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Serological Study of *Toxoplasma gondii* Infection using Lateral Flow Chromatographic Immunoassay in Pigs and Goats from Dhankuta, Nepal

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Abstract: *To estimate the seroprevalence of toxoplasmosis and risk factors for seropositivity in pigs and goats in Dhankuta district in Nepal in 2018, 300 sera samples (Pigs-200 and goats-100) were randomly selected and were tested by using Lateral Flow Chromatographic Immunoassay (Rapid Toxo Ab test card, China). Samples were collected from Pakhribas, Hile, Sidhuwa, Dhankuta of Dhankuta district and test was done at laboratory of Agricultural Research Station (ARS), Pakhribas, Dhankuta. The disease prevalence and p-value (<0.05) were calculated to analyze the influence of risk factors on seroprevalence of toxoplasmosis. Out of 300 tested sera, 21% samples were infected for T. gondii. Seropositivity for toxoplasmosis was found high 26% (52/200) in pigs whereas 11% (11/100) in goats. Considering risk factors, seropositivity for toxoplasmosis was statistically significantly higher in pregnant 41.3% (p=0.00013) followed by non pregnant 21.7% and 10.8% in other male and female. Similarly, females were found 2.6 times more (prevalence: 26.5%) seropositivity compared with males (prevalence: 10%). High prevalence 27.7% was found in 6-18 months old animals. Additionally, presence of cats in surrounding areas, the pasturing system and the hygiene in the farms were assessed as factors for contributing the disease. This study indicates that exposure of pigs and goats to oocysts of T. gondii is widespread, suggesting that the consumption of raw and undercooked pork and chevon might be a source of human toxoplasmosis. Risk factor information can be used to design control programs to reduce exposure.*

Keywords: *Toxoplasmosis, pigs, goats, rapid toxo kit, Dhankuta*

I. INTRODUCTION

Toxoplasmosis is a global zoonosis occurs in almost all warm blooded animals including human beings and birds, is caused by *Toxoplasma gondii*. Based on serological investigations, it is reported that upto one third of the world's human population has been infected to this parasite [1-3]. The parasite is known to cause congenital disease and abortion both in humans and livestock species [4,5]. In most countries, toxoplasmosis comes as the

second cause of abortion in prevalence after Chlamydial abortion [6]. Therefore, the infection has an economic and clinical significance in many sheep and goat producing countries.

A broad spectrum of animals can be infected by ingestion of raw or undercooked meat containing viable tissue cysts or by ingesting food or water contaminated with oocysts from the feces of infected cats [7,8]. People usually acquire infection via ingestion of tissue cysts in undercooked meat, consuming food and water that has been contaminated with sporulated oocysts, or by accidentally ingesting oocysts from the environment, or vertically by transplacental transmission of tachyzoites.

Once infected, humans may remain infected for the entire life. Clinically, patients may have headache, disorientation, drowsiness, hemiparesis, reflex changes and convulsions, and may become comatose [9]. Infection with *T. gondii* during pregnancy can result in fetal death, neonatal death or various congenital defects such as hydrocephalus, central nervous system abnormalities and chorioretinitis [10].

The majority of infections are asymptomatic and unapparent or latent, but in pigs, sheep and goat clinical toxoplasmosis most often reported. Infections during pregnancy can cause abortions, stillbirths, mummification or resorption of the fetus [11].

There are numerous serological procedures available for the detection of IgG and IgM antibodies; these include the Sabin–Feldman dye test (DT), indirect hemagglutination assay (IHA), indirect fluorescent antibody assay (IFA), modified agglutination test (MAT), latex agglutination test (LAT), enzyme-linked immunosorbent assay (ELISA) and complement fixation test (CFT) [12]. For the diagnosis of *T. gondii* infection, detection of the organism itself is confirmative but very difficult.

Seroprevalence in different populations may vary according to different environments, social customs and habits. In Nepal, seropositivity rates were 57.9% [13]; 30.6% [14] and 50.6% [15] in human using different serological tests. Nepal is a country of vast diversification in geotopography. The positive rate is reported to vary from place to place [16-18]. In addition to goat meat, there is increasing trend of people towards consumption of pork in Nepal.

Cats are the most popular pet in the world and are now found in almost every place where humans live [19,20]. No doubt Nepal has unknown number of domestic, stray and wild cats. Usually cats do not show the clinical signs even during shedding of oocysts. So cats have a key and crucial role in the epidemiology of toxoplasmosis. Therefore, expanding the basic knowledge about *T. gondii* infection in both animals and humans in Nepal is a matter of importance. Therefore, lateral flow chromatographic immunoassay (LFCIA) (Toxo Ab card, Rapid test®) was used in pigs and goats. Prevalence of toxoplasmosis investigation is very important in Nepal for surveillance and monitoring for future planning control strategy.

By considering these points, the present research work has been aimed with the following objectives: (1) To determine the seroprevalence of toxoplasmosis in pigs and goats in Dhankuta District and (2) To identify risk factors of *T. gondii*.

II. MATERIALS AND METHOD

2.1 Study Area

Study areas lie in Dhankuta district of Province No.1 of Nepal (Fig. 3). The studied areas were Pakhribas, Hile, Sidhuwa and Dhankuta of Dhankuta district. The altitude of study areas ranges from 1,315 meter to 2,025 meter above sea level. The area is located 27^0 02' 09.8" N latitude and 87^0 17' 61.2" E longitude. The average minimum temperature ranges between 4.5^0 C (January) to 18.5^0 C (July) with lowest 1.5^0 C in January and average maximum temperature ranges between 15.3^0 C (January) to 24.6^0 C (August) with maximum of 29.5^0 C in June. Average annual rainfall at this area occurred 1492.3 mm and Relative humidity was recorded between 57.8% (January) to 91.1% (September). The climate of Pakhribas is sub-tropical to temperate (ARS Pakhribas, Annual report, 2017/018).

2.2 Study population and collection of sample

Altogether 300 samples (200 pigs and 100 goats) were examined in a year 2018. The type of the study was cross-sectional. Random sampling method was used for collecting samples from animals. Blood samples of pigs and goats were collected from slaughter places as well as farmers' house and were brought to the laboratory of ARS Pakhribas. Blood samples were kept refrigerated (4 ^{0}C) in the laboratory and one-day later sera were harvested by centrifuging at 3000 x g for 30 minutes. Each serum was labeled to identify the animal and stored at -20 ^{0}C.

Basic relevant history and data like age, sex, status of body condition- pregnant, non-pregnant, availability of cats, occupation of human and hygienic condition of shed/farm etc were also obtained from attendants both from slaughter places and farmers' house during blood collection.

2.3 Lateral flow chromatographic immunoassay (LFCIA)

For rapid test, Toxo Ab test card®, Porcine Rapid Test kit, China was used (Fig. 1 and 2) in the experiment. This kit is a lateral flow chromatographic immunoassay and the test cassette consists of: 1) a burgundy colored conjugate pad containing recombinant *T. gondii* antigens conjugated with colloidal gold (*T. gondii* conjugates) and rabbit IgG-gold conjugates, 2) a nitrocellulose membrane strip containing one test line (T line) and a control line (C line). The T line is pre-coated with reagents for detection of Immunoglobulin (Ig) anti-*T. gondii* antibody and the C line is pre-coated with a control line antibody.

A drop of blood/serum (35 µl) was kept into well (S) of kit and development of color line was observed within 10 minutes. Presence of a burgundy colored on both T and C lines indicated a *T. gondii* positive test result. Absence of burgundy colored on test line (T) was considered a negative result.

2.4 Data Analysis

All the collected data were compiled in Microsoft Excel. Data were analyzed using R 3.4.2 packages. Seroprevalence percentage and P value at 95 % significance level were calculated to show the association of risk factors with disease and results were tabulated.

Figure 1. Toxo Ab test cards and serum/blood samples

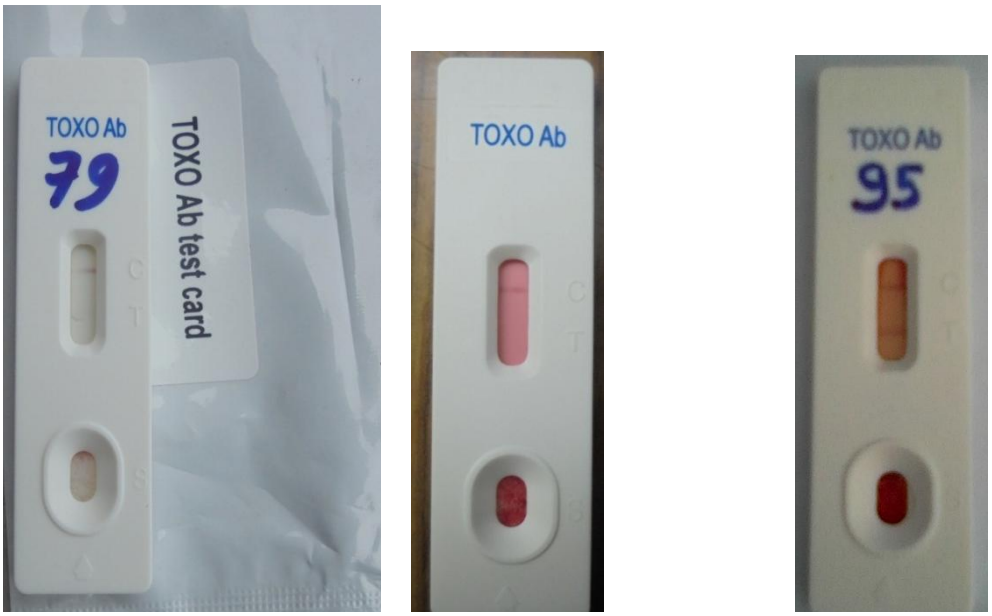

Figure 2. Toxo Ab test card, middle one- negative and right one- positive

III. RESULTS

3.1 Toxoplasmosis in different species

Pigs and goats in Dhankuta were found to be infected with toxoplasmosis and overall was 21%. The odds of toxoplasmosis was 2.3 times higher in pig than goat (Table 1 and Fig. 3).

Figure 3. Globe indicating Nepal; and Map of Nepal showing study area and seroprevalence of toxoplasmosis in pigs and goats in Dhankuta District

Table 1. Seropositivity of toxoplasmosis in pig and goat

Species	No. of tested samples	Positive samples	Prevalence %	Odds Ratio (OR)	P value at <0.05
Pigs	200	52	26	2.3	0.00269
Goats	100	11	11	Reference	
Overall	300	63	21		

3.2 Distribution of Toxoplasmosis in studied animals

The distribution of toxoplasmosis in studied animals (pigs and goats) is found statistically significant in relation to sex, age and condition of animals, presented in Table 2 and 3. The prevalence of toxoplasmosis was found higher in female (26.5%), in aged 6-18 months old (27.7%) and in pregnant (41.3%).

Table 2. Distribution of toxoplasmosis based on sex and age

Variables	No. of tested samples	Positive samples	Prevalence %	Odds Ratio (OR)	P value at <0.05
Sex					
Female	200	53	26.5	2.6	0.00091
Male	100	10	10	Reference	
Age					
Below 6 months	102	11	10.8	Reference	0.00147
6-18 months	184	51	27.7	2.6	
Above 18 months	14	1	7.1		

Table 3. Distribution of toxoplasmosis based on condition of animal

History of animal	No. of tested samples	Positive samples	Prevalence %	Odds Ratio	P value at <0.05
Pregnant	46	19	41.3	1.8	0.00013
Non pregnant	152	33	21.7	Reference	
Others (males and young)	102	11	10.8		

Additionally, cats were wandering for food and shelter in and around study sites. They were seen in pasture and other places like slaughter houses and butchers' places. Hygienic conditions of farms or sheds/pens were found poor in majority of livestock farmers.

IV. DISCUSSION

The overall seropositivity of toxoplasmosis we observed was 21% in Dhankuta district. The seroprevalence of toxoplasmosis has been reported to vary widely ranging from 3.3% in Mexico to 90% in The Netherlands [9]. The study revealed variable rate of seroprevalences of toxoplasmosis between pigs and goats in the study area. Although toxoplasmosis is considered harmless for non-pregnant, it is potentially harmful during pregnancy, especially at the first trimester [21].

It was found that anti-*T. gondii* antibody is prevalent in pig (26%) and goat (11%). These estimates are extremely low in case of goats compared to previous reports as 29.5% in Nepal [22], 16% in Bangladesh [20]

and 28.09% in India [23]. Our report is near to a review paper recorded 21.6% (n=920) seroprevalence of *T. gondii* in pigs in South Asian countries [24]. A high rate recorded 53.8% (n=91) in pigs in Bombay, India [25] whereas low 11.7% (n=712) in Kathmandu, Nepal in pig [26].

Many pig and goat raisers keep their animals in the house where they live, hence more contact between these animals and cats and so higher risk of contracting toxoplasmosis. Cats were wandering to and fro so that grazing areas and farm got contaminated and thus pasturing system and improper hygiene in the farm act as risk factors for the transmission of this parasite.

Surprisingly, in all the studied species of animals, highly significant and appreciable findings were that female, older age group (6-18 months old) and pregnant animals were found more susceptible to *T. gondii*. This finding resembles a report that older age and females are more prone to susceptible to toxoplasmosis [27]. The higher seroprevalence in female as compared to male might be attributed to the management system in those females are retained in the farm for longer periods for breeding purpose than males. Few males are retained for mating while the majority are culled and sold for cash purpose. The hormonal difference in relation to stress of lactation and pregnancy leading to immunosuppression may also increase susceptibility to toxoplasmosis in females [28].

Higher seroprevalence in older age group compared to young is consistent with earlier studies and is the result of higher likelihood of ingestion of oocysts with increasing age [9, 29-31].

Actually, the LFCIA (Toxo Ab rapid test) used in pigs and goats as a trial but the study found effective in pigs and goats although limited number of samples were examined. Therefore, LFCIA (Toxo Ab test card, Porcine toxo rapid kit, China) kit is good for screening of toxoplasmosis.

V. CONCLUSIONS

Based on LFCIA, the seroprevalences of toxoplasmosis were 26% and 11% in pigs and goats, respectively. With above results of the experiment, it is suggested that this assay is highly useful as a serodiagnostic tool in *T. gondii* infections. This study indicates that exposure of pigs and goats to oocysts of *T. gondii* is widespread, suggesting that the consumption of raw and undercooked pork, chevon might be a source of human toxoplasmosis. Risk factor information can be used to design control programs to reduce exposure. As this parasite has zoonotic importance, the knowledge should be disseminated to the people and animal raisers so that precautions can be taken in time.

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