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Original Research

Seroprevalence and Genotyping of *Toxoplasma gondii* in Horses Slaughtered for Human Consumption in Italy

Roberto Amerigo Papini*, Gloria Buzzone, Simona Nardoni, Guido Rocchigiani, Francesca Mancianti

Dipartimento di Scienze Veterinarie, Università di Pisa, Pisa, Italy

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ABSTRACT

This study aimed to investigate the seroprevalence of *Toxoplasma gondii*–specific immunoglobulin G antibodies, to detect *T. gondii* DNA and to genotype the parasite in horses (N = 153) slaughtered at two plants located in a province of Northern Italy. Blood samples were collected, and sera were examined by an indirect fluorescent antibody test (cutoff titer, ≥1:20). Portions of tongue, masseter muscle, and heart from seropositive horses were used for nested polymerase chain reaction (PCR). Genotyping of *T. gondii* DNA from nested PCR–positive tissues was performed by PCR-restriction fragment length polymorphism of 13 markers. Seropositivity for *T. gondii* was detected in 17.6% of slaughtered horses. Prevalence was higher in females than in males and in older (aged >9 years) than in younger horses. Grade horses were statistically more likely to be infected than purebred ones. Three (11.1%) randomly chosen heart samples harbored *T. gondii* DNA. Polymerase chain reaction-restriction fragment length polymorphism analysis showed type I, mixed II/III, and III genotypes. Our results suggest that consumption of raw or undercooked meat from horses slaughtered in the study area may represent a potential source for human and animal infection with *T. gondii* in Italy.

1. Introduction

Toxoplasmosis is a common zoonosis with worldwide distribution [1,2]. It is caused by *Toxoplasma gondii*, which is an obligate intracellular protozoan parasite of the phylum apicomplexa and is virtually capable of infecting all warm-blooded animals, including humans [1,2]. *Toxoplasma gondii* has an unusual clonal population structure consisting of three predominant lineages, namely types I, II, and III [1,2]. Felids, mainly domestic cats, are definitive hosts of this parasite and can eliminate millions of environmentally resistant oocysts in their feces [1]. Under favorable climatic conditions, oocysts develop infectivity in a few days by sporulation and may remain infectious for more than one year in unfrozen moist soil [2]. The infection can occur by ingestion of sporulated oocysts contaminating the environment (water, soil, fruits, vegetables), by eating raw or undercooked meat containing viable tissue cysts, and by transplacental transmission [1,2]. Toxoplasmosis rarely causes overt clinical symptoms in immunocompetent patients [2]. However, it can cause necrotizing encephalitis in immunocompromised persons, abortion and stillbirth in pregnant women, chorioretinitis, hydrocephalus, intracranial calcifications, and a variety of other serious congenital consequences in newborns [2]. In livestock, *T. gondii* is a potential problem for the goat and sheep husbandry as it may cause abortions, stillbirths, and neonatal death [1].

Toxoplasma gondii DNA was detected in the retina, choroid, and sclera of a 17-year-old pony [3] as well as in an equine placenta [4] and in an aborted foal [5], showing not only that horses are susceptible to *T. gondii* infection but

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* Corresponding author at: Roberto Amerigo Papini, Dipartimento di Scienze Veterinarie, Università di Pisa, Viale delle Piagge 2, 56124 Pisa, Italy.
E-mail address: roberto.amerigo.papini@unipi.it (R.A. Papini).

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also that vertical transmission may occur in pregnant mares. Horses can be given pet status, particularly in the United States [6], but they can also be used for animal feeding, including pet foods, or enter the meat supply chain [7]. In 2013, improperly used or undeclared horse meat became a source of scandal across Europe [8]. Horse meat has very high iron level, low quantity of fat and cholesterol, and good concentrations of polyunsaturated fatty acids such as linoleic and α-linoleic acids, indicating that it may have beneficial effects for human health [9]. Standardized and specialized European production systems for equine meat are lacking because its annual per capita consumption is small and limited mainly to Belgium, France, Italy, and Spain [10]. In these countries, horses are slaughtered when they are unsuccessful racehorses or finish their racing career, they become unwanted because of owner-related issues (financial constraints, loss of interest, inability to afford a horse), or they are no longer able to perform as their owner requires because of old age or health conditions (lameness, injury, illness, expensive veterinary care). In addition, a number of horses are imported from East Europe's countries and often are illegally slaughtered [10].

Large number of serologic surveys have previously demonstrated antibodies to *T. gondii* in equine serum samples worldwide [11], but few studies focused on slaughtered horses [12–16], and only one has investigated *T. gondii* genotypes in this host species so far [15]. In European countries, previous reports examined *T. gondii* seroprevalence in various equine populations [11,17,18], but no data especially related to slaughtered horses are available. The aims of the present study were to survey the prevalence of anti-*Toxoplasma* immunoglobulin G (IgG) antibodies in horses whose carcasses were processed at slaughterhouses in Italy as well as to detect *T. gondii* DNA and to characterize the parasite genotype in muscle tissue of seropositive animals.

2. Materials and Methods

2.1. Study Area and Horse Population Examined

From January to June 2014, a total of 153 horses were surveyed at two slaughterhouses located in the province of La Spezia (latitude: 44°06′39″ N, longitude: 9°50′10″ E), Northern Italy. Depending on the demand, not only horses were slaughtered at these facilities. All horses sent to the two slaughter plants during the time of sampling were included in the study. They were reported to come from Italy (n = 141) and Poland (n = 12). Before slaughtering, data regarding sex, breed, and age were collected. Intact and castrated male horses were considered to be a unique group of gender. The study population encompassed female (n = 105), male (n = 48), grade (n = 105), and purebred (n = 48) horses, including Italian Trotters (n = 30), Bardigiano (n = 15), and Freiberger, also known as Franches-Montagnes (n = 3). Their age ranged from 1 to 22 years. According to age, horses were classified as follows: yearling (1 or 2 years old), young (3 or 4 years old), adult (5–9 years old), and aged (9 years old). They were 54, 27, 24, and 48, respectively. No other information was available.

2.2. Sample Collection

After the horses had been stunned and exsanguinated, a blood sample from each of them was collected in 10-mL centrifuge tubes. Approximately 100 g of tongue, masseter muscle, and heart was also collected from each horse and kept separate in plastic bags. After clot retraction at room temperature, serum samples were placed in 1.5-mL microcentrifuge tubes. Serum and muscle samples were stored at +5°C in a cool box and transported to the laboratory. Sera were examined within 24 hours from collection, whereas the portions of tongue, masseter muscle, and heart were maintained at +5°C until the serum sample from the same animal had been tested for anti-*Toxoplasma* antibodies.

2.3. Serologic Analysis

The gold standard method for toxoplasmosis in horses still remains unknown because an accurate assessment of sensitivity and specificity for any serologic test has not been determined. In this study, the presence of anti-*Toxoplasma* IgG antibodies was detected by an indirect immunofluorescence antibody test (IFAT). *Toxoplasma gondii* tachyzoites as antigen (ToxoSpot; bioMérieux, Marcy-l'Etoile, France) and fluorescein-conjugated rabbit antihorse IgG (Sigma Chemical, St. Louis, MO) as well as positive and negative controls were used. Positivity for horse sera was defined as an antibody titer ≥1:20, starting from an initial dilution of 1:10. The samples were then tested in two-fold dilutions until reaching end point titer. The present cutoff for IFAT was determined based on serosurveys for *T. gondii* in horses where a cutoff titer ≥1:16 was used for this test [19,20]. It was slightly increased accordingly with the serologic technique routinely used in our laboratory.

2.4. Molecular Techniques

Portions of tissues from seronegative horses were discarded. Among horses that were found to be serologically positive for *T. gondii* by IFAT, a subgroup was randomly selected by picking every three samples. Portions of tongue, masseter muscle, and heart from this subgroup of seropositive horses were submitted to DNA extraction with EuroGold Tissue DNA Mini Kit (EuroClone, Milan, Italy) following the manufacturer's instructions. Extracted DNA was submitted to amplification by a nested polymerase chain reaction PCR (n-PCR) protocol, which amplifies a 193-bp fragment in the first round and a 96-bp fragment in the second round [21]. Results were visualized using 2% agarose gel electrophoresis stained with ethidium bromide. The DNA samples from horses testing negative in the n-PCR assay were discarded, whereas genotyping of DNA samples from horses testing positive was performed by multilocus PCR-restriction fragment length polymorphism analysis of 13 markers [22].

2.5. Statistical Analysis

Prevalence rates were calculated as the number of positive samples divided by the number of examined