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Biologic and genetic characteristics of *Toxoplasma gondii* isolates in free-range chickens from Nicaragua, Central America

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Abstract

The prevalence of *Toxoplasma gondii* in free-ranging chickens is a good indicator of the prevalence of *T. gondii* oocysts in the soil because chickens feed from the ground. The prevalence of *T. gondii* in 98 free-range chickens (*Gallus domesticus*) from Nicragua was determined. Antibodies to *T. gondii* were assayed by the modified agglutination test (MAT), and found in 84 (85.7%) of 98 chickens with titers of 1:5 in 10, 1:10 in eight, 1:20 in seven, 1:40 in nine, 1:80 in 11, 1:160 in one, 1:200 in 27, 1:400 in six, 1:800 four, and 1:3200 in one bird. Hearts and brains of 32 chickens with titers of 1:10 or less were pooled and fed to three *T. gondii*-free cats. Hearts and brains of 66 chickens with titers of 1:20 or higher were bioassayed in mice. Feces of cats were examined for oocysts. The cat fed tissues from eight chickens with titers of 1:10 shed *T. gondii* oocysts. The two cats fed tissues of 24 chickens with titers of 1:5 or less did not shed oocysts. *T. gondii* was isolated by bioassay in mice from 47 chickens with MAT titers of 1:20 or higher. All infected mice from six isolates died of toxoplasmosis. Overall, 41 of 170 (24.1%) mice that became infected after inoculation with chicken tissues died of toxoplasmosis. Genotyping of these 48 isolates (47 from mice and 1 from pooled tissues) using polymorphisms at the loci SAG1, SAG2, SAG3, BTUB and GRA6 revealed eight genotypes. Six isolates had Type I alleles, three isolate had Type II alleles and six isolates had Type III alleles at all loci. Four isolates had mixed infections. Two isolates have a unique allele at SAG1 locus and combination of I and III alleles at other loci. The rest 27 isolates contained the combination of Type I and III alleles and were divided into four genotypes. More than one genotypes were often isolated in chickens from the same household, indicating multiple genotypes were circulating in the same environment. This may explain the high frequency of mixed infections observed. High rate of mixed infection in intermediate hosts such as chickens may facilitate genetic exchange between different parasite lineages in definitive feline hosts. This is the first report of genetic characterization of *T. gondii* isolates from Nicragua, Central America.
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Keywords: *Toxoplasma gondii*; Chickens; *Gallus domesticus*; Free-range; Nicragua; Central America; Genotype

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1. Introduction

Toxoplasma gondii infections are widely prevalent in human beings and animals worldwide (Dubey and Beattie, 1988). Humans become infected post-natally by ingesting tissue cysts from undercooked meat, consuming food or drink contaminated with oocysts, or by accidentally ingesting oocysts from the environment. However, only a small percentage of exposed adult humans develop clinical signs. It is unknown whether the severity of toxoplasmosis in immunocompetent persons is due to the parasite strain, host variability, or to other factors.

T. gondii isolates have been classified into three genetic types (I, II, III) based on restriction fragment length polymorphism (RFLP) (Ajzenberg et al., 2002a,b, 2004; Aspinall et al., 2003; Boothroyd and Grigg, 2002; da Silva et al., 2005; Dubey et al., 2004a,d; Ferreira et al., 2004, 2006; Fuentes et al., 2001; Grigg et al., 2001; Howe and Sibley, 1995; Howe et al., 1997; Jungersen et al., 2002; Mondragon et al., 1998; Owen and Trees, 1999). The parasite was previously considered clonal with very low genetic variability. However, most of the information was derived from isolates from Europe and North America. Using newer markers for genetic characterization and using recently isolated strains from Brazil and French Guyana, higher genetic variability was revealed than previously reported (Ajzenberg et al., 2004; Lehmann et al., 2004).

We have initiated a worldwide study of *T. gondii* population structure. For this we have chosen the free-range chicken as the indicator host for soil contamination with *T. gondii* oocysts because they feed from the ground (Ruiz and Frenkel, 1980). Thus far, we have characterized strains from South America (Brazil [Dubey et al., 2002; Dubey et al., 2003a; Dubey et al., 2003d; Dubey et al., 2006a], Peru [Dubey et al., 2004b], Venezuela [Dubey et al., 2005h], Argentina [Dubey et al., 2003e; Dubey et al., 2005f]), Colombia [Dubey et al., 2005d], Chile [Dubey et al., in press-a]; Central America and the Caribbean (Guatemala [Dubey et al., 2005e], Grenada, West Indies [Dubey et al., 2005b], Costa Rica [Dubey et al., 2006c), North America (USA [Dubey et al., 2003c; Lehmann et al., 2003], Mexico [Dubey et al., 2004c]), Africa and Middle East (Egypt [Dubey et al., 2003b], Israel [Dubey et al., 2004e], Mali, Kenya, Burkina Faso, and Democratic Republic of Congo [Dubey et al., 2005a]), Asia (Sri Lanka [Dubey et al., 2005g], India [Sreekumar et al., 2003]), Europe (Austria [Dubey et al., 2005c], and Portugal [Dubey et al., 2005, 2006b]). These studies are still not complete, nevertheless, a pattern is emerging that isolates from Brazil are genetically distinct (Lehmann et al., 2004).

Before the recognition of three genotypes of *T. gondii* (Howe and Sibley, 1995), *T. gondii* isolates were phenotypically classified as mouse virulent or avirulent. Type I strains were considered mouse virulent whereas Type II and Type III strains were avirulent or mildly virulent for mice (Howe and Sibley, 1995); Type I strains killed all mice within 2 week post-inoculation (p.i.), irrespective of the dose. However, these data are based on isolates that have been maintained in mice for an unknown time (Howe and Sibley, 1995). There are very few data on mouse mortality based on primary isolations. We have started to accumulate such data based on isolates from chickens using a specified protocol (subcutaneous inoculation of tissue digest into four to five SW mice).

In the present paper, we report on biologic and genetic characteristics of *T. gondii* isolates from chickens from Nicragua, Central America.

2. Materials and methods

2.1. Naturally-infected chickens

In Nicaragua smallholder poultry production is wide-spread. Thus, 71% of 199,549 rural households kept 1,269,116 adult hens, 241,296 roosters, and 1,479,204 replacements in the most recent agricultural census (CENAGRO, 2002). These chickens are kept free-range without fencing and only housed at night. For the present study, samples ($n = 98$) were obtained from free-range chickens from the El Sauce municipality (Fig. 1) within a radius of 10–15 km in different directions from El Sauce town (latitude 12°53′13N and longitude 86°32′17 W). The chickens originated from 36 different households that were at least 500 m apart. Eighteen households provided one chicken, six provided two, three provided three, one provided four, two provided five, two provided six, three provided eight and one household provided eleven chickens (Table 1).

Chickens were purchased, identified and housed together until they were killed on 28 November 2005. Samples of brain, whole heart, and blood were collected from each chicken, and kept at 4 °C until sent with cold packs by air to Beltsville, MD. Two days elapsed between killing of chickens and receipt of samples at Beltsville. Samples were received in excellent condition.

2.2. Serological examination

Sera of chickens were tested for *T. gondii* antibodies using eight dilutions, from 1:5 to 1: 640 with the