

Research note

DETECTION OF ANTIBODIES AGAINST *Toxoplasma gondii* AND *Chlamydophila felis* IN MALAYAN CIVETS (*Viverra zangalunga*), PALAWAN BEARCATS (*Arctictis binturong whitei*) and ASIAN PALM CIVETS (*Paradoxurus hermaphroditus*) AT A WILDLIFE FACILITY IN QUEZON CITY, PHILIPPINES

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ABSTRACT

Twelve captive civets composed of two Malayan Civets (*Viverra zangalunga*), four Palawan Bearcats (*Arctictis binturong whitei*) and six Asian Palm Civets (*Paradoxurus hermaphroditus*) from the Protected Areas and Wildlife Bureau - Wildlife Rescue Center, Quezon City, Philippines were used in the study. Blood was collected and the presence of antibodies against *Toxoplasma gondii* and *Chlamydophila felis* were detected using an ELISA-based kit. Five out of 12 civets showed antibodies against *T. gondii* while two out of 12 showed antibodies against *C. felis*. The results of this study confirmed the presence of *T. gondii* and *C. felis* antibodies in captive civets in the wildlife facility. The zoonotic potential of these organisms should be investigated and management within the rescue center should be reviewed to minimize the occurrence of the organisms.

Keywords: *Chlamydophila felis*, civets, ELISA, *Toxoplasma gondii*, zoonosis

INTRODUCTION

Toxoplasmosis and chlamydiosis are among the most prevalent and widespread parasitic and bacterial infections in warm-blooded animals (Everett, 2000; Wolfe, 2003). In the Philippines, previous studies were done to detect their presence in different animal hosts. *Toxoplasma gondii* has been detected in macaques (Asai *et al.*, 1991), *Rattus* spp. (Salibay and Claveria, 2005), and among stray and household cats (Advincula *et al.*, 2010). *Chlamydophila* (*Chlamydia*) *psittaci* has been detected in macaques (Asai *et al.*, 1991) and captive birds (Maluping *et al.*, 2007).

Toxoplasmosis is a zoonotic infection in humans acquired by handling or

eating undercooked or raw meat contaminated by cysts or ingestion of water or food contaminated with oocysts (Sibley *et al.*, 2009). Chlamydiosis is caused by an obligate intracellular bacterium *Chlamydomphila felis*. It is usually disseminated by aerosol or by direct contact (Everett *et al.*, 1999). The cat is the primary host of the strains identified and the organism is endemic among housed cats worldwide.

Considering the zoonotic potential of *Toxoplasma* and *Chlamydomphila*, exposed and immuno-compromised individuals may be at risk of contracting the disease. In humans, antibodies against *T. gondii* has been detected in rural (Mindoro and Leyte), suburban (Cavite), and urban (Manila) residents (Auer *et al.*, 1995; Kawashima *et al.*, 2000; Salibay *et al.*, 2008) while there were no reports available for *C. psittaci*.

The Malayan Civet (*Viverra zibellina*) and the Asian Palm Civet (*Paradoxurus hermaphroditus*) are both widespread in Asian countries and listed by the International Union for Conservation of Nature and Natural Resources (IUCN) as "Least Concern" because of its stable population. The bearcat, on the other hand, is also found widespread in South and Southeast Asian countries. Special attention is given, however, to a subspecies endemic to the Philippines, the Palawan Bearcat (*Arctictis binturong whitei*). The species is listed by the IUCN as "Vulnerable."

The three species used in this study have been scheduled for release into the wild, hence the need for testing. The presence of *T. gondii* and *C. felis* in captive civets in the Philippines has neither been demonstrated nor confirmed and the data gathered will be helpful in the treatment and management of the animals at the rescue center, for future release activities and monitoring for zoonotic outbreak.

MATERIALS AND METHODS

Twelve captive animals composed of two Malayan Civets, four Palawan Bearcats and six Asian Palm Civets from the Protected Areas and Wildlife Bureau - Wildlife Rescue Center (PAWB-WRC), Quezon City, Philippines were used in the study. Purposive sampling was used for the Asian Palm Civets as these were animals scheduled for release. Convenience sampling for Palawan bearcats and the Malayan Civets was done because they represented the whole population at the PAWB-WRC.

Each civet was sedated with 2.5 mg/kg tiletamine-zolazepam (Zoletil 50[®], Virbac, Carros, France) intramuscularly. Manual restraint using a net was employed. The area over the cephalic vein was shaved and disinfected and 1 ml of blood was collected using 3 ml syringes with 23-gauge needle. Blood samples were transferred to a clean vial with EDTA as an anticoagulant and were properly labeled with sample numbers corresponding to each animal.

The Immunocomb[®] ELISA Feline *Toxoplasma* and *Chlamydomphila* antibody test kit (Biogal, Kibbutz Galed, Israel), a self-contained portable test kit for IgG antibodies that are specific for *Toxoplasma* and *Chlamydomphila*, was used in the study. The principle for this test kit is the phase immunoassay involving the antigen. The sensitivity for the *Toxoplasma* test is 92.3% while for the *Chlamydomphila* test is 94.7% with a specificity of 100% for both *Toxoplasma* and *Chlamydomphila* tests.

Manufacturer's directions were followed in running the ELISA test.

The CombScan 2000® (Biogal, Kibbutz Galed, Israel) software was used to provide both qualitative and quantitative results. It runs behind the principle of image analysis by detecting and reading the color results of the ELISA kit and translates it to numerical values. The software, through a scanner, translated the color results of the ELISA kit into numerical values with corresponding interpretations.

RESULTS AND DISCUSSION

Out of 12 captive civets, five demonstrated antibodies against *T. gondii*. Seven civets were seronegative (Table 1). Antibodies against *C. felis* revealed two civets had a CombScan® reading of 1 (suspicious) while ten civets were seronegative (Table 2).

Table 1. CombScan reading of blood samples from the Malayan Civet, Asian Palm Civet and Palawan Bearcat tested for *Toxoplasma gondii*.

Reading	IgG (IF) titer	Malayan Civet		Asian Palm Civet		Palawan Bearcat		Total
		M	F	M	F	M	F	
0	0	-	1	3	2	1	0	7
1	<1:16	-	1	-	1	1	1	4
2	1:32	-	-	-	-	-	1	1
3	1:66	-	-	-	-	-	-	-
4	>1:128	-	-	-	-	-	-	-
Total		-	2	3	3	2	2	12

CombScan reading based on CombScan® 2000: 0 (Negative); 1 (suspicious); 2 (positive); 3 (medium positive); 4 (High Positive).

M: male; F: female.

The presence of antibodies against *T. gondii* and *C. felis* in the captive civets does not necessarily mean that these animals have clinical disease (Wolfe, 2003). However, it may indicate that these animals may have been exposed to *T. gondii* and/or *C. felis* either from the area or even before they were brought to the PAWB-WRC since these civets were confiscated, rescued or turned-over to the rescue center.

Table 2. CombScan reading of blood samples from the Malayan Civet, Asian Palm Civet and Palawan Bearcat tested for *Chlamydomphila felis*.

Reading	IgG (IF) titer	Malayan Civet		Asian Palm Civet		Palawan Bearcat		Total
		M	F	M	F	M	F	
0	0	-	2	2	3	1	2	10
1	<1:16	-	-	1	-	1	-	2
2	1:32	-	-	-	-	-	-	0
3	1:66	-	-	-	-	-	-	-
4	>1:128	-	-	-	-	-	-	-
Total		-	2	3	3	2	2	12

CombScan reading based on CombScan® 2000: 0 (Negative); 1 (suspicious); 2 (positive); 3 (medium positive); 4 (High Positive).

M: male; F: female.

Advincula *et al.*, (2010) demonstrated *T. gondii* antibodies in 11 out of 30 (18.33%) and 17 out of 30 (28.33%) stray and household cats, respectively. These cats may spread the infection to susceptible animals and more importantly to humans. Dans (2002), using the feral cat population at PAWB-WRC, reported 13 out of 20 (65%) seropositive animals for *T. gondii*. Although those cats were destroyed, the possibility that the present feral population may still harbor the parasite and infect other susceptible animals, including the civets, is likely. Thus, retesting the present feral cat population may be necessary.

Other warm blooded animals may also become alternative hosts, such as the *Rattus* spp. which showed 87 (55.0%) seropositivity and was confirmed through a bioassay in *Mus musculus* (Salibay and Claveria, 2005; Salibay and Claveria, 2006). A survey of toxoplasmosis in rats within the PAWB-WRC can be done since they can also become a source of the infection for the feline population, other susceptible animals and man.

Maluping *et al.* (2007) demonstrated antibodies against *C. psittaci* among captive birds in the area. On the other hand, Perez (2012), in the same institution showed seronegative antibodies against *C. psittaci* using the feline *Chlamydia* test kit in Philippine Eagle Owls (*Bubo philippinensis*) but with suspicious results on the avian *C. psittaci* test kit. Cross contamination occurs between strains of *Chlamydia* (Flammer, 2003); thus, detection of different strains may be helpful. The presence of *Chlamydomphila* in the area can be a source of exposure, antibody production and possibly infection among animals kept at the rescue center.

The cages of the different civets were situated next to each other. This can contribute to the possible transmission of the disease to healthy individuals. Feral cats have access to animal holding areas and may become carriers of the disease and become a source of future outbreaks.

Animals that are either confiscated or brought in for rehabilitation should be screened for toxoplasmosis and chlamydiosis. Animals that test positive for either disease should be given proper medication, or destroyed if the disease is in the advanced stages. Animals infected should not be released back into the wild because they may spread the infection to other susceptible wildlife species. Zoonotic potential of toxoplasmosis and chlamydiosis should also be considered. Visitors, workers, clinicians and veterinarians frequenting the area should uphold biosecurity measures. Proper quarantine facilities should be available and protocols should be set in place.

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