PROCEEDINGS

International Seminar

THE ROLE OF VETERINARY SCIENCE TO SUPPORT MILLENNIUM DEVELOPMENT GOALS and

8th ASIAN ASSOCIATION OF VETERINARY SCHOOLS CONGRESS

FACULTY OF VETERINARY MEDICINE
UNIVERSITAS AIRLANGGA
PROCEEDING
International Seminar
THE ROLE OF VETERINARY SCIENCE TO SUPPORT MILLENNIUM
DEVELOPMENT GOALS AND THE 12\textsuperscript{th} ASIAN ASSOCIATION OF
VETERINARY SCHOOLS CONGRESS

ISBN : 978-602-70438-0-0

@ FACULTY OF VETERINARY MEDICINE UNIVERSITAS AIRLANGGA 2013
Hak cipta dilindungi oleh Undang-Undang

Diterbitkan Oleh :
Faculty of Veterinary Medicine Universitas Airlangga
Kampus C Mulyorejo Surabaya 60115
Telp. (031) 5992785, 5993016
Fax. (031) 5993015
e-mail : fkh@unair.ac.id
Website : http://www.fkh.unair.ac.id
Semen Characteristics of Captive Sumatran Tiger (Panthera tigris Sumatran) 
NI Wayan Kurniati Karja, Mohamad Fahruddin, Mohamad Agus Setiadji, Licya 
IT A Tumbelaka, Retno Sudarwati, Yohana Tri Hastuti, Bongot Huaso Mulla-Ardyta 
Widianti, Keni Sultan, KAZUHIRO Kikuchi, Takeshige Otol

Rabies In Animals In Bali Province from 2008-2012 
I Ketut Ell Supartika, I Ketut Wirata, I Gede Joni Ullantara, I Wayan Masa Tenaya, I 
Ketut Diarmita

Effectiveness of Red Algae (Eucheuma Spinosum) as Pathogenic 
Antibacterial In Coastal Organisms and Human 
Fattah, Afhariman, Muslimin, L, R, W Andy Omar, S. Bin

Anti-Coxiella Burnetii Antibody Specific for Q Fever Diagnosis 
Immunohistochemically In Ruminant 
Agus Setiyono, Mawar Subangki, William Marea, Vivi Dwi Santi, Ila Elvira, Mutya 
Fadhillah and Sulhi Aufa

Identification of Pathogenic Bacteria Escherichia Coli O157:H7 and 
Staphylococcus Aureus from Pasteurised and Non Pasteurised Bovine Fresh 
Milk 
Lucia R. Winata Muslimin, Dwi Kesumasari, M. Aqshar Marsani, Nurul Inayah, 
Amin arsyinli, and A. Aswan Salam

Clinical Sign Pattern of Infection Microsporum Cants on Dogs 
Gerson Yohanes I Sakan, Puspa Wikan Sari, Yanuartono and Soedarman 
Indarjuntoro

Detection of Autoimmune Thyroiditis Diseases (AITD) : Based on Thyroid 
Peroxidase (TPO) Autoantibody by Immunochromatography Rapid Test 
Aulannlam, Agung Pramana, W. Marchhenda and Dyah Kinsalh Wuragil

The Effect of Probiotic on Autoimmune Thyroiditis Model (AITD) Rat (Rattus 
norvegicus) Induced Sodium Iodide (NaI) Supplementation 
Hendra Legatawa, Wakhidatius Inrya, Adib Musta'In, Ridkli Rosmallasari, Bayu 
Noviaji, Dyah Kinsalh Wuragil and Agung Pramana W. Marchhenda

Analysis of Salmonella spp. from Poultries Carcasses Industries In Malang, 
Indonesia 
Dyah Kinsalh Wuragil, Masdiana C. Padaga

Molecular Genetic Analysis of Indigenous Bima Horse (Equus Caballus) 
Based on Cytochrome B Sequences 
Yuriadi, Rini Widayanti, Wayan Tunes Artama, Charles Rangga Tabbu

The Study of Blahong Leaves Extract (Anredera cordifolia) Ointment 
Ethanol Fraction on Skin Incision Wound Healing Process In Dog (Canis 
familiaris) 
Slamet Raharjo, Sri Hartati, Agus Budi Santosa, Fajar Kurniawan
Improving Milk Quality and Udder Health of Etawah Crossbred Goat by Good Milking Procedure
Yuni SuraIndrayah, Sari Retno Diwanti- Ditto Aji Diantha- Nurliyani ................................................. 107

Blood Chemistry Parameters of Adult Female Turi Ducks
Irkhma Widiyono, Sri Hartati, Hary PurmanaNingsih .......................................................... 112

The Influence of Temu Hitam (Curcuma aeruginosa roxb.) Rhizomes Ethanolic Extract Against Total Intraepithelial Lymphocyte Small Intestine on Layer Chicken Which Infect by Ascaridia galli
Handayu Untari, Eka PramyRtha HestianaH .......................................................... 117

Potential of Beluntas (Pluca indica L.) in Animal Feed to Decrease the Ammonia, Hydrogen Sulfide and Water Levels on Broiler Excreta
Taufik Hidayatuloh, Anggun Rahmawati, Zakia Shella Faradilla ........................................ 121

The Xenobiotic Metabolism in Lead Intoxication Mice with Vitamin C Supplementation
Juliana Christyaningsih .......................................................... 127

The Analysis of Distribution of Mycobacterium bovis Infection with Conventional Techniques, Polymerase Chain Reaction (PCR) and Geographical Information System (GIS) in Dairy Cow Cattle in Enrekang Regency
Sartika Juwita, Moch. Hatta, Lucia Muslimin, Ahmad Nadif ........................................ 135

The Effect of Cigarette Smoke Exposure due to Placental Apoptosis and Gestation Outcomes at Gestation Disorders Mechanism in White Rat (Rattus norvegicus)
Portia Sumarsono, Sruti Listra Adrenalin, Ika Wahyuni, Bayu Digka, Christian Marco, and Widjati .......................................................... 143

Some Factors that May Increase the Potency of Trypanosomiasis that was Caused by Trypanosoma Evansi to Become Zoonosis: A Review
Herlini Susijianti, Fx. Satria Pinandiyta, Rian Hari Suharto ................................................ 148

Antibiotic Resistance in Staphylococcus intermedius Strain Isolated from Dogs with Dermatological Disorders
Mustofa Helmi Effendi, Ngakan Made Rai Widjaja and Ristin Riwayanti ........................................ 152

Combination of Spirulina and Fermented Rumen Content Meal As Substitution In Feed Toward Feed Efficiency of Male Brolle
Mirni Lamid .......................................................... 156

Potential of Vitamin E (α-Tocopherol) Against on Spermatogenetic Cells and Seminiferous Tubule Diameter Testes of Mice (Mus Muscular) Induced with 2, 3, 7, 8-Tetrachlorodibenzo-P-Dioxin (TCDD)
Rosida Achlis, Ismudiono, Hanif Plumerlastuti .......................................................... 160
THE ANALYSIS OF DISTRIBUTION OF Mycobacterium bovis INFECTION WITH CONVENTIONAL TECHNIQUES, POLYMERASE CHAIN REACTION (PCR) AND GEOGRAPHICAL INFORMATION SYSTEM (GIS) IN DAIRY COW CATTLE IN ENREKANG REGENCY

Sartika juwita, Moch. Hatta, Lucia Muslimin, Ahmad Nadif

1 Sekolah Tinggi Penyuluhan Pertanian (STPP) Gowa, 2 Bagian Imunologi dan Biologi Molekuler, Fakultas Kedokteran, Universitas Hasanuddin, 3 Bagian Mikrobiologi, Fakultas Kedokteran Hewan, Universitas Hasanuddin, 4 Stasiun Karantina Pertanian Pare-Pare

email: tika_drh@yahoo.co.id

ABSTRACT

Bovine tuberculosis is currently an important zoonosis worldwide. Mycobacterium bovis is the etiological agent of bovine tuberculosis, has an extraordinarily broad mammalian host range that includes cattle, domestic livestock, and wildlife. The aims of the research are to (1) analyze the ability of the number of test to detect Mycobacterium bovis, i.e. conventional tests with staining acid-fast bacilli (AFB) and culture, and molecular tests with Polymerase Chain Reaction (PCR), (2) find out the distribution of Mycobacterium bovis infection in the field with Geographical Information System (GIS). The research was an explorative study to analyze a number of tests used to detect Mycobacterium bovis and to find out the distribution of Mycobacterium bovis infection in the field. The sample was the milk of dairy cow cattle taken using random sampling method from two districts representing the research location. The data was analyzed using crosstabulation statistics continued with Chi-square test. The results of the research indicate that of the 60 samples of milk of dairy cow cattle done by staining acid-fast bacilli (AFB) to milk decontamination there are 2 samples (3.3%) which are positive of Mycobacterium bovis, 60 samples (100%) are negative of bacterial culture and 6 samples (10%) with PCR test which are positive of Mycobacterium bovis. The sensitivity of PCR testing is 100% and the specificity is 93.1% compared to staining acid-fast bacilli (AFB) to milk decontamination. The next six samples are positive of PCR testing by using Global Positioning System (GPS), two samples are clustering samples, while the other four ones are spreading samples.

Keywords: Mycobacterium bovis, Conventional technique, PCR, Dairy Cattle

INTRODUCTION

Bovine tuberculosis is currently an important zoonosis worldwide. Mycobacterium bovis is the etiological agent of bovine tuberculosis, has an extraordinarily broad mammalian host range that includes cattle, domestic livestock, and wildlife. Mycobacterium bovis is a member of the Mycobacterium tuberculosis complex, a group that includes also Mycobacterium tuberculosis, Mycobacterium africanum, and Mycobacterium microti (Al-Saqr et al, 2009; OIE, 2009).

United States most cases of Tuberculosis (TB) in humans is caused by Mycobacterium tuberculosis. Mycobacterium bovis is another Mycobacterium that can cause TB in humans (CDC, 2011), then a live-attenuated vaccine strains of Mycobacterium bovis Bacillus Calmette and Guerin (BCG) derived from Mycobacterium bovis isolates and vaccine use is widespread in the world (Elizabeth et al., 1997). Mycobacterium bovis has been reported to cause 6-30% of cases of TB in humans in the USA on unpasteurised milk, in the USA in 1995-2005 approximately 1.4% of cases of human tuberculosis caused by Mycobacterium bovis and in San Diego more than 45% of culture results confirm human TB cases in children and 8% of all cases of human TB caused by Mycobacterium bovis. Also reported in Western Ireland that Mycobacterium bovis causes 6.3% of cases of human TB. Research
conducted in New Zealand showed an increase in cases of bovine tuberculosis between 1983 (3.7%) and 1989 (14.6%). Besides, it turns out some areas in Latin America are still human tuberculosis diagnosis based on examination of staining acid-fast bacilli (AFB), reported about 7,000 new cases of human TB per year are caused by infection of Mycobacterium bovis (Juan et al, 1995; Deepti et al, 2012). In the United Kingdom reported that the incidence of human tuberculosis caused by Mycobacterium bovis reached less than 1% of the total cases of human tuberculosis (Anonimus, 2009). Mycobacterium bovis responsible for about 5% of cases of TB in humans in Brazil and reported prevalence of bovine tuberculosis (BTB) in cattle in Brazil is estimated to 1.3% from 1989-1999 (Cristina et al, 2005). Btb prevalence of dairy cattle in Central Ethiopia about 50% (Firdessa et al, 2012), whereas in Southeast Ethiopia btb prevalence in cattle of 2% (Gumi et al, 2012).

Mycobacterium bovis has been declared to be one of the etiologic agents causing significant economic loss in the cattle industry (Rose et al, 1999) and dairy cattle industry (Tejeda et al, 2006). Detection of Mycobacterium bovis from cattle samples is very important, milk and meat are the main source of protein and other nutrients can be contaminated by pathogenic agents and has the ability as infectious TB disease in humans and other mycobacterium infection from animals to humans. Infected animals has the potential to infect humans (zoonotic tuberculosis). Mycobacterium bovis thus potentially causing health hazards to both animals and humans (Al-Saqr et al, 2009).

Efforts needed to prevent zoonotic diseases proper measures to control and eradicate Mycobacterium bovis infection in cattle. Detection of tuberculosis lesions in the abattoir (slaughterhouse) to be followed by examination of the area of origin of cattle in order to identify further cases (John et al, 2012). Tests that can be performed to identify Mycobacterium bovis are tuberculosis lesions in slaughterhouses, microscopic examination by staining acid-fast bacilli (AFB), culture, PCR (Polymerase Chain Reaction) (John et al, 2012), blood tests by ELISA (OIE, 2009) and histological test (Chirtope et al, 2000; Selwyn, 2002).

Data from the Office International des Epizooties (OIE) in 2010 showed that in Indonesia, including the province of South Sulawesi, clinically never reported any cases of bovine tuberculosis. In 2013 in Bangli regency of Bali Province reported that the seroprevalence study of bovine tuberculosis was 2.22% (Pitu, 2013). So far have not found a report or case study on the spread of bovine tuberculosis in dairy cattle Enrekang based on information from the Department of Animal Husbandry and Fisheries Enrekang Regency South Sulawesi Province.

Based on the description above, researchers are interested to analyze a number of tests used for the detection of Mycobacterium bovis which are staining acid-fast bacilli (AFB), culture, and PCR on dairy cattle and determine the distribution of Mycobacterium bovis in the field.

MATERIALS AND METHODS
Location and Design Research
The research was conducted on a dairy farm Enrekang regency for sample selection and the Laboratory of Immunology and Molecular Biology Faculty of Medicine of Hasanuddin University for staining acid-fast bacilli (AFB), culture and PCR tests. This study was an exploratory study to analyze the ability of a number of tests used to detect Mycobacterium bovis and to determine the distribution of Mycobacterium bovis infection in the field.

Population and sample
The population was female dairy cattle that are on a dairy farm in the Enrekang regency. Samples are all reasonable populations that meet the study criteria. Sample size was
calculated using the formula disease detection assuming 5% prevalence of *Mycobacterium bovis* and 95% confidence level. The formula is based on the total sample of 60 head of dairy cattle (Buddharta, 2002).

**Decontaminated method using HS-SH methods**

10 ml of milk sample treated with 5 ml of 7% NaCl, 4% NaOH and the tube mixed for 15-20 second and then incubated at 37°C for 20 min after this period, phosphate buffer pH 6.8 was added and the tube centrifuged at 3000 rpm for 15 min. The Supernatant was discarded and the sediment used for preparing the smear and inoculating culture media.

**The Conventional method**

Staining acid-fast bacilli (AFB) by heating method (Ziehl Neelsen). Milk spreads on glass objects that have been marked, allowed to dry and were fixed with roads pass through the flame three times. Then place on racks available preparations and flood the carbol fuchsin dye to cover the entire sample. Then the flame is passed under preparation until steaming hot substance (not to boiling) three times, let stand for 5 minutes. Then washed with water and added HCl Alcohol leave for 2 minutes, then wash with running water. Last flood the methillen blue dye for 1-2 minutes, then wash with water and let dry.

Milk samples that have been decontaminated taken with a sterile pipette and inoculated on the surface of the media Lowenstein Jensen (LJ) with sodium pyruvate. LJ slants were incubated at 35°C-37°C. All cultures held for a minimum of 4-5 weeks.

**The Molecular Method (PCR)**

1.5 cc of milk samples were centrifuged with a speed of 16000 rpm for 15 min, then the supernatant was discarded. Furthermore *Mycobacterium bovis* DNA extraction with Method Wizard-Genome DNA Purification KIT (Promega ®).

Detection of *Mycobacterium bovis* DNA with Multiplex PCR technique. Fill in gobead ependorf tubes the material is ready for use, consisting of: (10x PCR buffer as much as 2.5 μl, 0.1 μl dNTPs, Taq DNA Polymerase (1 μl) as much as 0.1 μl, diluted with PCR grade water by 19.3 μl, 0.1 μl of each primer JB 21 (5'-TCGTCGCCGTGATGCAATGTCG-3) and JB 22 (5'-CGTTCGGCTACGTCAAGAAAAAG-3), enter each primer ATB1 0.15 μl (5'-ATGCCGGGCTTTGCATCGTCGTCGTCG-3) and ATB2 (5'-CGGTGTGCGGAGAACGCGGCG-3), then enter another 2.5 μl of DNA template (extracted DNA samples). Insert in ependorf tube destilate water 25 μl for the negative control, and then to the positive control using 2.5 μl extract DNA from *Mycobacterium bovis*.

PCR amplification was performed on a Hybird Omne machine. The first amplification at 95°C temperature for 10 min, and then performed a total of 30 amplification cycles, and each cycle consisted of denaturation at a temperature of 94°C for 1 min, annealing at a temperature of 67°C for 1 min, extension 72°C for 1 min and final elongation process at a temperature of 72°C for 10 minutes.

Amplification products (PCR results) was taken as 10 μl and mixed with 1 μl blue juice loading buffer (without markers), mixed well and then put in a 2% agarose gel submerged in a tank containing tris buffer acetid acid EDTA, which previously the first well put 13 μl marker, then electrophoresis of negative charge (Cathode) to a positive charge (Anode) at 100 A for 60 minutes.

**Geographical Information System (GIS) Method**

Sixty samples by using a Global Positioning System (GPS) is the next position of data points presented in map form.

**Data Analysis**

Detection by staining AFB results were analyzed by looking at the presence or absence of red rod-shaped bacteria on microscopy, culture detection analyzed by looking at the growth of the colony is flat, smooth, white, buff, moist, and grow slowly, whereas detection by PCR were analyzed by
electrophoresis pieces on the presence or absence of DNA bands (band DNA) were formed. Data are presented descriptively by using tables and figures.

RESULTS AND DISCUSSION
Examination of milk samples by conventional techniques and Molecular

Table 1 shows that of the 60 milk samples of dairy cows performed staining for acid-fast bacilli (AFB) to milk decontamination are 2 samples (3.3%) were positive *Mycobacterium bovis*, a total of 60 milk samples (100%) negative for bacterial culture, and 6 samples (10%) of the positive PCR test for detecting *Mycobacterium bovis*
Sensitivity 100%, spesificity 93.1%

Table 2 shows that of the 60 milk samples of dairy cows performed staining for AFB to milk decontamination and positive for PCR, 4 samples were positive for PCR test but negative for staining AFB to milk decontamination and 54 samples were negative for staining AFB to milk decontamination and PCR. Sensitivity of PCR testing for the detection of *Mycobacterium bovis* in milk samples compared with staining AFB to milk decontamination test reaches 100% while the specificity reached 93.1%. The test results showed a significant difference between the results of PCR testing with staining AFB to milk decontamination (p <0.05).

Sixty dairy cattle milk samples were examined by PCR was found six samples positive *Mycobacterium bovis* and by using GPS (Global Positioning System) it is found there are two samples of the six samples were seen in cluster. Where clusters are found in the position of S 03.56316 and E 119.76 555, while the other four samples appear to be spread. The research results indicate the possibility of two positive samples in a cluster that has a close relationship in the transmission of *Mycobacterium bovis*. It is necessary to perform genotyping of both the sample and determine the source of infection with *Mycobacterium bovis*.

Then four positive samples *Mycobacterium bovis* spread also needs to be tracking the source of infection.

This study examines 60 milk samples from dairy cattle farmers in several district. Milk is one of the important media in the transmission of bovine tuberculosis (btt) (Srivastava et al. 2008). Humans can be infected by *Mycobacterium bovis* that drinking raw milk or unpasteurised milk from infected cattle of btt. Estimated in some countries more than 10% of human tuberculosis associated with bovine tuberculosis (btt) (OIE, 2009).

This study shows that of the 60 milk samples were decontaminated and then performed Ziehl-Neelsen staining and examined under a microscope at a magnification of 10x100 there are 2 samples (3.3%) positive *Mycobacterium bovis*. 60 samples (100%) negative for culture and 6 samples (10%) positive for PCR testing. *Mycobacterium bovis* from milk samples can be detected by using the specific primers JB21 and JB22 which has long amplicon 500 bp region which is a specific marker of *Mycobacterium bovis* and is able to detect 20 pg of pure DNA (same as the 4000 genomes) (Shah et al, 2002). Research conducted by Juan et al (1995) JB21 and JB22 primers able to detect *Mycobacterium bovis* to 10 fg DNA. Juan et al (1995) also found that the components are inside the milk will not inhibit the PCR reaction. Research carried out by Al-Saqur et al (2009) of 68 dairy milk samples 3 samples showed positive results with staining AFB, culture positive samples 7 and 7 samples were positive by PCR.

This study shows that there are two positive samples of staining AFB to milk decontamination and positive PCR, 4 samples PCR positive but negative for staining AFB to milk decontamination. According to Al-Saqur et al (2009) Staining AFB of milk has a low sensitivity, it does not mean a negative staining AFB showed no infection.
Table 1. The Comparison between the number of positive and negative Staining AFB to milk decontamination, bacterial culture and PCR

<table>
<thead>
<tr>
<th></th>
<th>Staining AFB to milk decontamination</th>
<th>Bacterial culture</th>
<th>PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>2 (3.3%)</td>
<td>0 (0%)</td>
<td>6 (10%)</td>
</tr>
<tr>
<td>Negative</td>
<td>58 (96.7%)</td>
<td>60 (100%)</td>
<td>54 (90%)</td>
</tr>
<tr>
<td>Total</td>
<td>60</td>
<td>60</td>
<td>60</td>
</tr>
</tbody>
</table>

Table 2. Sensitivity and specificity of molecular testing compared with staining AFB to milk decontamination

<table>
<thead>
<tr>
<th>PCR Result</th>
<th>Results of staining AFB</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>Positive</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Negative</td>
<td>0</td>
<td>54</td>
</tr>
<tr>
<td>Total</td>
<td>2</td>
<td>58</td>
</tr>
</tbody>
</table>

Figure 1. Map location of PCR results of Mycobacterium bovis
Figure 2. Result of staining AFB to milk decontamination

Figure 3. PCR results of milk samples no. 46-60 of 500 bp amplification targets

Staining AFB confined to dairy cattle for the detection of infected btb positive indeed. In the culture test, all the samples showed negative *Mycobacterium bovis*. This is because the amount of bacteria is too little to be in the culture on LJ media. According to Sjahrurachman (2008) positive results for culture is needed in 1000 bacteria/mL, and the direct microscopic examination, positive results in the majority of new cases occur if the number of bacteria per milliliter of at least 5000 bacteria. If found positive in staining AFB and there is no growth in culture, this might be due to bacteria that nonviable. Factor that determines the success of culture is the composition of the medium used for the isolation of *Mycobacterium bovis*, the growth rates and the presence of associated microorganisms which would hinder the prospect of isolation of slow growers, the potential inability to adapt isolates in vitro culture conditions, especially in situations where the number of bacteria are limiting, which can result in false negative result by culture, the variability in the tedious process of identification, which has been reported to be a problem and the composition of the media used for primary isolation, particularly of *Mycobacterium bovis* from clinical isolates (Al-Saqur, 2009).

Based on the results obtained by electrophoresis visible DNA bands formed in well number 1, 3, 15, 17, 35, and 50 while the negative control DNA bands are not formed. DNA bands formed showed that the milk samples from dairy cows suspected positive bovine tuberculosis is suffering from bovine tuberculosis. By Hatta et al.
DNA bands formed showed varying thickness which depends on a small and large number of DNA to be amplified. The more DNA amplified makes the DNA that forms a thick ribbon. DNA bands were formed in the positive control it looks thin because bacterial colonies were isolated LJ media culture that is stored for long periods and did not cultured again will cause degradation of the bacterial DNA. PCR technique has a high sensitivity and specificity for detection of Mycobacteria spp. PCR technique is more accurate and faster than using conventional methods in the diagnosis of Mycobacterium bovis (Al-Saqr, 2009; Shah et al, 2002; Juan et al, 1995).

CONCLUSIONS
PCR technique has a high sensitivity and specificity in the detection of Mycobacterium bovis. PCR technique is more accurate and faster than conventional techniques in the diagnosis of bovine tuberculosis. Of the six samples positive PCR results with GIS methods seen further distribution pattern Mycobacterium bovis infection which group/cluster (2 samples) and spread (4 samples).

REFERENCES
27 Juni 2012.


