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Salmonella Sp. Contamination of Duck Meat In A Traditional Market in Banjarbaru City

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Abstract:
Alabio duck is the superior poultry germplasm of South Kalimantan, where the meat is much sought after, because of its deliciousness, rich in protein, fat and minerals needed by the body. One of the processed products of alabio duck meat that is much favored by the public is roasted duck, the delicacy of roasted alabio duck is not only favored by local people as well as tourists visiting South Kalimantan, because the quality of alabio duck meat must be considered. The purpose of this study was to determine whether or not the contamination of salmonella sp. on alabio duck meat sold at the Banjarbaru City Traditional Market. The samples examined were 48 in the form of breast and thigh meat. The method of testing for Salmonella sp is based on the Indonesian National Standard (SNI) number 2897 of 2008, starting with enrichment, pre-enrichment, isolation and learning to use Hektoen Enteric Agar (HE) and Xylose Lysine Deoxycholate Agar (XLD) media. Samples suspected to contain Salmonella sp. followed by biochemical tests and Analytical Profile Index using API 20E. Based on API 20E testing 48 samples were isolated and identified 17 (36%) samples containing Salmonella sp. It is hoped that the government's participation in the provision of ASUH food and education to actors regarding sanitation in handling alabio duck meat as an effort to prevent the spread of zoonotic diseases is expected.

Key Word*: Alabio ducks; market; germplasm; zoonoses.*

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I. Introduction

Diabetes Alabio duck meat like other poultry meat is a source of protein that can be used to meet the needs of animal protein for the community. Duck meat contains protein, fat, and minerals that are needed by the body and are very liked by all Indonesian people (Ambarwati *et al*., 2012). Poultry meat also contains complete essential amino acids in a balanced ratio. In addition, poultry meat is more in demand by consumers because it is easy to digest, can be accepted by the majority of people (Yashoda *et al*., 2001), and has a relatively low price (Cohen *et al*. 2007). According to Bintoro *et. al*. (2006) the chemical composition of meat in general consists of about 75% water, 19% protein, 2.5% fat, 1.2% carbohydrates, 2.3% non-protein soluble substances, including nitrogenous substances 1.65% and inorganic substances 0.65%, and vitamins that are soluble in fat and water, relatively very little.

Alabio duck meat is also identical to other types of meat, which includes food that is easily damaged because it has a high nutritional value and is completely coupled with high water content and a pH close to normal so that it is easily contaminated with bacteria and easily damaged (perishable food). The proliferation of bacteria in meat can cause a decrease in the quality of meat, so the nutritional content decreases and is not safe for consumption. Alabio duck meat that has ASUH criteria (safe, healthy, whole and halal) is in great demand by the general public for consumption.

The quality of alabio duck meat is influenced by the number of microbes contained in the meat consumed, from the Poultry Slaughterhouse (TPU) to the consumer, because the total number of microbes indicates the feasibility and safety of the meat for consumption. As we all know, alabio duck farms in South Kalimantan are generally community farms that have not been well coordinated, for example environmental sanitation is not considered and the feed provided does not pay attention to the safety of consumption by alabio ducks. So the contamination may come from the food and drinks provided as well as from the environment around where the alabio ducks are grazed. Alabio ducks are generally released in the morning, in the afternoon they have collected again for cages.

Bacterial contamination can also occur at the time of slaughtering poultry at the TPU and preparing meat, there are several ways that cuts of meat sold in traditional markets are as desired by consumers, this makes the number of pieces or incisions in the meat so that the surface area of the meat increases and makes it easier to grow. microbial flower

One of the microorganisms that can contaminate food is Salmonella sp. Salmonella sp bacteria can be the main cause of diseases that are transmitted through food (foodborne disease). Gastrointestinal disease is a disease caused by salmonellosis bacteria. Salmonella sp. is found in raw foods such as eggs and raw meat, or in foods that are not fully cooked. Symptoms caused by salmonellosis can include diarrhea, stomach cramps, and fever. Salmonella within 8 to 72 hours after eating food contaminated with Salmonella sp. Other symptoms that can be caused include headaches, nausea, and vomiting (Sinaga, 2016).

According to the Food and Drug Supervisory Agency (BPOM) of the Republic of Indonesia (2009), the content of Salmonella sp is negative/25 g of food, for more details can be seen in Table 1. below. If food is contaminated by Salmonella sp, then consumed by the community every day it will cause disease. According to Fatiqin *et al*. (2019) infection by Salmonella sp bacteria attacks the gastrointestinal tract which includes the stomach, small intestine, and large intestine or colon. Enteropathogenic bacteria are generally present in small amounts in food, but are highly infective (Saptarini, 2009).

Table 1. Maximum Limit of Microbial Contamination in Food

No.	Type	Requirements
1	*Total Plate Count*	$1x10^6$colony/g
2	*Coliform*	$1x10^2$colony/g
3	*Staphylococcus aureus*	$1x10^2$colony/g
4	*Salmonella sp*	**Negatif/25 g**
5	*Eschericia coli*	$1x10^1$colony/g
6	*Campylobacter sp*	Negatif/25 g

Source : National Standardization Agency (2009).

Traditional markets are one of the places that have a high possibility of contamination and a breeding ground for microbes. Traditional markets are usually synonymous with dirty and disorganized places, and the meat sold is usually placed without a bottom so that it encourages bacterial contamination (Maulitasari, 2014 and Ken *et al*., 2016). According to Windyartono *et al*. (2016), other factors that lead to the growth of various microorganisms can also be caused by the flow of the meat sales process that is not good, the arrangement of the merchant's place is not well organized and the lack of awareness of traders regarding the correct handling of meat, so that the meat in the market is easily contaminated with bacteria. and will harm the health of humans who consume it (Irmayani *et al*., 2019).

Contamination will also increase if the tools used for cutting such as knives and cutting boards are dirty. The cutting boards used by sellers in traditional markets are made of wood, so they are more easily contaminated by bacteria. When compared to cutting boards made of plastic, wooden cutting boards can easily absorb water, so even after washing it can leave water contaminated with bacteria in them (Ishaqi, 2013). In addition, the process of transportation or travel in the distribution of slaughtered chicken to sellers is one of the things that can potentially increase contamination, both transportation from slaughterhouses to distributors, as well as from distributors to retailers or consumers (Kholifah *et al*., 2016).

Based on the description above, it is necessary to research to detect the presence or absence of Salmonella sp bacterial contamination in alabio duck meat sold in the Banjarbaru traditional market. Information about Salmonella sp contamination in alabio duck meat products sold at traditional markets in Banjarbaru City will be able to increase public awareness in buying, processing, and consuming duck meat sold in these traditional markets.

II. Material And Methods

Research Location and Time

The research was conducted in a traditional market located in Banjarbaru City, South Kalimantan. Furthermore, the implementation of isolation and identification was carried out by the Banjarbaru Veterinary Center. Sampling of duck meat was carried out at 07.00 WITA and 12.00 WITA.

Research Material

The materials used in this study were: Alabio duck meat (thigh and breast), Lactose Broth (LB), Blood Agar (BA), Rappaport Vassiliadis Broth (RV), Tetrathionate Broth (TTB), Xylose Lysine Deoxycholate Agar (XLD), Hektoen Enteric Agar (HE), Nutrien Broth (NB), Triple Sugar Iron Agar (TSIA), Mac Conkey Agar (MC), Oxidation Fermentation (OF), Reagent Nit 1, Nit 2, TDA, James , VP 1, VP 2, Paraffin oil, Suspension medium 5 ml and Aquadest

Research Tools

The tools that will be used in this research are sterile petri dishes, analytical balance, measuring cup, 37 °C incubator, sterile pipette, test tube, cool box, stomacher, vortex, micro sterilizer, round and straight eye ose needle, sterile heat-resistant plastic. , sterile scissors and tweezers, aluminum foil, biosafety cabinet, colony counter, gloves, 3 ml and 10 ml syringes, masks, laboratory coats, permanent markers and API 20E KIT.

Research Design

This research was conducted by survey method and laboratory analysis. Implementation and survey activities carried out include interviews with sellers, documentation, and sampling of alabio duck meat sold at the Banjarbaru Traditional Market, then the implementation of laboratory analysis includes testing samples and observing the growth of Salmonella sp.

Research Implementation

Sampling: Sampling from each market was carried out at different times, at 07.00 WITA, then at 11.00 WITA. Samples were taken in 3 different traditional markets, while to determine the sample by random sampling method and used the formula to determine the experimental sample of Federer (1963) namely (t-1) (n-1) 15. Based on the above formula the sample used was as much as 8 and the number of groups used was 8 (morning and afternoon) so the total sample was 48 samples. The samples that had been taken were put in clear plastic, labeled, and put in a cooler to be then taken to the laboratory for examination.

Laboratory Examimination: The method of testing for Salmonella sp is based on the Indonesian National Standard (SNI) number 2897 of 2008 carried out in several stages, namely:

Pre-enrichment

Weighing a sample of 25 g of alabio duck meat and then putting it in a sterile bag and then adding 225 ml of Lactose Broth (LB) solution into the container containing the sample, homogenized with a stomacher for 1 minute. Incubate at 35^0 C for 24 hours.

Enrichment

Gently stir the pre-enrichment culture then take and transfer 0.1 ml each into 10 ml of Rappaport Vassiliadis Broth (RV). Vortex media Rappaport Vassiliadis Broth (RV) for 30 seconds, then incubate at 42^0 C for 24 hours.

Isolation with Selective Agar Media

The incubated RV solution was inoculated by streaking on Hektoen Enteric Agar (HE) and Xylose Lysine Deoxycholate Agar (XLD) media. Incubate at 35^0 C for 24 hours. Observe the HE colony medium, it is bluish-green with no black dot (H2S). Whereas in XLD the colony is pink with or without shiny dots or almost the entire colony is black. a colony that is suspected to be positive (+) is continued to biochemical tests.

Biochemical test

Using the ose, take the suspected positive colony from XLD or HE media. Inoculate on Nutrient Broth (NB) media. Incubate for 24 hours at 37^0 C. Then the colony from Nutrient Broth (NB) media was inoculated into Blood Agar (BA) media. Incubate for 24 hours with a temperature of 37^0 C. After that, colonies suspected of Salmonella sp were inoculated into MacConkey (MC) and Oxidation Fermentation (OF) media. There are 2 OF media used, one of which is added with paraffin oil (OF-F) without paraffin (OF-O). Incubation for 24 hours at 37^0 C. Then proceed with testing using the oxidase test by taking the colony using ose and then placing it on oxidase paper. Let stand a few moments, if the paper turns purple the sign is positive.

KIT API 20 E

The sample to be tested is coded and then each well is filled with distilled water. The strip to be used is then carefully placed in the incubation box. Make a suspension by taking the suspected Salmonella sp colony from BA media, then put the ose into the 5 ml suspension medium. stir until homogeneous. Using a suspension syringe, slowly insert into each strip well, making sure there are no bubbles. Fill up to half well except for CIT, VP, GEL fill to full. While ADH, LDC, ODC, H2S and URE. filled half of the height of the well and added paraffin oil. Incubate for 24 hours at 37^0 C.

Data analysis

The data analysis technique was carried out descriptively on the results of sample testing. The reference in this study is according to SNI 7388:2009 regarding the maximum limit of microbial contamination in food, Salmonella sp bacteria must be negative/25g.

III. Result

Overview of Research Market Locations

Based on the observations, it can be seen that the cleanliness and selling conditions at traditional markets A and B are better than traditional markets C. Sellers in traditional markets A and B already use aprons and have used a cleaner place to put duck meat. The duck meat in the three traditional markets is mixed in piles with other chicken meat and offal. At market locations A and B, the piles are separated between duck meat, chicken, and offal, while at traditional market C duck, chicken and offal are placed in piles, even the seller cleans the offal in that place.

Sampling

The samples of alabio duck meat used in this study came from 3 traditional markets in Banjarbaru City with a total sample of 48. The condition of the samples from 3 traditional markets is shown in Table 2 below.

Table 2. Number of Duck Meat Samples Taken In Three Locations With Different Times

Location	Time	
	Morning	Noon
A	Pp_1A	Ps_1A
A	Pp_2A	Ps_2A
A	Pp_3A	Ps_3A
A	Pp_4A	Ps_4A
A	Dp_1A	Ds_1A
A	Dp_2A	Ds_2A
A	Dp_3A	Ds_3A
A	Dp_4A	Ds_4A
B	Pp_1B	Ps_1B
B	Pp_2B	Ps_2B
B	Pp_3B	Ps_3B
B	Pp_4B	Ps_4B
B	Dp_1B	Ds_1B
B	Dp_2B	Ds_2B
B	Dp_3B	Ds_3B
B	Dp_4B	Ds_4B
C	Pp_1C	Ps_1C
C	Pp_2C	Ps_2C
C	Pp_3C	Ps_3C
C	Pp_4C	Ps_4C
C	Dp_1C	Ds_1C
C	Dp_2C	Ds_2C
C	Dp_3C	Ds_3C
C	Dp_4C	Ds_4C

Description: A, B, C= traditional market
P = thigh and D: breast
p = Morning dan s = Noon

Fresh alabio duck meat from 3 traditional markets is taken in the form of fresh cuts of meat consisting of pieces of meat that have a lot of flesh, namely the thighs and breasts. The samples that have been taken are put in sterile white plastic bags to prevent further contamination. The alabio duck meat is stored at cold temperatures using a chiller so that the alabio duck meat is more durable.

Detection of Salmonella sp.

Salmonella sp. is a pathogenic bacterium that can cause food poisoning. In this study, a complete test was carried out to detect the content of Salmonella sp. The aim is to determine the presence or absence of Salmonella sp. on alabio duck meat sold in traditional markets in Banjarbaru City. In accordance with SNI 01/6366/2000 which stipulates that fresh meat must not contain Salmonella (negative salmonella).

Detection of Salmonella sp. on alabio duck meat starting from the pre-enrichment stage using Lactose Broth (LB) media. The goal is to suppress the growth of other competitive bacteria so that Salmonella sp bacteria can grow. The next step is selective enrichment using Rappaport Vasilidiasis (RV) media. RV media was used as enrichment medium for the isolation of Salmonella sp. RV media selective compounds such as malachite green and magnesium chloride combined with a low pH (5.2 ± 2) inhibited the growth of natural microbes originating from the digestive tract other than Salmonella (D'Aoust, 2001). The growth of Salmonella sp is also supported by the presence of soy peptone contained in RV media which functions as a source of nitrogen, carbon, and amino acids for Salmonella sp (Oxoid, 2011). The test results showed that of the 48 samples on LB media that were inoculated into RV media, only some showed changes in the form of turbidity on RV media.

Samples from RV media then streaked onto XLD and HE media. After incubation with the media, if the samples on XLD and HE media show the characteristics as shown below, then the sample is suspected of being positive for Salmonella sp. XLD and HE media are selective media types, which are able to suppress the growth of other bacteria that may grow in the medium. XLD media for testing Salmonella sp. bacteria, because it can ferment xylose, decarboxylate lysine and produce hydrogen sulfide from sodium thiosulfate. XLD media is also believed to be a coloring agent that provides the best visualization of color changes to detect Salmonella sp. The results of the fermentation can change the pH of the XLD media to become alkaline so that it can change the color of the media to pink (pink) and black colony is produced from hydrogen sulfide (Samiea *et al.*, 2019). HE media for detecting Salmonella typhimurium for 72 hours, while for detecting *Escherichia coli* 24 hours is sufficient (Warsiki, 2016). HE media which is classified as a differential medium because it can distinguish Salmonella sp bacteria from other bacteria by giving three types of carbohydrates (lactose, glucose, and sucrose with the highest lactose composition) in the media. Salmonella sp cannot ferment glucose alone. This causes the colony of Salmonella sp. to have a bluish-green color because the acid it produces reacts with the indicator in the HE medium. The nutrient content in these two mediums is different but both have the same selectivity ability so that these two mediums are used to compare the results of colonies grown in the medium. Based on the test results on XLD and HE media, the results obtained showed that the 48 samples taken were 17 samples suspected of being contaminated with (+) Salmonella sp. (Table 3.)

Table 3. Test Results of Alabio Duck Meat Samples With XLD and HE

No	Sample Code	XLD	HE
1	Ds_1B	Positif	Positif
2	Pp_1C	Positif	Positif
3	Pp_2C	Positif	Positif
4	Pp_3C	Positif	Positif
5	Pp_4C	Positif	Positif
6	Dp_1C	Positif	Positif
7	Dp_2C	Positif	Positif
8	Dp_3C	Positif	Positif
9	Dp_4C	Positif	Positif
10	Ps_1C	Positif	Positif
11	Ps_2C	Positif	Positif
12	Ps_3C	Positif	Positif
13	Ps_4C	Positif	Positif
14	Ds_1C	Positif	Positif
15	Ds_2C	Positif	Positif
16	Ds_3C	Positif	Positif
17	Ds_4C	Positif	Positif

Description:

B = Market 2 Pp = Thigh taken at morning Ps =Thigh taken at noon

C= Market 3 Dp = Breast taken at morning Ds= Breast taken at noon

Isolates whose identity has been known through biochemical tests were confirmed using the API 20E Kit. API 20E Kit is an identification system of Enterobacteriaceae and Gram-negative bacteria using 20 standard miniatures of biochemical test incubation 18-24 hours 35 – 37oC. After the incubation is complete, the strip is read based on the reading table provided in KIT API 20E. The addition of reagents was carried out for readings for the TDA, IND, VP, and GLU tests. After everything is done nine-digit numbers are obtained. The determination of the numerical profile value on the result sheet is divided into three groups of values, namely values 1, 2, and 3 for each type of test that will indicate certain results. The assessment of each group based on the positive reactions shown during the test will get a seven-digit profile number for 20 types of tests.

The seven-digit number is not sufficient to identify bacteria in some cases, so additional tests are needed such as nitrate NO2 reduction, glucose oxidation (OF-O), glucose fermentation (OF-F), motility (MOB), McConkey (McC), and Oxidase. NO2 test on GLU medium adds 1 drop each of NIT 1 and NIT 2 reagents. Wait for 2 to 5 minutes, the red color indicates a positive NO2 (Nitrogen Dioxide) reaction. The negative reaction (yellow color) can be concluded that there is a reduction of N2 nitrogen gas. In OF-O media when the color changes to yellow, the microorganism metabolizes carbohydrates oxidatively, while in OF-F media when it turns yellow indicates that the microorganisms metabolize carbohydrates fermentatively. Positive OF-F and OF-O media will change color to yellow and on the part where the colony is pierced spreads lik a root, the MOB is positive.

Then continued testing with MacConkey media, this medium is only selective and differentiation is only on Gram negative bacteria that ferment lactose or do not ferment lactose. The color change to yellow indicates that it cannot ferment lactose, one of which is Salmonella sp. colony on the media will be transparent.Oxidase test by taking the colony using an ose or sticking paper into a cup containing the colony, then wait for a while. The result will appear if the purple color indicates positive.

After everything is done so that nine digit numbers are obtained. Determination of the numerical profile value on the result sheet is divided into three groups of values, namely values 1, 2, and 4 for each type of test that will indicate certain results. The assessment of each group based on the positive reaction shown during the test will get a seven-digit profile number for 27 types of tests. The identification results can be identified using the apiwebTM identification software (Figure 1).

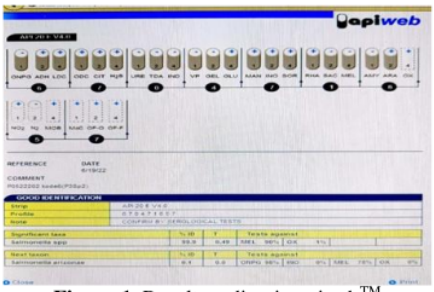

Figure 1. Result reading in apiweb^{TM}

Based on the test results from 48 samples of alabio duck meat taken from 3 traditional markets, it shows that from the 17 suspected samples, after being tested, 17 samples were positive for Salmonella sp. The data can be seen in Table 4. below:

Tabel 4. Distribution of the frequency of examination results for Salmonella sp. on chicken meat in traditional markets and modern markets

Sample Origin	Number of Samples	Salmonella sp . examination results		Total
		Positif	Negatif	
A	16	0	16	0
B	16	1	15	1
C	16	16	0	16

IV. Discussion

The results of the analysis based on (Table 4) can be seen that the results of the examination of 16 samples of cut Alabio duck meat at Traditional Market A did not contain Salmonella sp. so all meat originating from the traditional market meets the requirements set by SNI 7388:2009 which states that food products are not allowed to contain Salmonella sp. this is due to conditions that do not support the growth of Salmonella sp. meat and the presence of other bacterial contamination. The results of the analysis of 16 samples from traditional Market B, there was 1 sample (6.3%) that was positively contaminated with Salmonella sp. Meanwhile, the results of the analysis of 16 samples from Traditional Market C were all positive samples (100%), this was due to a lack of attention to environmental sanitation that resulted in the discovery of Salmonella sp. on alabio duck meat sold in the market, both taken in the morning and afternoon. In general, conditions in Market C are still not good, such as there are still puddles of dirty and muddy water when it rains. In addition, the alabio duck meat sold in Market C at the sales stall is in an open condition and there are lots of flies, because of the piles of garbage in the Market, this also has a high risk of contamination compared to Markets A and B with better market arrangements, there is a drainage channel and the location is not muddy and watery.

In general, market conditions and the method of selling alabio duck meat are still very minimal. Alabio ducks and live chickens are under the table selling alabio ducks and chickens that have been slaughtered. contamination by microorganisms of meat can occur either through the air, soil, touch, and the environment before and after slaughter (Pascual *et al*., 1999). According to Aftab *et al*. (2012), Salmonella spp contamination in chicken slaughterhouses has a higher incidence, followed by bacterial contamination on slaughter equipment such as knives and other equipment. The results of the study by Lee and Middleton (2003) stated that cases of typhoid fever due to Salmonella spp contamination in chicken meat in Ontario were reported at 37.3%. According to Pang *et al*. (2017) stated that poor sanitation conditions in tropical countries are a serious threat to society, especially children, against typhoid infections caused by contamination of Salmonella spp. on materials of animal origin such as chicken meat or other poultry meat. The results of Doaa's research (2013), stated that Salmonella typhimurium contamination was found in 44% of chicken meat marketed at Assiut Markets, Egypt. Nida *et al*. (2016) stated that chicken meat marketed in an open market without refrigeration has the greatest chance (44%) of being contaminated with Salmonella spp. In general, the three markets are traditional people's markets. Market A is one of the markets located in the District of North Banjarbaru where the market

arrangement is well organized, garbage disposal sites are available while Market B, which is located in East Banjarbaru, has good waste management, it's just that the selling place is located on the side of the main road, so it is easily contaminated bacteria. Market C located in South Banjarbaru is a former Banjarbaru Market that has been evicted, but there are still many traders who are reluctant to move and still use the back of the Market to sell. The point is that Market B and C generally have the same conditions, are very close to the city center, and do not yet have the facilities of a modern market, all types of merchandise are in one location which is only separated by a row of types of merchandise. Clean water facilities are minimal, relying on drilled well water, where the water is yellow, especially if it's a rainy day, and the market conditions are very muddy and dirty. Generally, traders peddle their wares as is without adequate protection or security. Clean water facilities are almost non-existent, buyers and sellers can directly touch or touch meat without any protection or water to wash their hands. A model of selling alabio duck meat that is sold to consumers. The market is open for almost 10 hours, this is also very supportive of the proliferation of microorganisms, especially zoonotic pathogenic bacteria such as Salmonella spp. Fitri (1999) Chicken meat that is sold until it runs out, by keeping it on the display table with environmental temperature and high humidity strongly support the proliferation of contaminant bacteria. Poultry meat is very suitable as a medium for microbial growth because poultry tends to be in a dirty environment. According to Nugroho (2006) the bacteria Salmonella sp. Usually found in foods that contain high enough protein as a good medium for the growth of microorganisms. In addition, bacterial contamination of poultry meat is also caused by the low level of knowledge of farmers, cleanliness of cages, and sanitation of water and feed.

Another factor that causes the presence of Salmonella sp bacteria in alabio duck meat is thought to be caused by conditions that strongly support the growth of Salmonella sp bacteria, namely sampling was carried out in the summer where the room temperature conditions were relatively high at around 37ºC, thus accelerating the growth of these bacteria. The development of Salmonella sp bacteria is very fast, each cell is able to divide every 20 minutes at warm temperatures. Therefore, Salmonella sp infections are more common in the summer. The research results of Nida *et al*. (2016) showed that Salmonella spp contamination in chicken meat sold in the open market reached 44%. When compared with the results of this study of 36%, the market conditions in the Banjarbaru area are actually still better. The results of the study are expected to make people pay more attention to environmental hygiene and sanitation, and to store meat in a clean and sterile place to minimize contamination of pathogenic bacteria (Long *et al*., 2016).

V. Conclusion

Based on the results of this study, it can be concluded that 36% of salmonella sp contamination has been found in alabio duck meat sold in 3 traditional markets in the city of Banjarbaru. From the results of this study, the authors suggest that further research is needed on the identification of species in Salmonella sp. as well as increased supervision from the local government on foodstuffs of animal origin as well as education to traders regarding sanitation and handling of alabio duck meat or other poultry meat.

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