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Prevalence Study of Infectious Bovine Keratoconjunctivitisin Dairy cattle under the Ladang Angkat Programme of University Veterinary Hospital of the Universiti Putra Malaysia.

[1]Faez Firdaus Jesse Abdullah*, [2,3]Muhammad Abubakar Sadiq,[1]Deva Darshini Thinakaran[2,4]Abdulnasir Tijjani, [2,5]Yusuf Abba, [1,4]Konto Mohammed, [1]Eric Lim Teik Chung,[1]Mohd Jefri Mohd Norsidinand[2]Abdul Rahman Omar,

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[1]Department of Veterinary Clinical Studies, Faculty of Veterinary Medicine, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia.[2] Department of Veterinary Pathology and Microbiology, Faculty of Veterinary Medicine, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia.[3]Department of Veterinary Public Health and Preventive Medicine, Faculty of Veterinary Medicine, University of Maiduguri, PMB1069, Borno State, Nigeria.[4]Department of Veterinary Microbiology and Parasitology, Faculty of Veterinary Medicine, University of Maiduguri, PMB1069, Borno State, Nigeria.[5]Department of Veterinary Pathology, Faculty of Veterinary Medicine, University of Maiduguri, PMB1069, Borno State, Nigeria.

Abstract: *Moraxella bovis is the causative agent of a highly contagious disease, infectious bovine keratoconjunctivitis (IBK) that affects cattle of all ages and that occurs worldwide. It is one of the examples of the diseases that may cause production losses in dairy farms in many countries. Infectious bovine keratoconjuctivitis, also known as pink-eye, contagious ophthalmia and New Forest eye disease is clinically characterized by corneal ulceration, oedema, blepharospasm, photophobia, ocular pain, lacrimation, corneal perforation and permanent blindness in severe cases. The aim of this study was to determine, by polymerase chain reaction (PCR) detection, , the prevalence of Moraxella bovis infections in dairy farms in 'Ladang Angkat' Program of the Faculty of Veterinary Medicine, UPM; and to ascertain the role of flies as a vector of Moraxella bovis. A cross sectional study was conducted, three dairy cattle farms under the 'Ladang Angkat' Program of the Faculty of Veterinary Medicine, UPM were selected and 50 animals were selected randomly from all the three farms according to proportions. The overall prevalenc of IBK amongst dairy farms in 'Ladang Angkat' Program of the Faculty of Veterinary Medicine, UPM was 2.0%. Farm specific prevalence varies from 0 to 5.50% and there was no statistically significant association was found between occurrence of prevalence and dairy farms sampled (p>0.05). Only nine (9) common house fly (Muscadomestica) were trapped from the three farms, none of the bacterial DNA extract made from the crushed fly suspension yielded amplicon via polymerase chain reaction (PCR) detection method. Improved farm management with enhanced fly repellent measures to reduce vector transmission and prompt identification, isolation and treatment of all infected cattle is hereby recommended.*

Keywords*: Prevalence, Infectious bovine keratoconjunctivitis (IBK), Dairycattle, Polymerase chain reaction (PCR)*

I. Introduction

Malaysian dairy sectoris still considered small with about 9% sufficiency in milkproduction level, but there is a deliberate effort by the government to double local milk production in the short term as well as the long term and to increase self-sufficiency to higher level (Mohd Karim et al., 2014). Despite these efforts the dairy industry in Malaysia is currently facing many challenges particularly in terms of production level. Outbreaks of diseases are among the main challenges faced by most of the dairy farms. Infectious bovine keratoconjunctivitis (IBK) is one of the examples of the diseases that may cause production loss in dairy farms in many countries. Infectious bovine keratoconjuctivitis, also known as pink-eye, contagious ophthalmia and New Forest eye disease, is a highly contagious bacterial disease of the eye that affects cattle of all ages and of worldwide occurrence (Chandler et al., 1979; Snowder et al., 2005). It is clinically characterized by corneaulceration, oedema, blepharospasm, photophobia, ocular pain, lacrimation, corneal perforation and permanent blindness in severe cases(Abdullah et al., 2013; Alexander, 2010; Kizilkaya et al., 2013). Although IBK is rarely fatal, however the associated impaired vision results inadverse economic impact of decreased weight gain, low calf growth rate, decreased milk production, increased treatment costs, and market discounts due to eye disfigurement and blindness (O'Connor et al., 2012; Postma et al., 2008).It has been estimated that

IBK costs cattle producers 150 million US$ in the United States and 22million AUD$ in Australia per annum as a result of inappetence and poor weight gain in affected animals suffering from ocular pain and visual impairment (Hansen, 2001; Kizilkaya et al., 2013).The primary etiologic agent of infectious bovine keratoconjunctivitis (IBK) is *Moraxellabovis* a Gram negative coccobacillus bacterium of the family Moraxellaceae (Pettersson et al., 1998).*M. bovis*is transmitted by direct contact with an infective material, such as nasal and ocular discharges, and viaconjunctival exudates bymechanical vectorsmost commonly by the face fly, *Musca autumnalis*(Brown et al., 1998).Calves are more susceptible to infection than adults but immunologically naïve cattle can be severely affected when the herd has not been previously exposed (Kizilkaya et al., 2013; Postma et al., 2008; Takele and Zerihun, 2000).Variations, among cattle in breeds, the susceptibility to IBK have been demonstrated Hereford cattle were found to be more susceptible compared with all other purebreds (Angus, Simmental, Charolais, Braunvieh, Limousin, Gelbvieh, Pinzgauer and Red Poll) and *Bos Indicus* breeds (Kizilkaya et al., 2013; Snowder et al., 2005). Polymerase chain reaction (PCR) has become an important tool for research and clinical diagnosis of infectious diseases. Multiplex real-time PCR assay was developed for the detection and differentiation of *Moraxella bovis* (*M. bovis*), *M. bovoculi* and *M. ovis*(O'Connor et al., 2012; Shen et al., 2011).There seem to be dearth of information on the prevalence of IBK among cattle population in Malaysia. Therefore this cross-sectional study was designed to study the prevalence of IBK infection and the role of fly vectors in selected dairy farms in 'Ladang Angkat' Program of the Faculty of Veterinary Medicine, UPM.

II. Materials And Methods

Three dairy cattle farms under the 'ladangangkat' programme of Faculty of Veterinary Medicine, Universiti Putra Malaysia were randomly selected by balloting. Total of 50 animals were selected randomly from all the three farms. Sub conjunctival swabs from both eyes of each animal were obtained using sterile Amies transport swab (Labchem Sdn Bhd).The collected sub conjunctival swab samples were streaked on blood agar and incubated for 48 hours at 37^{o}C aerobically. A three dimensional design NZI fly traps were placed in all three dairy cattle farms for an average period of four hours in each farm. The fly trap was placed in such way it faces the cattle house. The fly samples were crushed with a few drop of sterile saline between two sterile glass slides and the samples was swabbed with a sterile cotton swab and streaked onto a blood agar plate. The blood agar plate was then incubated for 48 hours at 37^{o}C. After incubation, colonies were Gram stained and viewed under the light microscope. Colonies that were gram negative were chosen and sub cultured again on blood agar for 48 hours at 37^{o}C.

Tentative identification of *Moraxella* sppwere made based on the criteria that all those isolates that were found to be gram-negative, coccioccurring in pairs, non-motile, oxidase-positive, nitrate positive, indole negative and nonsaccharolytic.DNA extraction was done using DNAzol™ (Invitrogen) according to the manufacturer's instruction. All template DNA extracts were properly labelled with isolate codes and stored at -20^{o}C. Touchdown thermocycler(SENSOQUEST®Labcycler)according to the manufacturer's instruction was used to perform PCR. A set of primers that targets, *M. bovis*, species specific regions of the 16S rRNA to amplify PCR product of 1541bp. A 50 µL volume reaction with DNase/RNase free PCR grade water comprising of 5 µL genomic DNA template, 2 µL (2U) Taq DNA polymerase,2 µLof 10X top taq PCR buffer, 2 µL of 25 mM $MgCl_2$, 2 µL of 400 µM of each 10x dNTP and 0.5 µl of each of the primers (10mM).The PCR cycling conditions were 5 minutes at 95^{o}C,followed by 35 cycles of 40 s at 95^{o}C, 40 s at 55^{o}C and1 min at 72^{o}C and finally extension at 72^{o}C for 7 min minutes before cooled down indefinitely at 4^{o}C.The amplicons obtained were analyzed by gel electrophoresis on a 1% agarose gel at 90 V for 30 minutes, visualized by UV irradiation using Flurosafe® DNA stain. The DNA template from a pure culture of *M.bovis* obtained from the Bacteriology Lab of Faculty of Veterinary Medicine of UPM was used as the positive control while DNase/RNase free water was used as the negative control in each run of the *M.bovis* conventional PCR assay.

Data obtained were analyzed by JMP 10Statisticssoftware (SAS®) to calculate the PearsonChi-SquareTesttodeterminewhether therewasanysignificantdifferenceintheprevalencefrom one farmto another.PearsonCorrelationtestwas usedtoseewhethertherewasanysignificantcorrelationbetweentheprevalenceand the number animals affected by pink eye disease annually.

III. Results

The overall prevalenc of IBK amongst dairy farms in 'Ladang Angkat' Program of the Faculty of Veterinary Medicine, UPM was 2.0%. The farm specific prevalence of IBK among the dairy cattle farms showed 0% prevalence in both farms A and C, Farm B 5.56% or 556 dairy cattle in every 100,000 (Table 1). No statistically significant association was found between occurrence of prevalence and dairy farms sampled ($p = 0.40$).

Table 1: Prevalence of infectious bovine keratoconjunctivitis (IBK) in each dairy cattle farm.

Farm	No. of Samples	No. of *M. bovis*isolated (%)
Farm A	16	0
Farm B	18	1 (5.56)
Farm C	16	0
Total	**50**	**1 (2.0)**

Fisher's Exact test: 1.708, p = 0.40

A total of nine flies were trapped in this study, 3 from farm A, 4 from farm B and 2 from farm C. All the flies trapped were identified to be the common house fly (*Musca domestica*). None of the crushed fly suspension yielded any bacterial growth, and none of the bacterial DNA extract made from the crushed fly suspension yielded PCR amplicon.

Table 2: PCR detection of *M. bovis* from the fly samples trappedin each dairy cattle farm.

Farm	No. of Flies trapped	*M. bovis*detected (%)
Farm A	3	0
Farm B	4	0
Farm C	2	0
Total	**9**	**0**

IV. Discussion

The overall prevalenc of IBK, obtained in this study, amongst dairy farms in 'Ladang Angkat' Program of the Faculty of Veterinary Medicine, UPM was 2.0%. This prevalence was lower compared to the prevalence of 21.4% during the spring and 29.3% summer months and a peak in the fall at 45% among cattle farms(Schöttker-Wegner et al., 1990). This increased IBK prevalence in that study is attributed to the highest values of ultraviolet (UV) radiation which preceded their study period. However, Brown et al. (1998) noted that IBK can occur during any time of the year, but outbreaks are more common during summer months which coincides with increased UV radiation and face fly vector population. Similarly the prevalence obtained in this study was also lower compared to the study byO'Connor et al. (2012) who reported the overall IBK prevalence in 18 of the 77(23%) bovine eyes examined. The difference in the prevalence might be attributed to the fact that their study was based on isolation and detection of *M. bovis* in bovine eyes with IBK, while ours is cross-sectional survey of conjunctival swab of apparently healthy animals. Furthermore, our finding is also lower that the point prevalence of IBK 5/18 (27.7%) among 18 IBK affected eyes reported by O'Connor et al. (2012)at the beginning of their study. Our finding of 2.0% overall prevalence, amongst dairy farms in 'Ladang Angkat' Program of the Faculty of Veterinary Medicine, UPM, was similar to the IBK prevalence of 2.11% reported by Takele and Zerihun (2000) among 5221 local zebu and crossbreed dairy cattle farms located in different parts of Arsi region, south-east Ethiopia. Although the sample size in that study was extremely lager than ours, nevertheless both study population comprised of cross-breed dairy cattle. The farm specific prevalence of 5.56% in farm B obtained in this study was however higher than 2.11%prevalence and3.54% among cattle of 2-3years age group and 1.8% among those of over 3years reported by Takele and Zerihun (2000). Variations in the prevalence of IBK could be as a result of several factors, among which is the virulence of *M. bovis* which in turn is influenced by both host and environmental factors. These could include factors such as breed and age of the animal (Takele and Zerihun, 2000), host immune system, *M. bovis* strain, exposure to ultraviolet (UV) light, face fly population, concurrent pathogens, and climate and pasture conditions (Snowder et al., 2005). The role of these factors in the pathogenesis of IBK among cattle populations is well documented (Baptista, 1979; Postma et al., 2008).The only animal from which *M. bovis* was detected by PCR was from farm B, but we have found no statistically significant association between occurrence of prevalence and dairy farms sampled ($p>0.05$). This could be due to the limited sample size and dairy farms selected for this study and the fact that all the farms involved are small holder dairy farms. The other reason could be the improved managements in those farms under the 'Ladang Angkat' Program of the Faculty of Veterinary Medicine, UPM.We were able to trap only few flies, where 9 flies were trapped and all are of the *Musca domestica* species (common housefly). None of these flies yielded any *M. bovis* growth and none was also positive for 16S rRNA PCR detection. These could be due to the improve sanitary states of the farms under the 'Ladang Angkat' Program of the Faculty of Veterinary Medicine, UPM due to improved management practices. Fly prevalence in dairy farms could be associated with transmission of IBK from infected to non-infected cattle. Rodríguez (2006) reported that only 5% of the calves developed IBK in the absence of other aggravating factors, in a study where calves were placed in a controlled environment in the absence of face flies and UV radiation. These findings indicate that contact transmission, in the absence of other factors such as face flies, is not significantly involved in the development of IBK and hence highlighting the importance of flies.

It can be concluded in this study that the overall prevalence of IBK among cattle under the 'Ladang Angkat' Program of the Faculty of Veterinary Medicine, UPM is 2.0%.The PCR detection of *M. bovis*, species specific regions of the 16S rRNA can be used in confirmation of *M. bovis* from conjunctival swabs cultured isolates. Direct contact transmission could be the significant mode of transmission among the dairy cattle under the 'Ladang Angkat' Program of the Faculty of Veterinary Medicine, UPM. Improved farm management with enhanced fly repellent measures to reduce vector transmission and prompt identification, isolation and treatment of all infected cattle is hereby recommended.

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