STUDY OF ESCHERICHIA COLI AND SALMONELLA SP. BACTERIAL CONTAMINATION FROM MEATBALL SELLER ON BANDAR BUAT MARKET IN PADANG CITY

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ABSTRACT

Meatball is a very popular food in Indonesia. Almost all levels of society like this food. Even though they have undergone food processing, meatballs are not completely safe from microbial contamination. The high protein content in meatballs can act as a substrate for the growth of microorganisms. The research has been carried out at the Padang Industrial Research and Standardization Center on 15 October-14 November 2019. Meatball samples are household and industrial production which is determined on purposive sampling in Padang City. The results showed that the sample of homemade meatballs contains the results of Escherichia Coli contamination at 4 APM/gram. The results of testing for Salmonella bacterial contamination were not found. Meanwhile, the meatball samples produced by the manufacturer showed that there was Escherichia Coli bacteria contamination of> 2,400 APM/gram and the results of testing Salmonella Sp. bacteria contamination on meatball samples were not found Salmonella Sp. bacteria. Based on the two test parameters, it can be concluded that the factory-produced packaged meat meatball samples and home-produced packaged meatballs are not suitable for consumption because one of the test parameters, namely the analysis of Escherichia Coli contamination on meatballs, does not meet SNI 3818: 2014 concerning meatballs.

Keywords: *meatballs; Escherichia Coli; Salmonella Sp.*

INTRODUCTION

Meat is a food ingredient that becomes the source of animal protein. The high level of meat consumption is due to the nutritional value contained in meat is more than other food ingredients. Besides, meat has essential amino acids that are more complete when compared to proteins of plant origin. Meat can be processed in a variety of attractive products with various shapes and flavors to extend the shelf life and increase economic value without reducing the nutritional value of the processed meat. Processed meat that has long been known and is very popular is meatballs (Firahmi et.al., 2015).

Meatballs are a very popular food in Indonesia. Almost all levels of society like this food, so it's no wonder that meatball sellers are spreading in every region. According to the Indonesian National Standard, the meat content in meatballs is at least 50%, but the reality is in the field to reduce production costs, many meatball sellers make meatballs that contain less than 50% meat (Fauziah, 2014)

Even though they have undergone food processing, meatballs are not completely safe from microbial contamination. The high protein content in meatballs can act as an intermediary or substrate for the growth of pathogenic microorganisms and other disease-causing organisms (Cahyadi, 2008), besides the high water content in meatballs due to processing can also cause microbial growth to be faster. Poor handling of processed food products and contamination can result in several dangerous diseases and even poisoning.

Foodborne illness by bacteria can be in the form of intoxication or infection. Intoxication through food is caused by the presence of bacterial toxins that are formed in food when the bacteria multiply, while foodborne infections are caused by the entry of bacteria into the body through contaminated food and the body reacts to these bacteria. Both of these will cause

gastrointestinal disease. The most common bacteria that cause foodborne infections are Salmonella and E. Coli (Arlita et.al., 2014).

The results of previous research conducted by Harsojo and Andini in 2003, in their research to determine Salmonella bacteria in meatball samples using the plate count method, it turned out that bacterial contamination was quite high (11.4 x 107 colonies/g) so that when it was related to the Indonesian National Standard (1995) these meatballs do not meet the requirements because they have exceeded the permissible threshold. Besides, from the results of the identification of Escherichia Coli which was carried out at the Bali Provincial Health Laboratory in June 2014, the results were: 3 negative samples and 1 positive sample Results of MPN values: sample 1 was 0/25 grams, sample 2 was 0/25 grams, sample 3 is 0/25 gram, and sample 4 is 240/25 gram

Escherichia Coli comes from human and animal feces, is transmitted into food due to unhygienic behavior of handlers, washing of unclean equipment, the health of food processors and handlers, and use of washing water containing Coliform, E. Coli, and Faecal Coliform (Susanna and Hartono, 2003). Salmonella Sp. are harmful bacteria that can contaminate meat and processed products (meatballs and sausages). These bacteria are excreted from the digestive tract of animals or humans along with feces. Therefore, products originating from farms are susceptible to contamination by Salmonella Sp. Pain caused by Salmonella bacteria is called salmonellosis. The main mode of transmission is by ingesting bacteria in food derived from infected animal foods (Dona, 2016).

Pathogenic bacterial contamination in food and beverages can cause various kinds of diseases including typhoid, diarrhea, food poisoning, and so on. These diseases will more easily infect people who experience decreased immune system due to internal (intrinsic) and external (extrinsic) factors (Siagian, 2002).

Therefore, to ensure the health and safety of consumers, periodic bacteriological laboratory examinations must be carried out. Therefore, it is necessary to monitor meatball products from bacterial contamination per SNI. The purpose of this study was to determine the comparison of Escherichia Coli and Salmonella Sp. Contamination in factory-produced and home-produced meatballs.

LITERATURE REVIEW

This research was conducted on 14 October - 15 November 2019 at the Microbiology Laboratory of the Industrial Standardization Research Institute (BARISTAND) in Padang.

Sampling

The samples analyzed were factory-produced packaged meat meatballs obtained from mini markets in the Bandar Buat area, and home-produced meatballs obtained from one of the stall sellers at Bandar Buat Market. The analysis that will be carried out on this sample is the identification of Escherichia Coli and Salmonella Sp.

Analysis of Escherichia Coli Bacterial Contamination

Escherichia Coli bacteria are bacteria that live in the digestive tract of humans and animals, Escherichia Coli is a facultative anaerobic bacteria that can grow in both aerobic and anaerobic conditions, bacteria classified as facultative anaerobes are common pathogenic bacteria. Escherichia Coli has a short stem (coccobacilli) with a size of 0.4-0.7 µm x 1.4 µm, is motile (can move), does not have a nucleus, external organelles, or cytoskeleton but has external organelles, namely villi which are thin filaments, and longer.

Foods that are often contaminated are chicken, pork, beef, seafood, eggs, and processed egg products, vegetables, fruit, and fruit juices. E. Coli is a bacteria that is sensitive to heat, can grow at temperatures between 10 - 40oC with an optimum temperature of 37oC. The optimum growth is at pH 7.0-7.5 and Aw minimum of 0.96.

Testing and calculation of Escherichia Coli are carried out using the APM (Most Possible Number) method, which is to count the number of microbes using a liquid medium in a test tube, which generally uses 3 or 5 series of tubes and is followed by a biochemical test, namely IMVIC testing and then referred to in APM table.

1. The tools
   1) Test tubes (small, medium, and large sizes)
   2) Durham tube
   3) Diluent bottle
   4) Measuring pipette
   5) Incubator 36 ± 1ºC
   6) Autoclave
   7) Ose needle
   8) Homogenizing apparatus
   9) Petri dishes

2. The materials
   1) Sample 0107 (factory-produced packaged meatballs)
   2) Sample 0304 (home-produced packaged meatballs)
   3) Lactose broth (LB)
   4) Brilliant Green Lactose Bile (BGLB) Broth 2%
   5) Violet Red-Bile Agar (VRBA)
   6) Methyl Red-Voges Proskauer (MR-VP) Medium
   7) Simmons Citrate Agar or Koser Citrate
   8) Kovacs reagent
   9) Methyl Red solution
   10) Voges Proskauer reagent
   11) Reagent for gram staining
   12) Indole reagent
   13) Alpha-naphthol solution
   14) Potassium hydroxide 40% solution
   15) Koser's Citrate Medium
   16) Buffered Peptone Water (PW)
   17) Escherichia Coli Broth (EC Broth)
   18) Eosin Methylene Blue (EMB) Agar
   19) Nutrient Agar (NA)

3. Work procedures
   1) Perform sample homogenization
   2) Pipette 1 ml of sample dilution 10-1 into each of the 3 tubes containing 5 ml of Lactose Broth in which there is an inverted Durham tube
   3) Do the same for the 10-2 dilution in the second 3 tubes and 10-3 in the third 3 tubes (each dilution uses a new and sterile pipette)
   4) Store all cylinders in an incubator at 36 ± 1ºC for 24-48 hours
   5) After 24 hours then record the number of cylinders forming gas at each dilution
   6) Insert 1 positive gas culture loop on Lactose Broth into a tube containing E.C Broth in which there is an inverted Durham tube
   7) Incubate at 44-45ºC for 24-48 hours
   8) Note the cylinder in which gas is formed (E. Coli is considered positive if gas forms inside the tube)
   9) Continue determination of E. Coli by inoculating the gas-forming culture to the EMB or VRBA hatchery in a petri dish
   10) Incubate at 35ºC for 18-24 hours

11) Select a dark red colony (VRBA) with a diameter of 0.5 mm or more, or a metallic luster colony (EMB) and inoculate the slanting Nutrient Agar in the tube, incubate at 35ºC for 18-24 hours
12) Perform IMViC tests (indole, methyl red, voges-proskauer and citrate) of Nutrient Agar culture in step (11)
    a) IMViC testing
        1. Indole test
           From a pure culture of slanting Nutrient Agar, inoculate 1 loop of culture into the tryptone broth. Incubate at 35 ± 1ºC. for 18-24 hours
           Add 0.2-0.3 ml of indole reagent to each tube and shake for 10 minutes. The dark red color on the surface indicates a positive indole reaction, the orange color indicates a negative indole reaction
        2. Methyl red test (methyl red)
           From pure slanting Nutrient Agar culture, inoculate 1 culture loop into the MR-VP hatchery
           Incubate at 35ºC for 48 hours, using a pipette, transfer 5 ml to the test tube, add 5 drops of methyl red and shake. The yellow color indicates a negative reaction, and the red color indicates a positive reaction
        3. VP test (Voges Proskauer)
           From a pure culture of slanting nutrient agar, inoculate 1 loop of culture into MR-VP hatcheries. Incubation at 36 ± 1ºC for 48 hours
           Using a pipette, transfer 1 ml of the suspension into the tube, add 0.6 ml of the alpha naphthol solution and 0.2 ml of the potassium hydroxide solution and shake. Let stand for 2-4 hours. Pink to dark red indicates a positive reaction, the color does not change to indicate a negative reaction
        4. Test for citrate
           From pure slanting Nutrient Agar culture, inoculate 1 cent of the culture into Simmons citrate or Koser's crat. Incubate at 350C for 48-96 hours. The blue color shows a positive reaction, the green color shows a negative reaction (in Simmons Citrate) and the turbidity in Koser's crat culture shows a positive reaction.

    b) The results are stated as follows
        1. Observe the formation of gas in the Durham tube. If a gas is formed, by showing the Most Likely Number table, the Most Likely Number (APM) can be stated as E. Coli.
        2. Confirm the results of the Gram stain test and the biochemical reactions. If the Gram stain shows the presence of rod-shaped bacteria and a pink (gram-negative) color and the biochemical reaction shows positive indole and methyl red tests, and the VP test and citrate test are negative, it can be confirmed that the presence of E. Coli.
        3. Calculate APM of E. Coli per gram or milliliter of the sample using the table

Analysis of Salmonella Sp.

Salmonella is a Gram-negative, non-spore-forming, facultative anaerobic rod, belonging to the Enterobacteriaceae family. These bacteria measuring 0.7-1.5 x 2-5 μm are motile with peritricus flagella, except for Salmonella Pullorum and Salmonella Gallinarum which are not motile because they do not have flagella. These bacteria grow optimally at 35-37°C, can catabolize various carbohydrates into acids and gases, use citrate as the sole carbon source, produce H2S, and can decarboxylate lysine to cadaverine and ornithine to turn into putrescin. Salmonella can grow at a maximum salt content of 8%.

Salmonella Sp. bacteria can be contaminated in food and beverages that have been contaminated by human feces, the most common transmission occurs due to ingesting food containing Salmonella sp. Bacteria. Salmonella Sp bacteria usually contaminate foods such as eggs, fish, and chicken meat. These bacteria can grow at a pH of 7.2 and an optimum

temperature of 35-430C but will stop growing at a temperature of 46.60C. cleanliness so it is not contaminated.

Salmonella testing and calculations were carried out by conducting selective hatcheries using XLD media followed by biochemical tests and serological tests.

1. The tools
   1) Diluent bottle
   2) Test tube
   3) Measuring pipette
   4) Petri dishes
   5) Incubator temperature 37ºC and 42-43ºC
   6) Autoclave
   7) Water bath
   8) Ose needle

2. The materials
   1) Sample 0107 (factory-produced packaged meatballs)
   2) Sample 0304 (home-produced packaged meatballs)
   3) Buffered Peptone Water (BPW)
   4) Bismuth Sulfite Agar (BSA)
   5) Brilliant Green Agar (BGA)
   6) Galactosidase reagent
   7) Indole reagent and indole seeding
   8) Lysine Decarboxylation Medium (LDC)
   9) Saline Solution
   10) Selenite Cystine Broth
   11) Semi-Solid Nutrient Agar
   12) Tetrahionate Brilliant Green Broth
   13) Salmonella Polyvalent o
   14) Salmonella Polyvalent H (Antisera Spicer Edwards)
   15) Salmonella Shigella Agar (SSA)
   16) Xylose Lysine Desoxxycholate (XLD)
   17) Nutrient Agar (NA)
   18) Triple Sugar Iron Agar (TSI Agar)
   19) Urea Agar or Urea Broth
   20) MR-VP Medium

3. Work procedures
   1) Sample preparation and homogenization
   2) Pre-enrichment
      a. Transfer the homogenized sample (1) aseptically to a sterile 100 ml vial
      b. Incubate at 36 ± 1ºC for 16-20 hours
   3) Enrichment
      a. Pipette 10 ml of pre-enrichment culture (2) into 100 ml of Selenite Cystine Broth
      b. Incubation at 35-37ºC for 24 hours
   4) Planting in selected/selective hatcheries
      a. Transfer the enrichment culture (3) by scraping each culture with an ose needle into a petri dish containing BGA and BSA or other selective hatcheries (XLD, HE agar, SS agar)
      b. Incubation at 37ºC for 24 hours
      c. Observe the suspected salmonella colonies on the media with the following characteristics:
         BGA : colonies that are pink to red in color or clear to opaque with pink to red circles

BSA : colonies are brown, gray to black, and sometimes a metallic luster. The color of the media around the colony is initially brown and then turns black as the incubation period increases
XLD : pink colony with a black spot in the middle
HE : blue-green colony with or without a black spot in the center
SSA : colony colorless to pink, clear to opaque

5) Affirmation
   a. Select 2-5 suspect colonies and scratch on the surface of the nutrient agar so that in a Petri dish that has been prepared in advance and incubate at 37ºC for 20-24 hours
   b. From the isolated colony on nutrient agar, transfer it to the media as follows:
      5.1 TSI Agar
         a) Suspects of salmonella colonies are transferred to the TSIA slant hatchery by scratching their slants and stabbing their upright
         b) Incubation at 37ºC, for 24-48 hours
         c) Observe the changes as follows:
            In the upright section Salmonella will:
            - Ferments glucose, the color of the media changes from purple to yellow
            - Does not ferment sucrose, medium remains purple
            - Can form H2S gas, the color of the media changes from purple to black
            On the slant Salmonella will:
            - Can ferment lactose or sucrose, the color of the media becomes yellow
            - Cannot ferment lactose or sucrose, the color of the media remains red or does not change
      5.2 Urea Agar
         a) Scratch the suspect salmonella colony on the surface of the slanted Urea Agar
         b) Incubation at 37ºC for 24 hours, the appearance of pink color indicates a positive reaction and the color does not change the negative reaction
      5.3 Lysine Decaboxylation Medium
         a) Inoculation of suspected salmonella colonies in the liquid hatchery (Lysine Decarboxylase Broth)
         b) Incubate at 37ºC for 48 hours, the appearance of purple indicates a positive reaction
      5.4 Beta-Galactosidase reagent
         a) Suspend the salmonella colony suspect in 0.25 ml of saline solution in a test tube
         b) Add 1 drop of toluene
         c) Put in a water bath at 37ºC for a few minutes
         d) Add 0.25 ml of reagent, Beta-galactosidase, and shake
         e) Store again in a water bath at 37°C for 24 hours, the formation of yellow color indicates a positive reaction and if it does not change the negative reaction
      5.5 V.P Medium
         a) Put 1 loop of the suspect salmonella colony into 2 test tubes, each containing 0.2 ml of VP seed
         b) Incubate the 1st tube at room temperature and the 2nd tube at 37ºC for 48 hours
         c) Then in each tube add 2 drops of creatine solution, 3 drops of alphanaftol solution, and 2 drops of KOH reagent, shake each time you add the reagent
         d) Observe within 15 minutes. The formation of a pink to dark red color indicates a positive reaction and if it does not change a negative reaction
      5.6 Indole Medium
         a) Put 1 loop of suspect salmonella into the indole medium in the tube

b) Incubate at 37ºC for 24 hours, add 1 ml of indole reagent
c) The formation of a red bracelet indicates a positive reaction and if it does not change or a brownish yellow color a negative reaction

6) Serological test
Perform serologic tests if biochemical reactions reveal salmonella. Take 1 loop of culture from TSI Agar and apply it to the prepared glass. Then put little antisera next to the culture. By using a loop mix the drops of antisera with culture until homogeneous. Clumping that occurs shows a positive test. If the biochemical reaction shows the presence of salmonella and the serological test is positive, then the salmonella is positive

RESULTS AND DISCUSSION

Results

The results of research on the analysis of factory and home-made meatballs are presented in Tables 1 and 2 below.

Table 1. Analysis results of packaged meatballs produced by the factory

No	Parameter	Unit	Quality standards SNI 3818:2014	Test results	Test Method
1.	*Escherichia Coli*	APM/gram	< 3	4	SNI-19-2897-1992
2.	*Salmonella Sp*	/25 gram	negatif/25 gram	negatif	SNI-19-2897-1992

Table 2. Analysis results of home-produced packaged meatballs

No	Parameter	Unit	Quality standards SNI 3818:2014	Test result	Test Method
1.	*Escherichia Coli*	APM/gram	< 3	>2400	SNI-19-2897-1992
2.	*Salmonella Sp*	/25 gram	negatif/25 gram	negatif	SNI-19-2897-1992

Discussion

Meatball Packaging Factory Production

In the test results of Escherichia Coli bacteria contamination by APM method (using 3 tubes) on packaged meat meatball samples produced by the factory, it was found that there was 4 APM/gram of Escherichia Coli contamination. From these results it can be concluded that the meatball sample with code 0107 has been contaminated with Escherichia Coli bacteria according to SNI 3818: 2014 that the limit of Escherichia Coli bacteria contamination in meatballs is <3 APM/g.

Factors associated with the risk of Escherichia Coli infection in factory-produced packaged meatballs are contamination of raw materials by animal waste, food made not through the cooking process, contaminated food after cooking, sold as a ready-to-eat menu, and contact with sick people or animals. Cattle are the main reservoir for Escherichia Coli including raw meat (Melliawati, 2009).

While the test results of Salmonella Sp bacteria contamination with XLD selective hatchery method on packaged meat meatball samples produced by the factory showed that Salmonella Sp bacteria were not found. So it can be stated that the packaged meat meatball samples produced by the factory were negative from Salmonella Sp. Following SNI 3818:2014,

the limit of contamination of Salmonella Sp bacteria on meatballs is negative/25 g or cannot exist in food.

Home Production Packaged Meatballs

In the test results of Escherichia Coli bacteria contamination by APM method (using 3 tubes) on home-produced packaged meat meatball samples, it was found that there was Escherichia Coli contamination of> 2,400 APM/gram. From these results it can be concluded that the sample of homemade meatballs has been contaminated with Escherichia Coli bacteria according to SNI 3818: 2014 that the limit of Escherichia Coli contamination in meatballs is <3 APM/g.

Factors associated with the risk of Escherichia Coli infection in home-produced packaged meatballs are contamination of raw materials by animal waste, food made not through the cooking process, contaminated food after cooking, sold as a ready-to-eat menu, and contact with sick people or animals. Cattle are the main reservoir for Escherichia Coli including raw meat (Melliawati, 2009).

In home-produced packaged meatballs, it is usually stored in a refrigerator or a place mixed with other different food ingredients such as raw meat, fish, vegetables, and others. So that the meatball can be easily contaminated and contaminated with Escherichia Coli bacteria.

Escherichia Coli grows well at temperatures between 8°-46° C and the optimum temperature is 37°C. Bacteria that are kept below the minimum temperature or slightly above temperature, will not die immediately but are in a state of sleep or dormancy (Melliawati, 2009). This explains that meatballs that have been contaminated with Escherichia Coli bacteria, even though stored at a temperature below the minimum temperature or slightly above the temperature will not kill the bacteria.

While the test results of Salmonella Sp bacteria contamination with XLD selective hatchery method on home-produced packaged meat meatball samples (0304) showed that Salmonella Sp bacteria were not found. So it can be stated that the packaged meat meatball samples produced by the factory were negative from Salmonella Sp. Following SNI 3818: 2014, the limit of contamination of Salmonella Sp bacteria on meatballs is negative/25 g or not in food.

Comparison of Escherichia Coli and Salmonella Sp

Based on the results of the tests that have been carried out, the results show that the factory-produced meatball samples have less Escherichia Coli contamination than the home-produced packaged meat meatball samples, this can be a consideration for the feasibility of consuming factory-produced meatballs.

The small amount of Escherichia Coli contamination in factory-produced meatballs is due to the implementation of SOPs at the production site so that sterilization and sanitation are better maintained, while home-produced meatballs do not have SOPs or certain standards in the production process so that sterilization and sanitation are not maintained. According to Erna Sofiana (2012), the presence of Escherichia Coli and Salmonella Sp in snack food samples can be influenced by several things such as raw materials, water, serving, containers, and environmental cleanliness (Sofiana, 2012).

According to Motarjemi (2003), utensils and cooking utensils used in food preparation can also be a source of contamination. If the equipment is used again without being cleaned properly. The contamination of germs on cooking utensils and food is caused by inadequate washing facilities, the washing method does not comply with the provisions, there is no special place for storing cooking utensils, and is not carried out by disinfection. Wipes that are left wet can also spread widely on food and food surfaces when the rag is used.

Meanwhile, the results of Salmonella Sp bacteria contamination showed that the factory-produced packaged meat meatball samples (0107) and home-produced packaged meatballs (0304) did not contain Salmonella Sp bacteria. The absence of Salmonella bacteria in meatballs indicates that factory-produced and home-produced packaged meatballs in Padang City are free

from Salmonella because they do not contain bacteria that can interfere with health caused by Salmonella bacteria.

According to Handayani's research, 2018 The absence of Salmonella in meatballs shows that boiling affects the presence of Salmonella bacteria in food because it is known that meatballs undergo a heating process during handling, to kill Salmonella in food, generally for a minimum of 12 minutes at a temperature of 600C. Therefore, the longer the meatballs are boiled or heated, the number of Salmonella bacteria will be less or even none at all. This can occur because in the process of making meatballs, through the boiling process with boiling water (heating at 100oC) (Handayani and Wahyudi, 2018).

Based on the two test parameters, it can be concluded that the factory-produced packaged meat meatball samples and home-produced packaged meatballs are not suitable for consumption because one of the test parameters, namely the analysis of Escherichia Coli contamination on meatballs, does not meet SNI 3818: 2014 concerning meatballs.

CONCLUSION

The results of the analysis of Escherichia Coli contamination on the 0304 meatball sample showed a number> 2,400 APM/gram. This result was greater than the analysis result for the 0107 meatball sample, which was 4 APM/gram. While the analysis of Salmonella Sp bacteria contamination in meatball samples with code 0107 and 0304 was neither found nor negative. Based on the two test parameters, it can be concluded that the factory-produced packaged meat meatball samples (0107) and home-produced packaged meatballs (0304) are not suitable for consumption because one of the test parameters, namely the analysis of Escherichia Coli bacterial contamination on meatballs, does not meet SNI 3818: 2014 concerning meatballs.

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