UNIVERSITI PUTRA MALAYSIA

EPIDEMIOLOGY AND ECONOMIC IMPACT OF BOVINE BRUCELLOSIS IN PENINSULAR MALAYSIA

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FPV 2014 6

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By

MUKHTAR SALIHU ANKA

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Doctor of Philosophy

January 2014

DEDICATION

Dedicated to my mum Hajiya Safiya Salihu Anka for her love and support.

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

EPIDEMIOLOGY AND ECONOMIC IMPACT OF BOVINE BRUCELLOSIS IN PENINSULAR MALAYSIA

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January 2014

Chairman: Latiffah Hassan, PhD
Faculty: Veterinary Medicine

Bovine brucellosis is an important bacterial disease in cattle and other bovine species including bison, elk and buffalo and is mainly caused by the bacterium *Brucellaabortus*. Brucellosis is a zoonotic disease with serious economic impact to livestockindustry in both developed and developing countries. The present study was carried out to understand the epidemiology of bovine brucellosis in Peninsular Malaysia; to describe its occurrence and distribution, to evaluate spatial and temporal clusters pattern and distribution, to identify potential risk factors associated with bovine brucellosis, to assess economic impact of bovine brucellosis to farmers and costs of monitoring, control and eradication to government and finally to evaluate the effectiveness of culling policy of area-wise eradication program of brucellosis in Peninsular Malaysia.

A retrospective examination of surveillance data between 2000 and 2008 was carried out to investigate the presence and occurrence of bovine brucellosis in Peninsular Malaysia. The findings showed that bovine brucellosis is widely distributed across the peninsula. The annual seroprevalence for the 9-year study period was 2.7% and 21.7% for individual animal and herd level prevalence respectively. The herd prevalence fluctuated but remained high within the period of the study while animal prevalence was comparatively low but has a more discerning trend over the study period. In the second study, data on the surveillance activities was aggregated into district level due to lack of farm coordinate and confidentiality of the farm centriole of the districts was used as the coordinate to run spatial and temporal cluster, the ArcGIS and Sat Scan softwares were used for the analysis. The result showed that bovine brucellosis clustered in both space and time and cluster pattern confirmed the earlier finding of similar trend. Three clusters were detected: the first cluster around the state of Pahang and part of Johor covering 5 districts with 68 km radius, the second cluster was situated in Kelantan covering large area of 230 km with 51 districts and the third cluster wa in Selangor affecting 6 districts covering 34 km. However, the cluster was not statistically significant. High risk areas (hot spot) were also detected to be around the clusters identified.

A case-control study was conducted in four states of Peninsular Malaysia: Kelantan, Pahang, Negeri Sembilan and Selangor whereby 71 farmers were interviewed to investigate the potential risk factors associated with bovine brucellosis in Peninsular Malaysia. Blood and vaginal swabs from cattle, whenever permissible, were collected to detect *Brucella abortus.* Questionnaire on the herd-level risk factors was developedincluding information on general farm management, biosecurity, medical history and public health awareness. Serological tests as well as culture and identification test were performed on the samples using Rose Bengal Plate Test and *Brucella* agar with supplement respectively. Univariate analysis was conducted for the potential risk factors and disease status and variables significant on the analysis were modeled into multivariate logistic regression. Seroprevalence was 1.8% (n=282) but samples were cultured negative. The Potential risk factors included farmers with more than one species of animals, presence of wildlife and history of abortion. About 78.9% of the farmers participated in the survey reported assisting their animals during delivery and 71.8% had not been using basic personal protective equipments such as glove and face mask to protect them from potentially contracting the infection. Similarly, 2.8% reported consumed unpasteurized milk from their animals. In addition, 19.7% have had episodes of fever with one farmers experienced undulant fever, and was later diagnosed as infected with brucellosis.

A case-control study assessed the direct economic impact of bovine brucellosis in Peninsular Malaysia. This study incorporated the data from 71 farms based on the record of year 2010, surveillance data from DVS, data from DVS statistics on estimated cattle population and some conservative assumption based on proxy. The estimated direct economic cost of bovine brucellosis in Peninsular Malaysia was calculated using a simple spread sheet tool based on established method by Bennett et al 2003. The total economic loss in 2010 from sampled farms due to bovine brucellosis was estimated to be RM 1,445,367.30. Resources spent for the control and eradication accounted for 21% (RM302, 036) of the lost. This included the cost of vaccination of RM 13,736.00 and compensation cost of RM 288, 300.00 There were also significant devastating impacts to livestock farmers which accounted for 79% (RM 1,143,331.30) of the cost which included milk reduction losses of RM54, 581.30, partial carcass condemnation ofRM 961,000.00 and loss due to abortion accounted for RM 127,750.00.

A cross-sectional survey was carried out to evaluate the efficiency of the culling process prescribed for seroreactor cattle in Malaysia. Fourteen abattoirs in four states: Kelantan, Pahang, Negeri Sembilan and Selangor were visited and data on culled cattle from 2005 to 2008 were examined. The data was managed and analysed using Microsoft Excel. A total of 1,954 cattle were culled in the 14 abattoirs in the four states from 2005 to 2008. In 2005, 157.9% seropositive cattle were culled and for 2006, 2007 and 2008 only 51.5, 41.0 and 88.4% were culled respectively. The state of Selangor has the highest culling rate of 114.2%, followed by Pahang (94.0%), Negeri Sembilan (22.0%) while Kelantan had no record of culling within the study period. The overall culling rate for the studied states within the study period was 72.16%.

In conclusion, bovine brucellosis in Peninsular Malaysia is widely distributed with major clustering of high risk areas. The disease occurrence was associated with several factors such as system of management, other species of animal in the farm and history of abortion. The economic burden of the disease was significant and

estimated to be RM 1,445,367.30 in the year 2010. The area-wise eradication program of brucellosis was shown to be effective in reducing the prevalence. However, there is a need for improvement especially in area of enforcement of the culling policy.

Abstrak tesis yang dipersembahkan kepada senat University Putra Malaysia
Sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

EPIDEMIOLOGI DAN IMPAK EKONOMI BRUSOLOSIS BOVIN DI SEMENANJUNG MALAYSIA

Oleh

MUKHTAR SALIHU ANKA

January 2014

Pengerusi: Latiffah Hassan, PhD
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Bruselosis bovin merupakan penyakit yang disebabkan oleh bakteria *Brucella abortus* pada lembu dan spesies lain termasuk banteng, rusa dan kerbau. Brucelosis adalah penyakit zoonosis yang memberi kesan ekonomi yang serius kepada industri ternakan di negara maju dan membangun. Kajian ini telah dijalankan untuk memahami epidemiologi brucelosis lembu di Semenanjung Malaysia, untuk menggambarkan kejadian dan taburan, untuk menilai corak gugusan ruang dan masa dan taburan, untuk mengenalpasti faktor risiko berkaitan dengan brucelosis lembu, untuk menilai kesan ekonomi disebabkan oleh penyakit ini kepada petani dan kos kepada kerajaan untuk program pemantauan, kawalan dan pembasmian dan akhirnya untuk menila keberkesanan dasar pemusnahan program pembasmian bruselosis di Semenanjung Malaysia.

Pemeriksaan retrospektif data pengawasan antara tahun 2000 dan 2008 telah dijalankan untuk menyiasat kehadiran dan kejadian brucelosis lembu di Semenanjung Malaysia. Hasil kajian menunjukkan bahawa taburan brucelosis lembu didapati secara meluas di seluruh Semenanjung Malaysia. Seroprevalens tahunan bagi tempoh 9 tahun ialah 2.7% dan 21.7% masing-masing bagi haiwan individu dan kelompok. Prevalens kelompok berubah-ubah tetapi kekal tinggi dalam tempoh kajian manakala prevalens haiwan adalah agak rendah tetapi mempunyai tren yang berbeza dalam tempoh kajian.Dalam kajian kedua, data aktiviti pengawasan telah diagregatkan ke peringkat daerah kerana kekurangan data koordinat ladang dan bagi memelihara kerahsiaan ladang. Daerah digunakan sebagai koordinat untuk menjalankan analisis gugusan ruang dan masa menggunakan ArcGIS dan perisian Sat Scan.. Hasilnya menunjukkan bahawa brucelosis lembu adalah bergugusan mengikut ruang dan masa, corak gugusan mengesahkan trend yang hampir sama. Tiga gugusan depat dikesan: gugusan pertama di seluruh negeri Pahang dan sebahagian daripada Johor yang meliputi 5 daerah dengan 68 km radius, gugusan kedua terletak di Kelantan meliputi kawasan besar 230 km dengan 51 daerah, gugusan yang terakhir telah ditemui di Selangor melibatkan 6 daerah meliputi 34 km walaupun gugusan itu tidak ketara secara statistik. Kawasan berisiko tinggi (hot spot) juga dikesan di sekitar gugusan yang dikenal pasti.

Satu kajian kes-kawal telah dijalankan di empat negeri di Semenanjung Malaysia; Kelantan, Pahang, Negeri Sembilan dan Selangor di mana 71 petani telah ditemuramah tentang faktor risiko yang berpotensi berkait dengan bruselosis lembu

di Semenanjung Malaysia. Darah dan swab vagina lembu, bilamene dibenarkan, dikumpulkan untuk mengesan *Brucella abortus*.. Soal selidik faktor risiko kumpulan peringkat telah dibina termasuk maklumat mengenai pengurusan am ladang, biosekuriti, sejarah perubatan dan kesedaran kesihatan awam kepada petani. Serologi dan kultur dan pengenalpastian telah dijalankan ke atas sampel menggunakan Ujian Rose Bengal dan agar Brucella dengan makanan tambahan. Analisis univariat telah dijalankan untuk faktor risiko yang berpotensi dan status penyakit dan pembolehubah yang bekeertian pada analisis dimasukkan ke model regresi logistik multivariat. Seroprevalens yang diperolehi ialah 1.8% (n = 282) tetapi sampel dipepati negeri pada kultur. Faktor risiko potensi yang dikenalpasti adalah petani dengan lebih dari satu spesies haiwan, kewujudan hidupan liar dan sejarah keguguran. Lebih kurang 78.9% daripada petani mengambil bahagian dalam kajian melaporkan membantu haiwan mereka sewaktu proses kelahiran dan 71.8% didapati tidak pernah menggunakan peralatan asas perlindungan peribadi seperti sarung tangan dan topeng muka untuk melindungi mereka daripada dijangkiti oleh jangkitan tersebut. Begitu juga 2.8% dilaporkan minum susu dari haiwan merekayang tidak dipasteurkan. Di samping itu, 19.7% mempunyai episod demam dimana seorang daripada petani tersebut mengalami demam undulan, dan kemudiannya telah didiagnosis dengan bruselosis.

Kajian kes-kawal ini juga menilai kesan ekonomi langsung brucelosis lembu di Semenanjung Malaysia. Kajian ini menggabungkan data yang dikumpul daripada 71 ladang berdasarkan rekod tahun 2010, data pengawasan dari Jabatan Perkhidmatan Haiwan, data statistik dari Jabatan Perkhidmatan Haiwan mengenai anggaran populasi lembu dan beberapa andaian konservatif berdasarkan proksi. Anggaran kos ekonomi langsung bruselosis lembu di Semenanjung Malaysia telah dikira menggunakan kaedah yang dibina oleh Bennett et al 2003. Jumlah kerugian ekonomi ladang yang disampel disealibatabkan bruselosis lembu dianggarkan sebanyak RM 1,445,367.30.Sumber yang dibelanjakan bagi kawalan dan pembasmian menyumbangk sebanyak 21% (RM302, 036) daripada kerugian. Ini termasuk kos pemvaksinan sebanyak RM 13,736 dan kos pampasan sebanyak RM 288, 300. Kesan ekonomi buruk yang besar juga didapati kepada penternak yang menyumbangkan sebanyak 79% (RM 1,143,331.30) daripada kos termasuk kerugian pengurangan susu sebanyak RM54,581.30, kondemnasi separa karkas RM 961,000 dan kerugian akibat keguguran sebanyak RM 127,750 .

Satu kajian keratan-rentas telah dijalankan untuk menilai kecekapan proses pemusnahan yang ditetapkan untuk lembu yang seropositif di Malaysia, di mana sebanyak 14 rumah penyembelihan di empat negeri Kelantan, Pahang, Negeri Sembilan dan Selangor telah dilawati dan data mengenai lembu yang dimusnahkan dari tahun 2005-2008 diperiksa. Data tersebut diurus dan dianalisis menggunakan Microsoft Excel..Sebanyak 1,954 ekor lembu telah dimusnahkan dalam 14 rumah penyembelihan daripada empat negeri yang kami lawati dari 2005 hingga 2008. Pada tahun 2005, 157.9% lembu seropositif dimusnahkan and bagi tahun 2006, 2007 dan 2008 tedapat 51,5, 41.0 dan 88,4% dimusnahkan, masing-masing. Negeri Selangor mempunyai kadar pemusnahan tertinggi sebanyak 114.2%, diikuti oleh Pahang (94.0 %), Negeri Sembilan (22.0%) manakala Kelantan tidak mempunyai rekod pemusnahan dalam tempoh tersebut. Kadar pemusnahan keseluruhan bagi negeri-negeri yang dikaji dalam tempoh kajian ini adalah 72.16%.

Kesimpulannya, bruselosis lembu di Semenanjung Malaysia tertabur secara meluas dengan gugusan utama kawasan berisiko tinggi. Kewujudan penyakit dikaitkan dengan beberapa faktor seperti sistem pengurusan ladang, spesies haiwan lain di ladang dan sejarah pengguguran. Beban ekonomi penyakit bagi ladang yang disampel adalah dianggarkan sebanyak RM 1,445,367.30.pada tahun 2010. Program pembasmian brucelosis didapati berkesan untuk mengurangkan prevalen penyakit.Walau bagaimanapun, terdapat keperluan untuk penambahbaikan terutamanya dalam bidang penguatkuasaan menakai haiwan yang berpenyakit.

ACKNOWLEDGEMENTS

I wish to express my profound gratitude to Almighty Allah for giving me the opportunity and wisdom in pursuing through to this achievement. I am grateful to the Chairperson of my PhD committee, Assoc Prof Dr Latiffah Hassan for her continuous coaching and support throughout the years of my PhD, for burning the midnight oil reading, re-reading and revising my work, for asking insightful comments and questions, and for offering invaluable advice. Assoc Prof Dr. Siti Khairani Bejo, Professor Dr. Zaianal Abidin Mohamed and Dr. Ramlan Bin Muhamad for their invaluable guidance, advice, encouragement through the course of the study and in preparation of this thesis. Without their patience, support and contribution, I wouldn't have accomplished the goal of finishing this dissertation.

I am especially grateful to the Department of Veterinary Services Putrajaya and Veterinary Research Institute Ipoh, state and district veterinary department staff and owners of farms in the four states we visited for granting me the permission to collect samples and for their time in helping me interviewing the farmers. I thank the managers of the abattoirs in Pahang, Selangor, Kelantan and Negeri Sembilan for their assistance in providing us with data. Specifically, my gratitude goes to Dr. Azri Adzhar, late Dr. Roosevien Farida Nilawati Rachmat of the DVS Putrajaya for their help and assistance during the course of the study.

Additionally I appreciate the help of Dr. Annas Saleh, Dr. Muhamad Salim Bin Tahir for their kind assistance during our data collection, thank you for helping me converse with the farmers. Special thanks to the staff who accompanied me to the field during our farm sampling, individuals like En Mohd Faizal Bin Abdullah, En Amin bin Abd.Hamid, Puan Nazira Abu, En Mohd Helmy Bin Mahroob, En Azri bin Samin, Encik Wan Azaman bin Wan Mahmud, Encik Hassan Gua Musang, and many more that I may not have enough space to mention here.

To my colleagues and friends especially Dr. Ibrahim Abubakar Anka, Dr. Abdulrasheed Bello Aliyu, Dr. Hassan Ismail Musa, Dr. Adamu Abubakar Yarima, Dr. Khumran Armiyau Mada, Dr Kabir Sahabi Kalgo, Nasiru Matazu for their guidance from the inception to the completion of this study, thank you. They have been instrumental in helping me shape my thesis and providing me with insight to the joys (and stress) of academic research. My thanks are due to Encik Hafiz, Encik Hajar and Cik Krishnamma in the Bacteriology Laboratory, Department of Veterinary Pathology and Microbiology, Faculty of Veteriunary Medicine, Universiti Putra Malaysisa for their technical assistance.

I am especially grateful to my Director of Veterinary Service in Zamfara State Directorate of Livestock Development Dr. Auwal Bawa Moriki for his encouragement during my study. Special thanks also go to my colleagues Dr. Bello Mainasara Shinkafi, Dr. Muhammad Muhammad Bagudu and Dr. Usman Ismail Gusau all in Zonal Veterinary Clinic Gusau Zamfara State for their support.

I am especially gratitude to my parents specifically my mother Hajiya Safiya Anka and my late Father Alhaji Salihu Anka for giving me guidance, support and prayers. My heartfelt appreciation goes to my wife Miss Maryam Anka, whose dedication,

love and persistent confidence in me has taken the load off my shoulder. To my son Al-ameen Mukhtar Anka, you are the source of strength and perseverance. It was you who motivated me when times were difficult and I felt like there was no light at the end of the tunnel.One love to my brothers and sisters for their support and encouragement, especially Sani Salihu Anka for his motivation and assisance.

Finally, I would like to thank everyone who has contributed to the successful realization of this thesis, as well as expressing my apology that I could not mention them personally one by one.

I certify that a thesis examination committee has met on 08/01/2014 to conduct the final examination of Mukhtar Salihu Anka on his thesis entitle "Epidemiology and Economic Impact of Bovine brucellosis in Peninsular Malaysia" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 march 1998. The Committee recommends that the student be awarded the degree of Doctor of Philosophy

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Arc GIS	Geographic information system (GIS) software
AUSVETPLAN	Australian Veterinary Emergency Plan
CD4Tcell	Cluster of differentiation 4 timus dependent cells
CDC	Centers for Disease Control and Prevention
CFSPH	Center for Food Security and Public Health
CFT	Complement Fixation Test
CI	Confidence Interval
DNA	Deoxyribonucleic acid
DSM	Department of Statistics Malaysia
DVS	Department of Veterinary Services
EFSA	European Food Safety Authority
ELISA	Enzyme-linked immunosorbent assay
ESRI	Economic and Social Research Institute
EU	European Union
FAO	Food and Agricultural organization of united nation
FMD	Food and Mouth Disease
GDP	Gross Domestic Product
GIS	Geopraphical Information System
HPAI	Highly Pathogenic Avian Influenza
IgM	Immunoglobulin M
IH	Institute Haiwan
JHSPH	Johns Hopkins Bloomberg School of Public Health
JUPEM	Department of Survey and Mapping
LPS	Lipopolysaccharide
MOA	Ministry of Agriculture
MTM	Malaysia-Thailand-Myanmar peninsula
NPC	National Poison Centre
OIE	International Organization for Animal Health
OR	Odds ratio
PAHO	Pan American Health Organization
PCR	Polymerase chain reaction
PFGE	Pulsed field gel electrophoresis
PPE	Personal Protective Equipment
RB51	Strain RB51 vaccine
RBPT	Rose Bengal Plate Test
RM	Malaysian Ringgit
rRNA	Ribosomal ribonucleic acid
S19	Strain 19
SNP	Single nucleotide polymorphism
VRI	Veterinary Research Institute
WHO	World Health Organization

CHAPTER 1

INTRODUCTION

Bovine brucellosis (Bang's disease, Enzootic Abortion, Epizootic Abortion, Slinking of Calves, and Contagious Abortion) is an infectious disease in cattle and other species including bison, buffalo and elk caused by the bacterium *Brucella abortus.* This organism is a facultative intracellular pathogen with up to seven biovars (1-6 and 9) have been reported. Some of these biovars differ only slightly from each other biochemically (OIE, 2009). Other *Brucella* species that have been uncommonly associated with the disease in cattle including *B. melitensis* and *B. suis* (Carvalho et al., 2010).

Brucellosis is considered as a highly infectious zoonotic disease with a serious economic repercussion on both humans and animals (Radostits et al., 2000;Abernethy et al., 2006). Occupational exposure to *Brucella* has been reported in laboratory workers, farmers, veterinarians and others who are in contact with infected animals or tissues. Brucellosis is one of the most easily acquired laboratory infections (Robichaud et al., 2004). In addition, individuals who do not work with animals or tissues may become infected by ingesting unpasteurized contaminated milk or dairy products (Corbel, 2003). Infection in humans is manifested in the form of fever, lethargy and night sweats as initial symptoms. However, complication may set in as a result of chronic infection, which follows involvement of many organs and system such as liver, spleen, kidney and skeleton among others (Wright, 1997).

Major efforts have been undertaken around the world to control brucellosis in livestock due to its significant economic losses and the often debilitating consequences in humans (Halling & Boyle, 2002). The incidence of bovine brucellosis varies both within and between countries. Advances in the control and eradication practices have led to a significant reduction in the disease incidence or complete eradication in some countries, including the United Kingdom, Scandinavian countries, Australia, New Zealand, Canada, Finland, Norway and Sweden (Seleem et al., 2010). However, bovine brucellosis remains a significant threat in Africa, the Middle East, Central and South America, and Asia including Malaysia (Corbel, 2006). The disease is usually introduced into a herd from an infected animal, but may also enter the herd via semen from infected bulls and via contaminated fomites. Bovine brucellosis can be eradicated from a herd by test and culling or by depopulation. Other important measures for control and eradication are quarantine of infected animals and good surveillance system. Vaccination may be used to control this disease in endemic areas, or used as part of an eradication program (Nicoletti, 1984).

Spatial epidemiology has recently been used to aid in understanding of infectious diseases including those that are zoonotic in nature (Ostfeld et al., 2005). Understanding spatial distribution of diseases can provide an insight into their determinants and helps in their control (Ruankaew, 2005; JHSPH, 2006). Geographical distribution is a key element in the epidemiologic investigation, and several tools have been explored for spatial-related data (Auchincloss et al., 2012). The tools can be in the form of a simple map or more complex that includes modeling by which geographic distribution of diseases can be visualized and

analyzed in time. Therefore the map can reveal spatio-temporal trends, patterns, and relationships that are difficult to discover in tabular or other formats (Ruankaew, 2005). Spatial epidemiology of bovine brucellosis in Malaysia has not been explored. Knowledge about the spatial distribution is crucial for disease control and prevention.

Animal health economics is a growing discipline that is becoming more important as aid to decision making in livestock health at various stages. The stages of intervention and decision making range from individual animal to the national herd stage and lastly to international disease mitigation effort (Otte & Chilonda, 1987). In recent years, there has been an increasing interest in estimating the magnitude of financial losses or impact of livestock diseases and control strategies (Bennett, 2003) in both developed and developing countries (FAO, 1962). Estimation of the overall financial losses due to bovine brucellosis in the country will help to demonstrate the magnitude of the economic damage caused by the disease, particularly to farmers, livestock industry and the country. Most of the study conducted in Malaysia concluded that bovine brucellosis is of economic importance. However, none has performed economic analysis to estimate the impact of disease on the livestock industry or farmers, nor evaluate possible cost of controlling the disease.

The study aimed to enhance the understanding on the epidemiology of bovine brucellosis in Peninsular Malaysia so that disease control can be tailored to the local disease situation. In addition, to substantiate the claim that the disease is of economic significance, an economic analysis was performed based on the data that were obtained during this research work and supported by other data that were not able to be gathered during this work.

Three major hypotheses were

1. Brucellosis is clustered in several areas in Peninsular Malaysia.
2. Several factors (risk) play a vital role in the occurrence of bovine brucellosis.
3. Bovine brucellosis causes a significant economic losses in the livestock industry in Malaysia

The specific objectives of this study were to:

1. determine the distribution, patterns and trends of the seroprevalence of bovine brucellosis in Malaysia between 2000 and 2008.
2. identify spatio-temporal distribution of bovine brucellosis in Peninsular Malaysia.
3. identify potential risk factors associated with bovine brucellosis among cattle herds.
4. estimate the direct economic impact of bovine brucellosis
5. evaluate the efficiency of culling of bovine brucellosis seroreactors between 2005 and 2008.

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