

## Bacteriology of Vaccinated and Non-Vaccinated Eye of Cats

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### Abstract

Thirty cats were divided into two groups: group 1 (owner-kept cats) and group 2 (stray cats). Group 1 consisted of 11 cats in which the vaccination status was up to date whereas group 2 consisted of 19 cats in which the vaccination status was unknown. Conjunctival swabs were taken from these cats for bacteria isolation and identification. Giemsa staining of the swabs was also conducted to identify *Chlamydophila* sp. Two cats in group 1, one male and one female Persian which had bilateral watery discharge, were positive (18.18%) for bacteria in their eyes. The male Persian cat was positive for *Enterobacter* spp. and *Moraxella nonliquefaciens* bilaterally. However, in both eyes of the female Persian cat, only *Enterobacter* spp. was isolated. In group 2, 11 domestic short hair stray cats had only *Staphylococcus intermedius* in their eyes which did not show any ocular discharges. Three cats in this group had bilateral isolation and four had unilateral isolation of this bacteria. Of the 30 cats studied, the most predominant bacteria isolated was *S. intermedius* (36.67%) followed by *Enterobacter* spp. (6.6%) and *M. nonliquefaciens* (3.3%). None of the 30 cats studied showed the presence of *Chlamydophila* sp. in their eyes and there was no evidence of eye lesions in these cats. It is believed that the *S. intermedius* isolated was normal conjunctival flora of the eyes of these cats. *Enterobacter* spp. and *M. nonliquefaciens* could also be the normal flora of the Persian cats, since these bacteria were isolated from their eyes in the absence of lesions but with ocular discharge that may promote the growth of these microorganisms.

**Keywords:** bacteriology, vaccinated cats, non-vaccinated cats, conjunctiva

### Introduction

There are many studies on bacteriology of the eyes in many animal species such as in the Canadian beaver (Cullen, 2003), dogs (Marilena et al., 2005), elephants (Kodikara et al., 1999; Briksawan et al., 2004), horses (Andrew et al., 2003; Yagamata et al., 2005), opossum (Chantale et al., 2002), racoon (Chantale et al., 2002) and cat (Campbell et al., 1973; Shewen et al., 1980; Espinolaz et al., 2008). The most common bacteria isolated from variable animal species were *Staphylococcus* spp. and *Streptococci* spp. (Shewan et al., 1980; Kodikara et al., 1999; Chantale et al., 2002; Briksawan et al., 2004; Miller et al., 2005; Marilena et al., 2005;). Beta haemolytic *Streptococci* (Shewan et al., 1980; Kodikara et al., 1999), *Staphylococcus* spp., *Pseudomonas* spp., and *Streptococcus* spp. had been commonly associated with conjunctivitis or ulcerative keratitis. (Marilena et al., 2005).

Eye disease is one of the most common diseases diagnosed in domestic animals especially in cats and dogs. One of the most common eye diseases in cats and dogs is conjunctivitis (Martin et al., 1973; Peiffer et al., 1997; Mac Calla et al., 2001). Conjunctivitis is the inflammation of the mucous membranes of the eyes. Purebred cats are more prone to conjunctivitis. Conjunctivitis in cats usually proceeds to chronic stage. It usually occurs in young cats especially those below one year old. The most common agents causing conjunctivitis are Feline Herpesvirus, *Chlamydomphila felis*, Feline Calicivirus, *Mycoplasma* sp, trauma and allergy (Mac Calla, 2001). Some cats may develop secondary bacterial infection after severe conjunctivitis. Besides eye pathogens, previous studies had showed the isolation and identification of conjunctival flora in various animals including cats. However, studies on the types of bacteria, either normal flora or pathogens, that can be isolated from the eyes of cats is still very limited. This study has the following objectives: to identify the species of bacteria that can be isolated from the eyes of owner kept cats (vaccinated) and stray cats (non-vaccinated) and, to identify *Chlamydomphila* sp. in the eyes of cats (vaccinated and non-vaccinated cats) since *Chlamydomphila felis* is one of the common agent causing conjunctivitis and has high prevalence in cats and also a zoonotic potential to humans (Hartley et al., 2001).

## **Materials and Methods**

### ***Conjunctival Swab Samples***

The conjunctival swab samples were taken from both eyes of 30 cats from 16<sup>th</sup> until 24<sup>th</sup> November 2009. The samples were divided into two groups which consisted 19 of cats was Group 1 and 11 cats in Group 2. The vaccination status of Group 1 (owner kept cats) is up to date while for Group 2 (stray cats), the vaccination status is unknown. Conjunctival swabs from Group 1 were taken from cats kept by owners in Sri Serdang whereas Group 2 cats were taken from stray cats from residential colleges in UPM. The conjunctiva swabs were taken from cats in both groups, with or without eyes discharges in both eyes and regardless of age, sex, and breed using sterile cotton swabs. The cats were observed for eye lesions and details were recorded. After the conjunctival swabs were taken, the swabs were immediately transferred into transport media.

### ***Bacterial Culture***

The conjunctiva swabs from the transport media were streaked onto blood and McConkey's agar and then smeared onto two slides for staining with Giemsa and Gram stain. The slides were observed under light microscope for bacteria (Gram-positive or Gram negative) and *Chlamydomphila* identification. The plates that were streaked with the swabs were incubated at 37°C for 18-24 hours. After incubation, the bacteria colony morphology on the plates were observed and recorded. Then, the colony was stained with Gram stain and subcultured onto blood agar to obtain pure culture of the bacteria. The plates were then incubated for 18-24 hours at 37°C. After incubation, the colony morphology was recorded and the bacteria were Gram stained again to determine the cell morphology before biochemical tests were conducted.

### **Biochemical Test**

Biochemical tests were carried out depending on the gram staining results. Gram-positive cocci bacteria were tested for catalase, coagulase, blood broth, acetoin production, maltose, mannitol and indole. Gram-positive rod was tested for catalase, urease, glucose, nitrate, sucrose, hemolysin and trehalose. Gram-negative bacteria were tested for oxidase and then inoculated into triple sugar iron, sulphide indole motility, urea and nitrate media. Then, the results of biochemical tests from each colony were referred to the Diagnostic Manual of Veterinary Clinical Bacteriology for bacteria identification.

### **Results and Discussion**

No *Chlamydophila* sp. was identified from the conjunctiva swabs using the Giemsa staining technique (Table 1).

Two (male and female) out of 11 cats (18.18%) in Group 1 were positive for bacteria (Table 1). The male Persian cat was positive for both *Enterobacter spp.* and *Moraxella nonliquefaciens* in both eyes which were also watery whereas the female Persian cat was positive for *Enterobacter spp.* (pure growth) only and bilaterally present. The eyes of this female Persian were also watery. However, both Persian cats had no evidence of eye lesions.

In Group 2, 11 out of 13 cats (57.89%) were positive for *Staphylococcus intermedius* (Table 1). This bacteria was obtained in pure growth from the eyes of the 11 cats with no clinical signs of eye discharge or lesion (Table 2). Four out of these 11 domestic short hair stray cats (53.85%) had positive isolations of *S. intermedius* from both eyes (Table 2).

Overall, in the 30 cats studied, the most predominant bacteria isolated was *S. intermedius* (36.67%) followed by *Enterobacter spp.* (6.6%) and finally *Moraxella nonliquefaciens* (3.3%).

**Table 1.** Bacteriology of cats

<b>Group</b>	<b>Vaccination</b>	<b>Total</b>	<b><i>Chlamydophila</i></b>	<b>No of cats with positive bacterial growth</b>	<b>Type of bacteria</b>	<b>Positive growth (%)</b>
1	Up To Date	11	-	2	<i>Enterobacter sp</i> and <i>Moraxella nonliquefaciens</i>	18.18%
2	Unknown	19	-	11	<i>Staphylococcus intermedius</i>	57.89%

**Table 2.** Clinical signs and lesion in cats

<b>Group</b>	<b>Vaccination status</b>	<b>Total</b>	<b>Breed</b>	<b>Eye Lesion</b>	<b>Eyes discharges</b>	<b>Unilateral (Right/Left)</b>	<b>Bilateral</b>
<u>Group 1</u>							
Male	All Vaccinated	1	Persian	-	Watery	-	1
Female		1	Persian	-	Watery	-	1
<u>Group 2</u>							
Male	All	4	DSH	-	-	-	4
Female	Unknown	7	DSH	-	-	3R,1L	3

In the present study, 43.33% of the cats in both groups had positive bacterial growth. Unlike the previous study (Campbell et al., 1973), where the species of bacteria was not documented, in this study, *Staphylococcus intermedius* was isolated from Group 2 cats only with unknown vaccination status.

Many factors such as sampling technique, geography, season and ambient temperature at the time of collection may influence the prevalence of the different species of bacteria from the eyes of cats (Gerding and Kokamo., 1990). However, this is debatable when studies reported that some of the factors did not have any influence statistically (Marilena et al., 2005; Li Wang et al., 2008). Breed, age and sex of dogs did not influence the species of bacteria that can be isolated from the conjunctiva, but which season of the year the samples were taken do have significance on the isolation of bacteria from dogs' eyes in Beijing (Li Wang et al., 2008). A study of conjunctival flora in Thoroughbred mare in Florida showed that there was no significant difference in which season sampling was conducted but age of the mare showed significance where young horses had higher percentage of isolation of gram negative bacteria from the eyes (Marilena et al., 2005)

Since *S. intermedius* had been isolated from stray cats in Group 2 only, the absence of this microorganism from Persian cats in Group 1 could be due to the lacrimal fluid which contains antibacteria such as lysozymes and wandering macrophages or rate of washing of the conjunctival membrane which influence the effective colonisation of the eyes by microorganism (Shewen et al., 1980). Also, possible previous treatment with antibiotic in Group 1 cats may explain why *S. intermedius* are present in stray cats only. However, in this study, we isolated *Enterobacter sp* and *Moraxella nonliquefaciens* in two Persian cats from Group 1 with watery eyes discharges. This can be due to the breed of the cats and also how the owner manages the hygiene practice of their cats especially litter tray management. Persian cats, especially purebred, are more prone to conjunctivitis. This is due to the anatomy of short tear duct that causes increase tear production. Tears do contain antibacterial substance but, it also can cause irritation to the conjunctiva. From the irritation, it can lead to mild inflammation of the mucous membrane and can further cause severe damage and even ulcerative keratitis due to

secondary bacteria infection. Tears also contain protein such as mucin which will favour the opportunistic bacteria to colonize the eyes (Michael et al., 2007)

In the present study, *Moraxella nonliquefaciens* was isolated from one Persian cat from Group 1. *M. nonliquefaciens* can be isolated from normal respiratory tract in humans. This bacteria rarely causes disease but there are two reports on *M. nonliquefaciens* in two immunocompromised human patients with endophthalmitis in Norway following posttrabeculectomy surgery (Laukeland et al., 2002). However, there are no reported cases of *M. nonliquefaciens* causing lesions in animal such as cats.

In this present study, no *Chlamydomphila* sp. was detected using Giemsa staining of conjunctival swabs. Since Chlamydia is an intracellular microorganism, adequate conjunctiva cells are needed to be obtained by scraping the conjunctiva with the animal under general anaesthesia to observe for inclusion body.

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