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Original scientific paper

Enhanced biosecurity measures may contribute to the reduction of Campylobacter incidence in slaughterhouses

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A B S T R A C T

As a preventive measure, biosecurity is the first line of defense against many pathogens. Applied biosecurity measures can reduce the prevalence of *Campylobacter* infection in commercial broiler populations. A systematic evaluation, encompassing at least annual monitoring of applied biosecurity measures and on-farm prevalence of *Campylobacter* infection is highly recommended.

This study was performed on three broiler farms with the aim to assess the effectiveness of the biosecurity measures applied. Broiler farms included in the study previously had problems with *Campylobacter* infections, and therefore, after the intervention through a risk-based scoring system and bacteriological testing of samples from the farm and the corresponding carcasses in the slaughterhouse, several biosecurity measures were implemented. Obtained results showed that after the intervention, farms increased their external biosecurity by 16.34%, internal biosecurity by 22%, and overall biosecurity by 18.34%. The major interventions concerned the removal of manure and carcasses, all improved measures taken for feed and drinking water, and measures in the subcategory of cleaning and disinfection protocols carried out between two production cycles. After the improvements, during the screening process on the farms, *Campylobacter* was not isolated from pooled fecal samples in any of the broiler houses. This indicates that at least six houses (two houses per farm) were *Campylobacter*-negative at broiler slaughter age. In pooled neck skin samples originating from studied farms, *Campylobacter* was not isolated after the improved measures were implemented.

The results showed that the assessment of biosecurity protocols on broiler farms is a useful tool, and *Campylobacter* can serve as a biomarker for the efficiency of the implemented biosecurity protocols.

1. Introduction

Biosecurity in animal production 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*, 2022). It is the cornerstone of preventive medicine aimed at preserving public and animal health, plants, and the environment. In such a way, biosecurity is part of the One Health concept (*Renault et al.,* 2022). In broiler production, good biosecurity protocols are very important to reduce the risk of introducing and spreading pathogens and to reduce the use of antimicrobials and consequent-

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ly the development of antimicrobial resistance in veterinary and human medicine. Applying stringent biosecurity measures may reduce flock infection, but sustaining such measures on the farms seems extremely difficult. Therefore, it is very important to regularly monitor and evaluate the current biosecurity status on the farms (*Newell et al.* 2011; *Gelaude et al.,* 2014; *Dewulf et al.*, 2018; *Caekebeke et al.*, 2021).

One of the major causes of foodborne bacterial gastroenteritis in Serbia is *Campylobacter*. It is the second most common intestinal infection with a high incidence in the last three years: 11.3 (2019), 6.4 (2020), and 6.4 (2021) in 100,000 people (*Institute of Public Health of Serbia "Dr Milan Jovanovic Batut"*, 2022). The most frequently identified source of human infection is fresh broiler meat contaminated during processing. A high incidence of *Campylobacter* infection in broiler flocks is widespread and the infections are age-dependent (*Evans and Sayers*, 2000; *Mullner et al.*, 2009; *EFSA*, 2020). When broilers become infected with *Campylobacter*, the infection is spread from the house, through the anteroom to the areas surrounding the broiler house. Vectors for transmission of the pathogen potentially could be farmers (boots, clothes, and equipment), rodents, and insects (*Newell and Fearnley*, 2003; *Battersby et al.*, 2016; *Royden et al.*, 2021). Studies showed that multiple interventions, hygiene, biosecurity measures, and additional and complementary interventions (probiotics, vaccination) help in controlling the incidence of *Campylobacter* infection in poultry broiler flocks (*Gibbens et al.*, 2001; *Newell et al.*, 2011; *Horvat et al*, 2022). A linear relationship between the flock prevalence and incidence of campylobacteriosis in humans is confirmed (*Rosenquist et al.*, 2003), so reducing the prevalence of positive flocks should contribute to the reduction of disease in humans (*Newell and Fearnley*, 2003; *EFSA*, 2011). The main goal should be to produce chicks free from infection at slaughter, and in this way, the possibility of creating a potential source for human infection would be reduced (*Evans and Sayers*, 2000; *Sibanda et al.*, 2018).

The aim of this study was to assess the effectiveness of the biosecurity measures on the broiler farms that historically had a problem with *Campylobacter* infections, being implemented after the intervention through the risk-based scoring system and bacteriology testing of samples from the farm and the corresponding carcasses in the slaughterhouse.

2. Materials and methods

The study was performed on three broiler farms (labeled from 1 to 3) in Serbia during 2022–2023. The participating broiler farms were 18, 15, and 2 years old. The capacity of the farms was 100000 broilers (large-scale commercial producers), placed in 2–4 houses. Houses were in a good state of repair. Participating farms were visited two times for biosecurity data collection and three times for sample collection.

During the first visit, data were collected regarding biosecurity levels and farm characteristics. In the face-to-face interview with the farmers, it was found that farms had problems with overall broilers' health, mainly related to intestinal disorders, poor weight gain, and high feed conversion rates in the previous period. The farms cooperated with one slaughterhouse for further processing. Slaughterhouse internal control quality results indicated that flocks' neck skin samples were colonized with *Campylobacter* at the final slaughter age, especially during the summer season (chicken carcass neck samples with more than 1000 CFU/g of *Campylobacter*).

Assessment of biosecurity measures on the farms was carried out based on the application of the appropriate questionnaire where farmers on a voluntary basis answered several questions regarding the implemented biosecurity measures. The checklists for the broiler farm comprised of 79 questions divided into 11 categories. External biosecurity was assessed within 8 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 categories: 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 were fully implemented). The study described biosecurity assessment in broiler farms using the risk-based Biocheck.UGent scoring system (http://www.biocheck.ugent.be/). Overall biosecurity was computed as the average of external and internal biosecurity scores. The final scores for each biosecurity category were obtained for every farm separately.

For every non-compliance, correction measures that should be done at the points of attention were suggested to the farmers. Attention was made to external (purchase of one-day-old chick, practice related to the removal of manure and all measures taken for the feed and drinking water), as well as internal biosecurity measures (monitoring of flock health and practice related to cleaning and disinfection).

The second farm visit was done 10 months later. During the visit, farmers on a voluntary basis answered several questions regarding the implemented biosecurity measures, and data were collected to assess the efficacy of the corrective measures they took. Also, flocks were screened for *Campylobacter* presence over one production cycle, with two flocks included from each farm, about two weeks before slaughtering. In agreement with the farm owner and with respect to all ethical principles, a total of 60 chicken fecal samples were collected. Fecal samples from 10 birds were pooled by mixing and placed into sterile fecal containers. Each container consisted of pooled feces from five different broiler chickens on the same farm.

Fecal samples were transported at 3°C and cultured within an hour of collection onto a *Campylobacter* selective media for isolation. Approximately 0.2Xg of feces was added to 1 ml of the enrichment medium Preston broth and incubated at 41.5°C for 24h ± 2h. After incubation, the culture was transferred with a sterile loop of 10 µl on the surface of the isolation medium, mCCD agar, and plates incubate at 41.5°C in a microaerobic atmosphere for 44h ± 4h. Suspect colonies were subcultured in *Campylobacter* blood-free selective agar base to get pure cultures according to ISO 10272–1 (*SRPS EN ISO 10272-1:2017*, 2017a**)**.

In addition, neck skin samples were collected after washing with chilled water and tested for concentrations of *Campylobacter*. From each flock, five pooled samples of neck skin (10 g of skin samples were taken) were obtained by blending three neck skins. In total 180 neck skin samples were tested. To obtain *Campylobacter* from the neck skin samples, detection and enumeration of *Campylobacter* (*SRPS EN ISO 10272-2:2017*, 2017b) was according to the Rulebook on general and special conditions of food hygiene at any stage of production, processing, and circulation (*Official Gazette of RS no. 72/10*, 2010) and Rulebook on amendments to the Rulebook on general and special conditions of food hygiene at any stage of production, processing, and traffic (*Official Gazette of RS no. 62/18*, 2018). We prepared an appropriate decimal dilution of the samples and transferred 0.1 ml of the initial suspension to an mCCD agar plate and incubated at 41.5°C in a microaerobic atmosphere for 44 h ± 4 h. The test results were interpreted as satisfactory for the skin-neck samples that had less than 1000 CFU/g.

Differences between obtained scores for subcategories of external and internal biosecurity were analyzed using parametric independent samples t-test for the normally distributed data. The alpha level for significance was 0.05. Statistical analyses were performed using Graph Pad Prims v 9.4.1 software.

3. Results

The results of the biosecurity assessment obtained during the first and the second visits are presented in Table 1. A graphical illustration of the biosecurity score in visit 1 and its post-intervention score are given in Figure 1.

During the initial visit, the average overall biosecurity score ranged from 60% to 63%, with an average score of 61.66 ± 1.25%. Results showed that external biosecurity scores ranged from 62% to 65%, averaging 63.33 ± 1.25%. Internal biosecurity score ranged from 56% to 64%, averaging 58.68 ± 3.77%.

The scores for subcategories varied between the farms. Noteworthily, before the intervention of removing manure and carcasses, the subcategory within the category of external biosecurity had the lowest mean score (a score of 5%). The farms left the manure for some time near the farm, and after 15–20 days, they brought it to the nearest fields. The carcass storage was placed inside the houses, being never disinfected after use, only washed. Also, no gloves were used during the manipulation of dead birds. This point is very important as dead birds have been identified as potential sources for various pathogens. Compared to the world scores (WS) obtained from the Biochek.UGent online survey database, lower overall scores were also obtained for steps of broiler depopulation (51%) and for the measures used for the feed and drinking water (45%). On all farms, there was no clear separation between clean and dirty sections of the premises, especially separation in the anteroom. Also, drinking water quality was never tested on a regular basis.

Concerning internal biosecurity, results obtained on the initial visit showed that the lowest score was obtained for the subcategory cleaning and disinfection protocols carried out between the two production cycles (43%).

Obtained data for biosecurity showed that the overall external, internal, and total biosecurity scores significantly differed between the first and the second visits (Figure 1.). Results were better on the second visit compared to the first visit. Biosecurity scores for subcategories feed and water and the removal of manure and carcasses were improved on all farms. Within internal biosecurity, the biggest

Table 1. The average biosecurity scores (%) of the farms for the different categories of the Biocheck. UGentTM survey in the first and second visits for biosecurity compliance. *p* values are provided for a comparison between the farms in the first and the second visits

	Visit 1 (%)	Visit 2 (%)	*p*-value	World score (%)
External biosecurity				
Purchase of one-day-old chicks	59.00	80.67	0.0266*	63.00
Depopulation of broilers (slaughterhouses, traders, individuals)	51.00	55.67	0.3735	58.00
Feed and water	45.00	81.00	0.0298*	57.00
Removal of manure and carcasses	5.00	77.33	0.0003*	56.00
Visitors and farmworkers	80.00	89.33	0.1161	69.00
Material supply	100.00	100.00	-	69.00
Infrastructure and biological vectors	96.67	95.00	0.4226	76.00
Location of the farm	52.00	57.00	0.7696	64.00
External biosecurity score	63.33	79.67	0.0490*	65.00
Internal biosecurity				
Disease management	63.67	98.00	0.0464*	76.00
Cleaning and disinfection	43.00	67.67	0.0234*	66.00
Materials and measures between compartments	82.00	82.00	-	73.00
Internal biosecurity score	58.67	80.67	0.0027*	71.00
Overall biosecurity score	61.66	80.00	0.0288*	66.00

* *p* values below 0.05 were considered statistically significant

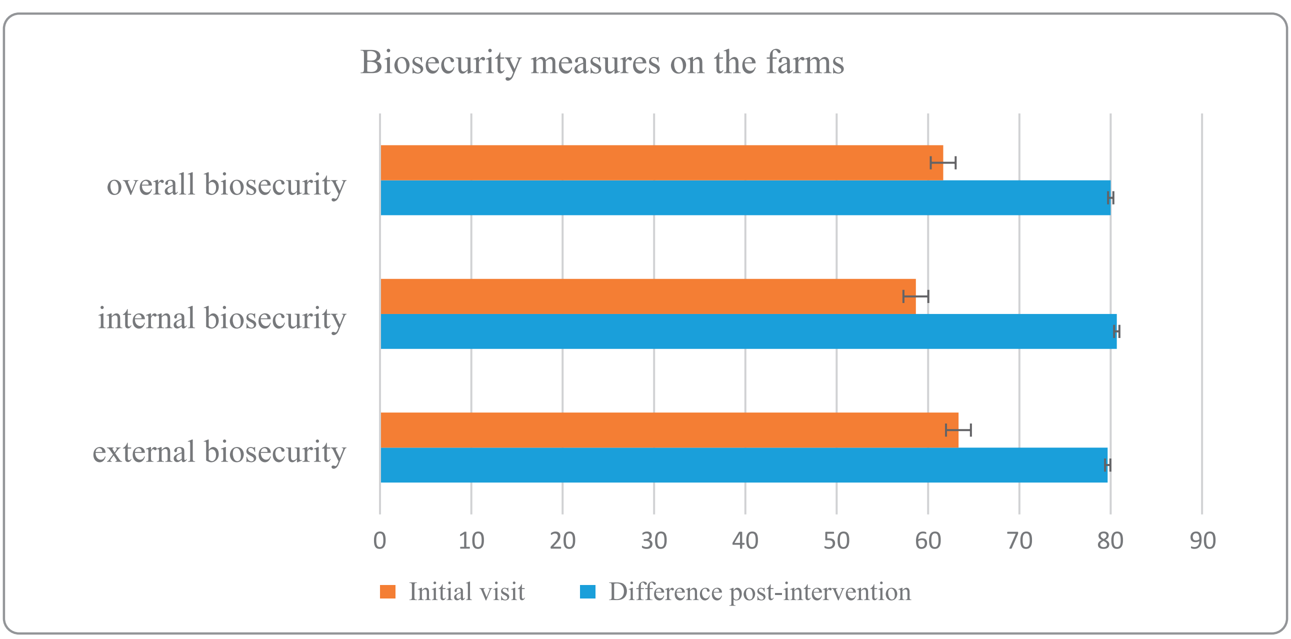

Figure 1. Comparison of the overall, external, and internal biosecurity scores (%) in the first and second visits

improvement was seen in the cleaning and disinfection protocols, with an increased score of 24.67%.

During the screening process of the second visit, *Campylobacter* was not isolated from pooled fecal samples in any of the broiler houses. This indicates that at least six houses (two houses per farm) were *Campylobacter*-negative when the broilers were of slaughter age. In pooled neck skin samples originating from the studied farms, 100% (180/180), i.e. all samples had less than 10 CFU/g *Campylobacter*.

4. Discussion

In order to identify the effectiveness of the biosecurity measures implemented after the intervention at all points of attention on the broiler farms, the investigation covered biosecurity assessment and *Campylobacter* analysis of the samples originating from farms and on the slaughterhouse level. The costs of the intervention were not high as farms had their own biosecurity policy that had to be slightly changed, since there were challenges for farm workers to persevere and consistently perform.

Major differences were observed concerning external biosecurity among all three farms. External biosecurity is fundamental to prevent the entry of the pathogens, and data obtained during the initial visit showed that external biosecurity had lower scores than internal on all farms.

The depopulation of broilers aims to optimize the use of the farm space. On the studied farms, the flocks were partially depopulated in two to three steps. The risk of partial depopulation for *Campylobacter* introduction and transmission has been proven, and this happens due to the contamination of harvesting equipment and materials used by the catching crew. As it is financially challenging to stop partial depopulation practices, it is suggested that the focus should be on external biosecurity to avoid the introduction of pathogens. Therefore, improved hygiene practices and sustainable biosecurity programs are important points of action (*Hertogs et al.*, 2021; *Sarnino et al.*, 2022).

Special attention was put on the removal of manure and carcass management as it was the cause of the low external biosecurity scores. Farms had practiced storing the manure prior to use, and then unprocessed poultry manure was spread as organic fertilizer on the land that surrounds the farms. That manure could be contaminated with pathogenic microorganisms, antibiotics, pathogenic microorganisms with antibiotic-resistant genes, heavy metals, growth and sex hormones, and pesticides. As shown, chicken litter can be a source of *Campylobacter jejuni* (*Wilkinson et al.*, 2011; *Kyakuwaire et al.*, 2019). Managing poultry manure requires a complex approach covering transportation, storage, and further handling and/or processing (*Dróżdż et al.*, 2020). In our study, after our visits, the farmers signed contracts with a bioenergy plant to accept the used poultry litter. Also, the farms increased the carcass storage hygiene and the frequency of carcass elimination from the farm by trucks. With those interventions, the subcategory removal of manure and carcass achieved better scores.

Although rodents were not a problem on the farms due to effective vermin-control programs, insects, including flies, were potential problems. Flies can serve as vectors for *Campylobacter* in broilers. These insects can transmit *Campylobacter* from outside farm livestock into broiler houses. Preventing fly entry during summer leads to a decrease in the prevalence of *Campylobacter* positive flocks at slaughter (*Hald et al.*, 2007). Generally, insect control interventions reduce the peak percentage of contaminated chickens and neck samples of chicken carcasses (*Horvat et al.*, 2022). The problem can be solved by regular removal of carcasses, repairing all leaking water lines and keeping the manure dry, and relocating used litter from the farm.

According to the risk-based scoring system, farms had low scores for feed and water management. They did not conduct microbiological analyses of water samples. The drinking water should be kept at a microbiologically safe level as contaminated feed and water can easily serve as a transfer medium for pathogenic bacteria. The addition of disinfectants to drinking water gave promising results for *Campylobacter* control in chicken flocks (*Maharjan et al.*, 2016; *EFSA*, 2020; *Scollo et al*., 2023).

Sometimes farms made a change of feed supplier, but not during one production cycle. The feed silos are completely sealed against water, birds, and vermin. The feed suppliers did not have access to the houses where direct contact with the poultry would be possible. The major problem was a lack of clear separation between the clean and the dirty areas of the farm premises. The traffic that serves different companies uses the same road as the vehicles for internal movements at the farm. There were no protocols for fully cleaning and disinfecting the trucks at the farm entrances.

Concerning internal biosecurity measures, attention was made to cleaning and disinfection. Farms owned special protocols for cleaning and disinfection between flocks, but they did not perform routine controls, such as bacteriological testing, to verify the efficacy of their applied protocols. Visual inspection of cleaning alone is not reliable to assess the hygiene status of broiler houses (*Luyckx et al.*, 2015). In Serbia, there is no official requirement for periodic control of the general hygiene status of broiler houses after cleaning and disinfection. The downtime was short (approximately seven days) after each production cycle. *Agunos et al.* (2014) reported that inadequate cleaning and disinfection and short downtime between flocks can be a potential source of *Campylobacter* colonization due to the carryover of strains from one flock to the next. Longer periods (over 10 days) between sequential flocks reduce residual bacterial contamination in or around a previously positive house from spreading to a new flock in the same house (*EFSA*, 2020). Every poultry house had an anteroom and a hygiene lock present, where farm workers can wash and disinfect their hands, but this does not necessarily imply their implementation. Farmers and visitors can also transmit *Campylobacter* to chickens, bringing the bacteria through contaminated shoes or boots, contaminated tools, or through contaminated clothes and hands (*Horvat et al*., 2022). A standard hygiene protocol followed by all staff who entered into populated broiler farms could reduce the risk of a flock getting infected with *Campylobacter* by 50%. Hygiene protocols should include a strict procedure with boot dips before entering the farm and houses and specific clothing (*Gibbens et al.*, 2001). As was noted by *Van Limbergen et al.* (2018), better education of the staff could help to improve the overall biosecurity on broiler farms. Continuous training and motivating the staff in combination with the results of monitoring of farm-specific critical points could help to increase efficiency and prevent staff from becoming inattentive due to routine (*Scollo et al.*, 2023).

Studies showed that the transportation crates can be a reservoir of *Campylobacter* if the washing and disinfection processes of crates in the slaughterhouse are not sufficient to eliminate all bacteria (*Horvat et al.*, 2022). The studied farms received transport crates that were disinfected by the slaughterhouse.

Campylobacter is often detected in broiler flocks at 3 to 4 weeks of age. In this study, during the screening process of the broiler houses, *Campylobacter* was not isolated in any of the poultry. This is probably the result of a stronger commitment to biosecurity practices by breeding farms (*Hertogs et al.* 2021). Additionally, farms started with the acidification of drinking water with a buffered organic acid basis, which is proven to reduce the risk of *Campylobacter*-positive flocks (*EFSA*, 2020).

Flocks that are infected with *Campylobacter* can be a source of the bacteria in the corresponding carcasses. Cross-contamination during the transportation and slaughter process is also important, as is the cross-contamination rate between carcasses and processing equipment (*Zhang et al.*, 2018; *Perez-Arnedo and Gonzales Fandos*, 2019). On-farm control of *Campylobacter* in poultry would reduce the risk of human exposure to this pathogen and have a significant impact on food safety. The aim should be to decrease environmental exposure through improved biosecurity measures (*Lin*, 2009).

This study indicated that improved on-farm biosecurity measures can play an important role in reducing the prevalence of *Campylobacter* infection in poultry broiler flocks. The results further indicated the importance of good biosecurity protocols applied during broiler production. Each intervention can be quantified with the continuous assessment of implemented biosecurity programs. On-farm testing of birds can also help to predict the exposure risk of the hazard *Campylobacter* in resultant food.

5. Conclusion

As a preventive measure, biosecurity is the first line of defense against many pathogens. A systematic evaluation, encompassing at least annual biosecurity monitoring and on-farm prevalence of *Campylobacter* infection is recommended. The risk of spreading the pathogen to humans in close contact with broilers, means moving attention to the healthcare of farm workers and slaughterhouse staff as a part of the One-health approach. Our results show that the assessment of biosecurity protocols on broiler farms is useful and that *Campylobacter* can serve as a biomarker for the efficiency of the implemented biosecurity protocols. It is very important that farmers consistently follow the biosecurity rules in their daily work. This study also had some limitations, as it was focused only on one slaughterhouse and three farms, so future studies should include an increased sample size from the farms and slaughterhouses for analysis.

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