

**PREVALENCE OF FELINE IMMUNODEFICIENCY VIRUS, FELINE LEUKEMIA VIRUS AND TOXOPLASMA GONDII IN CAPTIVE TIGERS (*Panthera Tigris*) IN A WILDLIFE FACILITY IN THE PHILIPPINES**

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**ABSTRACT**

The aim of the study is to determine the prevalence of Feline Immunodeficiency Virus (FIV), Feline Leukemia Virus (FeLV) and *Toxoplasma gondii* in the captive tigers of a wildlife facility in the Philippines. Nine of the ten captive tigers (*Panthera tigris*) in the facility were used: six were adults and 3 were juvenile. Furthermore, five of these nine animals were males and four were females. There have been no reports of disease outbreaks and all the animals were apparently healthy during the time of the study as the mean ( $\pm$ SD) hematologic values were within the normal range. Of the nine samples, 33% (3/9) were exposed to *T. gondii* but none of the animals had evidence of exposure to either FIV or FeLV. Control of stray domestic short haired cats within the wildlife facility should be maintained to control disease spread and maintain the health of the captive animals.

Key words: Feline immunodeficiency virus, feline oncovirus, *Toxoplasma gondii*, tigers

**INTRODUCTION**

One of the highly pathogenic viruses that infect both domestic and wild felids is the FIV (Hayward and Rodrigo, 2008). FIV is closely related to the Human Immunodeficiency Virus (HIV) in structure, life cycle and pathogenesis (Mosallanejad *et al.*, 2010) making FIV an ideal model in the study of HIV infection (Faure, 2008). The prevalence of the infection in animals in the wild and those in captivity are of equal importance, with the latter suggested to be closely monitored (Troyer *et al.*, 2005). This is transmitted through bites.

Feline Leukemia Virus, on the other hand, is one of the most common and important infectious diseases of cats worldwide (Little *et al.*, 2009). The virus is fatal to felids and is important in the conservation of the wild felids worldwide (Filoni *et al.*, 2008). Although it is a major pathogen of domestic cats throughout the world, the infection and resulting disease are rare in wild felids (Williams and Barker, 2001). FeLV is transmitted vertically.

*Toxoplasma gondii* is a protozoan parasite that is found worldwide (Aiello and Mays, 1998). It affects a wide variety of warm blooded animals (Thompson *et al.*, 2009) including man (Little, 2008). Toxoplasmosis is an important zoonotic disease in the world, recognized by the National Institute of Health (Bethesda, USA) as a category B priority pathogen (Weiss and Dubey, 2009). The parasite is transmitted via feco-oral route, as felids are the only host to excrete environmentally viable oocyst (Marchiondo *et al.*, 1976).

This study will determine the prevalence of the aforementioned disease-causing agents in the captive tigers of a wildlife facility in Metro Manila. Captive felids rarely encounter these diseases, thus, may exhibit more grave symptoms once they got infected (Fowler, 1986). It is important to identify pathogenic agents in these animals to control infection and prevent its spread to other susceptible hosts, including man. The results of this study will also aid in the efforts to monitor the health and conserve these animals, hoping to preserve their populations for the appreciation of future generations.

## MATERIALS AND METHODS

Nine tigers (*Panthera tigris*) (six adults and three juveniles; five males and four females), currently sheltered at a wildlife facility in Metro Manila, Philippines were used in the study. A 10<sup>th</sup> tiger (adult, female) was present in the facility but was not included in the study to prevent stress to the animal since at the time of the study, it was lactating.

As a government-ran wildlife facility, most of the animals within are confiscated from or surrendered by private collectors whose permit to care for exotic animals are either missing or lapsed. Thus, the tigers arrive in the facility at different ages and in different conditions. The three juveniles were born within the facility 3 months prior to the time of the sample collection, and at that time, all adult tigers have been in their care for at least one year. The animals are fed with a mixture of raw carabeef and live chicken once a day. They are also given a carabao's leg every 15 days. Drinking water is provided *ad libitum*. Fecal examination and deworming (using Ivermectin) are done twice a year and vaccination (vs. Rabies and Feline Panleukopenia virus) is conducted annually. The individual holding cages are cleaned daily and disinfected using sodium hypochlorite. There is no apparent disease outbreaks reported yet and all the tigers were apparently healthy during blood collection.

The methods used in this study were approved and permitted by the University's Institutional Animals Care and Use Committee. Prior to blood collection, the adult tigers were placed in individual holding cages. The adult animals were chemically restrained using tiletamine hydrochloride- zolazepam hydrochloride (Zoletil® 50mg/ml, Virbac Laboratories, Carros, France), administered using a dart. The juvenile tigers, on the other hand, were physically restrained.

Approximately 5ml of blood was collected from the lateral saphenous vein of the adult animals and 3ml from the juveniles. The blood collected was divided equally for placement in sterile lavender capped vacuum tube with ethylene diaminetetraacetic acid and red capped vacuum tube. The former was used to examine the packed cell volume (PCV), total white blood cell count (tWBC), relative and absolute differential white blood cell count (dWBC), following standard hematology processes (Coles, 1986). The latter was used for the collection of serum by allowing the tube to stand for at least 30 minutes. Once clot has formed, the samples were centrifuged at 2000 rpm for 10 minutes. The serum was transferred into several aliquots to clean, labelled Eppendorf® tubes. The sera were processed using the test kit.

Commercial self-contained portable test kits for FIV (ImmunoRun® Antibody Test Kit, BioGal Laboratories, Israel), FeLV (ImmunoRun® Antigen Detection Kit, BioGal Laboratories, Israel) and *Toxoplasma gondii* (ImmunoComb® IgG Antibody Test Kit, BioGal Laboratories, Israel) were used in this study. The antibody test kits are based on

a solid phase immunoassay principle on which purified FIV and *Toxoplasma* antigens are attached. Sensitivities of the tests for FIV, FeLV and *Toxoplasma* are 96.8%, 94.7% and 92.3%, respectively; with specificities of 99.7%, 99.7% and 100%, respectively. The test kits were used as per manufacturer's recommendations. Digital scanning software (BioGal CombScan® 2000, BioGal Laboratories, Israel) was used to interpret the results of the test kits for antibodies, while the results of the antigen test kit was interpreted based on the manufacturer's qualitative interpretation.

## RESULTS AND DISCUSSION

Table 1 shows the mean ( $\pm$ SD) values of the hematologic profile of the nine captive tigers used in this study. These values are within the normal range, confirming that the animals used in the study were apparently healthy and that these animals were not incubating an infection at the time of the study.

Table 2 shows the summary of results of the study. Results show that none of the nine captive tigers have serologic evidence of exposure to either FIV or FeLV. The absence of antibodies indicates that these animals have neither previous exposure to, nor active infection by, these viruses.

Since all of the nine tigers tested negative for both FIV and FeLV, the study cannot determine nor generalize on whether age and sex are predisposing factors in the contraction of infection. Other authors (Birchard and Sherding, 1994; Mosallanejad *et al.*, 2010) have found these pathogens to be most common in cats within the ages of four to seven years old. Males, according to Lee *et al.* (2002), are at increased risk of exposure to the viruses as they more often exhibit roaming and territorial behaviors (Lee *et al.*, 2002). Since the subject animals are kept in a concrete enclosure that prevents the animals from roaming, contraction of infection from an outside source is minimal. Furthermore, because these animals have been reared together, territoriality is minimally observed thus aggression, biting and fighting are also minimized.

**Table 1. Hematologic values of the captive tigers (*Panthera tigris*) (n=9) from the wildlife facility in Manila, Philippines.**

Parameters	Mean ( $\pm$ SD)	
	Adult (n=6)	Juvenile (n=3)
PCV (%)	37.5 (0.36)	33.67 (0.38)
tWBC ( $\times 10^3$ cells/ $\mu$ L)	14.00 (0.74)	11.16 (0.56)
dWBC ( $\times 10^3$ cells/ $\mu$ L):		
Neutrophils	11.87 (0.74)	9.33 (0.50)
Lymphocytes	1.49 (0.25)	1.38 (0.20)
Monocytes	0.32 (0.10)	0.11 (0.07)
Eosinophils	0.25 (0.17)	0.18 (0.06)
Basophils	None observed	None observed

**Table 2. Frequency distribution of captive tigers (*Panthera tigris*) (n=9) with serologic evidence of exposure to three feline pathogens using commercially available antibody (Ab) and antigen (Ag) test kits.**

	Adult (n=6)		Juvenile (n=3)		Total (n=9)
	Male (n=4)	Female (n=2)	Male (n=1)	Female (n=2)	
FIV Ab	0	0	0	0	0 (0%)
FLV Ag	0	0	0	0	0 (0%)
<i>T. gondii</i> Ab	1	2	0	0	3 (33%)

Three of the nine (33%) tigers tested had serologic evidence of exposure to the parasite *Toxoplasma gondii*. Of the three tigers, one was male and the other two were females, all of which were adults. The test which detects IgG confirms previous exposure or infection, but not clinical disease. One possible source of the parasite is the population of stray domestic short haired cats found within the vicinity of the same wildlife facility that Reyes *et al.* (2013) reported to be 47% positive (14/ 30) for the said parasite.

None of the tigers showed signs of the disease. *T. gondii* usually invades the hosts without producing clinical signs and rarely cause severe clinical manifestations (Samuel *et al.*, 2001). However, clinical toxoplasmosis and oocyst shedding has been reported in captive wild felids including tigers (Marchiondo *et al.*, 1976; Lukesova and Literak, 1997; Silva *et al.*, 2001; Gonzales *et al.*, 2007; De Camps *et al.*, 2008). This is the first study that demonstrated the seroprevalence of *T. gondii* in captive tigers in the Philippines. The result suggests a significant exposure rate in these tigers and is of great importance because felids are capable of shedding oocysts on the environment (Gonzales *et al.*, 2007).

Since the captive tigers in this facility has been previously exposed to *T. gondii*, care should be taken not to induce disease outbreak via stress since these diseases are immune mediated. The felines in the facility are currently FIV- and FeLV-free, and this should be maintained.

Quarantine of newly acquired felids should be carried out. These animals may be from other wildlife facilities or those animals coming from private collectors or rescued animals with no medical history available. This is done to minimize possible disease transmission. Routine physical examination and routine serologic testing may be performed to fully assess the condition of the animals. Animals found with serologic evidence of exposure to any of these agents should be separated, or better yet, transferred to a different facility, equipped to provide the necessary care for these animals.

The domestic cat population within the area should also be considered. Although the possibility of tigers acquiring the infection from domestic cats is minimal, the possibility should not be ignored. The population control, elimination and methods to prevent the replacement of removed domestic cats should be strictly implemented. Preventing the contact of domestic cats with the tigers and other felids in captivity can also help reduce cross contamination within the facility.

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