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Brief Report

Use of filter papers to determine seroprevalence of *Toxoplasma gondii* among hunted ungulates in remote Peruvian Amazon

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Lowland tapir (*Tapirus terrestris*)

ABSTRACT

Toxoplasmosis is a zoonosis caused by the protozoan *Toxoplasma gondii*, and it is found worldwide. To determine whether ungulates are reservoirs of *T. gondii* in an isolated and remote region of the northeastern Peruvian Amazon, antibodies to *T. gondii* were determined in 5 species of ungulates by the modified agglutination test (MAT). These animals were hunted by subsistence hunters along the Yavarí-Mirín River, in the northeastern Peruvian Amazon. Blood samples were collected by hunters on filter papers. For determination of *T. gondii* antibodies, blood was eluted from filter papers, and a titer of 1:25 was considered indicative of exposure to *T. gondii*. Antibodies to *T. gondii* were found in 26 (31.0%) peccaries (*Pecari tajacu*, *Tayassu pecari*), six (17.1%) brocket deer (*Mazama americana*, *Mazama gouazoubira*), and four (40.0%) lowland tapir (*Tapirus terrestris*). We also introduced a modification to the MAT protocol that allows the extraction of fluid samples from several types of laboratory-grade filter paper, thus enabling researchers to easily adapt their approaches to the materials presented to them.

1. Introduction

Toxoplasmosis is a zoonosis caused by *Toxoplasma gondii*, an obligate intracellular protozoan that infects warm-blooded animals (Montoya and Liesenfeld, 2004; Dubey, 2010). The two most important modes of transmission of the parasite are the consumption of raw or undercooked meat contaminated with tissue cysts and ingestion of oocysts shed by felids in the environment (Dubey, 2010). Felids are the definitive hosts of *T. gondii* and consequently are the only source of oocysts found in the environment (Dubey, 2010). Infection with *T. gondii* can also occur via congenital transmission and organ transplantation (Montoya and Liesenfeld, 2004). In immunocompetent humans, toxoplasmosis is generally asymptomatic but may result in transient fever and lymphadenopathy. Especially in immunocompromised patients, however, toxoplasmosis may be life-threatening, and infection of pregnant women with *T. gondii* may lead to congenital defects (Montoya and Liesenfeld, 2004).

The collection of whole blood on filter paper has been demonstrated in a variety of serological studies around the world and has been proven to be a cost-effective method for surveillance because it eliminates the need for storage and transport under cold conditions; therefore, this quality may appeal especially to low-income countries, especially if samples are taken in remote areas (Andriamandimby et al., 2013).

Little is known of the epidemiology of *T. gondii* infections and clinical toxoplasmosis in animals in Peru (Dubey, 2010). The objective of this study was to determine the occurrence of *T. gondii* exposure among 129 hunted ungulates in the Peruvian Amazon and to introduce a modification to the modified agglutination test (MAT) protocol that allows the extraction of fluid samples from several types of laboratory-grade filter paper. Because the study

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was conducted in an isolated and remote area of the Peruvian Amazon, data obtained from this study are likely to shed light on the maintenance of *T. gondii* in its natural setting.

2. Materials and methods

2.1. Study area

The Yavarí-Mirín River in the Peruvian Amazon is on the international border between Peru and Brazil, between the Tamshiyacu-Tahuayo Conservation Regional Area and the Lago Preto Conservation Concession, opposite the world's largest indigenous reserve in Brazil (Fig. 1). This area harbors the highest diversity of animals in the Amazon, being especially rich in primates and amphibians, is a source area for game animals, which disperse to more hunted areas, and is home to Yagua indigenous communities and other indigenous people in voluntary isolation. Nueva Esperanza (04°19′53″S; 71°57′33″W; UT-5:00), an indigenous Yagua community, is the only permanent settlement located along the Yavarí-Mirín River.

The climate in the region is typically equatorial with an annual temperature from 22–36 °C, a relative humidity from 80–100%, and an annual rainfall from 1500–3000 mm. Seasons are defined as dry (January–February and July–September) and wet (March–June and October–December).

2.2. Sample collection

From 2007 to 2012, blood samples were collected by subsistence hunters, who were trained by the researchers in proper post-mortem blood collection techniques during the first hunts involved in the study. Upon return of the dead animal to the community and during removal of the visceral organs, a filter paper was saturated in blood, which was then sealed in a plastic bag with desiccant and left inside the house in indoor conditions from seven days to four months before transfer to storage in Lima at −70 °C.

Samples included dried blood spots on filter paper from 84 peccaries (*Pecari tajacu*, *Tayassu pecari*), 35 brocket deer (*Mazama americana*, *Mazama gouazoubira*), and 10 lowland tapir (*Tapirus terrestris*). These species consume diets of fruit supplemented by browse (Black and Vogliotti, 2008; Gongora et al., 2011; Luxenberg, 2011; Arkive, 2013b; Keuroghlian et al., 2013). Habitat destruction and hunting pressure are important threats to their survival (Black and Vogliotti, 2008; Naveda et al., 2008; Keuroghlian et al., 2013).

These species were selected for the study because they are considered by Carme et al. (2002) as terrestrial, or ground-dwelling, species; the prevalence of *T. gondii* for terrestrial species was shown to be significantly higher than for arboreal or arboreal/terrestrial animals. Their feeding habits suggest that these species are similarly predisposed to infection with *T. gondii* oocysts found in the environment, which are excreted from wild felids and may contaminate water sources and wash up on stream banks, where they may remain infectious for years (Carme et al., 2002). To further focus the study, the sample population included only ungulates, which comprise a sizeable proportion of animals hunted near Nueva Esperanza. Samples were collected subject to the subsistence needs of the community and the availability of game, which explain the large variation in sample size among species.

2.3. Elution procedures and serological testing for T. gondii antibodies

Four types of filter papers by Whatman™ GE Healthcare Life Sciences (Uppsala, Sweden) were used: FTA™ cards, 903 Protein Saver Snap-apart cards, and cellulose filters (Grade 2: 8 μm, Grade 3: 6 μm). The manufacturer provided the blood concentration for FTA™ and Protein Saver (PS) Snap-apart cards; the blood

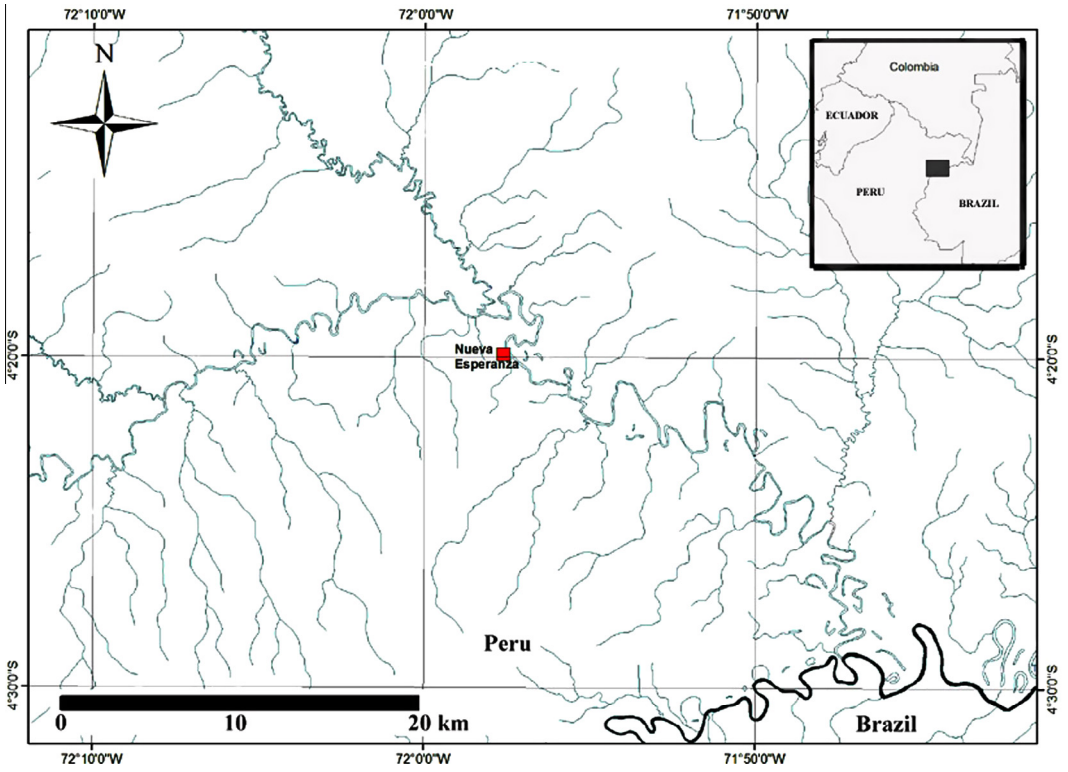

Fig. 1. Animals were hunted in the area surrounding the community of Nueva Esperanza, the only permanent settlement located along the Yavarí-Mirín River. The study site's geographic remoteness reflects extremely limited contact with domestic animals and humans, which eliminates the effect of spillover from domestic animals as a potential source of infection for wildlife. The data from this study are likely to shed light on the maintenance of *T. gondii* in its natural environment.