurity measures. The questionnaire for the broiler farms comprised 79 questions divided into 11 subcategories. External biosecurity was assessed within eight subcategories: purchase of one-day-old chicks, depopulation of broilers (slaughterhouses, traders, and individuals), feed and water, removal of manure and carcasses, visitors and farm workers, material supply, infrastructure and biological vectors, and location of the farm. Internal biosecurity was assessed with questions from three subcategories: disease management, cleaning and disinfection, and materials and measures between compartments. Every category was scored from 0 (absolute lack of biosecurity on the farm) to 100 (when the measures are fully implemented). This study described biosecurity assessment in broiler farms using the online risk-based Biocheck.UGent scoring system (<https://biocheckgent.com/en/questionnaires/broilers>). be/). General biosecurity was computed as the average of external and internal biosecurity scores. The final scores for each biosecurity category were calculated for each farm. During the authors' visits to the different farms, we compared the answers with the realistic situation on the farm or in the flock and ensured the right answer was chosen in the questionnaire.

Differences between the external and internal biosecurity scores obtained were tested by independent samples *t*-test (mean). The same test was applied to compare differences in the farm scores and world scores (WS) obtained from Biocheck.UGent online survey database. The alpha level for significance was 0.05. Statistical analyses of the results obtained in the study were performed using Graph Pad Prims v 9.4.1 software.

RESULTS

The results of the biosecurity assessment are presented in Table 1. The average general biosecurity score was 84.3%, but a wide extent of scores was found among the participating farms. Results showed that external biosecurity scores ranged from 57% to 93%, averaging $83.6 \pm 10.7\%$. Internal biosecurity scores ranged from 48% to 98%, averaging $85.7 \pm 15.1\%$. Differences between total external biosecurity scores and total internal biosecurity scores from participating farms were not significant ($p = 0.6583$) with an acceptable variation of total scores on the different farms (CV external = 12.75%, CV internal = 17.5%) (Fig. 1).

Table 1. Biosecurity scores and statistical markers for the different subcategories of internal and external biosecurity in 16 broiler farms

	WS ¹	Mean	SD	Median	Min	Max	CV
External biosecurity							
Purchase of one-day-old chicks	63	69.6	8.2	69.0	47.0	90.0	11.9
Depopulation of broilers (slaughterhouses, traders, individuals)	57	72.4	12.7	73.0	44.0	86.0	17.5
Feed and water supply	57	90.2	4.3	92.0	78.0	92.0	4.8
Removal of manure and carcasses	55	79.8	28.8	88.0	12.0	100.0	36.0
Visitors and farm workers	69	88.1	12.8	94.0	57.0	100.0	14.6
Material supply	71	91.8	17.7	100.0	56.0	100.0	19.3
Infrastructure and biological vectors	75	93.5	9.3	100.0	74.0	100.0	9.9
Location of the farm	64	81.8	22.8	100.0	44.0	100.0	27.9
<i>External biosecurity score</i>	64	83.6	10.7	87.0	57.0	93.0	12.8
Internal biosecurity							
Disease management	75	86.5	14.6	92.0	56.0	98.0	16.9
Cleaning and disinfection	65	84.0	17.0	90.0	39.0	98.0	20.2
Materials and measures between compartments	75	88.1	20.5	100.0	29.0	100.0	23.2
<i>Internal biosecurity score</i>	71	85.7	15.1	89.5	48.0	98.0	17.6
Overall biosecurity score	67	84.3	11.9	88.5	56.0	95.0	14.1

¹WS – world score

The scores for subcategories varied between the farms. Noteworthy, removing manure and carcasses, a subcategory within the category of external biosecurity, had the lowest mean score (farm 9 and farm 10 both had a score of 12%), with high variation between farms (CV = 36%). Relatively low scores were also obtained for the number of steps in broiler depopulation (farm 9 had a score of 44%) and for the location of the farm (farm 2 and farm 3 both had a score of 44%). From the different subcategories of external biosecurity, high scores were obtained in the subcategory of

infrastructure and biological vectors, with a mean score of 93.5%. This subcategory includes an appropriate protocol for rodent control and prevention of direct contact between flocks and wild birds. According to the results described in the present study, farms have an excellent solution for all measures they are taking for the biosecurity of feed and drinking water ($90.2 \pm 4.3\%$). Most of the farmers submitted water samples for quality analyses once per year and usually, they took samples at the source, while some of them took samples both at the source and at the end of the waterline.

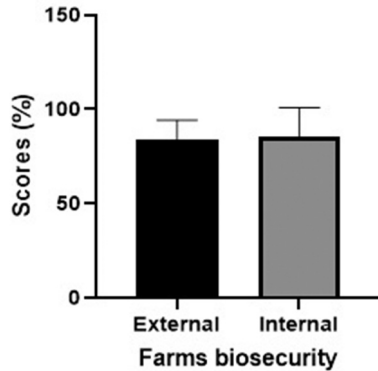


Figure 1. External biosecurity score and internal biosecurity score averaged from 16 broiler farms in the Belgrade city region

The world scores (WS) for external biosecurity measures differ significantly ($p < 0.05$) in comparison to the results from farms 8, 12, and 13 (Fig. 2).

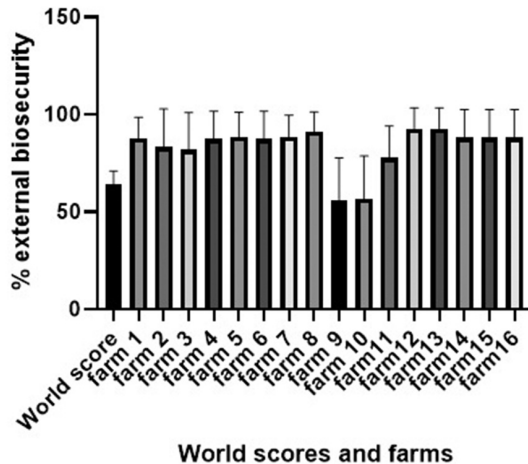


Figure 2. Comparison of the average world score and external biosecurity scores from 16 broiler farms in the Belgrade city region

Concerning internal biosecurity, the lowest score (farm 10 had a score of 29%) was obtained for the subcategory material and measures applied between compartments

(stable-specific equipment and farm clothing). However, ten farms had very good scores (100%). It is recommended that different equipment and clothing are used in different houses. A relatively low score was obtained for the cleaning and disinfection protocol on two farms carried out between two production cycles (farm 9 had a score of 39%, and farm 10 had a score of 47%).

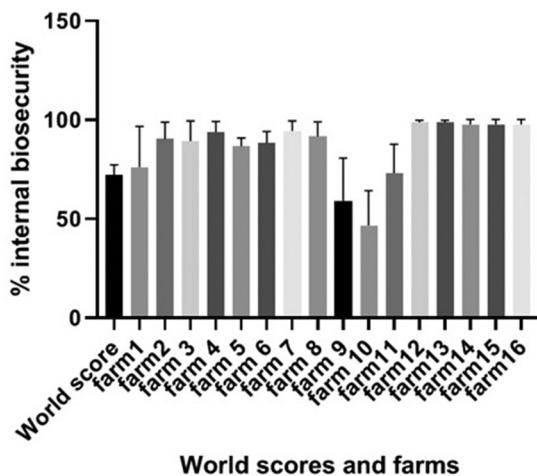


Figure 3. Comparison of the average world score and internal biosecurity scores from 16 broiler farms in the Belgrade city region

DISCUSSION

In the Belgrade city area, there are 20 broiler farms in total, whereas in this assessment, 16 were included. The farms were selected randomly, so the investigation included broiler farms that were willing to review the situation regarding biosecurity protocols that are in use.

The average overall biosecurity score was higher than the average world score obtained from the Biocheck.UGent database, but it should be kept in mind that the database includes the results from less standardizable farming systems (backyard flocks). Also, the average external and internal biosecurity scores obtained were higher than the average world scores. According to the results of previous research (Gelaude et al., 2014; Limbergen et al., 2018), the results obtained showed the internal biosecurity score was higher in most of the farms in our study than the external biosecurity score.

External biosecurity scores of the different farms for certain subcategories (especially, broiler depopulation, means for manure and carcasses removal, and location of the farm) showed large variations when compared at the individual farm level. In order to decrease stocking density, the flocks were partially depopulated on the studied farms in three or more steps. The vehicle driver, the catching team, and their equipment bring a risk of pathogen introduction with each new depopulation step (Lister, 2008;

Gelaude et al., 2014). According to Agunos et al. (2014), novel *Campylobacter* strains can be transferred to the remainder of the flock via transport equipment and catching personnel. This can contribute to increasing *Campylobacter* contamination in birds before they arrive at the slaughterhouse (Agunos et al., 2014).

Poultry production farms accumulate a large quantity of litter (feces, feathers, bedding materials, spilled feeds, drugs, and water). Chicken litter is considered a great source of important (nitrogen, phosphorus) elements and trace elements, and in most cases, it is applied to agricultural land (Bolan et al., 2010). However, it should be kept in mind that poultry litter can be contaminated with different pathogenic microorganisms, including those (like *E. coli*) that carry antibiotic-resistance genes, antibiotics, heavy metals, hormones, and pesticides. Also, chicken litter can be a source of human pathogens frequently associated with foodborne outbreaks, such as *Salmonella*, *Campylobacter jejuni*, and *Listeria monocytogenes* (Wilkinson et al., 2011; Kyakuwaire et al., 2019). In this study, the observed external biosecurity subcategories related to manure management methods and practices and carcass removal had very low scores on some farms (farm 9 and farm 10). These are medium-sized farms, more than 25 years old, and on those farms, they have the practice to leave the untreated litter close to the house for a long time after the production cycle. On the other hand, some among the studied farms were large and modern, with more economic inputs and with a high level of biosecurity practices, as disease outbreaks on such farms are more costly. Those farms have a contract with a rendering plant for the disposal of carcasses and a bioenergy plant for used litter.

In the last few years, in Serbia, it was recorded that avian influenza virus HPAI subtype H5N8 has emerged in wild birds (Božić et al., 2016; Djurdjević et al. 2023). H5N8 avian influenza virus was detected within the mute swan population in the Belgrade city area indicating a deteriorating epidemiological situation (Maletić et al., 2022). When this information is taken into consideration, critical control points for the prevention of pathogen introduction should be implemented on all poultry farms, especially those near large still water surfaces (FAO, 2008). All farms from the study have houses designed and maintained to prevent access by wild birds and rodents. The farms that were located close to lakes, and farms that were located close to other broiler farms and public roads, had low scores for the location subcategory. It has been reported that the risk of the pathogens spreading is higher in the case of the presence of another broiler farm in the neighborhood (Lister, 2008; Gelaude et al., 2014).

Disease management obtained the highest score concerning internal biosecurity. The farms undertake cleaning and disinfection regularly after each production cycle, but they do not perform routine control, such as bacteriological testing, to verify the efficacy of implemented measures and steps. In Serbia, there is no official requirement for periodic control of the general hygiene status of broiler houses after cleaning and disinfection. Also, in some farms, the sanitary break after each production cycle lasts between 3 and 8 days. Short sanitary breaks like this and inadequate cleaning and disinfection procedures can cause *Campylobacter* colonization on the farm prior

to the arrival of a new flock at the broiler houses (Agunos et al., 2014.). Concerning the subcategory related to the materials and measures that are undertaken between compartments, the scores ranged from 29% to 100%, and the reason for such a wide variation is a lack of awareness that in different houses, the same material cannot be used without being previously disinfected. It is recommended the use of the same equipment and clothes in different sections is avoided in order to decrease the risk of pathogen transmission (Gelaude et al., 2014). According to Alarcón et al. (2021), farm personnel are a crucial element of every farm. They are responsible for managing daily events on the farm, which requires them to work on a tight labor schedule. Biosecurity protocols should be realistic for implementation on the farm, and farm personnel must understand the importance of each measure from the disease transmission aspect (Scollo et al., 2022). In general, there is a lack of training for poultry farmers and farm personnel regarding biosecurity. Farmers who are educated on the outbreak, prevention of transmission, and spread of infectious diseases will be aware of the benefits that a good biosecurity protocol can bring (Nöremark et al., 2009).

These findings gave an overview of the current situation regarding the biosecurity status of broiler farms from the Belgrade city region. The study should help decision-makers and flock veterinarians to set feasible targets and sustainable biosecurity programs to improve biosecurity, the flock's health status, and farm profitability.

CONCLUSION

Despite the lack of regulation in the field of biosecurity, broiler farmers in the Belgrade region of Serbia are mostly aware of the importance of good policy regarding biosecurity. Periodic and systematic biosecurity evaluation is necessary if we want to secure the preservation of animal health. In this study, we tried to identify biosecurity factors that can be improved. The awareness of veterinarians and farmers about the importance and beneficial implication of good management and biosecurity measures would be raised by participation in the study. There are several areas for improvement regarding farm management, such as the removal of manure and carcasses, depopulation procedures, and finding an appropriate geographical location for the farm, especially with regard to the proximity to water surfaces. The number of steps involved in depopulating the farm should also be considered. The results showed that farm managers should think about better solutions regarding the manipulation of materials and equipment between different sectors of the farm, as these items can serve as mechanical vectors for pathogen transmission. However, the preliminary results are promising, and further steps should be to investigate whether improved biosecurity can help to improve broiler performance and reduce the use of antimicrobials in the Belgrade city area.

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Ethical approval

According to the Law on Protection of Animal Welfare from the Ministry of Agriculture and Environmental Protection of the Republic of Serbia and Directive 2010/63/EU, animal welfare was not violated and permission from the Ethical Committee of the Faculty of Veterinary Medicine University of Belgrade (or similar committee) for this study was not issued. Farm owners signed an informed consent form approving that the questionnaire will be used for publishing a scientific paper.

Authors' contributions

JM and BK contributed to the conception and design of the study. JM, LJS, and DG performed the farm visits and collected all data. VM and JK organized the database. MM performed the statistical analysis. JM and BK wrote the manuscript. LJS, VM, JK, and DG contributed to manuscript revision, reading, and adjustments. All authors approved the submitted version.

Competing interests

The authors declare that they have no competing interests. The authors have no relevant financial or non-financial interests to disclose.

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OCENA BIOSIGURNOSNIH MERA IMPLEMENTIRANIH NA FARMAMA BROJLERA NA BEOGRADSKOM PODRUČJU

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Kratka sadržaj

Sprovođenje biosigurnosnih mera, kao prve linije odbrane, suštinski je preduslov za sprečavanje pojave i širenja infektivnih oboljenja kod živine. Procena biosigurnosnih mera na farmama izvršena je primenom odgovarajućeg upitnika, gde je farmer odgovorio na brojna pitanja u vezi sa sprovedenim biosigurnosnim merama. Istraživanje je sprovedeno putem onlajn ankete Biocheck.Ugent na 16 farmi brojlera u regionu grada Beograda. Kapacitet farmi je bio 25000-100000 brojlera, smeštenih u 2 - 4 objekta,

u zavisnosti od farme. Rezultati su pokazali da se eksterna biosigurnost kretala od 57 % do 93 %, sa prosečnom ocenom od 83.6 %. Rezultat interne biosigurnosti se kretao od 48 % do 98 %, sa prosečnom ocenom od 85.7 %. Krajnji rezultat procene biosigurnosti kretao se od 56 % do 93 %, sa prosečnom ocenom od 84.3%. Rezultati za potkategorije su varirali između farmi. Uklanjanje stajnjaka i leševa, potkategorija u okviru kategorije eksterne biobezbednosti, imala je najnižu srednju ocenu (farme 9 i 10 su imale ocenu od 12 %). Niže ocene takođe su dobijene za potkategorije broj koraka depopulacije brojlera (farma 9 je imala ocenu 44 %) i za lokaciju farme (farme 2 i 3 su imale ocenu od 44 %). Što se tiče interne biosigurnosti, najniža ocena je dobijena za potkategoriju materijal i mere koje se primenjuju između odeljaka (farma 10 imala je ocenu od 29 %). Ovi nalazi trebalo bi da budu korisni donosiocima odluka i veterinarima i farmerima u postavljanju izvodljivih ciljeva i održivih biosigurnosnih programa, za poboljšanje biobezbednosti, zdravstvenog statusa jata i profitabilnosti farme.

Ključne reči: ocena, biosigurnost, brojleri, farme, Beograd