Risk analysis of critical control points of *Staphylococcus aureus* in layer farms and chicken egg distributors

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Abstract

Microbiological criteria play a role in verifying the critical control points (CCP), which become part of the hazard analysis, and the CCP system that guarantees quality, considering possible danger points or stages in the food production chain. Studies about *Staphylococcus aureus* in chicken eggs more extensively discuss the path, source, and level of prevalence of contamination at the final distributor or consumer. Therefore, this study investigates CCP contamination of *S. aureus* in chicken eggs and their potential consumption, which could endanger human health from the layer farm until the final distributor. This study is critical in health, public health, and veterinary medicine for preventing and controlling consumers' security. This study done for CCP on the chain distribution of chicken eggs starts with preparing production and distribution process flow diagrams for livestock, agents/wholesalers, and retailers. Confirmation of operational production in the field/location study is based on the flow chart that has been arranged, identifying all potential dangers associated with each stage and analyzing potential risks considering every action for controlling identified hazards. The next step involves pinpointing the CCP to counteract the identified threat. An application tree decision defines the CCP, and the logical and final approach is the determination limit critical to the CCP. Analysis results in the determination of CCP contamination of *S. aureus*, indicating that chicken eggs, personal hands (farm workers, wholesalers, and retailers), shelf eggs, and feces are CCP on farms.

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Introduction

The demand for quality food security for livestock products continues to increase with the implementation of free trade (Owusu-Sekyere *et al*., 2014). Therefore, it is necessary to improve the requirements of ISO 22000:2018 (ISO, 2018) through the implementation of the Hazard Analysis Critical Control Point (HACCP) system as a method to anticipate the occurrence of food poisoning and foodborne diseases due to contamination by pathogenic microbes, including viruses and bacteria, parasites, and fungi (Awuchi, 2023). HACCP is a system that internationally guarantees recognized quality based on possible hazards at various points or each production stage in the food industry. The system acknowledges, assesses, and limits significant food safety hazards to minimize the risk of food safety hazards. HACCP is used in the food industry to make products that meet human health and safety conditions. The paramount importance of HACCP is the anticipation of hazards and the priority identification of preventive control measures rather than final food product testing. One of these food problems is related to the production of laying hens before harvest, so it must concern producers, entrepreneurs, and policymakers. *Staphylococcus aureus* contamination of eggs generally occurs because this bacterium is naturally spread. *S. aureus* produces enterotoxins, which can cause the risk of food poisoning with symptoms of headache, vomiting, diarrhea, and even death. In the egg and poultry industry, it is necessary to study the critical point of possible *S. aureus* contamination because *S. aureus* is a microbe that is always present in nature in contact with

the environment. Several cases of egg-based food poisoning have been reported, and once detected, they were due to the presence of toxins produced by *S. aureus*. Therefore, the possible critical points for contamination in the egg supply chain for consumers have been explored.

A critical control point (CCP) is a point/stage/procedure in the food production system and is one of the parts of HACCP. CCP control is excellent and can prevent, eliminate, or reduce existing dangers to food security and public health (Viator *et al*., 2016). CCP identification is necessary for managing risky food and ensuring that the public consumes product-safe food. Because of that, this system can identify potential sources, dangerous materials, and food products. CCP monitoring and control was adopted by the Codex Committee on Food Hygiene, which was later ratified to adopt regulations applicable in each country worldwide. CCP monitoring is meaningful for action prevention, monitoring control dangers to ensure effectiveness, and verifying the whole system. The presence of *S. aureus* in food is an indicator of deviation towards i) implementation of personal hygiene; ii) sanitation equipment; and iii) sanitation environment (Zeaki *et al.*, 2019; Birgen *et al*., 2020; Pérez-Boto *et al*., 2023), which is a CCP. Based on the results of the study, known reasons leading to poisoning food are pathogenic microorganisms (Fernandes *et al*., 2022) and materials chemistry (Gupta *et al.*, 2022; Thi *et al.*, 2023). One of the microorganisms that are the causative pathogen poisoning food is *S. aureus*.

S. aureus is a capable pathogenic bacterium that produces various toxins that cause food poisoning. This bacterium can adapt to multiple types of food, such as stand heat (68.2°C) and protease enzymes such as pepsin inside digestive tracts (Kadariya *et al*., 2014). Gastrointestinal symptoms characterize staphylococcal foodborne intoxication and occur after consumption of *Staphylococci Enterotoxin* (SE) in food. Not all SE can form in each food because factors such as water activity, pH, potential redox, temperature, and others can influence its formation (Bianchi *et al*., 2014). Several outbreak reports show clinical symptoms of enterotoxin after consuming animal products, including Asao *et al*. (2003), who published that as many as 13,420 people in Kansai, Japan, were affected after consuming skim milk and yogurt contaminated with *S. Enterotoxin* A as much as 20-100 ng per individual. Chicken egg is one of the possible sources of animal protein-contaminated *S. aureus* through two main methods, namely transovarial and trans-shell. Contamination begins on the surface shell, followed by penetration by microorganisms into albumen, but in several cases, it is directed to the yolk egg (Pondit *et al*., 2018). Although a known path and source contamination study regarding analytical design risk CCP contamination exists, *S. aureus* starts from the layer farm and continues distributing. Bacteria still need to be considered for their outstanding contribution as a foodborne pathogen from chicken eggs compared to *Salmonella* spp.

Based on existing facts, it is necessary to identify and analyze CCP regarding the presence of *S. aureus* contamination in chicken eggs to provide safe animal protein nutrition for society and its use in food products. Then, there would be a guarantee of the security of chicken eggs, and this product would compete and be accepted in the market and increase the farmer's economy. This study can also be done to support the application method of raising good livestock, especially in layer farms, and minimize contamination of microbes in the food chain.

Materials and Methods

Location

The study was held from July until August 2023 in Regency Sidrap, Bulukumba, and Gowa, South Sulawesi Province, the biggest chicken egg supplier in this province (BPS Provinsi Sulawesi Selatan, 2021). This study comprises a population of layer farms, wholesalers, and retailers. Sample study as many as one farm with a population of 20,000-25,000 egg-laying hens, one wholesaler taking eggs from a layer farm, one retailer taking eggs from wholesalers in three districts, three agents/wholesalers from each farm, and three retail traders from each agent/wholesaler. The sampling technique used in the study is purposive sampling. The data collection techniques used in this research were observation and interviews. Data was collected through direct observation to confirm the flow chart and structured interviews with parties involved in the activities of the target location. This observation confirms chart flow production and interviews structure (Fani, 2010; Ministry of Agriculture of the Republic of Indonesia, 2020) (Table 1) with the parties involved in the activity destination location (Figure 1). Figure 2 shows the chart flow of the method for viewing and analyzing risks. At this point, controlling *S. aureus* cases in the egg supply chain is necessary. This matrix explains the severity and possible levels at the sample investigation level. The flow chart was made and adapted to implementation in the field during interviews with farm workers, wholesalers, and retailers. Figure 3 illustrates the examination of *S. aureus* using biochemical tests, which have been confirmed by DNA testing.

Study design and determination of risk level and critical control point

Identification and confirmation of chicken egg production process stages and distribution flow

The study used a cross-sectional design, where the variables included factors, risks, and observed effects simultaneously. It was measured only once with a representative sample (Thiese, 2014). This study consists of three stages (Figure 4). Stage 1 is to compile a chart flow that includes all stages of the production process: layer farms, wholesale distribution processes, and retailer distribution processes. Stage 2 confirms the operational on-site production based on the chart flow that has been arranged. Stage 3 identifies all danger potentials associated with each stage, then analyzes dangerous potentials and considers every action for controlling identified hazards (ISO, 2018). The stage analysis of potency danger is quantitatively done with a combination of opportunity (probability) and severity (severity).

The probability level consists of three indicators: the occurrence of more than one case of *S. aureus* infection in one year (value 3), the occurrence of one case of *S. aureus* in one year (value 2), and the occurrence of no cases in one year (value 1). The severity level for consumers (severity) consists of three indicators: high severity level, which means critical illness or death (value 3); medium severity level, which means illness (not a critical illness but still requires treatment) (value 2), and low severity level which means mild illness that can still carry out activities (value 1). The level of risk (significance risk) indicator is based on a combination of the effect possibility of happening risk and the level specified severity, which is a base action control that is low-risk (green), medium-risk (yellow), and high-risk (red). Stage 4 is the determination of a CCP, where control is applied to overcome the same danger. CCP is determined using a decision tree with a logical approach, referring to

guidelines established in Indonesia and internationally (FAO, 2020). Stage 5 is the determination of critical limits for the CCP (Table 2) (National Standardization Agency, 2000).

Results

Stages 1 and 2: identification and confirmation of chicken egg production process stages and distribution flow

Based on these results, the stages of the chicken egg production process on the farm, wholesale distribution, and distribution processes at retailers in the three districts were identified. Figure 1 explains the supply chain points, starting at the farm, wholesaler, and retailer. At the farm level, it begins with harvesting, then collecting, sorting, cleaning, grading, packaging, storage, and transportation. At the wholesaler level, it starts with collecting eggs from the farm, sorting and grading them, storing them, and transporting them to consumers. Meanwhile, retail starts with collecting either from the farm or the wholesaler, sorting and grading, and storing it for consumer transportation.

Stage 3: identification and potential danger analysis

The qualitative classification is based on observations, interviews, and an article study at layer farms, wholesalers, and

Figure 1. Retail research locations sourced from Sidrap (**A**), Bulukumba (**B**), and Gowa (**C**) Regencies

Table 1. Operational definition of critical control points in layer farm, wholesome, and retailer.

Variables	Operational definition	How to measure	Measurement scale	Category
Critical control point (X)	Point/stage/procedure in a food production system	Interviews by questionnaires; observation	Intervals	Yes = 1 No = 0
Laying chicken farming (Y1)	Farms that carry out laying hen cultivation activities to produce chicken eggs for consumption	Interviews by questionnaire; observation	Intervals	Yes = 1 No = 0
Wholesaler (Y2)	Traders who collect chicken eggs for consumption from distributors or breeders to distribute to retail traders	Interviews by questionnaires; observation	Intervals	Yes = 1 No = 0
Retailers (Y3)	Traders who collect consumption chicken eggs from agents/wholesalers to sell directly to consumers	Interviews by questionnaire; observation	Intervals	Yes = 1 No = 0

Table 2. *Staphylococcus aureus* has limited critical tolerance values in layer farms, wholesalers, and retailers.

No	Critical control point	Contamination limits	Reference
1	Fresh eggs	$<1\times10^2$ CFU/g	National Standardization Agency, 2000
2	People's Hands	20 – $\leq10^4$ CFU/g	Health Protection Agency, 2009
3	Shelves Egg	20 – $\leq10^4$ CFU/g	Health Protection Agency, 2009
4	Feces	20 – $\leq10^4$ CFU/g	Health Protection Agency, 2009

retailers. It describes the severity level from the lowest to the highest and the probability that it occurs in the supply chain. The classification also discusses the possibility of *S. aureus* contamination in chicken eggs from interviews with farms, agents, wholesalers, and retail traders.

Stage 4: determination of the critical control point

In this research, stage 4 is the determination of CCP using tree decision to high-risk (red) based on matrix determination risk from people's hands, eggs, shelves, and feces are CCP in this research.

Stage 5: determination limit of critical control point

Microbial criteria play a role in verifying CCP. Based on the existing CCP, the determination mark limit critical tolerance for *S. aureus* was set.

Discussion

Identification and confirmation of chicken egg production process and distribution channels

Based on observation, it is known that there are differences in production processes on farms and lines of distribution to wholesalers and retailers across stages of sorting and grading. The third location sorts from separated eggs to chicken, intact and damaged, while wholesalers and retailers are in the ninth grade.

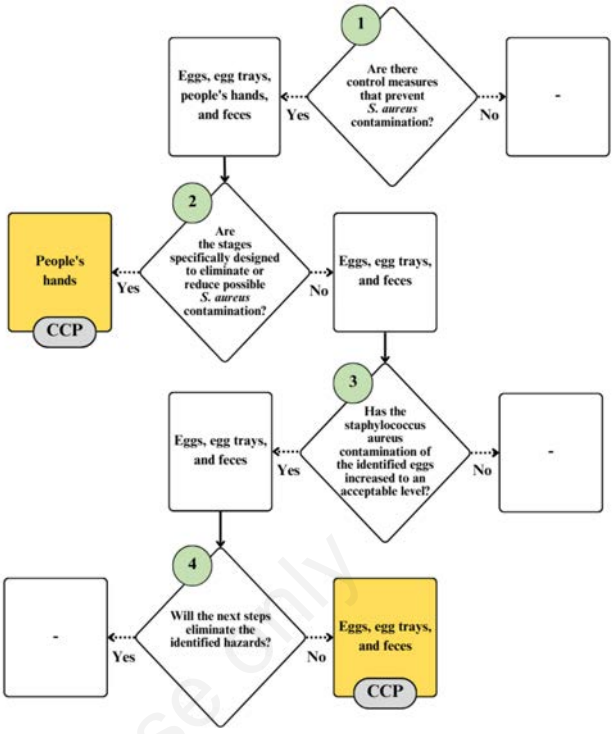

Figure 2. Determination of critical control points (CCP) based on the highest risk of contamination.

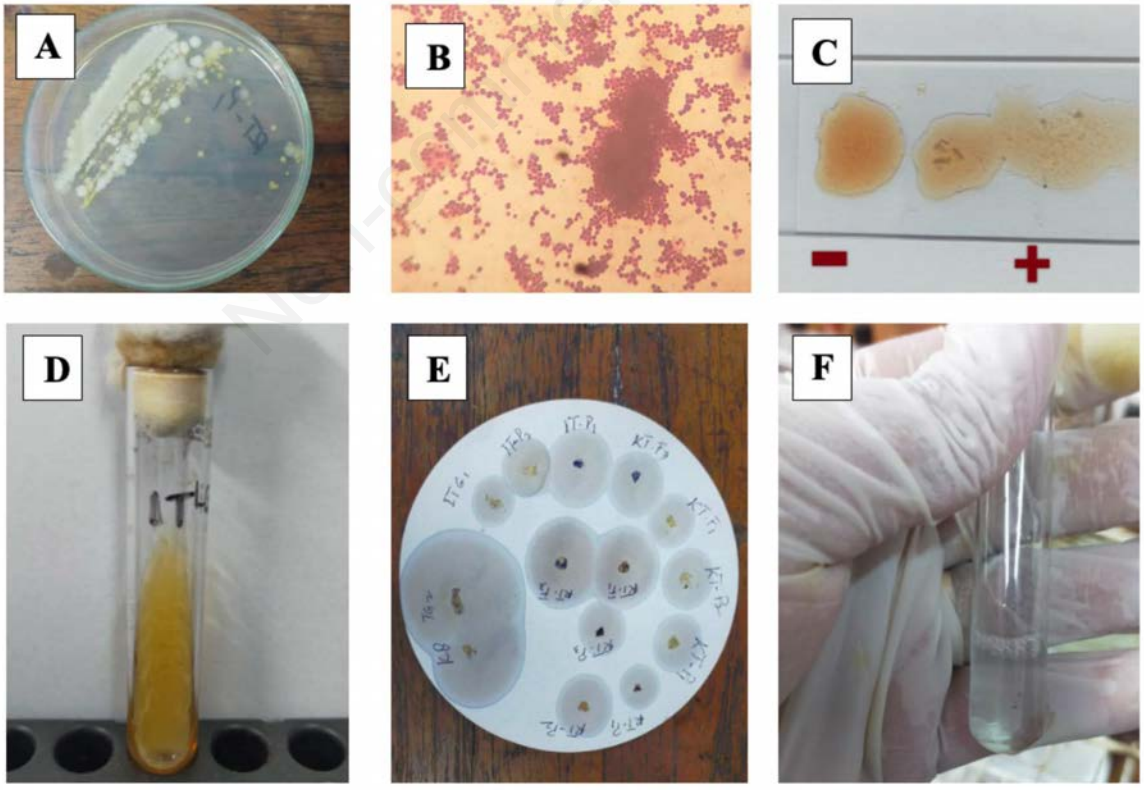

Figure 3. Positive results of testing for Staphylococcus aureus bacteria on nutrient agar media (**A**), gram staining (**B**), coagulase test (**C**), mannitol salt agar media (**D**), oxidase test (**E**), and catalase test (**F**).

Layer farms have seven stages, whereas wholesalers contain eight stages, and retailers comprise nine stages. The average time an egg is saved on the third farm before being sold to the trader or wholesaler ranges between 24 and 96 hours. The longer the stages are in each chain supply, the longer the egg is on point, and therefore, the bigger is the risk of the egg being contaminated by surrounding microorganisms.

Potential danger identification and analysis

At this stage, operational production in the field is confirmed according to chart flow. The third stage identifies all danger potentials with matrix determination risk (*Supplementary Tables 1-3*). It is known that chicken eggs, hand personnel (farm workers, wholesalers, and retailers), shelf eggs, and feces risk *S. aureus* contamination. Chicken eggs are contaminated with *S. aureus* by two main methods, *i.e.*, transovarial through channel egg or transshell, which involves contamination beginning with the surface of the egg, followed by penetration next by microorganisms to albumen or, in several cases, directly to the egg yolk (Awny *et al*., 2018; Pondit *et al*., 2018).

Figure 3 explains the limited critical tolerance values of *S. aureus* at layer farms, wholesalers, and retailers, as quoted from several references. Egg contamination can also happen horizontally through cloaca and shell contact with dirty surfaces such as feces, which will increase the risk of penetration of microbes into the egg (Sánchez *et al*., 2019; Damena *et al*., 2022). Several sources of contamination include litter material, crate eggs, feces, tools for packing and storing eggs, clothes and hands, worker cages, dust, and the environment (Anosa *et al.*, 2019). Research results (Hermana *et al.*, 2021) also found that *S. aureus* isolates obtained from cloacal swabs of farm chicken broilers and layers in Bogor are resistant to tetracycline, ampicillin, oxytetracycline, erythromycin, and nalidixic acid. Corrective actions must be taken for potential hazards with high risks, while preventive actions are taken for medium and low risks.

Determination of the critical control point

Based on the analysis results to determine the CCP of *S. aureus* contamination, it is known that chicken eggs, personal hands (farm workers, wholesalers, and retailers), shelf eggs, and feces are CCP throughout the chain supply. HACCP is a system that identifies, evaluates, and controls significant food security dangers (FAO, 2020). This system ensures zero-risk or risk-free food and aims to reduce risk and danger. The HACCP system is implemented to control food's dangerous biological, chemical, and physical contamination and identify ways to improve approaches based on effectiveness and economic efficiency.

Human hands (farmers, workers, cages, and traders), eggs, shelves, and feces from poultry are considered CCP because action control is carried out to minimize contamination of *S. aureus*. Washing hands with soap and running water and applying hand sanitizer are routine planned actions for removing or reducing the contamination from *S. aureus*, thereby reducing the risk of the human hand becoming a CCP on the chain supply of chicken eggs. Eggs, shelf eggs, and feces from poultry are also critical points because the amount of contamination by *S. aureus* can produce toxic and harmful consumers if it exceeds the level that can be accepted, *i.e.*, >10^5 CFU/g (Salasia *et al*., 2009). Because it is in the next stage, the required method for removing or reducing contamination is to pay attention to egg handling chicken in a way

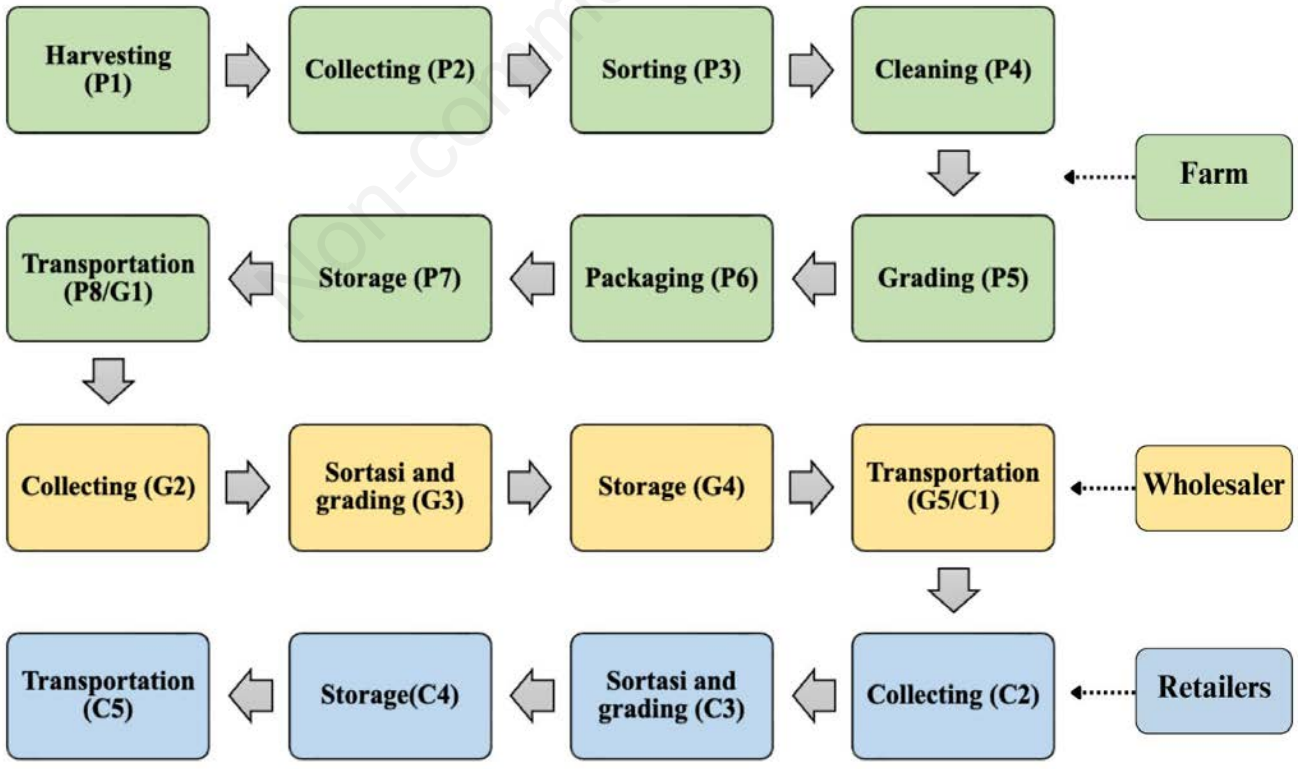

Figure 4. Stages of producing chicken eggs at layer farms, wholesalers, and retailers.

appropriate in the form of storage time, temperature storage egg, attention to hygiene sanitation shelves eggs, and improve sanitation hygiene farm.

Implementation of HACCP in the layer farm can guarantee that every chain provision starts with providing raw or processed food to the consumer. In each industry, food uses the HACCP system in a tight connection with other systems, such as good manufacturing practices, ISO, and other standards applicable in the country that are concerned with ensuring quality food (ISO, 2018).

Establishing critical limits at critical control points

Enterotoxin levels that cause enterotoxicosis are relatively low, with insufficient doses of 1.25 g in contaminated food. The dose of toxin can be achieved if there are 10^5 CFU/g *S. aureus* per gram of food (Salasia *et al*., 2009). In addition to testing samples at critical points, observations were carried out on aspects of sanitation hygiene at farms, wholesalers, and retailers. Implementation of sanitary hygiene in laying chicken farms ranges from 42.5-52.5%, based on seven indicators, including the sanitary hygiene of farm workers, visitor/guest sanitation hygiene, cage sanitation, egg storage warehouses, feed warehouses, egg handling hygiene, and livestock sanitation. It is known that the implementation of sanitation hygiene at the wholesale level for eggs originating from Gowa, Bulukumba, and Sidrap Regencies ranges between 29-32% (*Supplementary Table 2*). Meanwhile, implementing sanitation and hygiene at the retailer level is 41.5-55.5% (*Supplementary Table 3*) (Health Protection Agency, 2009). This is based on nine indicators: environmental cleanliness, storage warehouses, egg sorting, handling broken or dirty eggs, egg storage and distribution/delivery, egg packaging, employee hygiene, pest control program (rodents and insects), and sanitation and waste handling.

Conclusions

CCP of *S. aureus* contamination in egg production management on layer chicken farms is the chicken eggs, personal hands (farm workers, wholesalers, and retailers), egg racks, and feces.

The CCPs for *S. aureus* contamination in the management of egg production on laying hen farms are chicken eggs, personal hands (farm workers, wholesalers, and retailers), egg racks, and feces. The sanitation and hygiene levels at the sample locations are still between 29% and 55.5%.

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Online supplementary material:
Supplementary Table 1. Implementation of sanitation hygiene in laying chicken farms.
Supplementary Table 2. Implementation of sanitation hygiene at wholesalers of laying chickens.
Supplementary Table 3. Implementation of sanitation hygiene at retailer level.