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Salmonella detection from Bali cattle faeces

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Abstract
Salmonellosis is one of the diseases that can infect Bali cattle. This disease is caused by *Salmonella* bacteria that can give multiple symptoms from fever, diarrhea, abortion and Food Borne disease that can harm humans. In order to conduct preliminary diagnoses, see the prevalence and prevent the spread of this bacteria, faecal isolation from Bali cattle should be researched. 30 samples of Bali cattle faeces from various locations were used for this research. The samples were transported using Stuart Transport media and then enriched in Selenite Broth media. The samples were continued to be isolated to Xylose Lysine Deoxycholate (XLD), Triple Sugar Iron Agar (TSIA), Simmon's Citrate Agar (SCA), and Sulfide Indole Motility (SIM) media. The result showed that *Salmonella* can be isolated from faeces. From 30 faecal samples, 11 samples (36%) show signs of *Salmonella* bacteria morphology. After further testing with SIM media test, from 11 samples, 3 confirmed *Salmonella* morphology and 8 showed *Edwardsiella* characteristic, this found need to further investigate.

Keywords: *Salmonella*, Bali cattle, Feces

Introduction
Bali cattle (*Bos sondaicus*, *Bos javanicus*, *Bos/Bibos banteng*) are one of the most important types of cattle for industrial development to support tourism in Bali and Indonesia in general. This is because Bali cattle have excellent potential for the provision of meat and seeds. After all, they have good adaptability with good quality reproductive characteristics (Suranjaya *et al*., 2010) [1]. The development of strategic diseases in livestock is very worrying, Bali Cattle is an idol for breeders in Bali, they are a source of animal protein that is still not sufficient to meet the needs of the community (Yulastri *et al*. 2018) [2]. One of the strategic diseases that threaten Bali cattle is Salmonellosis, this disease is a bacterial disease caused by *Salmonella* sp. This bacteria is a facultative anaerobic gram-negative belonging to the *Enterobacteriaceae* family. There are 2 species in the genus, *Salmonella enterica*, and *Salmonella bongori*. The most relevant infection in dairy cattle is caused by *Salmonella enterica* (Smith, 2009) [3]. Adult cows suffering from acute salmonellosis will show symptoms of fever, lethargy, lack of appetite, and decreased milk production followed by diarrhea, in which watery stools contain blood and mucus. Animals that are pregnant can suffer abortion with symptoms or without showing other signs of illness. Common symptoms of salmonellosis are fever, lack of appetite, lethargy, dehydration, and emaciation. Death can occur 3-4 days after suffering from illness and can heal by itself after a few weeks or months (Pudjiatmoko, 2014) [4]. *Salmonella* is also the cause of Food Borne diseases or diseases caused by contaminated food. Symptoms of the disease experienced include nausea, diarrhea, stomach cramps, fever, chills, headache, and vomiting caused 8-72 hours after consuming contaminated food (Jay *et al*, 2005) [5].
Animals suffering from *Salmonella* infection can become persistent carriers. Other than being found in faeces, *Salmonella* can also be isolated from soil, water, and sewage contaminated with faecal material from salmonellosis patients (Ray, 2001) [6]. The isolation of *Salmonella* from the faeces of dairy cows or calves as well as the environment on dairy farms is increasingly common, faecal samples were collected from approximately 30 healthy cows on each of 121 dairy operations across 17 states. Forty percent of the dairy operations had at least 1 cow that was *Salmonella* positive (United States Department of Agriculture, 2007) [7]. In Bali cattle, this data still needs further research, so it is necessary to research to detect these bacteria so that prevention and treatment can be carried out immediately and the disease does not spread to harm animals and humans.

The purpose of this study was to determine whether *Salmonella* bacteria could be isolated through faecal examination of Bali cattle. The benefit of this study is to provide preliminary data on whether *Salmonella* bacteria can be isolated through Bali cattle feces and to see how much *Salmonella* prevalence is in the field for easier and faster handling.

Materials and Methods

Sample

Samples were 30 faecal swabs from Bali cattle and were taken randomly in various locations in Bali.

Methods

The faecal swab was isolated and transported using Stuart Transport media and then enriched in Selenite Broth media for 24 hours, and then they were transferred to Xylose Lysine Deoxycholate (XLD) media for further identification. *Salmonella* produces a black sphere in the middle of the colony as a result of H_2S gas production (Afriyani *et al*., 2016) [8]. Colonies suspected of being *Salmonella* on XLD media were continued at the confirmation stage through biochemical tests using Triple Sugar Iron Agar (TSIA), Simmon's Citrate Agar (SCA), and Sulfide Indole Motility (SIM) media. The results of the data from the identification of *Salmonella* bacteria are presented descriptively.

Result and Discussion

The result shows from 30 faecal samples, 11 samples (36%) show positive on XLD media, with signs of *Salmonella* bacteria colony growth as shown in table 4.1. and another 19 samples have no growth in the media.

Table 4.1: *Salmonella* Bacteria Identification Result

Sample Code	XLD Media	Gram	Shape	Sulfide Indol Motility Test	Simmon's Citrate Agar	Triple Sugar Iron Agar
S1	-	-	-	-	-	-
S2	+	Negative	Rod	Indole -, Motile	+	+/ H_2S+
S3	-	-	-	-	-	-
S4	-	-	-	-	-	-
S5	-	-	-	-	-	-
S6	+	Negative	Rod	Indole -, Motile	+	+/ H_2S+
S7	-	-	-	-	-	-
S8	-	-	-	-	-	-
S9	-	-	-	-	-	-
S10	-	-	-	-	-	-
S11	-	-	-	-	-	-
S12	+	Negative	Rod	Indole +, Motile	+	+/ H_2S+
S13	-	-	-	-	-	-
S14	+	Negative	Rod	Indole +, Motile	+	+/ H_2S+
S15	-	-	-	-	-	-
S16	-	-	-	-	-	-
S17	-	-	-	-	-	-
S18	+	Negative	Rod	Indole +, Motile	+	+/ H_2S+
S19	-	-	-	-	-	-
S20	+	Negative	Rod	Indole -, Motile	+	+/ H_2S+
S21	+	Negative	Rod	Indole +, Motile	+	+/ H_2S+
S22	-	-	-	-	-	-
S23	-	-	-	-	-	-
S24	-	-	-	-	-	-
S25	-	-	-	-	-	-
S26	+	Negative	Rod	Indole +, Motile	+	+/ H_2S+
S27	+	Negative	Rod	Indole +, Motile	+	+/ H_2S+
S28	-	-	-	-	-	-
S29	+	Negative	Rod	Indole +, Motile	+	+/ H_2S+
S30	+	Negative	Rod	Indole +, Motile	+	+/ H_2S+

The colony shows black colonies on XLD media, where the bacteria can produce H_2S that makes the centre of the colony will be black (Afriyani *et al*, 2016) [8]. *Salmonella* on XLD agar appears as red or pink colonies with or without a black centre (Wallace & Hammack, 2007) [9]. The gram stain shows gram-negative with rod-shaped bacteria according to *Salmonella* morphology (Yang *et al*. 2020) [10]. *Salmonella* has two layers of cell walls, an outer layer composed of lipopolysaccharide and protein, and an inner layer composed of peptidoglycan which is thinner than Gram-positive bacteria (Darmawan, 2017) [11].

Sulfide Indol Motility media showed mixed results, 8 samples showed positive indole, with positive H_2S, and were motile, while 3 samples showed negative indole results with positive H_2S and were motile. *Salmonella* sp gave positive results in the motility test which was characterized by the growth of bacteria that spread, motile, and the presence or absence of H_2S (Afriyani *et al*, 2016) [8]. According to Cappuccino & Sherman (1987) [12], the *Salmonella* test result will show negative in the indole test, with no red ring-formed appearing on the surface of the media, and positive in the motility test, in which root-liked form appears in media. The 8 samples that have positive result seems to refer to the *Edwardsiella* bacteria, but they need further identification.

In SCA media, the results are all samples were positive because the media were able to change their colour from green to blue. This test confirms that the isolated bacteria are *Salmonella* bacteria but besides *S.typhi* and *S.paratyphi A*. because they have different results (Safitri *et al*., 2019) [12]. The change colour in media means that bacteria use citrate as the only carbon source (Tilleand & Scott's, 2013) [14]. On this research 11 from 30 samples show positive result.

Salmonella growth on the TSIA media shows positive result and with the present of H_2S. The media's colour shifted from red to yellow as a result of the positive TSIA test findings. This tint of yellow denotes glucose fermentation by the bacteria. The presence of black precipitate at the butt indicates the present of H_2S gas. *Salmonella* sp. bacteria employ sodium thiosulphate as a source of sulfur to create H2S, as evidenced by the ensuing black colour (Budiarso *et al*., 2009) [15]. *Salmonella* should cause the top part of TSIA media turn red and the bottom turn yellow. If H_2S is created, it combines with ferrous sulfate (III), resulting in the formation of a dark precipitate of ferric sulfide FeS (II) at the tube's bottom (Midorikawa *et al*, 2014) [16].

All test result that conducted in this research showed the morphology of *Salmonella* bacteria even though there is some doubt in the SIM media, that showed from 11 positive samples, 3 samples are *Salmonella* and 8 samples is most likely *Edwardsiella* bacteria but as general result, *Salmonella* bacteria is able to detected through Bali cattle faeces using basic media test. The samples collection was carried out in traditional Balinese cattle cage, with a poor level of cleanliness, so it makes positive results. However, the cattle not showing *Salmonella* symptom when the data was collected. It is possible that the farmer bring the bacteria and contaminate the cage, due to the cleanliness of the cage and poor biosecurity, this bacteria can be found in the faeces that spread around the cage. *Salmonella* can survive in water, soil, and on a variety of surfaces, and can persist for at least one year in soil (Davies & Wray, 1996) [17]. These abilities of *Salmonella* will be dangerous if we still not obeying the biosecurity and the cage cleanliness. The present of this bacteria in the environment will increase the risk of infection to the cattle when their immune system are low.

Conclusion

Salmonella detection from Bali cattle faeces is possible to do. From 30 faeces samples that collected from Bali cattle, 11 samples (36%) show *Salmonella* morphology in XLD media, but after further testing with SIM media test, from 11 samples, 3 confirmed *Salmonella* morphology and 8 showed *Edwardsiella* characteristic.

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Conflict

The authors declare no conflicts of interest regarding the publication of this paper.

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