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SERODIAGNOSIS OF *TOXOPLASMA GONDII*, ASSOCIATED RISK FACTORS IN DOMESTICATED CATS: PREVENTING ZONOSIS IN HUMANS AND IMPLICATIONS FOR LIVESTOCK EXTENSION

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ABSTRACT

Toxoplasmosis, an important zoonotic parasitic disease-causing infection in humans and contaminating the environment. Cats are the sole source of spreading infection. Current study was designed to serodiagnoses in the flooded area, Jhang, Pakistan. Using rapid identification pen side strip test 316 pet cats were examined at CVAS, Jhang and other private clinics in that area. Rapid ID Chromatographic immune assay was performed to detect the antibodies for Feline toxoplasma IgM and IgG in serum. Related associated risk factors like breed, sex, contact with other livestock animals, deworming, location type (urban or rural area), diet (nature of food either cooked or uncooked meat), and outdoor access for wandering were also interviewed by owner. Total seroprevalence of cats was 10.4% (33/316). IgG antibodies were found 9.09 (29/316) while IgM antibodies were 2.21% (7/316) in cats. Seroprevalence was significantly high in cats older than one year. No significant difference was recorded between males and females. Cats from peri urban areas showed higher prevalence. Cats having access to outside, contact with other animals and eating uncooked food showed high seroprevalence. The current study confirms that *Toxoplasma gondii* is widespread in pet animals in district Jhang, Pakistan. This study urges pets lower to be aware about the Toxoplasmosis diseases. Whereas, the livestock extension sector should disseminate the precautionary awareness among the public.

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INTRODUCTION

Parasitic diseases cause serious threat to humans and animals all over the world (Abdel-Sattar *et al.* 2019; Hassan *et al.* 2020; Tammam *et al.* 2020). Among parasitic diseases, *Toxoplasmosis*, an infectious parasitic disease, is caused by an obligatory intracellular

protozoon *Toxoplasma gondii* belonging to phylum apicomplexan. It is of great zoonotic importance, infecting humans and other warm-blooded animals which are intermediate host (Awad and Barakat, 2019). It is distributed worldwide, infecting one third of world's human population and contaminating the environment

with resistant oocytes (Zhang *et al.*, 2016; Dubey, 2001; Okewole and Akpan, 2002). Cat either wild or domesticated is the definite host for shedding of oocytes in feces. The transmission of infection is possible through both vertical (via placenta) and horizontal (fecal oral route, ingestion of uncooked meat containing oocysts and via carnivorous route). Cats (*Felis catus*) as pets, are the sole source of infection transmission to human community from contaminated environment through feces and mechanically by flies, cockroaches, dung beetles, and earthworms (Dubey, 2004). Toxoplasmosis is also known as water-borne disease as spreading through sewage water and infecting humans in urban areas where it is difficult to treat water with process of chlorination and decantation (Meireles *et al.*, 2004). Serious losses are observed in different species of livestock and in pets (dogs and cats) of different ages due to mortality, embryonic death and resorption, fetal mortality and mummification, abortion, stillbirth, neonatal death and immunosuppression (Dubey, 2002). While in humans, it causes asymptomatic disease in adults, and the infection depends on the stage of pregnancy, developmental abnormalities and blindness in fetus that may arise from infected mother (Ahmad *et al.*, 2019). Oocysts are more dangerous to cause disease than cysts (Dubey, 2009). But the major losses are socioeconomics as long period treatment and children's care (Roberts *et al.*, 1994). Direct smear method is used to diagnose this disease through the feces concentration but it is less sensitive and difficult to differentiate *Hammondia hammondi* oocysts (Györke *et al.*, 2011). Antigen is detected through molecular technique by PCR but it is time consuming. In current study serologically Abs has been diagnosed in infected cats which is rapid ID test and less expensive as well.

MATERIALS AND METHODS

Study Area

This research study was approved by the Departmental Research Ethics Committee and was conducted following the ethical guidelines. This study was conducted in the district Jhang of the Punjab province. Jhang district, land of two rivers Jehlum and Chenab, is situated in the central Punjab, Pakistan. Population according to census 2017, is 2835000 at the area of 33097 km.

The climate of district is mild warm and temperate. More rain falls in summer than the winter. Average

temperature is 21.35 °C (32.5-10.2°C) annually while the annual rain fall is recorded 679mm. Maximum rain of 166mm is recorded in August and minimum rain of 9mm in November.

Study Animals and Sampling

316 cats of both sex, and different breeds and ages were examined at the outdoor clinic of College of Veterinary and Animals Sciences during the period of 2019. Clinical signs and symptoms of toxoplasmosis were observed including fever, loss of appetite, and lethargy. Blood samples were collected from the saphenous and jugular vein of examined pet cats with minimal doses of sedation according to Animal Welfare protocols (Ahmad *et al.*, 2014). Serum from collected blood was separated by centrifugation at 3000 rpm for 1 minute and stored at -20°C until analysis was performed.

Epidemiological Information

A questionnaire was designed to obtain the epidemiological data regarding age, breed, sex, contact with livestock animals, deworming, location type (urban or rural area), diet (nature of food either cooked or uncooked meat) and outdoor access for wandering (Sedlak and Bartova, 2006) and direct contact with soil.

Serodiagnosis

Rapid ID Chromatographic immune assay was performed to detect the antibodies for Feline toxoplasma IgM and IgG in serum (Luo *et al.*, 2018). Single step pen side test kit was used for antibodies detection against Feline toxoplasma, supplied by Bionote, Korea. Results were interpreted according to the manufacturer's instructions.

Statistical Analysis

Univariate and Pearson Chi-Square Tests were used to find out the relationship between serodiagnosis of toxoplasmosis and various risk factors (like sex, age, area, diet etc.) by using IBM SPSS Statistics 21. Different aspects of study were found like odd ratio, percentage and prevalence. Results were considered significant at $P < 0.05$.

RESULTS

The results obtained via rapid ID chromatographic assay are shown in the (Table 1). A total 316 samples were collected, out of which 33 were positive and 283 were

negative. Four study groups were made for serological interpretation of IgM and IgG antibodies. Each group has different percentage of total number of examined cats. Seroprevalence for first group [IgG (-ev) IgM (-ve)] 89.6%, for second group [IgG (+ev) IgM (-ve)] 8.22%, for third group [IgG (-ev) IgM (+ve)] 1.27% and fourth last

group [IgG (+) and IgM (+)] 0.94% were found. A total seroprevalence of IgG and IgM are 9.09 and 2.21 respectively. But overall prevalence of *T. gondii* in domesticated cats was 10.4%. Here it was seen that percentage of negative samples were high and low for positive.

Table 1. Number and percentage of IgM and IgG positive domesticated cats.

Sero-group	Cats	
	N	%
IgG (-) and IgM (-)	283	89.6
IgG (+) and IgM (-)	26	8.22
IgG (-) and IgM (+)	4	01.27
IgG (+) and IgM (+)	3	0.94
Total IgG (+)	29	9.09
Total IgM (+)	7	2.21
Overall Positives	33	10.4
Total Samples	316	--

Related associated risk factors were observed as shown in (Table 2). It was seen that the cats of age more than one year, living in the peri urban areas and access to outside were more infected with *T. gondii* 10.92%, 22.35% and 40, respectively. No significant difference was observed in the prevalence of both sexes ($P>0.05$, $\chi^2=1.918$). Seroprevalence of uncooked meat (12.50%) was higher than cooked (5.43%) ($P>0.05$, $\chi^2=2.481$). Also, non-dewormed cats and contact with other animals showed high seroprevalence ($P>0.05$, $\chi^2=2.11$). Soil exposure to pet cats is another factor enhancing the chances of infection. Overall prevalence (10.4%) of the current study shows that cats of District Jhang are exposed to *T. gondii*.

DISCUSSION

Parasitic diseases are continuously affecting both humans and Livestock population of both developing and advanced countries (Ali *et al.*, 2020; Li *et al.*, 2020). Toxoplasmosis, a common disease of cats, is difficult to diagnose upon its clinical signs Diagnosis is necessary for prevention and treatment of infection, and plays important role in transmission to human and contaminating the environment with oocytes. Cats are the definite hosts for shedding of oocytes in feces and cannot be diagnosed as eggs of other parasites are similar (Awad and Barakat, 2019). For rapid and accurate diagnosis, antibodies plays a major role in serological detection either acute or chronic form (Awad

and Barakat, 2019). Detection of two types of antibodies IgG and IgM confirms *T. gondii* which is more reliable as compared to another pen side test (Luo *et al.*, 2018; Dubey *et al.*, 2003). For more confirmation tissue sampling is required for which killing of animal is required (Ahmad *et al.*, 2014).

Epidemiological studies on prevalence of *T. gondii* in cat have been conducted in many parts of world. It occurs worldwide including Pakistan contaminating France (Afonso *et al.*, 2006), Brazil (Pena *et al.*, 2006), Czech Republic (Sedlak and Bartova, 2006), China (Zhang *et al.*, 2009), and Iran (Raeghi *et al.*, 2011). In Pakistan, Ahmad *et al.* (2001) and Ahmad *et al.* (2014) found 60% in Faisalabad and 26.43% prevalence in subtropical Arid parts in cats, respectively. The current study was designed to check the prevalence and risk factors favoring *T. gondii* in domesticated cats brought at College clinic for checkup and other veterinary clinics in District Jhang. For rapid identification serological test four groups were made and results were obtained.

Cats living in urban community were more affected than cats living alone (peri urban), and this factor is of vital in transmission of disease to humans due to congested population. Access to outside and soil exposure in rural and preurban areas are more as compared to urban which is the source of environmental contamination having oocytes. Cats either pets or stray play with soil get more chances of infection agreed by the Györke *et al.* (2011). One more thing, stray felids and livestock

populations are high in rural areas so, there is a high risk of getting disease. Also other cats in same area may be susceptible to toxoplasmosis as described earlier by Lopes *et al.* (2008) and Györke *et al.* (2011).

Table 2. Factors associated with *T. gondii* in cats.

Factors	Categories	N	(+)	%	P	Odd ratio (CI 95%)
Area	Urban	231	14	6.06	.011	Reference
	Peri urban	85	19	22.35		2.536 (1.214-5.296)
Access to outside	Yes	45	18	40	.000	Reference
	No	271	15	5.53		8.243 (3.766 - 18.043)
Age	>1 year	238	26	10.92	.950	Reference
	<1 year	78	07	8.97		0.974 (0.420-2.257)
Sex	Female	146	17	11.64	.166	Reference
	Male	170	16	9.41		1.667 (0.804 - 3.456)
Diet	Uncooked	224	28	12.5	.115	Reference
	Cooked	92	05	5.43		0.530 (0.238 - 1.180)
Deworming	Yes	226	11	4.86	.000	Reference
	No	90	22	24.44		0.135 (0.0610 - 0.298)
Contact with other animals	Yes	264	29	11.00	.000	Reference
	No	52	4	8.00		0.166 (0.069 - 0.398)
Soil exposure	Yes	129	20	15.50	.011	Reference
	No	187	13	6.95		0.344 (0.754 - 0.291)

As the age is concerned, more in geriatric cats of age more than one year or above (10.92%), they have more exposure of *T. gondii* in their life than younger one (8.97%). Infected intermediate hosts (rat, birds and small mammals) may be the carrier of tissue cyst, and cats (feral and stray) get infection during carnivorism. High prevalence has been already determined by Pena *et al.* (2006) based on including this factor as well. According to current study high seroprevalence in the cats, fed with uncooked meet and having outdoor access outdoor, were observed (Gauss *et al.*, 2003). According to current study it was found that there is no significance difference between seroprevalence of male and female as described by (Lopes *et al.*, 2008). Significant differences were found in the area, access to outside, age and diet as earlier reported by Awad *et al.* (2018).

CONCLUSION

Toxoplasmosis is a zoonotic disease, known as water borne zoonosis. It is the source of environmental contamination to cause the infection in humans. It is impossible to diagnose upon clinical examination so we move to serological techniques which are economic and less time consuming as compare to other molecular techniques. Here, rapid identification is the sole source

which confirms antibodies against toxoplasma at the spot in domesticated pet cats. This study confirms that the cats were the source of zoonosis. The current study further confirms that *Toxoplasma gondii* is widespread in pet animals in district Jhang, Pakistan. This study urges pets lower to be aware about the Toxoplasmosis diseases. Whereas, the livestock extension sector should disseminate the precautionary awareness among the public. The livestock extension department should critically examine this vulnerability of community, should arrange different awareness creating activity among the public in order to prevent from the zoonosis.

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