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SHORT COMMUNICATION

Serological examination of Philippine bats for *Histoplasma capsulatum*

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ABSTRACT

Histoplasmosis is a disease of medical and veterinary concern. Bats are considered as reservoir hosts of the etiologic agent *Histoplasma capsulatum*, and are therefore used as animal subjects in experimental studies. Thirty six bats, consisiting of nine bats (Microchiroptera: Vespertilionidae) from Aklan, 22 bats (Megachiroptera: Pterodidae) from Quezon City and another five bats (Microchiroptera: Vespertilionidae) from Quezon City, Philippines were tested for the presence of precipitating antibodies to *H. capsulatum* using immunodiffusion assay. Results revealed that none of the bats tested were positive for *H. capsulatum* in agar gel precipitation assay. This may be due to no previous exposure of the bats to the pathogen, exposure to the pathogen with insufficient time for seroconversion (infection time < 3 - 5 weeks) prior to capture or the bats were resistant to infection. Any one of the aforementioned factors may have contributed to the absence of antibodies or low undetectable levels of circulating antibodies for *H. capsulatum* in the tested bats. This is the very first attempt at field collecting and monitoring for precipitating antibodies for *H. capsulatum* in Philippine wildlife, particularly in Philippine bats.

Key words: Immunodiffusion assay, wildlife, antibodies, Aklan, Quezon City

INTRODUCTION

Bats are associated with important public health hazards and serve as an important wildlife reservoir of many emerging and re-emerging infectious agents. One of these infectious agents is *Histoplasma capsulatum* which causes asymptomatic to chronic disease in bats and humans. Among all the mammals infected, only bats are considered to manifest a significant role in the epizootiology of this mycosis (Hoff and Bigler, 1981). At least 70 species of the 1,001 species of bats known in the world (Mickleburgh *et al.,* 2002) inhabit the Philippines. Studies on detection of *H. capsulatum* in bats have not been carried out previously in the Philippines. Histoplasmosis has emerged in the Western Hemisphere and is highly endemic in the Ohio River and Mississippi River valleys of the United States (Lyon *et al.,* 2004). In Asia, prevalence of histoplasmin hypersensitivity has been rarely reported. In some parts of Southeast Asia, a reaction rate of 30% has been documented (Navy, 2005) while it ranged from 0-2% in China, Japan, and Hongkong and from 63-86% in Indonesia and Burma (Navarro *et al.*, 1992). Malaysia has a lower prevalence (4-43%) compared to Thailand (Houston, 1994). However, reported cases in Malaysia suggested a higher prevalence of histoplasmosis than in the published literature (Navy *et al.,* 2005). Most cases of histoplasmosis in non-endemic areas are underreported due to generalized non-specific symptoms observed among cases (Navarro *et al.*, 1992).

In the Philippines, cases of exposure to *Histoplasma* species were reported in poultry. A study on the incidence of histoplasmin hypersensitivity in long-term residents in Manila, specifically among the Manila Electric Company workers, implied that histoplasmosis is very rare in the Philippines but adequately present to be able to infect 25% of the test population (Bulmer and Bulmer, 2001). Serodiagnostic tests for antibodies in mycotic diseases which include immunodiffusion test and complement fixation are routinely used as screening tools. This preliminary investigation was carried out to detect the presence of antibodies in the sera of Philippine bats through immunodiffusion test.

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MATERIALS AND METHODS

Study animals

A total of 36 apparently healthy bats, 19 females and 17 males, were used in the study. Nine bats were collected from Pangihan caves in Barangay Pablacion, Malay and Libertad caves in Barangay Libertad, Nabas, Aklan using mobile nylon mist nets (3 m long and 1.5 m high with 35 mm mesh size) that were set up inside and at the entrance to the caves. Another 27 bats were captured in Diliman, Quezon City using seven nylon mist nets (12 m long and 2 m high with 35 mm mesh size) placed near swampy areas for two nights.

Species identification

The 36 bats used in the study were identified as belonging to seven species according to Philippine bat identification key of Ingle and Heaney (1992). These were *Miniopterus australis* (Tomes, 1858), *M. schreibersii* (Kuhl, 1817), *Cynopterus brachyotis* (Müller, 1838), *Eonycteris spelaea* (Dobson, 1871), *Rousettus amplexicaudatus* (Geoffroy, 1810), *Ptenochirus jagori* (Peters, 1861) and *Scotophilus kuhlii* (Leach, 1821).

Collection of blood samples

The body weight of each captured bat was recorded and the dosage of anesthetic drug was computed accordingly (0.45 ml of 5% zolazepam-tiletamine per 30 g body weight). The anesthetic was administered intramuscularly and the bats were euthanized through intracardiac exsanguination. The body parameter measurements of the carcasses were recorded for specimen identification.

Each carcass was placed on a necropsy board where the skin over the thorax and abdomen was reflected. The thorax was opened and the internal organs were collected by research collaborators from abroad for other investigative works. For the present study, blood was transferred into 1.5 ml pre-labeled microtubes and centrifuged for 5 min to collect the serum. The sera were transferred to new microtubes, stored in liquid nitrogen during transport and then stored at -40°C in the laboratory.

Immunodiffusion assay

The immunodiffusion assay was carried out according to manufacturer's instructions (Immunomycologics Inc., Norman, USA). Briefly, 34 µl of sera from each bat was inactivated by heating at 56°C for 30 min. Inactivation was done to inhibit the lytic activity and to increase precipitation as well as turbidity for better visualization in the presence of identical antibody-antigen complex. The sera were allowed to cool at room temperature (26°C). Two wells in each plate were filled with approximately 30 µl of the positive control (goat antisera) containing antibodies for both H and M antigen of *H. capsulatum.* One well was filled with 30 µl of phosphate buffer saline as negative control. This was repeated for the rest of the plates and the plates were incubated for 30 min. The central well was filled with 30 µl of a mycelial phase culture filtrate of *H. capsulatum* with H and M antigens. The plates were incubated inside a moisture chamber for 24 h and checked for precipitation bands. Results were confirmed after 48 h of incubation.

RESULTS

The 36 bats collected comprised 19 females and 17 males that belonged to seven species under two families, Vespertilionidae (3/7) and Pteropodidae (4/7). These were represented by two species of insectivores, *Miniopterus australis* and *M. schreibersii* captured from Aklan, four species of frugivores, *Cynopterus brachyotis*, *Eonycteris spelaea*, *Rousettus amplexicaudatus*, *Ptenochirus jagori* and one species of insectivore, *Scotophilus kuhlii* captured from Diliman, Quezon City.

An evident immune-precipitation reaction (exemplified by the appearance of bands) between positive control and H and M surface glycoproteins or antigens of *H. capsulatum* represent the expected positive test for the presence of precipitating antibodies to *H. capsulatum.* No precipitating antibodies to *Histoplasma capsulatum* were observed in any of the 36 bat sera tested using the agar gel precipitation method.

DISCUSSION

Histoplasmin (HMIN) is the antigenic component from the mycelial phase of *H. capsulatum* and is composed of H, M and C antigens. HMIN is widely used to assess both humoral and cell-mediated immune responses in patients with histoplasmosis (Guimaraes *et al.*, 2006). The H and M antigens are highly specific to *H. capsulatum* antibodies that equates to its usefulness in the diagnosis of the disease (Hook and Fife, 1967).

The M antigen is an immunodominant antigen that appears first in acute phase of

infection and is present throughout the progression of the disease (Guedes *et al.,* 2003). Hence, the antibodies to M antigen are present in both active and chronic histoplasmosis while the antibodies to H antigen appear only during an active infection (Yeo and Wong, 2002). The presence of antibodies to M antigen also signifies a past infection, a recovery stage or a result of histoplasmin hypersensitivity test (Green *et al.,* 1976).

Non-culture methods have been developed to aid in the diagnosis of diseases in a shorter time possible. These tests include detection of antibody or antigen which offers presumptive diagnosis. The detection of antibodies is considered to be a major tool currently in use. Two of the routine methodologies used are complement fixation and immunodiffusion. The latter is a rapid and excellent qualitative test that provides presumptive diagnosis for histoplasmosis. It is highly specific and almost as sensitive as complement fixation test. It has a sensitivity of 70-100% and specificity of 100% (Guimaraes *et al.,* 2006).

In the study, a total of 36 apparently healthy bats were serologically examined for the presence of precipitating antibodies to *H. capsulatum.* However, none of the tested samples from bats captured from urban and rural collection sites was positive for the presence of antibodies for M and H antigens of *H. capsulatum.*

There are no previous records of infection with *H. capsulatum* in the seven bat species tested in this study, although some reports are available for other bat species (Klite and Young, 1965; Hoff and Bigler, 1981; Mok *et al.,* 1982; Taylor *et al.,* 1999; Taylor *et al.,* 2000; Ulloa *et al.,* 2006). It is possible that the seven species of bats tested were either resistant or not susceptible to the pathogen. All species of bats in Philippines have not been tested against histoplasmosis. The absence of precipitating antibodies against *H. capsulatum* suggests that the tested bats had no previous exposure to the pathogen. However, the presence of infection in other bats in the natural population in the Philippines cannot be ruled out.

Literature on cave ecology have correlated cave conditions with the presence of *H. capsulatum* in bats (Taylor *et al.,* 1999). However, only 25% of the tested bats in the study were cave dwellers. The absence of antibodies to *H. capsulatum* in test bats suggests that they have not mounted an immune response to the pathogen or may not have been previously exposed to the pathogen. In case of previous exposure, it may be that the bats have not passed the three to five-week period needed for the development of detectable levels of antibodies.

Immunodiffusion assay is a diagnostic test acceptable as a screening method. However, other methods of detection such as enzyme-linked immunosorbent assay, radioimmunoassay and polymerase chain reaction assay are recommended for confirmation.

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