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IDENTIFICATION OF *SALMONELLA* SP. FROM ANIMAL PRODUCTS IN SITUBONDO, INDONESIA

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ABSTRACT

The aim of the study is to identify *Salmonella* sp, from animal products especially beef at traditional markets in Situbondo. By using the superficial part of *M. gastrocnemius*, 31 sample pieces was taken randomly from 14 traditional markets in 3 different regions in Situbondo for then to be tested referred to SNI 2897:2008 and ISO 6897:2002. Through the pre-enrichment process, each sample was rinsed by using Pepton Water medium and continued with enrichment in Tetra Thionate Broth Base medium (TTB). The colonies in TTB were then isolated using SS (*Salmonella shigella*) agar. Growth of colorless bacterial colonies by showing the presence or absence of black spots for then carry out the re-culture process to obtain pure isolates of *Salmonella* sp. After the isolation processes is done, a typical bacterial colonies were then identified and confirmed by using TSIA, Urea agar, SIM and MR-VP. Through a series of testing processes, showed that there is one sample (3.22%) positively contaminated with *Salmonella* sp. from 31 pieces of the total samples that used for.

KEYWORD : *Salmonella sp, Animal product, Situbondo*

INTRODUCTION

Food is one of the basic human needs to support the fulfillment of the needs in nutrition intake, maintaining a healthy body, and increase intelligence. The availability of adequate food of animal origin, both in quality and quantity, nutritious, as well as fulfill the ASUH standards (safe, sound look healthy, wholesome and halal) was an absolute matter to be a special concern for the Indonesian government in order to actualize national food security.

But in fact, some food products of animal origin, including beef may contaminate some pathogenic bacteria that can cause various diseases to humans as their consumers. *Salmonella* is one of the pathogenic bacteria that has the ability to cause illness in humans or so-called Food-Borne Pathogen and its zoonotic or can be transmitted from animal to human.

To be able to infect, *Salmonella* requires a certain amount of contaminants media, one of them through the food product, in this case in the form of beef. Ingestion of *Salmonella* into the human body can cause *Salmonellosis* or disease caused by *Salmonella*. The occurrence of *Salmonellosis* in humans of animal origin is generally caused by *S. enteritidis* and *S. typhimurium*, which both of serotype belong to the *Non-Typhoidal Salmonellosis* group. Common clinical symptoms of their infection are shown in the form of enteritis, diarrhea, vomiting and sometimes accompanied by septicemia. Both of these serotypes is able to infect humans and animals (The Center for Food Securuty and Public Health, 2013). *Salmonella* contamination in beef can be caused by many factors, such as inadequate sanitation of cutting environmental hygiene and the use of unclean meat cutter. In most of the traditional markets in Situbondo, the surrounding environment can be categorized quite cleanly, but some of them are at the level or level of slums. It's seen from the condition of each stall of meat from each seller and grouping system based on the food commodity.

MATERIALS AND METHODS

This research was conducted at Bacteriology and Microbiology Laboratory Faculty of Veterinary Medicine Universitas Airlangga. The object in this study are *Salmonella* sp. Obtained from test process by using thirty one cutlet beef (not carcass) by using the superficial part of *M. gastrocnemius* weighing 1 gram. This research was conducted from May – August, 2018.

The equipment used in this study include meat samples, petri dish, test tube, pipet, knife, scissors, tweezers, needle inoculation (ose), rlenmeyer, stomacher, Bunsen burner, digital scale, vortex, incubator, stove, autoclave, clean bench, refrigerator and freezer. Materials used in this study were pepton water (Merck 1.07228), tetrathionate broth (TTB)(Merck 1.05285), *Salmonella Shigella* (SS) agar (Merck 1.07667), triple sugar iron agar (TSIA) (Merck 1.03925), urea agar (Merck 1.08492), MR-VP broth (Merck 1.05712), SIM agar (Merck 1.05470), *Kovac*'s reagent, α-*Naphthol* solution and KOH 40%.

Each sample weighed as much as 1 g, then added 9 mL *Peptone Water* medium into reaction tube and homogenized with stomacher for 1-2 minutes. Incubated at 35 °C for ± 24 hours(Arifin, 2015). Samples that have been through the Pre-Enrichment stage are stirred gently, then taken the suspension. Transferred each 1 mL to 10 mL of *Tetra Thionate Broth* TTB medium. Incubated at 42 °C for ± 24 hours. From each enrichment medium that has been incubated, taken up 2 or more colonies with ose needles and inoculated in *Salmonella Shigella* (SS) agar by spread plate. Incubated at 37 °C for ± 24 hours. The isolates of the SS agar were then inoculated on TSIA (*Triple Sugar Iron Agar*) by thrusting the needle ose to the bottom of the agar medium which then scrawled on the slant medium. Incubated at 35 °C for ± 24 hours.

The cultures of the SS agar are inoculated into Urea broth using ose and incubated at 35 °C for ± 24 hours.Colonies of the SS agar were inoculated on SIM (*Sulfide Indole Motility*) and incubate at 35 °C for ± 24 hours. Then added some 0.2 – 0.3 mL *Kovac*'s reagent to the medium. The cultures of the SS agar are inoculated into a tube contained 10 mL of MR (*Methyl Red*) medium uses ose and incubated at 35 °C for ± 24 hours. Then added 5-6 drops of *Methyl Red* to the tube. The cultures of the SS agar are inoculated into a tube contained 10 mL of MR (*Methyl Red*) medium uses ose and incubated at 35 °C for ± 24 hours. Transferred as much as 5 mL of MR-VP into the test tube and added up 0.6 ml *á-Naphtol* solution and 0.2 ml 40% KOH.

Results of laboratory tested of *Salmonella* sp. In the form of quantitative data presented descriptively by calculated the percentage of positive samples to be compared with the total number of samples, then referenced according to the maximum limit of microbial contamination in food based on SNI 7388:2008. The sample stated to be positive if the medium shows constant results at all stages based on *Salmonella* biochemical reaction.

RESULTS AND DISCUSSION

Obtained from fourteen traditional markets in three different regions of Situbondo, samples are gained in fresh condition and hung at each beef stall. The meat looked fresh in red with elastic consistency, not stiff, doesn't feel sticky if it held off and still feels wetness on the hand.

The research conducted using qualitative methods refers to SNI 2897:2008 and ISO 6897:2002 about testing methods of microbial contamination in meat, eggs, milk and its processed products. *Salmonella* sp. detection method consisted of five standard processes, they are pre-enrichment, enrichment, isolation, identification and confirmation.

Fig. 1. Beef samples obtained in several traditional market. (a) Mimbaan market, (b) Asembagus market

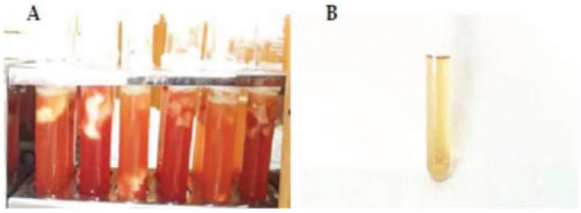

Fig. 2. Sample suspension in pepton water. (a) positive control, (b) negative control

After the incubation process is carried out, the entire medium containing sample pieces appeared cloudy. This stage is useful in order to recover *Salmonella* which indicated of injury during transportation, so that it's expected to increase the

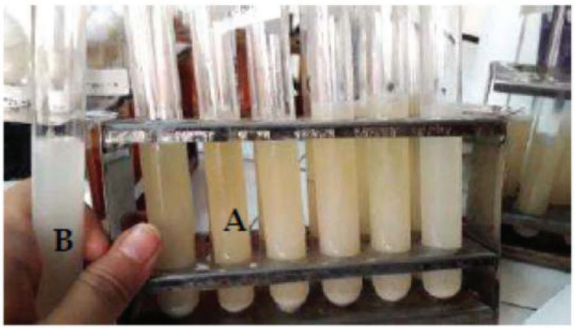

Fig. 3. Sample reactions in *tetrathionate* broth. (a) positive control, (b) negative control

number of *Salmonella* colonies itself.

After the incubation process is done, the TTB medium appeared cloudy with the presence of medium granule at the bottom of the reaction tube. Through one of the components of the TTB, bile salts, this ingredient used to stimulate the growth of *Salmonella* sp. while inhibit coliform and other enteric bacteria. It's according on the existence of Tetarthionate Reductase enzyme that owned by *Salmonella* which can make it resistant to tetrationate toxicity during the enrichment stage. Unlike the other gram-positive bacteria, where their growth is suppress by brilliant green and novobiocin (Merck, 2012).

Fig. 4. Culture on ss agar. colorless colonies with or without black spot, suspected as *Salmonella* sp. after re-culture (blue arrow).

The growth of suspected *Salmonella* sp. colonies on SS agar has a characteristic in the form of colorless colonies with or without black spot at its surface. Colonies that show these characteristic are selected to be re-cultured to obtain pure *Salmonella* sp. isolates as suspected. From 31 samples to be used, there's only 18 samples that shown up the identical characteristic of *Salmonella*'s colonies at the medium. While the other 13 samples are identified as non-*Salmonella*.

At this stage, a number of positive samples showed a color change only in the form of yellow on the butt, but not for the slant side (red). Between

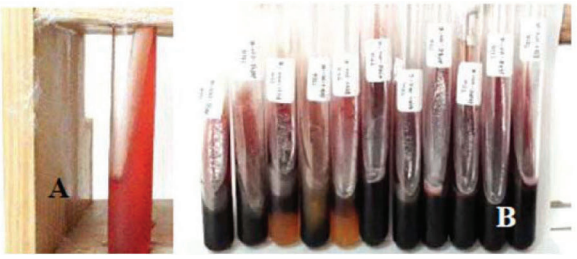

Fig. 5. Results of inoculation on tsia. (a) negative controled; (b) positive controled.

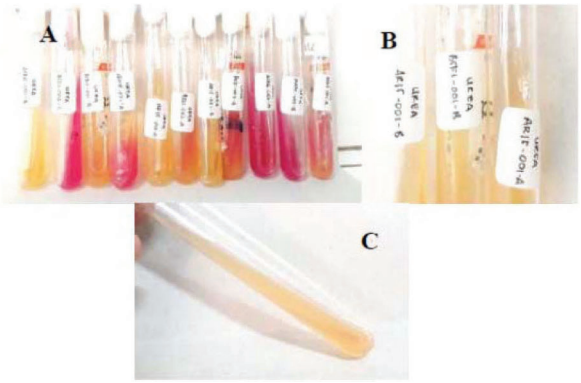

Fig. 6. Samples reaction in urea medium; (a) positive controled; (b) samples suspected of positive *Salmonella* shows negative reaction; (c) negative controled

these two parts, formed H_2S without the existence of gas formation. It happened in 2 from 11 samples, while on 9 other samples showed that H_2S production dominated in butt, so that no visible color changes in this part.

After being incubated for ±24 hours at 37 °C, obtained varied results. Counted from 11 samples that had been tested, there were 7 samples showed positive reaction, while another 4 samples showed negative and 1 sample isolate were stated not to grow. Specific *Salmonella* sp. marked by the negative reaction to the Urea in the form of no color change to the medium.

Three samples were identified negative *Salmonella*, indicated by the formation of the indol red ring, inexistence of H_2S and microbial motility

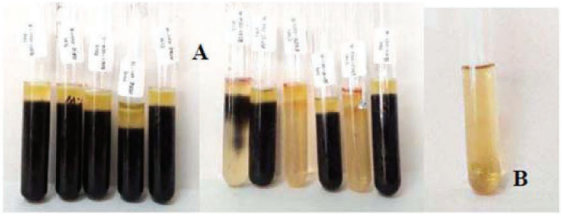

Fig. 7. Samples reaction in sim medium. (a) positive controled, (b) negative controled.

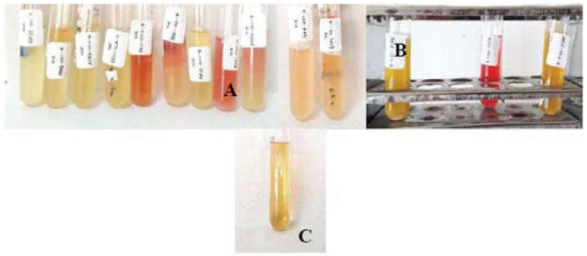

Fig. 8. Samples reaction in mr medium (a) positive controled; (b) further test results on positive suspected samples (c) negative controled.

through the ose needle puncture in the medium. While on the other 8 samples indicated positive *Salmonella* sp. It is deduced through the existence of yellow ring on the medium surface. Then in the vertical side of this semisolid medium, there is a trace of microbial motility through the appearances of H_2S.

Through this test, obtained as much 8 sample tubes showed a negative reaction, while the other 3 samples gave positive results on the *Methyl-Red* reagent. Those three samples that are indicated positive then retested to confirm the presumption. Through the re-test, showed that only one sample tube can be mentioned as had been contaminated with *Salmonella* sp. While the other two sample tubes gave negative results in the absence of discoloration on MR medium.

Through this test section, the whole samples showed a negative reaction. The occurrence of negative reaction to the medium caused by *Salmonella*'s inability in producing acetone (acetylmethylcarbinol) through the glucose fermentation process. Its because the final product produced by *Salmonella* is in the form of mixed acid (lactate, formate, succinate, ethanol and acetate) (Merck, 2012). Based on the test results that have been carried out, was obtained 1 of 31 samples (3.22%) which carried out from fourteen traditional markets in Situbondo positively contaminated with *Salmonella* sp., that is WDR-002 from Widoropayung traditional market in the Kecamatan Besuki. Having identified a single positive sample contaminated with *Salmonella* sp. indicates that the sample is not suitable for being consumption. While the quality of beef sold in thirteen traditional markets has met the safety standards of *Salmonella* sp. free contamination.

It referred to SNI 7388:2009 about the maximum limit of microbial contamination in food, that every food products which in this case are beef have must

Fig. 9. Samples reaction in vp medium. (a) positive controled, (b) negative controled.

not contain *Salmonella* per 25 grams of meat weight. Considering that *Salmonella* potentially causing foodborne disease in humans through animal products.

When obtained, a positive sample with the meat code of WDR-002 appears red typical of beef, elastic in consistency, but the condition of the meat feels dry when it held off and being sold together with the commodity of chicken meat. The fat color looks darker and at the stall table placed mosquito coils to avoid flies and other flying insects. The meat stall looks clean, is in an closed area, the surrounding environment made of paving blocks and located in front of the chicken meat stall. *Salmonella* etiologically as an agent of diarrheal infection with a systemic infection in humans, which generally caused by secondary contamination through food; foods of animal origin; or the environment.

Nowadays, *Salmonella* is divided into two species they are *S. enterica* and *S. bongori*, with serotypes that often infect animals generally caused by *S. enteritidis* and *S. typhimurium*, where both are included in the *S. enterica* subspecies.

Salmonella contaminates animals through endogenous infection when animal live and contaminate meat after the death as exogenous infection. Endogenous infection for*S. enteritidis* and *S. typhimurium* infect animals (cattle) transmitted through fecaloral route (The Center for Food Security and Public Health).

Animals can become infected by consuming fecal-contaminated food and drinking water or through close contact with infected animals, also because of the presence of parasitic vector agents that contribute in spread of that *Salmonella* sp. microbes.

The exogenous infections can caused by several factors like, the occurrence of ccross-contamination in cutting process and the use of equipment, eviiceration, inexistence of cold chain and low in personal hygiene by traders. Nowadays, eviceration have been suspected as a stage with a high risk of

cross-contamination on a carcass (Restika, 2012).

This eviceration process can be done manually or automatically by using a machine. The contamination in eviceration processs may come from workers, equipment and lvestock coditions such as the gastrointestinal tract that is still filled with feed or animal in pain conditions, such as diarrhea (Restika, 2012). In addition, icrobes contained in beef can come from livestock area, slaughter locations and contact with mechanical vectors (Syarifah and Novarieta, 2015).

Salmonella spp. are able to survive in a monthly period or even years on food products with low water content (Food Standards Australia New Zealand, 2017). *S. enterica* can be found in meat processing equipment. It's due to *Salmonella*'s ability to attach on the surface of the tools, cells can be easily transferred between other surfaces and ends on at the meat surfaces (Waldner *et al.*, 2012).

Besides that, the other factors that play an important role in *Salmonella* sp. contamination on food products can comes from dust, environmental surface (cracks of walls, corners of the floor), rodents, flies and slaughtered animals feces. Since it is known that *Salmonella* are able to form *rdar morphotype* on their villous in low humidity conditions, it is important adaptation to support *Salmonella*'s ability to be attached, have endurance and survive in the environment (Waldner *et al.*, 2012).

Traditional Market Hygiene Conditions

This research who have been conducted at fourteen traditional markets in Situbondo showed a fairly good sanitation. Each stall condition is fairly clean and located on a beef stall block and has a counter table with a flat surface. Carcass is carried out by hanging, except for a few pieces of carcass and

Table 1. Results of detection and isolation of *salmonella* sp. on beef at traditional markets in Situbondo

Regions	Sample Origin	Beef Code	Results	Contamination Percentage
West	Jatibanteng	JTBG-001	Negative	0%
		JTBG-002	Negative	
	Widoropayung	WDR-001	Positive	50%
		WDR-002	Negative	
	Besuki	BSKI-001	Negative	0%
		BSKI-002	Negative	
		BSKI-003	Negative	
	Suboh	SBH-001	Negative	0%
		SBH-002	Negative	
Middle	Sumberkolak	SMBR-001	Negative	0%
		SMBR-002	Negative	
		SMBR-003	Negative	
	Kilensari	KLNS-001	Negative	0%
		KLNS-002	Negative	
	Wringinanom	WRGN-001	Negative	0%
		WRGN-002	Negative	
	Ardirejo	ARDJ-001	Negative	0%
		ARDJ-002	Negative	
	Mimbaan	MMBN-001	Negative	0%
		MMBN-002	Negative	
	Mangaran	MGRN-001	Negative	0%
		MGRN-002	Negative	
East	Ketowan	KTWN-001	Negative	0%
		KTWN-002	Negative	
	PG Asembagus	PGAS-001	Negative	0%
		PGAS-002	Negative	
	Asembagus	ASBG-001	Negative	0%
		ASBG-002	Negative	
		ASBG-003	Negative	
	Jangkar	JGKR-001	Negative	0%
		JGKR-002	Negative	

visceral placed on a special plastic container on one side of the counter table. In several market (Besuki, Kilensari, Mimbaan, Ardirejo, Mangaran, Widoropayung and Asembagus) the stall floor made of paving blocks and whole meat stalls are located in a closed building.

Unlike the case with the other seven traditional markets (Jatibanteng, Suboh, Sumberkolak, Wringinanom, Ketowan, PG Asembagus and Jangkar), it's located in an open area, beef presentation is not hung and has been packaged using transparent plastic bag weighed 250 g each, except in the Sumberkolak. All of these traditional markets, the stalls floor made by ground. This condition allows the contamination occurrence by mechanical vectors, either by flies or dust from the soil around the stalls. In addition, personal hygiene which in this case concern to the sellers also plays a role in maintaining the beef hygiene.

The operator ability in beef processing and visceral organ removal is the key to hygienic slaughter. Clean meat, hygienic work and supported by operator expertise in hygienic slaughter can prevent carcass contamination by pathogenic bacteria (Winata, 2011).

Traditional markets that located in the middle and eastern regions, all of beef supply comes from slaughterhouses. Sumberkolak slaughterhouse supplies most of the traditional markets in the middle area, while Asembagus slaughterhouse supplies meat for traditional markets in the eastern part of Situbondo.

However, unlike the case with traditional markets in the west, cattle slaughtering is done at the back of the Besuki market with each beef stallholder take their cutting process by itself, with the number of cattle slaughtered is quite varied, one cow for one stall or one cow for two beef stalls. In other words, Besuki market supplies beef for all of traditional markets in the western region of Situbondo.

Although the meat supply comes from different abattoirs, but all of the cattle that used for are generally originate from various regions in Situbondo, with a small amount of them are comes from Prajekan (Bondowoso), the closest area to Situbondo. Where all cattle were slaughtered for are obtained through animal market at each regions.

CONCLUSION

Based on this research, it could be concluded that founded one positive sample (3.22%) with meat code WDR-002 located in PasarWidoropayungfrom total samples 31 pieces.

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