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Prevalence of Moraxella ovis Infection in Goats under the Ladang Angkat Programme,Universiti Putra Malaysia: A Cross-Sectional Study.

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Abstract: *Infectious keratoconjunctivitis (IKC) commonly known as pink eye disease is an inflammationofthecorneaandtheconjunctivathatarecaused by wide range of infectiousbacteriathatarehighlycontagiousingoats.The disease is characterized by blepharospasm, epiphora, corneal opacity and ulceration, and conjunctivitis in both domestic and wild ruminants. The aim of this study was to determinethe prevalence of Moraxella ovis infections among goats under the Ladang Angkat Program of University Veterinary Hospital, Universiti Putra Malaysia, by polymerase chain reaction (PCR); and to ascertain the role of flies as a vector of Moraxella ovis. A cross-sectional study was conducted ona total of sixty (60) goats from 4 farms with 15 goats from each farm were randomly recruited in this study. Subconjunctival swab samples were collected using a sterile swab and flies were collected using ~~an~~ NZI flytrap. Both the subconjunctival swabs and flies samples were cultured on blood agar. A conventional PCR assay targeting the Moraxella ovis specific 16S rRNA gene was carried out. Eighteen (30.0%) of the subconjunctival swabs samples were positive for Moraxella ovis. The farm specific prevalence of M.ovis showed that farm A has the prevalence of 33.33%, farm B 40.0%, farm C 20.0% and 26.67%in farm D. None of the fly samples tested positive for bothPCR and culture techniques.Improved farmpracticeslike reducing overcrowding of animals to reduce contact transmission and fly control are recommended for prevention. Prompt isolation and treatment of all infected animals can reduce transmission rate and spread ofinfestation.*

Keywords*: Infectious keratoconjunctivitis, Pink eye, prevalence,Moraxella ovis, goats.*

I. Introduction

Infectious keratoconjunctivitis (IKC) also known as pink eye disease is a term usedto describethecombinedinflammationofthecorneaandtheconjunctivathatarecaused by a wide range of infectiousbacteriathatarehighlycontagiousingoats(Schoenian, 2009).Pink eye is a worldwide, highly contagious disease of considerable economic importance (Giacometti et al., 2002; Njaa and Wilcock, 2013). Thedisease cause clinical signs such as blepharospasm, epiphora, corneal opacity and ulceration, and conjunctivitis in both domestic and wild ruminants (Dubay et al., 2000). Usually, there are cloudiness and redness of the cornea andthe conjunctiva; hence, the name pink eye disease(Tschopp et al., 2005). The eye discharges areserosanguinous originally and turns to mucopurulent and in severe cases which may culminate in temporary or permanent blindness(Plummer, 2014).The painful eyes, coupled with bilateral opacity and ulceration of the eye will lead to loss of body condition, reduced lactation(Abdullah et al., 2013). Loss of productions are compounded by the cost of keratoconjunctivitis for producers in terms of incurring additional labour and treatment costs (O'Connor et al., 2012). If steps to control and treat the disease are not taken, it may spread in the flock and blindness may result and blind animals on range may subsequently die (Smith and Sherman, 2011). A wide range of etiologic agents are responsible for this disease in goats and they are *Mycoplasma spp* (esp. *Mycoplasma conjunctivae*), *Moraxella bovis,Moraxella (Branhamella orNeisseria) ovis, Colesiota (Rickettsia) conjunctivae,Chlamydophila pecorum, Coxiella burnetii, Listeria monocytogenes* and

Acholeplasma oculi(Smith and Sherman, 2011) and *Staphylococcus aureus*(Abdullah et al., 2013).*Moraxella ovis* infection hasbeen implicated in epizootics of infectious keratoconjunctivitis in domestic sheep and goats (Dagnall, 1994b). This organism has been reported to be isolated from both healthy sheep and goats and those with keratoconjunctivitis, with higher occurrence of isolation rate in diseased animals (Dagnall, 1994a). Various diagnostic laboratories, for many years, have reported the recovery of *Moraxella ovis* (*M. ovis*) from infectious bovine keratoconjunctivitis (IBK) lesions (O'Connor et al., 2012). *Moraxella ovis* is one of four species of Gram negative diplococci which constitute the subgenus *Branhamella*. The same microorganism has thus been named *Neisseria ovis*, *Moraxella ovis* or *Branhamella ovis*(Elad et al., 1988). Predisposing factors such as age, breed, season, mechanical irritation (dust, grass, weeds, etc.), host immune response, eye pigmentation, and concurrent presence of pathogenic bacteria in the environment, strain of the organism involved and prevalence of flies were believed to influence the prevalence of this disease (Bath et al., 2005; Snowder et al., 2005; Takele and Zerihun, 2000). There is usually no mortality rate associated with IKC; however, the morbidity rate can be as high as 80% (Pugliese et al., 2008; Radostits et al., 2007). The aim of this study was to determine the prevalence of *Moraxella ovis* infections in goats by polymerase chain reaction (PCR), under the Ladang Angkat Program of University Veterinary Hospital, Universiti Putra Malaysia; and to ascertain the role of flies as a vector of *Moraxella ovis*.

II. Materials And Methods

Four (4) goat farms under the Ladang Angkat Programme of Faculty of VeterinaryMedicine, UPM were selected randomly for this study. Fifteen (15) goats were then selected randomly from each of the farm and subconjunctival swab samples were collected using a sterile swab. NZI flytrap wasset atasuitablelocationonthefarmand trapped flies were harvestedafter2 hours. Theflysampleswere placedinbetweentwo glassslides andcrushedwithafewdrops of sterile saline;andtheresultantmixturewasswabbedwithasterilecottonswab and streakedontoabloodagarplate. Both the subconjunctival swabs and fly samples were cultured on blood agar. All those isolates that are gram-negative coccioccurring in pairs, non-motile, oxidase-positive, nitrate positive, indole negative and nonsaccharolytic were tentatively identified as *Moraxella spp*. Bacterial DNA extraction was done using DNAzol™ (Invitrogen)kit according to the manufacturer's instruction. A conventional PCR assay targeting the *Moraxella ovis* specific 16S rRNA gene was carried out using oligonucleotide primersOvi16S1F; 5′-GAACGATGAGTATCCAGCTTGCT-3′ and Ovis1849R; 5′-CTCTTTACTTTGGTTAATTATTTTGTTGGA-3′(Shen et al., 2011). A PCR reactionmaster mix of 50 µL containing 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 µ*l* of each of the primers (10mM) was prepared and ran in a Biorad® thermocycler.The PCRcycling conditions were initial denaturation at 95ºC for 5 minutes, followed by 35 cycles of 95ºC for 30 seconds,55ºC for 40 seconds, and 72ºC for 1 minute; and a final extension at 72ºC for 7 minutes before holding at 4ºC. The amplified PCR products were analyzed by gel electrophoresis on a 1% agarose gel at 90 V for 30 minutes, stained with Flurosafe® DNA stain and visualized by UV irradiation. The DNA extracted from a pure culture of *Moraxella ovis* 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 *Moraxella ovis* conventional PCR assay. Data obtained were analyzed by IBMSPSSStatistics20.0software to calculate the PearsonChi-SquareTesttodetermine (relationship)whether therewasanysignificantdifferenceintheprevalencefromfarmtofarm. PearsonCorrelationtestwas usedtoseewhethertherewasanysignificantcorrelationbetweentheprevalenceand the number of animals affected by pink eye diseaseannually.

III. Results

The PCR detection of *M. ovis* from subconjunctival swabs showed an overall prevalence of 30.0%. Farm A accounted for the 8.33% of the overall prevalence; farm B contributed 10.0%, farm C 5.0% while farm D accounted for 6.67% of the overall prevalence of *M. ovis* (Fig. 1).

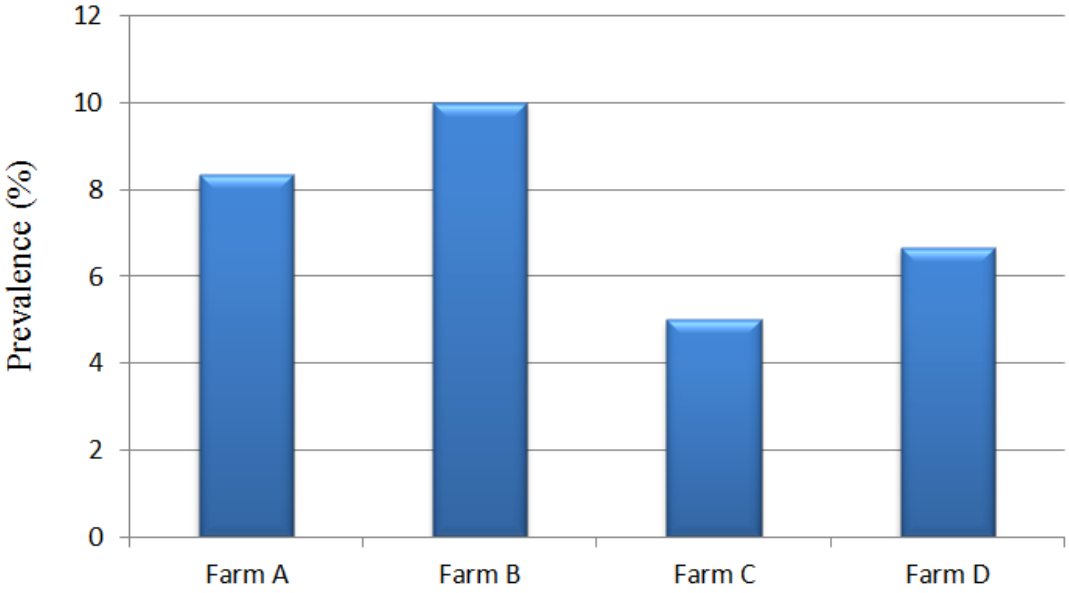

Fig. 1: the distribution of Prevalence of *M.ovis* by PCR detection among the 4 farms

The farm specific prevalence of *M.ovis* among goats by PCR detection revealed that farm A has the prevalence of 33.33%, farm B 40.0%, farm C 20.0% and farm specific prevalence of 26.67%farm D (Table 1).

Table 1: Distribution of farm-specific prevalence of *M. ovis* by PCR detection among the 4 farms.

Farm	Number Positive (prevalence)	Number negative
Farm A	5 (33.33)	10
Farm B	6 (40.0)	9
Farm C	3 (20.0)	12
Farm D	4 (26.67)	15
Total	**18**	**46**

$\chi^2 = 1.50$, df = 3, p = 0.662

Overall only 5 house flies (*Musca domestica*) were trapped with 3 from farm B and 2 from farm C; no flies were trapped in either of farms A and D. Although all the swabs from crushed captured flies yielded culture growth butnone of these samples tested positive for *M. ovis* specific16S rRNA.

IV. Discussion

The overall prevalence of *M. ovis* infection in this study was 30.0%. The animals sampled in this study were apparently normal, however,Dagnall (1994a)reported the isolation of *M. ovis* significantly more often from eyes affected by IKC than from unaffected eyes. The prevalence obtained in this study was slightly similar to prevalence reported by Akerstedt and Hofshagen (2004)where *Moraxella (Branhamella)* ovis was isolated from 28% of 85 sampled animals in affected herds and from 10% of the 50 sampled animals in healthy herds. Out of the overall prevalence farm B contributed 6 (10.0%) the highest among the four farms sampled. The lowest prevalence of 3 (5.0%) was recorded in farm C. this could be the due to differences in hygiene standards that varies from one farm to another. This could also be the reason for the variation in the farm specific prevalence recorded where farm B showed the highest prevalence of 6 (40.0%). The lowest farm specific prevalence of 3 (20.0%) was recorded from farm C. The prevalence obtained in this study was higher as compared to the report of O'Connor et al. (2012) reported the prevalence of 23% of 77 with the identification of *M. ovis* in only one of the IBK-negative eye. This could be attributed to the fact that this study was based cross-sectional design while their study was based on longitudinal cohort study.The animals sampled in this study are not currently suffering from IKC. Based on the available records some of the animals sampled in the study have suffered from IKC hence the detection of *M. ovis* could be associated the recent occurrence of the disease.From 6 cases of infectious keratoconjunctivitis (IKC) in 3 mule deer (*Odocoileus hemionus*) and 3 moose (*Alces alces*), Dubay et al. (2000) reported the isolation of *Moraxella ovis* from two mule deer and two moose. In a similar manner, Dagnall (1994a)reported the isolation of *M. ovis* significantly more often from eyes affected by IKC than from

unaffected eyes. Similar result was obtained in another study in which Vaid et al. (2014) reported the isolation of 3 *M. ovis*isolates out of the 6 cases of IKC in sheep that were randomly sampled. Although flies, especially the *Musca spp*, face fly (*Musca autumnalis*) and the house fly (*Musca domestica*), were believed to play a significant role in the transmission of this disease,*M. ovis* was not detected in any of the fly samples in this study. Transmission of this organismis by direct contact, nasal and ocular discharges, and most commonly by the face fly (*Musca autumnalis*) and hence control of fliesis the general approach to IKC prevention (Snowder et al., 2005). Overall only 5 flies were trapped in this study with 3 of which were from farm B and 2 from farm C; no flies were trapped in either of farms A and D. Apparently farm B which has the highest prevalence of 6 (40.0%) was the farm that has the highest number of flies trapped, however that was not statistically significant. The absence of flies from farms A and D and the lower number of flies trapped in this study could be attributed to the improved hygiene standard of the farms under the Ladang Angkat Program of University Veterinary Hospital, Universiti Putra Malaysia. It can be concluded that the overall prevalence of *M. ovis* infections among goats in farms under the Ladang Angkat Program of University Veterinary Hospital, Universiti Putra Malaysia is 30%. The farm specific prevalence vary from 5 (33.33%) in farm A, farm B 6 (40.0%), farm C 3 (20.0%) and 4 (26.67%) in farm D. *M. ovis* specific 16S rRNA was not detected from the 5 fly samples obtained from two farms out of the four. Improved farm sanitation with focus on reducing overcrowding of animals to reduce contact and fly control and repellant measures are recommended for prevention. Prompt isolation and treatment of all affected animals can reduce transmission rate by direct contact and by fly infestation.

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