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Genetic and biologic characteristics of *Toxoplasma gondii* infections in free-range chickens from Austria

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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 free-range chickens (*Gallus domesticus*) from 11 Biofarms in Austria was determined. Antibodies to *T. gondii* assayed by the modified agglutination test (MAT) were found in 302 of 830 (36.3%) chickens with titers of 1:10 in 50, 1:20 in 69, 1:40 in 53, 1:80 in 40, 1:160 or higher in 90. Hearts of 218 chickens with MAT titers of 10 or higher were bioassayed individually in mice. Tissues from 1183 chickens were pooled and fed to 15, *T. gondii*-free cats. Feces of the cats were examined for oocysts; 11 cats shed *T. gondii* oocysts. *T. gondii* was isolated from 56 chickens by bioassay in mice. Thus, there were 67 isolates of *T. gondii* from these chickens. Genotyping of these 67 isolates using the SAG2 locus indicated that all 33 were Type II. Phenotypically and genetically these isolates were different from *T. gondii* isolates from Brazil. None of the isolates was virulent for mice. This is the first report of isolation of *T. gondii* from chickens from Austria.
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Keywords: *Toxoplasma gondii*; Chickens; *Gallus domesticus*; Free-range; Austria; Genotype

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,

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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) (Howe and Sibley, 1995; Howe et al., 1997; Mondragon et al., 1998; Owen and Trees, 1999; Fuentes et al., 2001; Grigg et al., 2001; Ajzenberg et al., 2002; Boothroyd and Grigg, 2002; Jungersen et al., 2002; Aspinall et al., 2003; Ajzenberg et al., 2004; Dubey et al., 2004a,d; da Silva et al., 2005). The parasite used to be considered clonal with very low genetic variability. However, most of the information was derived from isolates from Europe and North America. Based on newer markers for genetic characterization and using recently isolated strains from Brazil and French Guiana revealed higher genetic variability 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. Thus far, we have characterized strains from South America [Brazil (Dubey et al., 2002, 2003a,d), Peru (Dubey et al., 2004b), Venezuela (Dubey et al., in press-f), Argentina (Dubey et al., 2003e; Dubey et al., in press-c)], Central America and the Caribbean [Guatemala (Dubey et al., in press-b), Grenada, West Indies (Dubey et al., in press-e)], 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)], and Asia [Sri Lanka (Dubey et al., in press-d), India (Sreekumar et al., 2003)]. These studies are still not complete, nevertheless, a pattern is emerging that isolates from South America are genetically distinct (Lehmann et al., 2004).

Little is known of the characteristics of isolates of *T. gondii* from animals from Austria. In the present paper, we attempted to isolate and genotype *T. gondii* from chickens from Austria.

2. Materials and methods

2.1. Naturally-infected chickens

Samples were obtained from slaughter houses that catered to chickens from Bio-farms that were at least 2 km apart (Table 1). Samples were collected from the assembly line that processed 4000 chickens per hour. Therefore, it was not possible to match blood and heart from many chickens. From many chickens only hearts were collected. All chickens sampled were more than 1-year-old. Samples were obtained in three batches in July and August 2004, and January 2005. In the first batch (farms A–D) there were 276 chickens from four farms (Table 1). Blood and hearts were collected from 190 chickens; from the remaining 86 chickens from farms A and B blood and fluids were squeezed out of the hearts. In the second batch samples were collected from 936 chickens from five farms (farms E–I); from 396 chickens there were matching hearts and blood samples and only hearts were collected from 540 chickens (Table 1). In the third batch sera and matching hearts from 158 chickens and 225 hearts without sera were collected (Table 1). From two farms (J,K) sera and hearts were sent cold by air to the Animal Parasitic Diseases Laboratory (APDL), USDA, Beltsville, MD. Three or 4 days elapsed between killing of chickens and receipt of samples at Beltsville.

Chickens on these organic farms were maintained free-range; they all used centrally distributed feed (Bio food, Garant Co., Klagenfurt, Austria) and same type of management (outdoor access of 4 m^2 each animal with plants, stocking rates must not exceed 6 layers/m^2, 18 cm perches for each animal, and a maximum of 4800 chickens or 3000 layers in each poultry house). Chickens were kept indoors until 20-week-old and then roamed free on the pasture. The pastures had only wire fences and were not cat proof. Cats were present on farms, especially on farm H and cats from the neighbor hood had access to chicken pastures. All egg layers from a given farm were sent to slaughter and the premises were cleaned before starting a new batch. Chicken carcasses were not sold directly to consumers and were used mainly for baby food and soups.