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Research Article

Detection of *Toxoplasma Gondii* in Cats and Dogs Brains in Buenos Aires City Argentina

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Abstract: The aim of the present study was the detection of Toxoplasma gondii by direct and indirect immunofluorescence technique in dogs and cats from Buenos Aires city. The identification of *Toxoplasma gondii* was carried out in 50 clinically compatible cases, with predominant neurological symptomatology by Direct and indirect immunofluorescence techniques. *Toxoplasma gondii* was detected in 14 cases (28%) (CI 95% 16.19-42.52). Ten detections corresponded to cats (71.42%) and 4 corresponded to dogs (28.65%), out of a total of 35 cats and 15 dog brains tested. We concluded that direct immunofluorescence is a sensitive and rapid technique for the direct diagnosis of *Toxoplasma gondii* in brain samples, as well as a useful tool in live animals (ganglion biopsies, body fluids, aqueous humor).

Keywords: Toxoplasma gondii, brains, cats, dogs, detection, direct immunofluorescence.

INTRODUCTION

Toxoplasmosis is a parasitic disease caused by an obligate intracellular zoonotic protozoan, *Toxoplasma gondii* (*T. gondii*). It is distributed worldwide, affecting different mammals and even humans. The definitive host is the feline species which maintains the disease in the wild environment. Infection is acquired mainly through the digestive tract, with the ingestion of infective oocysts by consumption of raw meat. Infections could be asymptomatic, but there may be a generalized intestinal, encephalic, respiratory and ocular form (especially in young and immunosuppressed animals (Sparkes, 1993)). In cats, the most frequent is the respiratory form, which can occur with fever, cough and atypical pneumonia. Nervous signs may also be present manifesting as ataxia and convulsions. In dogs, the infection could depict variable signs, similar to other diseases such as distemper.

In United States of America, Memphis city, out of a total of 140 cat brain samples showed 23.33% positive results (Acha and Syfres, 1988). In another study carried out in USA, the prevalence was 11% (Dubey, 1973). The same author found 80% positive cases in a sample of 100 cat brains (Dubey, 1993). In 2010, Dubey reported that in Mexico was found 2% using Toxoplasma modified Agglutination test (MAT). In addition, Alvarado-Esquivel *et al*., (2014) found in Mexico 67% using MAT test.

In Argentina, three tests, the indirect immunofluorescence technique (IIF), direct agglutination test (DA) and indirect hemmagglutination (IHA) are used to diagnose this disease. A study carried out in Córdoba province (central region of Argentina) 73 wild felines were analyzed out of which 59% were positive using IHA. In this report, oocysts were detected in 34.74% of fecal samples (Pizzi *et al*., 1978). Other study encompassing canine samples of the Corrientes province (northeastern region of Argentina) in 1978 a total of 46. 6% positive cases were found using IHA technique (Acha and Szfres., 1988). More recently, Venturini *et al*., (2008) found 30.3% positive cases using IIF in dogs.

In Buenos Aires city the prevalence of *T. gondii* was 59.9% and 60.6% in dogs and cats, respectively (Gury Dohmen, 1995). The study was carried out using DA and IFI test. Recently, several studies have been carried out using traditional tests such as latex agglutination and indirect immunofluorescence to detect *T. gondii* in cats (Bartova *et al*., 2018; Sroka *et al*., 2018).

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To our knowledge, there are no other studies using Direct immunofluorescence (DIF) for detection of this parasite in Argentina, therefore, the aim of this study was the detection of *T. gondii* in cats and dogs brains in Buenos Aires city (CABA) by this method.

MATERIALS AND METHODS

Studied population

The cases selected from 1994 to 2012 for this study were 15 dogs and 35 cats of CABA and Gran Buenos Aires (the metropolitan area surrounding CABA). According to the data obtained from clinical records, studied animals presented compatible symptoms with toxoplasmosis, such as predominant nervous symptoms (tonic-clonic seizures, paralysis, blindness, pleural effusion). As an inclusion criterion, all animals were tested negative for the direct immunofluorescence Rabies test. Animals that had trauma-related nervous symptoms were not included in the study

Blood collction

Blood was obtained by cardiac puncture using a 50/12 needle, allowing coagulation for 20 minutes and then separating the serum by centrifugation within 4 hours post-mortem. The organs selected for isolation were the brain, lymph nodes, and lung.

Microscopic identification

Direct immunofluorescence was used to perform the microscopic identification of the parasite in brain samples, (Grossi *et al*., 1968, Matossian, 1977). It should be noted that this is actually an indirect immunofluorescence technique although some authors described it as direct in previous studies (Grossi *et al*., 1968), due to the direct visualization of the parasite (positive sample). It is based on the methods described for the detection of *Toxoplasma* in human tissues (Gury Dohmen, 2004), modified for veterinary use. It was carried out as follows: brain touch impressions were made and dried at room temperature; challenged with a high title feline or canine serum (1/2048) at a 1:4 dilution incubated in humid chamber at 37° C for 30 minutes. Then, slides were washed in distilled water for five minutes, dried and stained with feline or canine anti gamma globulin – conjugated to Fluorescein isothiocyanate (FITC) + for 30 minutes at 37°C in a humid chamber. Finally, slides were washed for five minutes, dried and observed with an epifluorescence microscope.

Serology

The previously described indirect immunofluorescence (IFI) technique was used. This technique was described in human medicine by Tchoulamjan *et al*., (1984) and adapted for Veterinary Medicine in our laboratory, using smears of *T. gondii* RH strain inactivated with formaldehyde. Titers higher than 1/128 were considered positive. All cats and dogs were tested by this technique except for four cats (table 1) and one dog (table 2).

Biological isolation

Biological isolation of *T. gondii* was performed in those cases in which the reading of the DIF technique was doubtful (Tchoulamjan *et al*, 1984). All experiments in white mice were carried out following the guidelines laid down by the Institute of Laboratory Animal Resources Commission on Life Sciences National Research Council. Mice were inoculated intraperitoneal with fluids from lymph nodes, lungs and cerebrospinal fluid puncture in an amount of 0.5 to 1.5 cm^3. Fluids were previously grounded in a mortar and diluted in physiological solution (1/5 dilution) with sodium penicillin 10,000 IU/cm^3 and streptomycin $5mg/cm^3$. After 4 days of incubation, ascetic and pulmonary fluid were taken for the detection of the parasite. This technique was performed in three cats showing positive results and two dogs showing negative results (table 1 and 2).

RESULTS

A total of 14 out of 50 (28%) samples analyzed, tested positive to the presence of *T. gondii* in brain tissue by DIF technique (Tables 1 and 2). Most animals presented neurological symptomatology as a predominant sign but, in some cases, also respiratory and ocular signs. Of the total 14 positive cases diagnosed by DIF, 10 corresponded to cats (71.42%) and 4 to dogs (28.57%). Nine of the cats presented predominant neurological signs as the main symptom, except for one (case 23) which presented pulmonary symptoms. In this case, the IIF showed a 1/1024 titer and the parasite was detected in lungs. Two other positive cases corresponded to cats with feline immunodeficiency virus (FIV), depicting an acute decrease in lymphocytes, showing a titer lower than 1/512 and higher than 1/1024. Four cats of the seven remaining cases tested positive by IIF (Table 1). In four cases the IIF was not performed since in two of them the parasite could be isolated by intraperitoneal inoculation. The other two cases the DIF were positive.

Table 1. DIF, IIF data and symptomatology obtained in cats

.		DIF	IIF (Titre)	Symptoms /Observations
1	Pos	-	1/1024	Nervous Symptoms Compatible with Toxoplasmosis
2	-	Neg	1/4	Nervous Symptoms Compatible with Toxoplasmosis
3		Neg	1/4	Nervous Symptoms Compatible with Toxoplasmosis
4	Pos	-	1/1024	Virus VIF (+) hemogram with marked lymphopenia
				Nervous symptoms compatible with toxoplasmosis
5	Pos	-	Not performed	Nervous Symptoms Compatible with Toxoplasmosis
6	-	Neg	1/4	Nervous Symptoms Compatible with Toxoplasmosis
7	Pos	-	Not performed	Nervous Symptoms Compatible with Toxoplasmosis
				With, predominantly tonic-clonic seizures
8	-	Neg	1/4	Nervous Symptoms Compatible with Toxoplasmosis
9	-	Neg	1/4	Nervous Symptoms Compatible with Toxoplasmosis
10		Neg	1/4	Nervous Symptoms Compatible with Toxoplasmosis
11	*Pos	-	Not performed	Nervous Symptoms Compatible with Toxoplasmosis Isolation by
				inoculation in mice entered dead without known data
12	Pos	-	1/256	Nervous Symptoms Compatible with Toxoplasmosis
13	-	Neg	1/4	Nervous Symptoms Compatible with Toxoplasmosis
14	-	Neg	1/4	Nervous Symptoms Compatible with Toxoplasmosis
15	-	Neg	1/4	Nervous Symptoms Compatible with Toxoplasmosis
16		Neg	1/4	Nervous Symptoms Compatible with Toxoplasmosis
17		Neg	1/4	Nervous Symptoms Compatible with Toxoplasmosis
18		Neg	1/4	Nervous Symptoms Compatible with Toxoplasmosis
19		Neg	1/4	Nervous Symptoms Compatible with Toxoplasmosis
20		Neg	1/4	Nervous Symptoms Compatible with Toxoplasmosis
21	*Pos		Not performed	Nervous Symptoms Compatible with Toxoplasmosis
				Inoculation to mice with positive isolation
22		Neg	1/1024	Nervous Symptoms Compatible with Toxoplasmosis
23	Pos	-	1/1024	They were identified from lung stamps belonging
				to interned cats in cages with pulmonary symptoms.
24		Neg	1/1024	Nervous Symptoms Compatible with Toxoplasmosis
25	Pos		1/1024	Nervous Symptoms Compatible with Toxoplasmosis
26	Pos	-	0	Nervous Symptoms Compatible with Toxoplasmosis
				FIV(feline immunodeficiency virus) (+)
27		Neg	1/4	Nervous Symptoms Compatible with Toxoplasmosis
28		Neg	1/4	Nervous Symptoms Compatible with Toxoplasmosis
29		Neg	1/4	Nervous Symptoms Compatible with Toxoplasmosis
30		Neg	1/4	Nervous Symptoms Compatible with Toxoplasmosis
31		Neg	0	The serology was made in life animal
32		Neg	1/4	Nervous Symptoms Compatible with Toxoplasmosis
				tonic-clonic seizures
33		Neg	1/4	Nervous Symptoms Compatible with Toxoplasmosis
34		Neg	1/4	Nervous Symptoms Compatible with Toxoplasmosis
35		Neg	1/4	Nervous Symptoms Compatible with Toxoplasmosis
* Weak positive				

In cats where DIF diagnosis was weak positive the isolation was performed by intraperitoneal inoculation in mice confirming the presence of the parasite (cases number 11 and 21). In three dogs with previous negative identification of the parasite by DIF, suspicious materials were inoculated and isolation was not achieved.

TABLE 2. DIF, IIF data and symptomatology obtained in dogs

CASES		DIF	IIF (Titre)	Symptoms / Observations
36	-	Neg	1/1024	Nervous Symptoms Compatible with Toxoplasmosis
37	Pos	-	1/1024	Tonic – clonic seizures
38		Neg	1/64	Tonic – clonic seizures
39	Pos	-	1/2048	Epilepsy
40		Neg	1/512	Nervous Symptoms Compatible with Toxoplasmosis
41	Pos		1/512	Nervous Symptoms Compatible with Toxoplasmosis
42	-	Neg	1/4	Nervous Symptoms Compatible with Toxoplasmosis
43		Neg	1/64	Nervous Symptoms Compatible with Toxoplasmosis.
				Intraperitoneal inoculation to mice with negative isolation
44	Pos		1/512	seizures

45		Neg	1/2048	Nervous Symptoms Compatible with Toxoplasmosis
46		Neg	1/4	Intraperitoneal inoculation to mice with negative isolation
47		Neg	Not performed	Inoculación intraperitoneal a ratones con aislamiento negativo
48		Neg	1/4	Nervous Symptoms Compatible with Toxoplasmosis
49		Neg	1/16	Nervous Symptoms Compatible with Toxoplasmosis
50		Neg	1/256	Tonic-clonic seizures

The four dogs which tested positive by DIF presented neurological symptomatology and also tested positive by IIF (Table 2). Two animals corresponded to a mother and a puppy, the mother depicting a titer above 1/2048. One of her puppies also developed a positive serology (lower than 1/1024), presenting no symptoms, which would be explained by transplacental transmission (HCN º 5723) (See case 39). The other two cases presented neurological signs with tonic-clonic seizures before dying (epilepsy diagnosis) and positive serology with titer between <1/512 and 1/1024. The parasite identification was performed post-mortem (cases 44, 38).

DISCUSION

While performing antigen detection, the sensitivity of the new DIF technique was similar or higher to that reported by other authors with isolation techniques or immunohistochemical methods in cats infected with *T. gondii* (Dubey, 1973; Dubey *et al*., 1993). Congruently, in another study, similar results were found using brain samples of 34 animals which were also inoculated in mice to isolate the parasite (Da Silva *et al*., 2002). In cases where it was not possible to perform IIF, the comparative method was the biological assay, which allows the identification of samples with viable parasites as well as enhancing the detection of *T. gondii* (Becerra *et al*., 2012).

Among cats, nine cases presented neurological symptoms and one presented pulmonary symptoms (case number 23).In this case, the IIF showed a 1/1024 titer and the parasite was detected in lung . Although this cat was not subjected to FIV diagnosis, it is likely that it was infected with this virus because of its symptomatology and since, for example, in humans affected by AIDS, the parasite can be identified in brain and lung post mortem biopsies. Cats acquire long-term immunity after infection (Gury Dohmen, 2004), with no clinical symptoms of the disease in most cases. When toxoplasmosis coexists with diseases such as FIV (cases 26 and 4) there is a condition that stops the antibodies conversion from IgM to IgG (Lappin, 1990) presenting clinical symptomatology, most frequently neurological symptoms. Those are related to diffuse or focal non-suppurative encephalitis in the usual form of multiple abscesses, as observed in humans. In these 2 cases (4 and 26), Toxoplasma "nests" were observed by DIF similar to those seen in humans. These “nests” were widely distributed, although some authors claim an irregular distribution in the brain of felines with feline immunodeficiency (Tchoulamjan *et al*., 1984).

Toxoplasmosis is frequent in canines with distemper, but sometimes animals with this disease died before we could diagnose toxoplasmosis due to the action of the virus. The cases found had a prolonged course with a diagnosis of idiopathic epilepsy. The lack of concordance between symptomatology and the identification of the parasite could be due to neosporosis, which produces similar symptoms. This disease is not studied in our Institute because it is not a zoonosis.

Those cases in which animals depicted neurological symptoms and showed a positive post-mortem IIF but were negative by DIF could be explained due to an irregular distribution of parasites in the brain, as seen in chronic forms, difficulting its detection.

Fluorescent examination of the parasites in brain revealed a semilunar form that is observed at the first stage of infection with extracellular localization. Also, the oval form of intracellular localization that is described in a secondary stage sometimes presents agglomerates that are observed in the third stage of the infection (chronic cases) (Tchoulamjan *et al*., 1984). This was found in two cases with (FIV).

DIF has shown to be highly sensitive and specific and has a great value in the direct diagnosis of toxoplasmosis since it has a high agreement with serology and parasite isolation in positive cases (Tchoulamjan *et al*., 1984). Additionally, this technique yields results faster than the isolation of the parasite since it can be performed directly from live animals (lymph nodes, biopsies, pleural fluid, and ascites).

CONCLUSION

As a conclusion, despite the low number of samples in this report, direct immunofluorescence is a sensitive and rapid technique for the direct diagnosis of *T. gondii* in brain samples, as well as a useful tool in live animals (ganglion biopsies, body fluids, aqueous humor). In addition, DIF / IIF both techniques should be considered together in conjunction with clinical symptomatology.

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