

Detection of *Mycobacterium bovis* and *Klebsiella pneumoniae* at Bali Cattle Slaughterhouse by culture analysis and PCR

Deteksi *Mycobacterium bovis* dan *Klebsiella pneumoniae* pada Rumah Potong Sapi Bali melalui Analisis kultur dan PCR

I Gede Oka Darsana¹, I Nyoman Dibia², Hapsari Mahatmi^{3*}

¹Magisterial Program of Veterinary Medicine, Udayana University, Denpasar, Bali

²Animal Disease Investigation Centre, Denpasar, Bali

³Laboratory of Microbiology, Faculty of Veterinary Medicine, Denpasar, Bali

*Corresponding author: hmahatmi@yahoo.co.id

ABSTRAK

Penelitian mengenai prevalensi bovine tuberculosis dan *Klebsiella pneumoniae* pada rumah potong sapi di Bali dilakukan di Rumah Potong Sapi Pesanggrahan dan Mambal dari Bulan Januari sampai dengan Maret 2015. Pengamatan terhadap lesi dilakukan pada organ paru-paru dan limfaden yang selanjutnya diperiksa melalui analisis DNA untuk *Mycobacterium bovis* dan kultur untuk isolasi *Klebsiella pneumoniae*. Dari seluruh sampel, tidak ada sampel yang ditemukan lesi yang mengindikasikan menderita tuberculosis di kedua rumah potong hewan. Namun, *Klebsiella pneumoniae* teramati pada 1/4513 sampel (0,02%) hanya di Rumah Potong Sapi Mambal. Penelitian ini merupakan laporan pertama adanya *Klebsiella pneumoniae* di Bali. Karenanya, dibutuhkan kehati-hatian terhadap *Klebsiellosis* yang bersifat zoonotik ini dan formulasi manajemen kontrolnya.

Kata kunci: sapi Bali, *Mycobacterium bovis*, *Klebsiella pneumoniae*, prevalensi

ABSTRACT

A study to determine the presence and prevalence of bovine tuberculosis and *Klebsiella pneumoniae* at cattle slaughterhouse in Bali was carried out in Pesanggrahan and Mambal abattoirs from January to March 2015. The Lungs and lymph nodes were inspected for lesions and then examined through DNA analysis for *Mycobacterium bovis* and culture for isolation of *Klebsiella pneumoniae*. Of the samples examined, no one had lesions suggestive of tuberculosis in two abattoirs. However, *Klebsiella pneumoniae* was observed 1/4513 sample (0.02%) only in Mambal abattoir. This study is the first report of the presence of *Klebsiella pneumoniae* in Bali. Therefore, a aware of this zoonotic *Klebsiellosis* and its control management formulation are need.

Keywords : Bali Cattle, *Mycobacterium bovis*, *Klebsiella pneumoniae*, prevalence

INTRODUCTION

Bali cattle plays an important role in supplying beef in Indonesia because it has good quality, high fertilization and low fat percentage (Merliana *et al.*, 2014). The Bali cattle is considered to be well adapted to the country's harsh environmental tropical conditions. Despite it is superior qualities this breed has weakness. The Bali cattle has a susceptible to Jembrana diseases and Malignant Catarrhal Fever. Another disease, such as Bovine tuberculosis (BTB) and *Klebsiellosis* until recent years there was a

little concern about these infection in bali cattle.

Bovine tuberculosis is caused by *Mycobacterium bovis* (bovine tubercle bacillus) which is a member of *Mycobacterium tuberculosis* complex. This disease is a chronic infectious disease and contagious zoonotic disease of domestic animals, wild animals, and humans. Sometimes this disease can be acute and progressive, specially on calves (Poeloengan *et al.*, 2014). And, *Klebsiellosis* is a disease that caused by *Klebsiella pneumoniae* bacteria (Rahmawati, 2009). *Klebsiella pneumoniae* is

an opportunistic pathogen on human and also animal (Younan *et al.*, 2013).

In Bali, there have been limited studies to determine the prevalence of Bovine tuberculosis and Klebsiellosis. According to Putra *et al.* (2013), seroprevalence of bovine tuberculosis in Bali cattle from Bangli region was 2.22%. Moreover, former report stated that Klebsiellosis event was found on the cattle lung samples that show the sign of pneumonia from cattle slaughterhouse in Gorontalo (Retnowati & Nugroho, 2015).

This study was aimed to determine the prevalence of BTB at cattle slaughterhouse based on polymerase chain reaction (PCR) techniques and Klebsiellosis based on cell culture methods.

MATERIALS AND METHODS

Sample

Samples were collected from Pesanggaran Abattoir, Denpasar and Mambal Abattoir, Badung. The selection of the cattle sampled at each abattoirs was strictly based on the clinical signs, following postmortem observation of typical granulomatous lesions of Bovine tuberculosis in the lungs or the lymph nodes.

Bacterial Culture and Identification

Culture was conducted in category III containment laboratories. Samples were transported in refrigerated condition, and processed within 24h of collection. *Klebsiella pneumoniae* bacteria were tested by culturing on MacConkey media. Confirmatory testing of presumptive positives was by Gram colouring test, biochemical and sugar test.

Polymerase Chain Reaction (PCR) Method

The tissue samples showing clinical signs of Bovine tuberculosis were collected and transported on ice pack to laboratory. Genomic DNA were extraction using *genomic DNA mini kit*. DNA were amplified under standard conditions as described Geneaid Product Information. The primers were 5'-CAGGGATCCACCATGTTCTTAGCGGGT TG-3' (forward) and 5'-TGGCGAATTT

CTTACTGTGCCGGGGG-3' (reverse) (Nahar *et al.*, 2011). The reaction was performed in a final volume of 25 µL. After an initial denaturation step (at 94°C, for 3 min), 40 amplification cycles were performed as follows: denaturation at 95°C for 15 second, annealing at 63°C for 15 sec, and extension at 72°C for 30 sec. A final extension was performed at 72°C for 15 min. The amplified PCR products were electrophoresed in 1.5% agarose gel.

Data Analysis

The obtained data were analyzed descriptive statistically using SPSS Program for Windows.

RESULTS AND DISCUSSION

Results

A total of 4513 Bali cattle from slaughterhouses were examined. Two tissue samples were collected from cattles that show suspected respiratory lesions. The lungs inspection found pathologic changes such as pneumonia. While, the lymph nodes were showed no pathologic changes. Further confirmation using PCR technique, the both samples were negatif for *Mycobacterium bovis* (Figure 1).



Figure 1. PCR amplification of gene specific for *M. bovis*. Electrophoresis 1.5% agarose gel. 1,2 the samples, + positive control of *M. bovis*

Culture analysis demonstrated that there was *Klebsiella pneumoniae* in one sample from two sample that suspected respiratory lesions. The biochemical test and sugar test of the sample were presented in Table 1.

Table 1. The Biochemical and Sugar Test for *Klebsiella pneumoniae*

Biochemical and sugar test	Result to Sample A	<i>Klebsiella pneumoniae</i> , Cowan (1974) and Sharma <i>et al.</i> , (2014)
Motilitas	-	-
Sitrat	+	+
Gas dari		
Glukosa	+	+
Laktosa	+	+
Sukrosa	+	+
Methyl		
Red	+	+
Voges		
Proskauer	-	-
Indol	-	-
Urea	+	+
TSIA	Acid/Acid ; H ₂ S - ; Gas +	Acid/Acid ; H ₂ S - ; Gas +
Oksidase	-	-
Katalase	+	+

Discussion

The result of PCR technique in this study confirmed negative for *Mycobacterium bovis*. Refers to the epidemiology concept that a disease is due to the interaction of agent, host, and environment factors (Martin *et al.*, 1987). The negative result of *Mycobacterium bovis* as the cause of BTB in this study can be overviewed from those 3 factors. Poeloengan *et al.* (2014) stated that the susceptible hosts of BTB infection is so wide in range, such as guinea pig, rabbit, mencit, hamster, monkey, horse, dog, cow, pig, cockatoo and fowl. Wild animal that is a permanent reservoir from the *Mycobacterium bovis* infection is fox (*Meles meles*) in Britain, possum (*Trichosurus Vulpecula*) in New Zealand and monkey in

Indonesia (Poeloengan *et al.*, 2014). BTB agent have the ability to live a couple of days outside of its source (Tarmudji and Supar, 2008), this indicates that in the amount of time the BTB agent still have the ability to infected the host. *Mycobacterium bovis* infection can spread rapidly towards the cattle especially through the aerosol inhale, from the cough or sneezing of the animal that have tuberculosis or from the dust particle which contain the agent. The disease also spread rapidly in a very dense location of cattle (Cousins, 2001), or when a wild animal and cattle herd in the same field (Cosivi *et al.*, 1998). While the infection in human, it is usually through the drink of fresh milk and consuming the raw animal product. Besides, the farm worker that keep the animal inside the cage can increase the risk of the infection in aerosol way, from human to animal, or the opposite (Cosivi *et al.*, 1998).

The major finding in the present study is the presence of *Klebsiella pneumoniae* in cattle. The animal that susceptible to Klebsiellosis infection caused by *Klebsiella pneumoniae* bacteria are buffalo and cow (Sayed dan Zaitoun, 2009), dog, monkey, guinea pigs, muskrats (Brisse *et al.*, 2006). Horse and camel (Younan *et al.*, 2013). While the red swallow (*Milvus milvus*), Egypt vulture, Antarctic skua (*Catharacta spp.*), Red-billed chough (*Pyrrhocorax pyrrhocorax*), wild turkeys (*Cathartes aura*), peregrinefalcon (*Falco peregrinus*) were suspected as the carrier agent of *Klebsiella pneumoniae* (Sharma *et al.*, 2014).

Factors that may influence the presence of *Klebsiella pneumoniae* in cattle in Bali are due to environmental factor. *Klebsiella pneumoniae* can grow under the aerob condition at a temperature of 12-43°C with the optimal growth at a temperature of 35-37°C (Rahmawati, 2009). Besides, the environmental factor that causing the Klebsiellosis such as the stress of carriage, the polluted water and environment from the waste of the paper factory and wood finishing textile, waste of the plants product and sugar cane (Brisse *et al.*, 2006). Although the prevalence of Klebsiellosis is small, a proper

postmortem inspection should be practiced effectively at the abattoir, before taking out beef to the public. Besides, the zoonotic agent, *Klebsiella pneumoniae*, can also cause an economical loss on Bali cattle.

CONCLUSION

The prevalence of *Klebsiella pneumoniae* in cattle was very low (0.02%). *Mycobacterium bovis* was not found in cattle slaughtered based on PCR technique.

REFERENCE

- Brisse S, F Grimont and PAD Grimont. 2006. The Genus *Klebsiella*. Chapter : 3.3.8, Vol 6: 159–196.
- Cosivi O, JM Grange, CJ Daborn, MC Raviglione, T Fujikura, D Cousins, RA Robinson, HFAK Huchzermeyer, IDE Kantor and FK Meslin. 1998. Zoonotic Tuberculosis due to *Mycobacterium bovis* in Developing Countries. *Emerging Infectious Diseases*, Vol. 4, No. 1.
- Cousins DV. 2001. *Mycobacterium bovis* infection and control in domestic livestock. Australian Reference Laboratory for *Bovine tuberculosis*. *Rev. Sci. Tech. Off. int. Epiz.*, 20 (1): 71-8.
- Merliana MR, IN Wanida and IK Puja. 2014. Polimorfisme Lokus Mikrosatelit BM1329 dan Hubungannya dengan calving interval pada sapi bali. *JIKH*, 2(2) 117-125.
- Martin SW, A Meek and P Willeberg. 1987. *Veterinary epidemiology*. Iowa : Iowa State University Press.
- Nahar Q, Pervin M, Islam M, Khan MAHNA. 2011. Application of PCR for the Detection of Bovine tuberculosis in Cattle. *J. Bangladesh Agril. Univ.* 9(1):73-78.
- Poeloengan M, I Komala and SM Noor. 2014. Bahaya Dan Penanganan Tuberculosis. Lokakarya Nasional Penyakit Zoonosis.
- Putra PGW, NK Besung and H Mahatmi. 2013. Deteksi Antibodi *Mycobacterium tuberculosis bovis* pada Sapi di Wilayah Kabupaten Buleleng, Bangli, dan Karangasem Provinsi Bali (Tesis). Denpasar : Universitas Udayana.
- Rahmawati D. 2009. Pengaruh Vaksinasi Kultur *Klebsiella pneumoniae* Hasil Inaktivasi Pemanasan Dan Iradiasi Sinar Gamma Terhadap Kondisi Fisik Serta Profil Protein Serum Darah Mencit (Skripsi). Jakarta : Universitas Islam Negeri Syarif Hidayatullah.
- Retnowati Y, and TAE Nugroho. 2015. Pemeriksaan Mikroba Dan Patologi Organ Paru-Paru Sapi Yang Mengalami Pneumoni di Kota Gorontalo. Gorontalo : Universitas Negeri Gorontalo.
- Sayed SM and AMA Zaitoun. 2009. Aerobic Bacterial Pathogen of Pneumonic Feedlot Buffalo-Calves, In Assiut Governorate, Egypt. *Ass. Univ. Bull. Environ. Res.* Vol. 12 No. 1.
- Sharma SK, P Sharma and BN Shringi. 2014. Phenotypic and Genotypic Characterization of *Klebsiella pneumoniae* Obtained from Egyptian Vultures and Steppes Eagles from India. *Israel Journal of Veterinary Medicine*. Vol. 69 (3).
- Tarmudji and Supar. 2008. Tuberculosis Pada Sapi, Suatu Penyakit Zoonosis. *Wartazoa*, Vol. 18 No. 4.
- Younan M, IV Glücks, R Podschun and S Bornstein. 2013. *Klebsiella pneumoniae*: A Commensal And Cause Of Septicaemia In Camel Calves (*Camelus Dromedarius*). *Journal of Camelid Science*.