

Identification of Bacteria in Aceh Cattle with Repeat Breeding

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Abstract. Initial studies report that the incidence of repeat breeding (RB) in Aceh cattle is associated with bacterial infection in the reproductive tract, particularly in the uterine cornual. Therefore, it is necessary to conduct research to identify the type of bacteria in the uterus of Aceh repeat breeding cows. The objective of this study was to identify the types of bacteria that infect the uterus of Aceh cows that experiencing RB. In this study, 16 Aceh cows were used: 7 fertile Aceh cows and 9 RB ones, all aged 3–8 years with a body condition score (BCS) of 3–4. Uterine swab samples were taken from all cows for examination and identification of bacteria. In the uterine samples of two groups of Aceh cows (RB and fertile), 41 bacterial isolates from 11 different types of bacteria were found. The results showed that the most prevalent bacteria in fertile cows was *E. coli* (26.7%), while no *Salmonella* was found. In RB Aceh cows, the most common bacteria were *Salmonella* sp. (29.6%), isolated from 8 samples, and *E. coli* (22.7%) isolated from 6 samples. In conclusion, RB Aceh cows showed higher numbers of bacterial isolates than fertile Aceh cows, with the most dominant bacterial isolate being *Salmonella* sp. (29.6%).

Introduction

Repeat breeding (RB) is a clinical reproductive disorder which can cause infertility. It can decrease reproductive efficiency and livestock productivity, especially in cattle (Prihatno et al., 2013; Thasmi et al., 2020). Early diagnosis of RB disorders minimizes economic losses among farmers (Bonneville-Hébert et al., 2011; Carneiro et al., 2016). Subclinical endometritis in cattle is considered an important etiological factor for RB disorders (Bedewy and Rahaway, 2019; Jeremejeva et al., 2016).

During the reproductive period in cows, the uterus can be exposed to infectious agents, especially during mating and after birth (Yilmaz et al., 2012). Uterine infection can be caused by microorganisms originating from the posterior genital organs (vulva, vagina, and cervix) or from the uterine tract (Bhat et al., 2014; Yilmaz et al., 2012). Of cows with RB, 71.2% were reported to have bacterial infection (El-Khadrawy et al., 2011). Uterine infection causes 20% of RB cases in cattle (Azawi, 2010).

Isolates from the early postpartum uterus include anaerobic bacteria and both Gram-positive and Gram-negative aerobic bacteria (Nath et al., 2014). The bacteria commonly found in cows with RB are *Staphylococcus* spp. (21.0%), *E. coli* (18.4%), *Bacillus* spp. (13.1%), *Corynebacterium* spp. (13.1%), *Pseudomonas* spp. (10.5%), *Proteus* spp. (10.5%), *Klebsiella* spp. (7.9%) and *Streptococcus* spp. (5.3%) (El-Khadrawy et al., 2011). Bacteria that infect the uterus are divided into 3 categories, namely pathogenic bacteria, potential pathogens, and contaminant

bacteria. Pathogenic bacteria include *E. coli*, *Trueperella pyogenes*, *Prevotella* spp., *Fusobacterium necrophorum*, and *Fusobacterium nucleatum*. Other potentially pathogenic bacteria include *Acinetobacter* spp., *Bacillus licheniformis*, *Enterococcus faecalis*, *Haemophilus somnus*, *Mannheimia haemolytica*, *Pasteurella multocida*, *Peptostreptococcus* spp., *Staphylococcus aureus* (coagulase +), *Streptococcus uberis*, and *Fusobacterium* sp. Contaminant bacteria include *Aerococcus viridans*, *Clostridium butyricum*, *Clostridium perfringens*, *Corynebacterium* spp., *Enterobacter aerogenes*, *Klebsiella pneumoniae*, *Micrococcus* spp., *Providencia rettgeri*, *Providencia stuartii*, *Proteus* spp., *Propionibacterium granulosum*, and *Streptococcus acidominimus* (Carneiro et al., 2016).

Pathogenic microorganisms isolated from the infected uterus are commonly found in livestock environments. They are capable of infecting other tissues and organs (Moreno et al., 2016). These microorganisms can cause changes in the pH of the cervical, vaginal, and uterine mucus (Bedewy and Rahaway, 2019; El-Khadrawy et al., 2011). They can inflame and denude the uterine mucosa, interfering with the implantation process (Siregar et al., 2017). Generally, uterine infection can lead to endometritis (Carneiro et al., 2016; Melia et al., 2014), failure of pregnancy, and infertility in cattle (Yilmaz et al., 2012). It is suspected that abnormal uterine conditions can also cause failure of pregnancy and endometritis in Aceh cattle.

Several causes of reproductive disorders in Aceh cows have been reported. Low progesterone levels at the onset of the luteal phase do not appear to cause RB among Aceh cows (Jeon et al., 2015; Thasmi et al., 2017). Rather, initial studies report that the incidence of RB in Aceh cattle is associated with

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bacterial infection of the reproductive tract, especially in the uterine cornual (Dolezel et al., 2010; Rafika et al., 2020). The objective of this study was to identify the types of bacteria that infect the uterus of Aceh cows experiencing RB.

Materials and methods

Experimental animals

In this study, 16 Aceh cows were used: 7 fertile Aceh cows and 9 RB Aceh cows, all aged 3–8 years with a body condition score (BCS) of 3–4. Uterine swab samples were taken from all cows for examination and identification of bacteria.

Isolation and identification of bacteria from the uterine mucosa

Bacterial samples were collected from the uterine tract using a sterile cotton swab attached to the tip of an AI gun (Hasan et al., 2015). The swab sample was steeped in nutrient broth (NB) media and incubated at 37°C for 24 hours. Bacteria were cultured in nutrient agar (NA), blood agar, Mac Conkey agar (MCA), and mannitol salt agar (MSA) (Oxoid Ltd.).

It was incubated at 37°C for 24–48 hours; following the procedure of Yilmaz et al. (2012) and Hasan et al. (2015), bacteria were identified by morphology with Gram staining, catalase test, haemolysis, and biochemical tests including motility, indole and urease test (MIU test), triple sugar iron agar (TSI-A), and Voges Proskauer Test (VP) (Oxoid Ltd.) (Hasan et al., 2015; Joy and Faruk, 2011).

Data analysis

The observations resulting from identification of morphology, types of bacteria and intensity of the isolates in this study were analysed descriptively.

Results

The results showed that all Aceh cows sampled showed bacterial isolates in the uterine mucosa (100%). The intensity of bacterial isolates found in Aceh cattle is presented in Table 1. The results showed that the most common bacteria found in uterine samples of fertile Aceh cows was *E. coli* (26.7%). In RB Aceh cows, the most common bacteria found were *Salmonella* sp. (29.3%), isolated from 8 samples, and *E. coli* (22.7%), isolated from 6 samples. No *Salmonella* sp. was found in fertile Aceh cows.

The results of bacterial isolation showed that all Aceh cows had bacterial isolates (100%) in the uterine mucosa. All 9 uterine samples from RB cows contained 3 types of bacterial isolates, including 6 isolates of *E. coli* (22.2%); 8 isolates of *Salmonella* sp. (29.6%); 4 isolates of *Corynebacterium* sp. (14.8 %); *Enterobacter* sp., *Pseudomonas* sp., *Staphylococcus* sp., *Shigella* sp., each 2 isolates (7.4%); and 1 isolate of *Citrobacter freundii* (3.7%) as presented in Table 2.

Discussion

In the RB and fertile Aceh cows, 42 bacterial isolates were found from 11 different types of bacteria, as presented in Table 1. Based on Table 1, the greatest mean of bacteria is isolated from the uterus of RB Aceh cows. Previous reports stated that the highest average number of microorganisms, mainly pathogenic, was found in cows with uterine infections. About 60% of uterine bacteria were found in RB cows and sick cattle, a much higher frequency of isolates than in fertile cows (Gani et al., 2008; Kather et al., 2012; Sumiarto, 2013).

In fertile Aceh cows ($n = 7$), only 2 samples (28.6%) contained single isolates, while 3 samples (42.9%) contained 2 types of bacterial isolates. Two samples

Table 1. Frequency distribution of bacterial isolation from uterus of fertile and RB Aceh cows

No	Bacterial type	Number of bacterial isolates	
		Fertile, n = 7 (%)	RB, n = 9 (%)
1	<i>Escherichia coli</i>	4 (26.7)	6 (22.2)
2	<i>Salmonella</i> sp.	0	8 (29.6)
3	<i>Corynebacterium</i> sp.	3 (20.0)	4 (14.8)
4	<i>Enterobacter</i> sp.	2 (13.3)	2 (7.4)
5	<i>Pseudomonas</i> sp.	2 (13.3)	2 (7.4)
6	<i>Staphylococcus</i> sp.	1 (6.7)	2 (7.4)
7	<i>Shigella</i> sp.	0	2 (7.4)
8	<i>Bacillus</i> spp.	1 (6.7)	0
9	<i>Klebsiella</i> sp.	1 (6.7)	0
10	<i>Citrobacter freundii</i>	0	1 (3.7)
11	<i>Streptococcus</i> sp.	1 (6.7)	0
		15	27
	Total	42	

Table 2. Intensity of bacterial isolates found in endometrial mucosa of fertile Aceh cows and RB Aceh cows

Parameter	Fertile Aceh cows		RB Aceh cows	
	n	(%)	n	(%)
Number of samples with a single bacterial isolate	2	28.6	0	0
Number of samples with 2 bacterial isolates	3	42.9	0	0
Number of samples with 3 bacterial isolates	2	28.6	9	100
Number of samples with bacterial isolates	7	100	9	100

contained 3 bacterial isolates (28.6%). The uterine bacterial isolates from fertile Aceh cows included *E. coli* with 4 isolates (26.7%), *Corynebacterium* sp. with 3 isolates (20%), *Enterobacter* sp. and *Pseudomonas* sp., each with 2 isolates (13.3%). Other bacteria observed were *Staphylococcus* sp., *Bacillus* spp., *Klebsiella* sp., and *Streptococcus* sp., each with 1 isolate (6.7%). Most of the bacterial isolates found in this study greatly resembled those in the report mentioned above. The identified bacterial isolates included *Staphylococcus* spp. (37.8%), *Bacillus* spp. (35.1%), *E. coli* (29.7%), *Pseudomonas* spp. (18.9%), and Gram-negative rod-shaped bacteria (24.3%) (20). Another report found that the most common bacterial isolates from the bovine uterus were *E. coli* (38.3%), followed by *S. aureus* (20.0%), *Proteus* spp. (10.0%), and *Pseudomonas* spp., *Klebsiella* spp., *Bacillus* spp., 5.0%, 5.0% and 6.7%, respectively (Kusumastuti, 2014).

RB cows had more bacterial isolates than fertile cows (62.5% vs. 28.6%). Bacterial isolates found include *Bacillus* sp. (50%), *Staphylococcus* sp. (40%), *E. coli* sp. (20%), and *Streptococcus* sp. (10%) (Kusumastuti, 2014). Several types of bacteria isolated from the uterus of dairy cows were *Bacillus* sp., *Staphylococcus* sp., *E. coli*, and *Streptococcus* sp. (Singh et al., 2000), *E. coli*, *Streptococcus* spp., *Arcanobacterium pyogenes*, *Bacillus licheniformis*, *Prevotella* spp., and *Fusobacterium necrophorum* (Azawi, 2008; Földi et al., 2006; Petit et al., 2009; Singh et al., 2000; Yavari et al., 2007). This shows that uterine bacteria are closely related to the incidence of endometritis. Uterine infection and endometritis in Aceh cattle are probably caused by bacterial infection.

The number of bacterial isolates in RB Aceh cows (2.45 ± 2.58) was higher than in fertile Aceh cows (1.36 ± 1.28), but it was not statistically significant ($p > 0.05$). However, the number of bacteria isolated from the uterus of Aceh RB cows was higher (64.3%) than that of fertile Aceh cows (35.7%). The most common bacteria found in fertile Aceh cows included *E. coli* (26.6%) and *Corynebacterium* sp. (20%), and in RB Aceh cows, it was *Salmonella* sp. (29.6%), *E. coli* (22.2%) and *Corynebacterium* sp. (14.8%). The discovery of *E. coli* bacteria in the uterus of Aceh cows in this study was probably due to faecal contamination containing these bacteria. Bacterial contamination can also occur during the implementation of artificial insemination, embryo transfer, uterine biopsy, and

intrauterine drug infusion that does not pay attention to aspects of hygiene (Casarin et al., 2018).

Bacterial contamination causes abnormalities or infections of the uterus (Jun et al., 2008). Uterine infections are always associated with the presence of *Arcanobacterium pyogenes*, *E. coli*, *Fusobacterium necrophorum*, and *Prevotella melaninogenica* (Frazier et al., 2002; Gani et al., 2008; Jun et al., 2008; Källero, 2010; Petit et al., 2009; Yavari et al., 2007), and *Corynebacterium pyogenes* (Abere and Belete, 2016).

In the genital organs of domestic animals, there are several bacteria that are harmful and pathogenic to susceptible animals. Bacteria can travel from the vagina to the uterus. In the cervix and uterus, it can multiply, leading to contamination and infection of the uterus (Chapwanya et al., 2012). When the uterus is infected, inflammatory cells infiltrate the endometrium and cause acute and chronic inflammation, followed by necrosis, hyperaemia, increased numbers of neutrophils, lymphocytes, and macrophages, cystic dilation or atrophy of the uterine endometrium (Bajaj et al., 2016; Chethan et al., 2015; Thasmi et al., 2018), endometrial hyperplasia, endometrial atrophy, and pyometra (Källero, 2010). A cow's uterus with endometritis shows exudates in the endometrial lumen that vary, such as serum, mucus, and purulence that fill the inside of the uterus (Frazier et al., 2002; Källero, 2010).

Conclusion

The RB Aceh cows showed higher numbers of bacterial isolates than fertile Aceh cows, with the most dominant bacterial isolate being *Salmonella* sp. (29.6%). The RB Aceh cows tend to be infected by more than 3 bacterial isolates.

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Conflict of interests

The authors declare that they have no conflict of interests.

Author's contribution

Cut Nila Thasmi and Husnurizal Husnurizal participated in performing uterine swabs, selecting samples, and writing the initial manuscript. Sri Wahyuni performed manuscript revision and

assisted bacterial identification. Tongku Nizwan Siregar performed practical experiments and sample collection. Hafizuddin Hafizuddin developed the original idea and protocol and revised the final manuscript.

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