Identification of listeriosis and potency of antimicrobial probiotics by in vitro test on beef cattle

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Abstract. The study aimed to identify listeriosis in cattle by using conventional and molecular diagnostic methods and to test the implementation of probiotics as inhibitors of listeriosis in cattle in vitro. Laboratory tests conducted to diagnose listeriosis and antimicrobial activity. Parameters included the color, shape, size, and surface of the colony, shape, and gram staining. Molecularly, the observed variable: the size of the molecular weight of PCR products. Results showed that the conventional method diagnoses of Listeria monocytogenes isolate having the green colonies, round shapes, 1 mm size, shiny convex surfaces, gram-positive, 1-2 µm x 0.5 µm short stem and Listeria innocua isolate had white colonies, round shape, slippery surface, 1-2 mm size, gram-positive, short stem size 0.3 - 0.5 µm. Additionally, conventional method diagnosis found 12 listeriosis isolates [37.5%]. Molecular diagnosis using specific PCR to Listeria monocytogenes and Listeria innocua, produce a product with 1500 bp in size and found in 8 listeriosis isolates [25%]. The highest inhibition zone was obtained from Weissella paramesentroides LAB strain 259 with 22 mm clear zone diameter in the Listeria monocytogenes test bacteria and LAB Pediacoccus pentosaceus CTSPLI strain with a 24 mm clear zone diameter in the Listeria innocua test bacteria. In conclusion, probiotics can inhibit the growth of Listeria monocytogenes and Listeria innocua from uterine isolates.

1. Introduction
Reproductive disorders in beef cattle can be caused by various factors, including non-infectious agents and infectious agents. Especially for reproductive disorders caused by infectious agents or infectious diseases is also causes abortion. The previous study showed that the infection causes abortion in cattle, particularly Listeria monocytogenes. From 150 cases of abortion or about 4.66% in Tunisia after being identified with Polymerase Chain Reaction [PCR] is caused by Listeria monocytogenes [1]. In Indonesia, so far there is no research on abortion associated with Listeria monocytogenes. This could be caused by lacking information regarding Listeria monocytogenes compared to developed countries. A survey on abortion cases conducted in the Padangsidimpuan even did not report any abortion caused by listeriosis. Furthermore, surrounding the farm at Padangsidimpuan, listeria has been found to influence the reproductive aspect of cattle.

Listeria monocytogenes causes abortion in ruminants since it can invade intracellularly and multiply in bovine trophoblast cells. This capacity is addressed to contribute to the occurrence of abortions. Characterization of bovine listeriosis exhibited information that Listeria monocytogenes belongs to the gram-positive bacterium, found in almost all places such as soil, water, silage [animal
feed made from green leaves preserved by fermentation and other natural sources [2]. *Listeria monocytogenes* does not form spores and lives on facultative anaerobic conditions [can live with or without oxygen]. These bacteria are psychrotrophic and can last for months in a wet environment, and can compete with other organisms, especially at the medium temperature. Besides, these bacteria are more heat resistant than vegetative pathogenic bacteria, grow between -2 °C and 50 °C, with optimal growth between 30 °C and 37 °C [3].

Probiotics are living microorganisms that can grow in the intestine and can benefit their hosts either directly or indirectly through their metabolites. A source of probiotics comes from lactic acid bacteria [LAB]. This species can produce various forms of compounds that allow inhibiting the growth of pathogenic bacteria; in the form of peptides or proteins that can inhibit other certain bacteria [4]. Hence, bacterial probiotics can be used as an alternative antibiotic for inhibiting pathogenic bacteria.

2. Materials and Methods

Samples of research were obtained from the uterus of 32 female cows collected from the slaughterhouse [RPH] in the city of Padangsidimpuan. The cows diagnosed with a reproductive disorder. For probiotic sources used curd, buffalo fresh milk, and commercial antibiotics [Penicillin, Ampicillin, and Kanamycin]. Molecular observations were carried out using **electrophoresis** and PCR.

2.1. **Identification of listeriosis with conventional diagnose [modified from [5]]**

The evaluation of listeriosis was tested using a selective medium *Listeria Enrichment Broth* [LEB] and *Agar Listeria Ottaviani Agosti* [ALOA]. Characterization of listeria isolate was done based on gram staining. The enrichment process was conducted with LEB and incubation for 24 hours at 37 °C. After the incubation process, bacteria were planted using ALOA and purified before the DNA extraction process. Identification of listeriosis is represented using a formula as follow:

$$\frac{F}{N} \times 100\%$$

Where: F: measured sample with a positive profile, N: total number of the measured sample.

2.2. **Identification of listeriosis with molecular technique**

Identification of samples was carried out using specific primers, namely listeria Primer hyl A [F] [5 ‘-GG AGG TTC CGC AAA AGA 3’] and hyl A [R] [5 ‘-CCT CCA GAG TTA TTG ATG TT - 3’]. The observation steps included extraction of DNA bacterial genomes through KIT Promega [USA], agarose gel, **electrophoresis** of DNA genome extraction and running of PCR. The number of the identified fragment was calculated using a formula:

$$\frac{F}{N} \times 100\%$$

Where: F: measured sample with a positive profile, N: total number of the measured sample.
3. Results and discussion

3.1. Quantity of identified listeriosis with conventional and molecular method

Microscopically, *Listeria monocytogenes* is found in an irregular Y or coccus shape. According to Baek [6] and Donelly [7], *Listeria monocytogenes* is sometimes found as a coccus shape so that it can be misidentified with *Streptococcus* spp. Their cell forms sometimes appear to be elongated therefore can be imitated with *Corynebacterium* spp. The difference in color absorption during gram staining can be caused by peptidoglycan and membrane permeability of gram-positive organisms with gram-negative [8]. Table 1 and Table 2 show the proportion of *L. monocytogenes* which is higher than *L. innocua*. Based on conventional identification, the proportion of *Listeria* is lower which is around 37% [9]. The percentage of *Listeria*, however, is higher compared to the normal counts of 3.3% to 14.8% [10]. Overall, the level of cases of listeriosis in bovine uterus based on conventional identification is higher than the molecular one.

![Table 1](image1.png)

**Table 1. Identification of listeriosis on cattle using the conventional method**

| Data                      | Total Count | Proportion [%] |
|---------------------------|-------------|----------------|
| Detected *Listeria monocytoogenes* | 7           | 21.90          |
| Detected *Listeria innocua*   | 5           | 15.60          |
| Undetected Listeria        | 20          | 62.50          |
| Total Number               | 32          | 100.00         |

![Table 2](image2.png)

**Table 2. Identification of listeriosis on cattle using the molecular method**

| Data                      | Total Count | Proportion [%] |
|---------------------------|-------------|----------------|
| Detected *Listeria monositogenes* | 6           | 18.75          |
| Detected *Listeria innocua*   | 2           | 6.25           |
| Undetected Listeria        | 24          | 75.00          |
| Total Number               | 32          | 100.00         |

![Figure 1](image3.png)

**Figure 1. PCR product of *Listeria monocytoogenes* [a], and *Listeria innocua* [b].**

3.2. Antimicrobial activity of LAB

Antimicrobial probiotic activity [LAB] test exhibited 6 isolates of bovine uterus samples that were positive infected by *Listeria monocytoogenes* and 2 *Listeria innocua* isolates. The inhibition zone produced from 4 LAB isolates originating from buffalo milk from Agam Regency and curd from Lintau and Sijungjung Districts [*Weissella paramesentroides* strain 259, Pediococcus pentosaceus strain CTSPLI, *Lactobacillus plantarum* strain LGP, and *Lactobacillus fermentum* strain L23] are presented in Table 3. They are superior compared to positive controls with kanamycin, ampicillin, and penicillin. This indicates that the 4 isolates of LAB have strong antimicrobial properties against 8 isolates of listeria causing bacteria originating from uterus cows. According to Siegumfeldt [11], the
antimicrobial mechanism of LAB includes the production of organic acids, hydrogen peroxide, diacetyl, and broad-spectrum antimicrobial compounds such as reuterin and bacteriocin.

| Observed Bacteria of Listeria | The Diameter of LAB Inhibiting Power |
|-------------------------------|-------------------------------------|
| Codes                        | Pediacoccus pentosaceus | Weissella paramesentroides | Lactobacillus plantarum | Lactobacillus fermentum |
| K2                           | 10                     | 10                         | 14                       | 10                       |
| K3                           | 18                     | 22                         | 18                       | 18                       |
| K4                           | 15                     | 10                         | 14                       | 15                       |
| K6                           | 14                     | 11                         | 16                       | 14                       |
| K7                           | 16                     | 13                         | 17                       | 12                       |
| P6                           | 18                     | 19                         | 17                       | 12                       |
| K5                           | 16                     | 11                         | 17                       | 12                       |
| S8                           | 24                     | 12                         | 19                       | 13                       |

Information: K2, K3, K4, K6, K7 dan P6 : Species of Listeria monocytogenes; K5, S8 : Species of Listeria innocua

4. Conclusion

The conventional diagnosis could identify more listeria isolates compared to the molecular diagnosis. Natural probiotics could produce clear zones and have potential inhibition to the growth of Listeria monocytogenes and Listeria innocua while commercial antibiotics showed no inhibitory response to Listeria spp.

5. References

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