SEROPREVALENCE OF *TOXOPLASMA GONDII* IN SMALL RUMINANTS AND PIGS IN AND AROUND RANCHI.

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DEPARTMENT OF  
VETERINARY PARASITOLOGY

Faculty of Veterinary Science & Animal Husbandry

BIRSA AGRICULTURAL UNIVERSITY  
RANCHI-834006  
(JHARKHAND)  
2011
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IN PARTIAL FULFILMENT OF THE REQUIREMENTS
FOR THE DEGREE OF
Master of Veterinary Science

IN
VETERINARY PARASITOLOGY

2011
Dedicated
To
My Beloved Parents
Acknowledgements

With great sense of modesty and reverence I wish to express my sincere gratitude to my reverend guide and major advisor Dr. A.R. Deb, University professor & Chairman, Department of Veterinary Parasitology, Ranchi Veterinary College, Ranchi for his meticulous planning, inspiring guidance, valuable suggestions, untiring surveillance, constant encouragement, constructive and inspirational criticism and keen interest in the execution of the present work and painstaking critical examination of the manuscript.

I would also like to express my sincere gratitude to all the esteemed members of my advisory committee Dr. Ashok Kumar, Ex-University Professor, Department of Veterinary Parasitology, Dr. R.L. Prasad, University Professor and Chairman, Department of Veterinary Biochemistry, Dr. K.K. Singh, University Professor and Chairman, Department of Veterinary Pathology, Dr. L.B. Singh, University Professor and Chairman, Department of Animal Genetics for all the help, counsel and encouraging support during the period of research work.

I feel immense pleasure in expressing my profound regard and deep sense of gratitude to Dr. A.K. Tewari, Senior Scientist, Division of Parasitology, IVRI, Bareilly for the amicable supervision, timely and indefatigably help, moral support, sincere and right advice, continual moral support, sincere and right advice, constructive suggestions during the thick and thin of my research period.

I would also like extend my sincere thanks to Dr. D.K. Singh, Additional Director research, Birsa Agricultural University, Ranchi for helping me a lot for the provision of funds for my research work and providing me permission to collect the blood samples sheep from the farm.

I am extremely thankful to Dr. S.K. Singh, Dean of the Faculty, Ranchi Veterinary College, Ranchi for providing all the requisite facilities to carry out this work smoothly.

I must express my sincere thanks to Dr. Balraj Sinha, Ex-DRI-cum-Dean, Post Graduate Studies, Birsa Agricultural University, Ranchi for providing all the requisite facilities to carry out this work smoothly.
I am highly obliged to Dr. N.N. Singh, Hon’able Ex-Vice-Chancellor, Birsa Agricultural University, Ranchi for his valuable and kind help by providing necessary facilities and funds to execute this work successfully.

I express my sincere thanks to Dr. K. Jha, Deputy Registrar, Birsa Agricultural University, Ranchi for his co-operation and help.

I am highly obliged to my seniors Dr. Ram Krishna Bauri, Dr. Shyamal Soren, Dr. Dilip Kumar Jha, Dr. Hemant Kumar, Dr. Rustam, Dr. Subodh Kumar, Dr. Niraj Kant Kumar, Dr. Vijendra Pal, Dr. Santosh Kumar for their constant help and moral support.

I would also like to extend my sincere adoration and support to my colleague Dr. Ravuri Halley Gora, Dr. Akhileswar Murmu, Dr. Rahul Kumar, Dr. Lalit Vijay, Dr. Niraj Kumar for their selfless untiring help during entire period of my work.

Sincere gratitude is expressed to Mr. Hadish Ansari, Mr. Shibu, Mrs. Chotan, Mr. Mithlesh Kumar, Mrs. Pushpa, Mrs. Bajo and Mrs. Hafiza Khatun (all from Department of Veterinary Parasitology, R.V.C., Ranchi), for their co-operation during the research work.

I express my genuine appreciation and perpetual affection to my father Sri Ram Bachan Singh, Mother Smt. Nutan Kumari, sister Pallavi Bachchan, and to my younger brother Manbendra Bachchan for their blessing, moral support, helps and continuous encouragement which sustained me to cover through the real long and hard journey leading to the studies.

Above all, I offer my heartful devotion to the almighty god for his blessing to moreover the endeavour a great success.
ABSTRACT OF THESIS
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Abstract

Submitted to the

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IN

VETERINARY PARASITOLOGY
**ABSTRACT**

*Toxoplasma gondii*, an intracellular apicomplexan protozoan parasite, is prevalent worldwide. All the stages of lifecycle of *T. gondii*, viz. tachyzoites, bradyzoites and sporulated oocysts are potentially infective. Information on prevalence of toxoplasmosis in animals and man is scanty from India. Since there is no information available on the prevalence of toxoplasmosis among the economically important farm animals from the Jharkhand state, the present study was undertaken with the objective of studying the seroprevalence of *T. gondii* in small ruminants and pigs. In order to accomplish the objective, ELISA was laboratory standardized using standard reagents. A native protein based antigen was prepared from the purified tachyzoites of *T. gondii*, whole tachyzoite lysate antigen (WTLA) and
was used in the indirect ELISA for screening the Toxoplasma-specific IgG molecules present in the sera. The serodetection potential of the native WTLA based ELISA was further compared with a recombinant surface antigen 1(SAG1) protein based ELISA. The serum samples were collected randomly from the target animals maintained either as free range management system in rural house holds as well as from the organized Government farms of Ranchi and Chatra districts of Jharkhand state. While collecting the samples, the age, sex and breed of the animals were recorded for drawing correlations with the prevalence of toxoplasmosis, if existing.

A total number of 240 pig serum samples were collected from organized farm and slaughter house. The recombinant surface antigen1 based ELISA was superior in detection of the T. gondii specific IgG antibodies in 44.58 % of the serum samples tested over the crude whole tachyzoite lysate antigen (WTLA) which could detect the same in 40% of the samples. The prevalence of the disease was found more in the samples collected from slaughter houses i.e., 53.75% and 23.75% than that of farm i.e., 26.25% and 48.12% by rSAG1 and WTLA based ELISA, respectively.

Sera samples were collected from Muzaffarnagari, Shahabadi and Chotangpuri breeds of sheep from Chatra and Ranchi districts of Jharkhand. The rSAG1 showed diagnostic superiority over the native WTLA. Out of 444 samples analyzed, 188 samples (42.34%) were found positive by rSAG1 ELISA, whereas 131 samples (29.5%) were positive with whole tachyzoites lysate ELISA.

The seroprevalence of toxoplasmosis was studied in the Beetal and Black Bengal breeds of goats reared in the organized farm of RVC, Ranchi and Chatra as well as from the village conditions of Chatra and Ranchi districts of Uttarakhand. A total number of 445 serum samples were collected of which 120 samples were collected from Chatra farm maintaining Beetal breed of goats and 65 samples were
collected from Instructional farm of small ruminants, Ranchi maintaining Black Bengal breed of goats. Rest 260 samples were collected from Black Bengal goats reared at village conditions. The rSAG1 ELISA was superior in detection the toxoplasma specific IgG molecules and could detect 42.47% of the total serum samples as positive, whereas whole tachyzoites lysate ELISA could detect 29.44% of the samples as positive.

The *Toxoplasma gondii* specific polypeptides were resolved in the molecular range of 73.0 to 11 KDa in a 6-15% gradient SDS-PAGE under denaturing condition. The major polypeptides detected were in the molecular range of 73, 64, 55, 49, 40, 38, 37, 30, 25, 14 and 11 KDa. To identify immunodominant protein in whole tachyzoite antigen lysate of *T. gondii* western blotting was performed. The proteins in the molecular range of 73, 64, 49, 40, 37, 30 and 25 KDa showed immunoreactivity against goat serum collected from field, whereas 55, 49, 37, 25 KDa band shown a good immunoreactivity against sheep serum collected from field.

The ELISA based detection method applied in the present study involving pig, sheep and goat population reared either as free range husbandry system or in organized farms generated important data on seroprevalence of toxoplasmosis at Jharkhand region. A high seroprevalence of toxoplasmosis among several food animals warrants for adoption of control measures to prevent transmission to susceptible humans.
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Abbreviations

% : Percent
@ : at the rate of
X g : Gravity
µg : Microgram
µl : Microlitre
µM : Micromole
ELISA : Enzyme linked immunosorbent assay
Fig. : figure
g : Gram
h : Hour
HRPO : Horse radish peroxidase
i.e. : That is
IFAT : Indirect fluorescent antibody test
IgG : Immunoglobulin G
IgM : Immunoglobulin M
Kg : Kilogram
L or l : Litre
mg : Milligram (s)
Min : Minute (s)
ml : Millilitre
mM : Millimolar
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<tr>
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<tr>
<td>0°C</td>
<td>Degree celsius</td>
</tr>
<tr>
<td>OD</td>
<td>Optical density</td>
</tr>
<tr>
<td>O/N</td>
<td>Overnight</td>
</tr>
<tr>
<td>PBS</td>
<td>Phosphate buffer saline</td>
</tr>
<tr>
<td>PBS-T</td>
<td>PBS containing 0.05% Tween-20</td>
</tr>
<tr>
<td>pH</td>
<td>Log hydrogen ion concentration</td>
</tr>
<tr>
<td>Rec-ELISA</td>
<td>Recombinant ELISA</td>
</tr>
<tr>
<td>rpm</td>
<td>Revolution per minute</td>
</tr>
<tr>
<td>SDS-PAGE</td>
<td>Sodium dodecyl sulphate-polyacrylamide gel electrophoresis</td>
</tr>
<tr>
<td>SE</td>
<td>Standard error</td>
</tr>
<tr>
<td>sec</td>
<td>Second</td>
</tr>
<tr>
<td>TEMED</td>
<td>NNN’N’- tetramethylene diamine</td>
</tr>
<tr>
<td>APS</td>
<td>Ammonium Per Sulphate</td>
</tr>
<tr>
<td>U</td>
<td>Unit (s)</td>
</tr>
<tr>
<td>V</td>
<td>Volume</td>
</tr>
<tr>
<td>v/v</td>
<td>Volume/Volume</td>
</tr>
<tr>
<td>viz.</td>
<td>Namely</td>
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<tr>
<td>WTLA</td>
<td>Whole Tachyzoite Lysate Antigen</td>
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Toxoplasma gondii is a ubiquitous protozoan parasite with worldwide distribution. The parasite has no host specificity and infects virtually all nucleated cells of all warm-blooded mammals and birds (Dubey and Beattie, 1988; Tenter et al., 2000; Dubey et al., 2004; Dubey et al., 2005; Dabritz and Conard, 2008; Dubey and Jones. 2008). The zoonotic potential of the parasite has assigned it a special importance (Tenter et al., 2000). Felines serve as the definitive hosts where the sexual multiplication takes place leading to production of oocysts, while all other non-felines acts as intermediate hosts for the parasite in which an asexual multiplication takes place leading to formation of rapidly multiplying form called tachyzoites or bradyzoites, a slowly dividing form (Frenkel et al., 1970; Miller et al., 1972). All stages of lifecycle of the parasite are potentially infective. The ability of forming tissue cysts in any type of nucleated cells renders the infected animals as potential source of infection to all carnivores and omnivores (Tenter et al., 2000).

Toxoplasma infection has special significance for small ruminants and pigs. A primary infection established during pregnancy may result in apparent infertility or in stillbirths and abortion (Buxton, 1998; Esteban-Redondo and Innes, 1997; Pereira-Bueno et al., 2004) which accounts for substantial economic loss, the exact quantum of which is yet to be determined in India. The animals especially the herbivores generally pick the infection while grazing in pastures contaminated with oocysts shed by cats. The Toxoplama oocysts are significantly resistant to environmental factors (Yilmaz et al., 1972; Frenkel et al., 1975; Dubey and Beattie, 1988, Lindsay et al., 2002, 2003).

Humans get infected by ingestion of raw or lightly cooked meat containing live T. gondii tissue cysts or by ingestion of contaminated vegetables and fruits (Weinman et al., 1954; Desmonts et al., 1965; Sacks et al., 1983; Dubey, 1996; Choi et al., 1997; Kniel et al., 2002;
Dubey et al., 2002; Dubey et al., 2005; Belfort-Neto et al., 2007; Robertson, 2007; Dubey et al., 2008) besides transplacental transmission of tachyzoites from infected mother. Transmission of tachyzoites may also occur via contaminated blood products, tissue transplants or even through unpasteurised milk (Tenter et al., 2000). Toxoplasmosis in humans was circumstantially linked to drinking raw goat milk (Patton et al., 1990; Riemann et al., 1975; Sacks et al., 1982; Skinner et al., 1990) or human milk (Bonametti et al., 1997).

Normally the infection in immunocompetent individuals is asymptomatic and the bradyzoites remain in latent phase. The individuals particularly at risk of developing clinical illness include pregnant women and immunosuppressed individuals, viz. patients undergone tissue transplantation (Slavin et al., 1994; Renoult et al., 1997; Chandrasekar et al., 1997; Wreghitt et al., 2001), AIDS patients (Jones et al., 1996; Luft et al., 1983, 1993; Porter et al., 1992) or cancer patients undergoing immunosuppressive therapy (Israelski et al., 1993; Wreghitt et al., 2001). These individuals are at risk of developing acute lethal infection if left untreated. The parasite can pose a serious threat to the unborn child if the prospective mother becomes infected for the first time while pregnant. The very young and very old individuals may also be more susceptible to infection. Transplacental transmission of infection in humans may cause serious disease leading to mental retardation and loss of vision in congenitally infected children (Wallon et al., 2004).

The infection is widespread both in man and animals throughout the world. Environmental conditions play an important role in the degree of natural spread of *T. gondii* infection. Infection is more prevalent in warm climates and in low lying humid areas than in cold climates and dry mountain regions (Walton et al., 1966; Ghorbani et al., 1981). The humid and warm environment conditions are favorable for sporulation and prolonged survival of the oocysts. There is a greater need for identifying the status of the disease in common food animals such as
goat, sheep, pigs etc. In India, the parasite was first reported by Krishnan and Lal (1933) in rabbits. Later the parasite was isolated from pigs, sheep, goats, humans (Gautam et al., 1979; Dubey, 1987), cats and also from aborted ewes (Verma et al., 1989). Information on sero-epidemiology of toxoplasmosis is scanty from India. In India, most of the serological surveys in livestock were conducted more than 2 decades ago and were mostly based on convenience samples (Chhabra and Mahajan, 1978). According to available literature, seroprevalence of *Toxoplasma gondii* ranges from 7.9% to 80% in various species of domesticated animals studied with highest incidence in goats. *Toxoplasma* antibodies were detected in 23% of sheep and 12.5% of goats from Palampur (H.P.) region, whereas, 81.2% of Pashmina goats and 90% of the local goats in Kumaon region were found positive for *Toxoplasma* specific antibodies (Dubey et al., 1993).

Infected soil is a continuous source of infection for animals and humans and nothing is known about the extent of soil contamination with *T. gondii* from India. From a public health point of view, determination of *T. gondii* infection in roaming pigs would be useful in understanding the epidemiology.

With above background information the present work was planned with the following objectives:

1. **Seroprevalence of *Toxoplasma gondii* in small ruminants and pigs in and around Ranchi.**

2. **Production and characterization of native crude antigen of *Toxoplasma gondii* using SDS-PAGE and Western blotting.**
Toxoplasma gondii is prevalent worldwide with an exceptionally broad host range and is capable of infecting almost any type of cell. Domestic cat and other wild felids act as the definitive host and virtually all warm blooded animals including birds act as the intermediate hosts. The organism was first detected by Nicolle and Manceaux (1908, 1909) in the tissue of hamster like rodents, Ctenodactylus gundi. Earlier several species of Toxoplasma were named mainly in accordance with the host species in which they were detected (Levine and Baker., 1987; Ashburn., 1992; Tenter and Johnson., 1997), but later Toxoplasma gondii has been considered as the only valid species of genus Toxoplasma (Frenkel, 1977; Smith, 1981; Euzeby, 1987; Dubey and Beattie, 1988; Current et al., 1990; Evans, 1992; Tenter and Johnson, 1997; Literak et al., 1998; Frenkel, 2000).

The life cycle Toxoplasma gondii is indirect. In the definitive hosts, viz. cat and other wild felines, the sexual cycle takes place in the intestinal epithelial cells (Frenkel et al., 1970). Infected cats excrete oocyst which are infectious to a wide range of bird and mammals, which act as intermediate host, where the asexual life cycle takes place. There are three infectious stages of the parasite: the tachyzoites (tachos = speed in Greek i.e rapidly dividing form) in tissues, the bradyzoites (slowly dividing form) inside cyst in tissues and the sporozoites develop within the oocyst in cat faeces. The first two stages occur in intermediate hosts and the last one take place in cats.

During the course of evolution, a broad range of potential routes of transmission of T. gondii came to light. Studies on congenital toxoplasmosis in humans point out transmission of the parasites during pregnancy as an important mode of parasite transmission of T. gondii in nature between different host species, and the epidemiological impact of the different sources causing infection or diseases is scanty. Likewise,
the asexual stages of *T. gondii*, in particular the tachyzoites, has been the focus of many studies, while the sexual stages of the organism leading to sporozoites formation within the oocyst attracted much less attention. Moreover, only few studies have aimed at identifying the risk factor that may be associated with acquiring an infection of *T. gondii* postnatally (Tenter *et al.*, 2000).

### 2.1 Prevalence of toxoplasmosis

The worldwide reports on the prevalence of toxoplasmosis in domesticated animals are highly variable (Dubey and Beattie., 1988) and to cite a few, toxoplasmosis have been reported in 47% goats (Antonig *et al.*, 1998), in 92% sheep (Cabannes *et al.*, 1997), in 69% cattle (Cabannes *et al.*, 1997), in 43% pigs (Fernandez *et al.*, 1998) in 84.1% cats (Garcia *et al.*, 1999) and in 40% chickens (Devada *et al.*, 1998). The major factors responsible for the observed variation in the prevalence are the cultural and food habits of people of different countries, the host species involved and the strain of the organism involved, etc. In absence of data on economic loss attributed to toxoplasmosis from India, the annual economic losses associated with toxoplasmosis in the USA were estimated to be 5.26 billion dollars (Robert and Frenkel, 1990) and the losses for a developing country like Uruguay was assessed at 1.4 to 4.7 million (Freyre *et al.*, 1997).

Information on seroepidemiology of toxoplasmosis is scanty in India. According to available literature, sero-prevalence of *T. gondii* ranges from 7.9% to 80% in various species of domesticated animals studied with highest incidence in goats. *Toxoplasma* specific antibodies were detected in 23% of sheep and 12.5% in goats from Palampur (H.P). In Kumaon region, 81.2% of Pashmina goat and 90% of the local goats were found positive (Dubey *et al.*, 1993). In meat producing animals, tissue cyst of *Toxoplasma gondii* has most frequently detected in pigs, sheep, goats and less frequently in poultry, dog and horse. Tissue cysts are found only rarely in beef or buffalo meat, although antibodies were
detected in 92% of cattle and 20% of buffaloes studies serologically (Tenter et al., 2000).

2.2 Prevalence in India

Based on indirect haemaglutination and Sabin-Feldman dye test, Gill and Prakash (1969) tested positive 11.1 per cent sera samples by dye test involving 108 one humped camels (Camelus dromedaries) and 10.8 per cent of 231 sera samples were tested positive by indirect haemagglutination test. In a slaughter house based study on buffalo (Bubalus Bubalis), Gill (1972) recorded an overall incidence of 10.2 per cent by Sabin and Feldman dye test. Higher infection rate of 12.9 percent was found in adult animals when compared to 8.0 per cent in the younger animals in the age group of 1-2 years.

Sharma and Gautam (1974) reported the presence of toxoplasma specific antibodies in the sera of apparently healthy camels and pigs in Hissar on the basis of indirect haemagglutination test. The incidence of 13.08 % (25 out of 191) for Toxoplasma specific antibodies recorded among camels. Older animals showed a higher rate of infection (16.8%) in comparison to the younger stock. In pigs the incidence was noted to an extent of 14.8%.

Chhabra and Mahajan (1978) assessed the sero-prevalence of Toxoplasma specific antibodies in buffaloes by indirect haemagglutination test in Chandigarh. 23.7% of the 219 animals examined were reported to be positive. In equids, 71 (11.8 per cent) of 603 samples studied were reported positive with titres ranging from 1:8 to 1:512. Specific titres of 1:64 or more were found in 34 (5.6 per cent equids of Babugarh (Uttar Pradesh) by direct haemagglutination test (Chhabra and Gautam, 1980). It was opined that chances of infection increase with age, whereas the sex appeared to have little influence. Using indirect haemaglutination test in a flock of dairy goats, Chhabra et al., (1981) recorded the range of antibody titres between 1:4 to 1:1024 in 34% of 371 apparently healthy animals. A high antibody titre of 1:256,
suggestive of recent infection, was noted in 18 (4.9%) animals including 13 nannies.

By indirect haemagglutination (IHA) test Verma et al., (1988) screened 164 samples, collected from ewes aborted first time, and 7.93% of the samples were detected positive for *Toxoplasma gondii*. Out of 13 *Toxoplasma* positive sera samples, 3 had antibody titres between 1:128 to 1:256 and rest had titres ranging between 1:16 to 1:64.

Sera sample collected from 88 sheep and 95 goats from 2 localities in India were tested for *Toxoplasma gondii* specific antibodies by direct agglutination test at a serum dilution of 1:25 by Dubey et al., (1993). Specific antibodies were detected in 22.7% of sheep and 12.5% of 8 goats studied from Palampur and 81.2% of 48 Pashmina goats and 89.7% of 39 local goats from Kumaon region.

Bharathi et al. (2003) analyzed 165 goat sera samples collected from and around Chennai for *Toxoplasma gondii* specific antibodies by modified direct agglutination test (MDAT) and Indirect haemagglutination test (IHAT). The MDAT appeared to be more sensitive could detect 41.21% samples as positive, whereas 32.21% samples were positive by IHAT.

Sharma et al. (2008) screened sera samples from 186 sheep, 83 cattle and 103 water buffaloes from Punjab using a commercial ELISA kit. Low prevalence *Toxoplasma gondii* specific antibodies recorded in 7 sheep, 2 cattle and 3 buffaloes.

Presence of tachyzoites of *T. gondii* has been demonstrated in the milk of several infected animals including sheep, goats, cows and mice (Pettersen, 1994; Dubey., 1998; Tenter et al., 2000). Infection of human through ingestion of raw goat milk has also been documented (Sacks et al., 1982; Skinner et al., 1990) which gives credence to the lactational route of transmission in human as well (Bonametti et al., 1997). The observation of Dubey (1998) that tachyzoites are infective orally to cats and mice further support the lactogenic route of transmission of *Toxoplasma*. Powell et al. (2001) detected *T. gondii* in milk of experimentally infected queens by bioassay in mice, as well as by PCR.
2.3 Diagnostic techniques

The various laboratories techniques used in the diagnosis of toxoplasmosis have been extensively reviewed by Davis (1973), Dubey and Beattie (1988) and Barker and Holliman (1992). The common serological test employed for detection of *T. gondii* antibodies were dye test (DT) (Sabin and Feldman, 1948), indirect haemagglutination test (IHA) (Jacob’s and Lunde, 1957), complement fixation test (CFT), modified agglutination test (MAT) (Desmonts and Remington, 1980), latex agglutination test (LAT) and direct agglutination test (DAT) (Dubey and Desmonts, 1987; Devda *et al.*, 1998). Although, DT is said to be highly specific and the sensitivity levels correspond to IFAT, it is rarely used now a days because of the potential risk factor associated with the use of the live tachyzoites.

Gorman *et al.* (1986) detected 50% positive cases of toxoplasmosis out of 226 goats screened in Chile by IFAT. The IFAT has also been successfully used in toxoplasmosis diagnosis in birds (sparrow) with a cut of titre of 1:10 (Literak *et al.*, 1977).

Udai Kumar (2004) screened 150 field goat sera collected from the local abattoir of Bareilly by IFAT and detected *T. gondii* specific antibodies in 42.66% of animals. The earliest detection of antibody by IFAT using acetone fixed whole tachyzoite antigen could be possible on 8th DPI with mean titres ranging from 1:18 to 1:2594.

2.3.1 Modified Agglutination Test

The development of a simple direct agglutination test has aided tremendously in the serological diagnosis of toxoplasmosis in humans and other animals. In this test, no special equipment or conjugates are needed. This test was initially developed by Fulton and Turk (1959) and improved by Desmonts and Remington (1980) and Dubey and Desmonts (1987), who called it the modified agglutination test (MAT). The MAT has been used extensively for the diagnosis of toxoplasmosis in animals. Titers in the MAT parallel those in the DT both in human sera and in animal sera. The sensitivity and specificity of MAT has been validated by
comparing serologic data and isolation of the parasite from naturally and experimentally infected pigs (Dubey et al., 1995; Dubey, J. P., 1997). In the MAT, sera are treated with 2-mercaptoethanol to remove non-specific IgM or IgM-like substances. This test detects only IgG antibodies; therefore, it may give false negative results during early stages of acute infection. (Dubey, J. P., and Crutchley, C., 2008). The results obtained with MAT differ, depending on the preservative used to prepare the antigen. Thulliez et al. (1986) reported that using acetone (AC test) in place of formalin (HS test) can detect IgG present during acute infection. The AC test has been very useful in diagnosis of toxoplasmosis in AIDS patients, and acute glandular toxoplasmosis (Montoya et al., 2007 and Thulliez et al., 1986).

2.3.2 Enzyme-linked immunosorbent assay (ELISA)

ELISA studies characteristically utilize, crude soluble antigens absorbed on to the walls of microtitre plate wells and the antigen-antibody reaction is enhanced by the addition of a secondary enzyme linked antibody and the reaction can be assessed objectively by quantitation of the colour that developed by an ELISA reader. The procedure is simple to perform, economical and easily adoptable for field use (Anthony et al., 1980; Spencer et al., 1980). The original ELISA (Voller et al., 1976) uses a soluble antigen preparation made from Toxoplasma RH strain tachyzoites. Dubey (1995) used T. gondii tachyzoite lysate (9.2µg of protein/ml equivalent to 5x 10^8 tachyzoites/ml in ELISA and found 24% out of 1000 sows positive for the T. gondii antibodies with a sensitivity and specificity of 72.9% and 85.9%, respectively. In a study conducted by Tenter et al. (1992), the ELISAs based on the recombinant antigen H4/GST or a mixture of the two recombinant antigen H4/GST and H11/GST gave consistent results with those obtained with the ELISA based on traditional ELISA antigen (TEA). Both the recombinant antigen or their combination showed cent percent sensitivity, specificity, positivity and negative predictive value. Using an improved ELISA format, it is possible to target T. gondii specific IgM, IgG and IgA antibodies (Takahashi and
Ross, 1994). Therefore ELISAs are most commonly employed for the detection of IgM antibodies appearing one week post-infection showing recent infection and IgG antibodies which appear at 2 weeks post-infection and persisting throughout the life.

2.3.2 a. Diagnosis with native protein

Various sero-epidemiological studies were conducted in different animals and human beings by using native tachyzoites protein as a diagnostic antigen in ELISA, LAT, IHA and MAT. A comparative study of IHA, IFAT and ELISA tests were carried out to determine the prevalence of Toxoplasma gondii antibodies in goats. A high and significant positive correlation was found between the titers obtained by the IHA verses IFAT, IHA verses ELISA, and ELISA verses IFAT. Therefore, it can be concluded that the three analysed tests have shown to be highly concordant and appropriate for epidemiological surveys of Toxoplasma infection in goats (Figureiredio et al., 2001).

Meireles et al. (2004) tested sera samples from stray cats and dogs for antibodies to T. gondii by indirect IHA and ELISA. Antibodies to T. gondii were found by ELISA in 40% of the cats and less than 50.5% in dogs. Haemaglutination showed low resolution and concordance, precluding their use for diagnosis of T. gondii infection compared with ELISA.

The validation of T. gondii IgG avidity ELISA was reported by Sager et al. (2003) based on the use of an affinity-purified, native T. gondii SAG1 antigen. The assay was used to examine sera from sheep experimentally infected with T. gondii and found that all the experimental animals sero-converted into within 21 days post infection, beginning with avidity that was initially low but thereafter increased overtime, with all sheep reaching high IgG avidity within 10 weeks post infection. High IgG avidity was found in 80% of the seropositive lambs, about 90% of the clinically healthy ewes and in 97% of the ewes with abortion problems (Sager et al., 2003). Based on an enzyme linked immunosorbent assay (ELISA) in 103 sheep farm in Ontario Waltner-
Toews et al. (1991) observed 99% of the farms had some sheep serologically positive for *Toxoplasma gondii*.

Zimmerman et al. (1990) tested 2,616 swine from 104 Iowa swine herds for antibodies against *Toxoplasma gondii*, using an ELISA. Data were analyzed according to swine type, herd size, facility type, and season. The true prevalence of toxoplasmosis was estimated as 5.4% among finishing swine and 11.4% among sows and gilts.

Berends et al. (1991) used ELISA to detect finishing pigs seropositive for *Toxoplasma gondii*. The investigation involved 120 farms and three slaughter houses and a total of 23,348 serum samples were examined. The prevalence of seropositivity for *Toxoplasma gondii* was found to be 2.1%.

Garcia-Vazquez et al. (1993) carried out a serological survey against *Toxoplasma gondii* in cattle, swine and goats were in four Mexican states. A total of 1203 pig sera from Cuernavaca, Morelos abattoir, 490 cattle sera on 12 ranches from four munincipalities and 707 goat sera from nine farms were tested. Sera from 107 of 1207 pigs (8.9%) were positive. Five of the nine goat farms had *T. gondii* seropositive goats and the average seroprevalence was 3.2%. Antibodies to *Toxoplasma gondii* was found in 47 of 397 cattle (11.9%) tested.

Knapen et al. (1995) undertook an ELISA based serological survey on the prevalence *Toxoplasma gondii* amongst swine and cattle in the Netherlands. They recorded a seroprevalence of *Toxoplasma gondii* in 1.8% of the finishing pigs and in 1.2% of the fattening calves. In sows and dairy cattle, seroprevalence of 30.9% and 27.9% respectively, were recorded. Seroprevalence of *Toxoplasma gondii* in dairy cattle in the North and in the South of the Netherlands was 13.1% and 42.6%, respectively.

Rodriguez-Ponce et al. (1995) tested sera samples from 1052 Canary goats from the island of Grand Canary to determine the prevalence of antibodies to (IgG) *Toxoplasma gondii* using an indirect ELISA test. The total prevalence was 63.31%. Significant differences were found between the island’s four climatic zones. The highest
seroprevalence was recorded in animals from low altitude zones with very little rainfall, high temperatures and a predominance of trade winds.

Skjerve et al. (1998) found 44.3% of the 194 sheep flocks from different areas of Norway were seropositive for *Toxoplasma gondii* by ELISA and antibodies could be detected in 16.2% of the 1940 individual animals.

Saurez-Aranda *et al.* (2000) estimated the prevalence of *Toxoplasma gondii* in 396 sera from 5 months old pigs obtained from abattoirs in Sao Paulo, Brazil (300) and Lima, Peru (96). The Seroprevalence was higher in pigs from Peru (32.3%) as compared to Brazil (9.6%), as detected by ELISA and Western blot. The authors opined that haemagglutination gave poor resolution and was not useful for the diagnosis of *T. gondii* infection.

Figueiredo *et al.* (2001) compared indirect haemagglutination (IHA), immunofluorescence (IFAT) and immunoenzymatic (ELISA) tests to determine the prevalence of *T. gondii* specific antibodies in goats based on one hundred seventy-four serum samples collected from four goat herds from the region of Uberlândia, State of Minas Gerais. Serum samples were analyzed by IHA, IFAT and ELISA, considering the reactivity of the serum samples at dilution 1:64 as cut off titer for the three tests. An overall seroprevalence of 18.4% was observed, with significantly higher positivity rate in the older animals (> 36 months). A high and significant positive correlation was found between the titers obtained by the IHA versus IFAT, IHA versus ELISA, and ELISA versus IFAT. They reported that the three analyzed tests have shown to be highly concordant and appropriate for epidemiological surveys of *Toxoplasma* infection in goats.

Meireles *et al.* (2003) detected antibodies to *Toxoplasma gondii* were found in 31.00% (62/200) of sheep, 17.00% (34/200) of goat and 11.00% (22/200) of cattle, without positive sample in chicken State of Sao Paulo, Brazil by enzyme-linked immunosorbent assay (ELISA).

Damriyasa *et al.* (2004) undertook a cross sectional survey to estimate the prevalences of antibodies to *Toxoplasma gondii* (ELISA,
IFAT), *Sarcocystis* spp. and *Neospora caninum* (ELISA, immunoblotting) in sows from breeding farms in southern Hesse, Germany. A total of 2041 plasma samples of sows from 94 randomly selected farms were examined. Antibodies to *Toxoplasma gondii* were detected in 19% of the sows by ELISA. The prevalence of anti-*Toxoplasma gondii* antibodies was positively correlated with the age of sows. Within-herd seroprevalence was significantly higher in farms with reproductive disorders than in those without such problems.

Ciamak Ghazaei (2005) tested sera samples from cattle, goat, sheep and chicken for *Toxoplasma gondii* specific IgG antibodies by enzyme linked immunosorbent assay (ELISA) in Iran. Antibodies to *Toxoplasma gondii* were found in 30% (60/200) of sheep, 15% (30/200) of goats, and 9% (18/200) of cattle whereas all samples collected from chickens were negative.

Clementino et al., (2007) reported 29.41% of sera from 102 sheep intended for consumption were positive for *T. gondii* specific IgG by a *Toxoplasma*-ELISA test with an increasing number of positive animals with advancing age.

Caballero-Ortega et al. (2008) determined the frequency of specific antibodies in sheep from the coastal, mountain and hill region of Colima using 351 serum samples by a previously standardized indirect ELISA. The frequency of infection was estimated and the farm location and flock size, as well as the animals’ age and sex were analyzed as risk factors for toxoplasmosis. The frequency of antibodies depended on the altitude, being higher at sea level than at 1200 meters above sea level (OR=3·77, 95% CI=1·79-7·94, P<0·0001), and the size of the flock, being higher in the large ones (OR=2·23, 95% CI=1·35-3·71, P=0·002). Older animals were more frequently positive and with a stronger response than young ones (OR=1·77, 95% CI=1·07-2·93, P=0·016). No differences were observed between male and female sheep. They concluded that the variations were related to altitude, flock size and age.

Hotea et al. (2010) assayed 580 of serological samples by ELISA to establish the prevalence of *Toxoplasma gondii* infection in female sheep.
from Caras-Severin County. The average prevalence in Western Romania was 46.7%.

2.3.2 b. Commercial ELISA kits based studies

Rajamanickam et al. (1990) collected 132 cattle, 107 goats, 106 sheep, 122 pigs and 48 chicken blood samples from large-scale and small holder farms in Malaysia and tested for toxoplasmosis by IHAT. Seventy out of 515 sera were seropositive for *T. gondii* at 1:64 dilution. The highest prevalence was observed in sheep (22.6% infected) followed by goats, chickens and pigs. No evidence of *Toxoplasma* infection at a titre of 1:64 or above was observed in cattle. It was suggested that kitchen scraps including animal byproducts were a possible source of infection for the chickens. This coupled with the fact that many of the small holders also keep domestic cats is probably the reason for a high prevalence rate in the chickens (16.6%).

In the framework of the Dutch field trial 'Integrated Quality Control (IQC) for finishing pigs' Berends et al., (1991) used ELISA (screening) techniques to detect animals seropositive for *Toxoplasma gondii* and/or *Trichinella spiralis*. The aim was to determine whether farms which consistently delivered seropositive pigs could be detected and monitored (defined as 'problem farms'). The investigation involved 120 farms and three slaughterhouses and examination of a total of 23,348 serum samples. In addition, all pigs were also screened for the presence of *Trichinella spiralis* with the digestion method (pooled samples). The prevalence of seropositivity for *Trichinella spiralis* and *Toxoplasma gondii* was 0.3% and 2.1% respectively. Parasitological examinations concerning *T. spiralis* were negative. Considering the characteristics of the used methodology, the conclusion was drawn that there were no parasitological or serological indications for *T. spiralis* infections, and that with respect to *T. gondii* the infection rate seemed to be equally low for all farms involved. In addition, a longitudinal pilot study during a whole finishing period was undertaken at two finishing farms. Animals seropositive for *Toxoplasma gondii* were found from the
earliest days of the finishing period. Housing and management may (still) play an important role in the prevention of contact with this parasite.

Bartoszcze et al. (1991) applied ELISA to assess *Toxoplasma gondii* antibodies in pigs. Among 925 swine examined 36.4 per cent of the animals were seropositive. Serum titres ranged from 100 to 3,200.

Toxoplasmosis is one of the most prevalent parasitic infections of man and livestock, and its transmission has usually been attributed to ingestion of undercooked or raw meat from infected livestock, with the infection rate in those animals being an important risk predictor of human disease, high in Iran and Ardabil State.

Dubey et al. (1992) examined 509 pig serum samples from 31 farms in Oahu, Hawaii for antibodies to *Toxoplasma gondii* using the agglutination test in dilutions of 1:25, 1:50, and 1:500 and for *Trichinella spiralis* using the enzyme-linked immunosorbent assay. *Toxoplasma gondii* antibodies were found in 48.5% of pigs. Antibody titers were: 5.1% positive at 1:25 dilution, 28.6% positive at 1:50 dilution and 14.7% positive at 1:500 dilutions. The prevalence of *Toxoplasma gondii* antibodies in garbage-fed pigs (67.3% of 199 pigs) was higher than in grain-fed pigs (33.8% of 180 pigs). Antibodies to *Trichinella spiralis* were found on seven of 31 farms. On five of these farms only a single serum was positive for *Trichinella spiralis* antibodies. The two remaining farms each had three positive sera (three of ten and three of 25 pigs tested); both of these farms fed garbage to pigs.

Arko-Mensah et al. (2000) has done serological survey of toxoplasmosis in pigs in Ghana was carried out between October 1997 and April 1998 in the three ecological zones of Ghana: the Coastal Savannah, the Forest Belt and the Guinea Savannah. Antibody against *Toxoplasma gondii* was measured in pig serum using a microplate-ELISA which had a sensitivity and specificity of 90.2 and 92.3%, respectively when compared with IFAT. A national seroprevalence of 39% was obtained in pigs, with the ecological distribution being 43.9, 30.5 and 42.5% for the Coastal Savannah, the Forest belt and the Guinea Savannah, respectively. The age of the animal, the breed, the
environmental conditions and the management practices appeared to be the major determinants of prevalence of antibodies against *T. gondii*. The seroprevalence of *T. gondii* was found to increase with age (*P*<0.05). Pigs from the two Savannah zones had a significantly higher (*P*<0.05) antibody prevalence than those sampled from the Forest belt. Antibody prevalence (46.8%) in crossbreed pigs was significantly higher (*P*<0.05) than that of the exotic Large White breed (38.8%).

Only limited epidemiological information is available on the seroprevalence of *Toxoplasma gondii* in domestic livestock in sub-Saharan Africa. In Uganda, goats are important to the local economy and are also popular food animals. A high incidence of *T. gondii* infection in goats would have implications both for animal production and for public health, but no data is available on *Toxoplasma* infection in these animals. Bisson *et al.* (2000) estimated the seroprevalence of antibodies against *T. gondii* in goats located in both urban and rural environments and from different geographical regions within Uganda. Goat sera were collected using a random, two-stage clustering method. Of 784 samples analyzed by antibody-ELISA from various districts in Uganda, 240 were tested positive. The combined (cluster-adjusted) seroprevalence was 0.31 (31%) (95% confidence intervals 0.28, 0.34) indicating a substantial level of infection in these regions. Seroprevalence was significantly higher in goats from urban locations. A strong positive relationship between age and seroprevalence was demonstrated and a mathematical model based on continuous exposure proved generally accurate in predicting seroprevalence. Farm environments were identified as being suitable for oocyst survival and transmission, and the reported incidence of caprine abortion was high. The high seroprevalence was thought to have a significant impact and that the consumption of goat meat might play a role in zoonotic transmission to humans.

Van der Puije *et al.* (2000) used to detect anti-*Toxoplasma gondii* antibodies in 1258 small ruminants (732 sheep and 526 goats) sampled from 28 different locations in the three ecological zones of Ghana by enzyme-linked immunosorbent assay (ELISA) and recorded an
overall seroprevalence of 30.5% (384 of the total). Sheep had a higher overall prevalence (33.2%) compared to the goats (26.8%). Animals sampled from the Coastal Savannah and the forest zones had prevalence of 39.4% and 39.1%, respectively, which were significantly higher ($P<0.01$) than the prevalence recorded for the drier Guinea Savannah zone (20%). Prevalence of antibodies in female animals (35.8%) was significantly higher ($P<0.01$) than that for males (21.1%). Significant differences were also observed between breeds and age groups. The ELISA was found to be both highly sensitive (92%) and specific (91%) when compared to the IFAT, which was used as a reference test.

Wyss et al. (2000) gathered information on the prevalence of *Toxoplasma gondii* and *Neospora caninum* in consumable meat. The prevalence was investigated by PCR in muscle and brain tissue of slaughtered cattle, sheep, pigs and horses in Switzerland. At the same time, serum samples were tested by ELISA. PCR based prevalence was determined in adult cow (3%), young bulls (2%), heifers (6%), calves (1%), sheep (6%) and in horses and pigs as 0%. For *N. caninum*, the PCR prevalence was much higher for both parasite species and all animal groups, with the exception of fattening pigs. It was concluded that the consumption of meat from cattle and sheep, but not from pigs and horses, carries a potential risk of infection by *T. gondii* for humans.

The prevalence of *Toxoplasma gondii* infection in Swedish pigs was investigated by Lundén et al. (2002). They analyzed 807 meat juice samples collected in 1999 from 10 abattoirs in different parts of the country. Analysis with ELISA revealed 42 (5.2%) of the samples positive. The seroprevalence was 3.3% in fattening pigs ($n=695$) and 17.3% ($n=110$) in adult swine. It was concluded that the risk of contracting *T. gondii* infection as a result of eating undercooked pork from Swedish pigs, especially adult animals, was not negligible.

Karsten et al. (2003) studied the seroprevalence of Toxoplasmosis in pig herds by ELISA and the influence of management systems on the occurrence of this infection. Furthermore they examined the suitability of meat juice compared with serum samples for the antibody test.
Additionally samples of minced meat from pork were tested for the presence of antibodies against *Toxoplasma gondii*. ELISA provided well comparable results both with serum and meat juice samples. Depending on the management system the prevalence was between 7.1% and 52.0%. In the smallest herds (1 to 20 animals) the highest prevalence was found. In organic farms the results varied between 0% and 30.6%. 5.4% of the 240 samples of minced meat were positive. The results indicated the importance of widespread monitoring and further research on the risk of *Toxoplasma* infections caused by meat and meat products.

Negash et al. (2004) conducted a serological survey to detect antibodies to *Toxoplasma gondii* using the modified agglutination test (MAT) and ELISA Engygnost IgG test. They tested 116 sheep and 58 goats of over 6 months of age from Nazareth area (39.17° N, 8.33° E), Ethiopia. A near perfect agreement was found between the two serological tests. Using the MAT as a reference test, the sensitivity and specificity of the ELISA Tests were determined as 98.4% and 90.9% respectively.

Damriyasa and Bauer (2005) performed a cross-sectional survey to obtain current data on the seroprevalence of *Toxoplasma gondii* infection in breeding sows in Münsterland, Germany. Antibodies to *T. gondii* were detected by ELISA in 140 (9.3%) of the 1500 sows examined. Forty-two (56%) of the 75 surveyed farms harbored at least one seropositive animal. In 3 (5%) of 62 farms where at least 10 sows had been examined > or = 30% of the animals were seropositive.

Gamble et al. (2005) compared the modified agglutination test (MAT) and a commercially available enzyme-linked immunosorbent assay (ELISA) for detection of antibodies to *Toxoplasma gondii* in naturally-infected market-aged pigs. Infected pigs were obtained from commercial slaughter facilities and from farms where infection had previously been detected. Infection was confirmed by bioassay in cats. For 70 bioassay positive pigs, 60 were positive by MAT (85.7% sensitivity) and 62 were positive by ELISA (88.6% sensitivity). Of 204 bioassay negative samples 193 were negative by MAT (94.6% specificity) and 200 were negative by ELISA (98.0% specificity). Good correlation
was seen between MAT and ELISA results. The results suggest that the ELISA may be a good tool for epidemiological studies of *Toxoplasma* infection on pig farms.

Sawadogo *et al.* (2005) undertook ELISA based serology test for the detection of anti-*T. gondii* specific IgG confirming a past infection using 261 sera from sheep intended for consumption in Marrakech. Of the total sera tested 72 (27.6%) sera were positive for IgG. This result shows that the seroprevalence approaches the world average and is similar to what is found in other cities of Morocco.

During a study on this public health problem, Mulla, A. S., (2006) tested serum samples from cattle, goats, sheep and chicken from the State of Ardabil, Iran, for IgG antibodies to *Toxoplasma gondii* by enzyme-linked immunosorbent assay (ELISA). Antibodies to *Toxoplasma gondii* were found in 30% (60/200) of sheep, 15% (30/200) of goats and 9% (18/200) of cattle, and none were found in chicken sera. Despite the differences in feeding habits of each species, the rate of infection of the animals tested could be attributed to livestock management methods, whose improvement could reduce infection.

During the study period (2001 to 2004), Poljak *et al.* (2007) tested sera from 6048 pigs were with commercial enzyme linked immunosorbent assay (ELISA). Apparent prevalence at the pig level was 1.59% in 2001, 0.06% in 2003 and 0.26% in 2004. Apparent prevalence at the herd-level was 13.7% in 2001; 1.25% in 2003 and 3.75% in 2004. Similarly, posterior Bayesian estimates of true prevalence at the pig level were 1.7% in 2001, 0.2% in 2003 and 0.3% in 2004. At the herd level, posterior estimates of prevalence were 11.6% in 2001, 0% in 2003 and 1.2% in 2004 when the herd cut point ≥1 was used.

Samra *et al.* (2007) collected serum samples randomly from 600 sheep from 5 different provinces chosen in South Africa. Two sheep abattoirs (representing formal slaughter of sheep) and 1 rural location (representing informal slaughter of sheep) per province were also selected randomly. The serum samples were tested for anti-*Toxoplasma gondii* IgG antibodies using 2 different serological tests: an indirect
fluorescent antibody (IFA) test and an enzyme-linked immunosorbent assay (ELISA) test using a commercial kit. The overall national seroprevalence per province in sheep was found to be 5.6 % (IFA) and 4.3 % (ELISA), respectively. This was lower than in other countries, possibly because South Africa has an arid climate. Differences in seroprevalence in different areas studied suggested an association with the climate and a significant correlation ($P > 0.05$) was detected between the prevalence of *T. gondii* and the minimum average temperature. The seroprevalence was found to be significantly higher ($P < 0.01$) in sheep originating from commercial farms (7.9%) than in rural sheep in the informal sector (3.4%). Also, sheep managed extensively had a seroprevalence of 1.8%, which was significantly lower ($P < 0.05$) than the seroprevalence in sheep under semi-intensive or intensive management systems (5.3%).

Vesco et al. (2007) determined the burden of *Toxoplasma gondii*-infections in sheep in Sicily, southern Italy and the risk factors for infection using sera from 1961 sheep collected just before slaughtering from 62 farms located in 8 out of 9 Sicilian administrative districts. The sera were analysed for *Toxoplasma*-specific IgG antibodies using commercially available enzyme-linked immunosorbent assay. Sheep less than 4 weeks old were further analysed by ELISA for *Toxoplasma*-specific IgM-antibodies. The overall seroprevalence of *Toxoplasma*-specific IgG-antibodies were 49.9% (937/1876) by ELISA. Eighty-seven (54/62) percent of the farms had at least one *Toxoplasma*-positive animal. All the farms fed the animals outdoor on pasture and only one was claiming organic farming. Having cats on the farm, age of the animals, farm size and the use of surface water sources for drinking were all significantly associated with *T. gondii*-infected animals on the farm. The presence of cats on the farm, farm size and using surface water as drinking water for the animals were risk factors for infection in sheep, with age as a significant confounder.

Prevalences of parasitic infections in pigs from different housing systems may vary, due to their contact with the environment, and this
might have consequences for food safety. Van der Giessen et al. (2007) selected 40 organic, 9 free-range and 24 intensive farms and tested a total of 845 serum samples for antibodies specific for *Toxoplasma* and *Trichinella* using ELISA assays. The overall seroprevalence of *Toxoplasma* in the total number of 845 serum samples tested was 2.6%, ranging from 0.38% in intensively raised pigs to 5.62% in free-range pigs. Of the housing systems tested, 4% (intensive farms) to 33% (free-range farms) were infected with *Toxoplasma gondii*. The risk of detecting *Toxoplasma* antibodies in a free-range farm are statistically higher (almost 16 times higher) than in an intensive farm. The authors observed that the risk of detecting specific antibodies was twice as high as in free-range compared with organic farms. The serological study in pigs from different farming systems showed that the seroprevalence of antibodies specific for *T. gondii* was higher in outdoor farming systems than in indoor farming systems.

Anamaria et al. (2008) studied the prevalence of *T. gondii* infection in sheep slaughtered in two slaughterhouses in the north-western of Romania for public consumption. They tested 105 sera by ELISA using a commercial kit. The sheep were divided into two groups according to age: group one - 2-3-year-old sheep (51); group two - 7-8-year-old sheep (54). Of the 105 sera sheep processed 48 were positive, achieving a prevalence of 45.7%. The prevalence of anti- *T. gondii* antibodies in sheep from group one was 45% (23/51), while in group two was 46.3% (25/54). The increased prevalence obtained by ELISA techniques showed the presence of antibodies anti-*T. gondii* at 45.7% of examined sera and they predicted a real risk of human contamination following raw meat consumption.

In order to identify possible risk factors for *T. gondii* infection in goat herds in Ceará, Brazil, Cavalcante et al. (2008) tested 2362 serum samples by ELISA. The serological prevalence was 25.1%. The risk factors identified for *Toxoplasma gondii* infection in goat herds were age, number of cats, use of wooden feeding troughs and absence of feeding troughs. Goats older than 37 months had 2.01 (CI 95%; 1.55 - 2.61)
higher risk of infection than younger animals. Greater risk of infection was observed in farms with more than 10 cats (OR = 1.73; CI 95%; 1.01 - 3.33). The use of wooden feeding troughs represented a high probability of infection (OR = 7.81; CI 95%; 1.66 - 36.67). The lack of feeding troughs also represented a high probability of infection (OR = 5.50; CI 95%; 1.24 - 24.39).

Dubey et al. (2008) investigated the prevalence of *Toxoplasma gondii* on a poorly managed pig farm in Maryland. Serum and tissue samples from 48 of the 100 pigs on the farm were available for *T. gondii* evaluation. Serological testing was performed using both ELISA and the modified agglutination test (MAT). Antibodies to *T. gondii* were detected by ELISA in 12 of 48 animals, while antibodies were detected in 34 of 48 pigs by MAT with titers of 1:10 in 1, 1:20 in 4, 1:40 in 7, 1:80 in 3, 1:160 in 8, 1:320 in 3, 1:640 in 4, and 1:1,280 in 4. Hearts of 16 pigs with MAT titers of 1:10 or higher were bioassayed for *T. gondii* in cats; 11 cats shed *T. gondii* oocysts. Hearts of 22 pigs were autolyzed and bioassayed only in mice; *T. gondii* was isolated from 3 of these 22 pigs. Results document high prevalence of *T. gondii* in pigs on a farm in Maryland.

Carneiro et al. (2009) carried out a study on caprine toxoplasmosis in the state of Minas Gerais, Brazil. To determine the prevalence of toxoplasmosis in goats in Minas Gerais, 767 sera from goats were tested by ELISA (enzyme-linked immunosorbent assay) and IFAT (indirect fluorescence antibody test). The prevalence of antibodies to *Toxoplasma gondii* was 43.0% and 46.0% by ELISA and IFAT, respectively. It was observed that 26.8% of the goats show low-avidity IgG to *T. gondii*. These results suggest the presence of animals in recent phase of toxoplasmosis in Minas Gerais. The risk factors for toxoplasmosis in goats were: age over 36 months (OR = 1.21; IC 95% 1.02–1.44), use of pen (OR = 1.83; IC 95%1.01–3.31) and pure breed animals (OR = 2.49; IC 95% 1.11–5.59).

Carneiro et al. (2009) investigated serum samples from 711 sheep from 109 farms in Minas Gerais State, Brazil, for determining the
Toxoplasma gondii seroprevalence using ELISA (enzyme-linked immunosorbent assay) and IFAT (indirect fluorescence antibody test). The T. gondii seroprevalence was 31.1% and 43.2% by ELISA and IFAT, respectively. Among the positive ELISA sheep sera, 19% of samples contained low avidity IgG, suggesting the occurrence of the recent phase of toxoplasmosis in Minas Gerais. Animals’ age, sex, breed, geographic origin and others variables associated with T. gondii infection were analyzed as risk factors for toxoplasmosis but only the age was significantly associated with the parasite disease, the older animals (age over 36 months) being more frequently infected than young ones (OR = 1.45, 95% CI: 1.20 - 1.74).

Villari et al. (2009) determined the seroprevalence of Toxoplasma gondii infection in pigs raised and slaughtered in Sicily, Southern Italy using a commercial Enzyme-Linked Immunosorbent Assay and evaluated the risk factors associated with the infection. They collected samples from seven slaughterhouses and on-site on 274 raising farms across Sicily. For each sampled pig born and raised in Sicily, information was obtained on gender, age, origin, final destination of meat and farm management. Data on the farm included: farming type, presence of cats and dogs, rodent control by rodenticides, cleaning methods, water supply, altitude and herd size. Antibodies against T. gondii were found in 16.3% of Sicilian pigs. The lowest seroprevalence, 7%, was found in the age group 5-7 months (market pig) and the highest, 19%, in the age group >24 months. Risk factors for seropositivity were: age 11-24 months compared to younger (OR 5.62; CI 1.52-20.8); farrow-to-finish farming type (OR 6.85; CI 1.87-25.01) compared to finishing and farrow-to-breed type; less than 50 pigs on the farm (OR 6.8; CI 1.76-26.2); no use of rodenticides (OR 2.71; CI 1.10-6.64), use of water coming from private sources (especially wells; OR 2.8; CI 1.03-7.72). Pigs raised in Sicily showed a high prevalence of antibodies against T. gondii.

Bartova et al. (2009) examined serum from 547 sheep from nine farms of the Czech Republic for antibodies against Toxoplasma gondii
and *Neospora caninum* by ELISA. Antibodies against *T. gondii* were found in 325 sheep (59%) with prevalence ranging from 11% to 96% in different farms. Antibodies against *N. caninum* were found in 63 sheep (12%) with prevalence ranging from 4% to 21% in different farms. Mixed infections were found in 53 sheep (10%). It was the first evidence of *N. caninum* antibodies in sheep from the Czech Republic.

In order to determine the seroprevalence of *Toxoplasma gondii* and of 2 representative microsporidia (*Encephalitozoon cuniculi* and *Encephalitozoon intestinalis*) in wild boars (*Sus scrofa*) and to compare with the molecular detection of parasites by PCR, Luptkova *et al.* (2010) collected sera from 91 hunter killed wild boars from eight different regions of the Slovak republic and assayed for specific antibodies (IgG) using 2 enzyme-linked immunosorbent assays and the presence of parasites were investigated into seropositive animals by standard and real time PCR from blood for *T. gondii* and from stool samples for *Encephalitozoon* spp. Circulating antibodies targeted against *T. gondii*, *E. cuniculi* and *E. intestinalis* were detected in 18 (19.8%), 4 (4.4%) and 3 animals (3.3%) respectively. The frequency of the anti-*T. gondii* antibodies was significantly higher in young boars (< 1 year old) (37.9%) than in adults (11.3%) (P< 0.01) whereas only adult subjects were seropositive for the microsporidia. A DNA fragment (191 bp) from *T. gondii* was detected in only one positive boar in a relatively high number of copies (1.5 x 10^5) and the presence of *Encephalitozoon* spp. was not confirmed by PCR. These results indicate that wild boars can be contaminated by *T. gondii* and microsporidia with moderate and low seroprevalences respectively and that they do not represent a great (in quantity) reservoir of these parasites, but the risk of toxoplasmosis transmission to humans remains qualitatively important throughout consumption of meat from an effectively infected boar.

Hill *et al.* (2010) tested 6238 sera for *T. gondii* antibodies using a commercial ELISA assay. The seroprevalence was 2.6% with a herd prevalence of 21.6% and a mean within herd prevalence of 2.7%.
Czopowicz et al. (2011) surveyed a herd level seroprevalence of *T. gondii* and *Neospora caninum* infections in goats in Poland. Sera were collected from all 49 breeding goat herds, scattered over the entire country, with the vast majority of them located in the western, central and Northern provinces. Only adult females (≥12 months of age) were included in the study. A herd was recorded as infected if at least one seropositive female was detected. In each herd, simple random sampling was applied and sample size was determined in a way, which allowed to evaluate serological status of a herd at expected individual-level seroprevalence 10% and level of confidence 95%. In total, 1060 sera were tested using two commercial indirect ELISA kits. Sera positive to *N. caninum* were subsequently confirmed with IFAT. The true herd-level seroprevalence was 100% for *T. gondii* and 9.0% for *N. caninum* infection. Three herds positive to *T. gondii* infection were randomly selected and all adult goats were tested with an ELISA. Individual-level true seroprevalence in these herds ranged from 30.2% to 100%. This is the first time that antibodies to *N. caninum* have been detected in goats in Poland.

Toxoplasmosis and neosporosis were recognized as economically important diseases with considerable impact on the livestock industry. Considering the scarce information on the occurrence of *Toxoplasma gondii* and *Neospora caninum* infections in sheep from Uberlândia, Minas Gerais State, Brazil, Rossi et al. (2011) investigated the frequency of antibodies against these parasites in sheep sera from this region by using different serological methods. A total of 155 sheep serum samples were analyzed by the indirect fluorescence antibody test (IFAT) and enzyme-linked immunosorbent assay (ELISA) for the detection of IgG against *T. gondii* and *N. caninum*. Seroreactivity by IFAT showed 80% of samples with titers between 512 and 2048 for *T. gondii* (cutoff ≥ 64) and 78% presenting titers between 50 and 200 for *N. caninum* (cutoff ≥ 50). Seroreactivity by ELISA showed 75% of samples with ELISA index (EI) between 2.0 and 3.0 for *T. gondii* (cutoff ≥ 1.3) and 54% presenting EI between 1.3 and 2.0 for *N. caninum* (cut off ≥ 1.3). Discordant results by
both tests were analyzed by immunoblot, resulting in a total seropositivity of 61% for *T. gondii* and 23% for *N. caninum*, with 41% to *T. gondii* only, 3% to *N. caninum* only, and 20% to both parasites. There was a significant positive association between seropositivity to *T. gondii* and age over one year (*P* < 0.001), but such association was not found for *N. caninum* infection. The authors concluded, since *T. gondii* and *N. caninum* infections were simultaneously present in sheep flocks, it should be emphasized the importance to carry out a regular monitoring of *Toxoplasma* infection due to its high prevalence, its zoonotic potential and induction of reproductive disorders leading to economic losses.

### 2.3.2.c. Serodiagnosis of *Toxoplasma* specific antibodies with recombinant proteins

Since a number of the serological targets for the detection of *T. gondii* antibodies are sequestered within internal organelles, the method of the preparation of the tachyzoite antigen has a significant effect on assay performance, as shown by the differential agglutination assay (Dannemann *et al.*, 1990). The most recombinant proteins, which are presumed to be the candidates for the diagnosis as well as subunit vaccine development, are SAG1, SAG2, MIC3, ROP2, GRA1 and GRA7.

The *Toxoplasma gondii* major surface antigen, called SAG1 or p30, is a highly immunogenic protein which has been the focus of intensive research for use as a diagnostic reagent, as a potential subunit vaccine, and for its role in invasion (Marti *et al.*, 2001). The coding sequence of SAG1 of *T. gondii* was cloned and expressed in *E. coli* as glutathione-s-transferase (GST) fusion protein by Kimbita *et al.* (2001) and the recombinant SAG1 (rSAG1) was used in ELISA. The specificity of the recombinant protein was confirmed by western blot. Results of the ELISA were comparable with those of commercially available latex agglutination test (LAT) kit (Kimbita *et al.*, 2001). The diagnostic efficacy of recombinant proteins SAG1 and GRA7, either individually or as a cocktail, was assessed with 56 reference goat sera by ELISA. The immunoreactivity of the refolded
SAG1 and GRA7 appreciable by high OD values. The reactivity of the recombinant proteins as a cocktail preparation was higher than that of individual proteins in ELISA and could detect accurately the infection in goats. This was reported as the first report of serological detection of caprine toxoplasmosis by ELISA using a cocktail of recombinant Toxoplasma proteins (Velmurugan et al., 2008).

Considering bacterial recombinant SAG1 protein as grossly misfolded and not effectively recognized by antibodies to native SAG1 Kim et al. (1994) cotransfected CHO cells with SAG1 gene and mouse dihydrofolate reductase (DHFR) gene. The purified recombinant SAG1 was recognized by human sera known to be reactive to Toxoplasma proteins.

Aubert et al. (2000) have evaluated the diagnostic utility of eleven Toxoplasma gondii recombinant antigens (P22 [SAG2], P24 [GRA1], P25, P28 [GRA2], P29 [GRA7], P30 [SAG1], P35, P41 [GRA4], P54 [ROP2], P66 [ROP1], and P68) in immunoglobulin G (IgG) and IgM recombinant enzyme-linked immunosorbent assays (Rec-ELISAs). Following an initial evaluation, six recombinant antigens (P29, P30, P35, P54, P66, and P68) were tested in the IgG and IgM Rec-ELISAs with four groups of samples which span the toxoplasmosis disease spectrum (negative, chronic infection, acute infection, and recent seroconversion). The results suggest that the combination of P29, P30, and P35 in an IgG Rec-ELISA and the combination of P29, P35, and P66 in an IgM Rec-ELISA can replace the tachyzoite antigen in IgG and IgM serologic tests, respectively. The relative sensitivity, specificity, and agreement for the IgG P29-P30-P35 Rec-ELISA were 98.4, 95.7, and 97.2%, respectively. The resolved sensitivity, specificity, and agreement for the IgM P29-P35-P66 Rec-ELISA were 93.1, 95.0, and 94.5%, respectively. Relative to the tachyzoite-based immunocapture IgM assay, the IgM P29-P35-P66 Rec-ELISA detects fewer samples that contain IgG antibodies with elevated avidity from individuals with an acute toxoplasmosis.
Gamble et al. (2000) assessed five recombinant *Toxoplasma gondii* antigens, designated B427, C51, C55, V22, and MBP30 for their potential use in an enzyme-linked immunoassay (EIA) for detection of *T. gondii* infection in swine. The antigens were evaluated with sera from young pigs that had been fed 1–10,000 *T. gondii* oocysts of the VEG or GT-1 strains. Results were compared with an EIA using a native *T. gondii* antigen extract. All 5 recombinant antigens, as well as native antigen, detected antibody responses in pigs inoculated with 1 or 10 oocysts of the VEG strain as soon as 3 week post infection. This antibody response persisted, at varying levels, for 14 week when the experiment was terminated. All antigens also detected antibody responses in pigs 4 week after inoculation with 10,000 oocysts of the GT-1 strain. The antibody response recognized by native antigen remained high through 51 week after inoculation. However, there was considerable animal-to-animal variation in responses to the individual recombinant antigens. The results suggested that these antigens might be useful for the serological detection of *T. gondii* infection in pigs.

Kimbita et al. (2001) cloned SAG1 into the plasmid pGEX-4T-3 and subsequently expressed in *Escherichia coli* (*E. coli*) as a glutathione-S-transferase (GST) fusion protein. The ELISA with rSAG1 was able to differentiate very clearly between sera from cats or mice experimentally infected with *T. gondii* and sera from normal cats or mice. The ELISA detected no cross-reactivity with sera from mice experimentally infected with the closely related parasite *Neospora caninum*. Some 193 cat sera were tested for antibodies to *T. gondii*, out of which 40 (20.7%) reacted positively by ELISA with the rSAG1 while another 79.3% cats reacted negative to the assay. Both positive and negative sera were confirmed by Western blot analysis. The results of ELISA were in agreement with those of a commercially available latex agglutination test (LAT) kit, although the former had higher titers than the latter.

Abu-Zeid YA (2002) used ELISA was for detection of antibodies against the immunodominant surface antigen 1 of *Toxoplasma gondii* and peroxidase-conjugated protein G instead of commercially
unavailable enzyme-conjugated anti-dromedary antibody. A latex agglutination test was employed to select 20 seronegative control animals, and peroxidase-conjugated protein A was used for comparison with protein G. The overall seroprevalence rate was 31.4%; males had to some extent higher seropositive rate than females (P = 0.077). Seropositive camels sampled in winter had significantly higher-antibody levels than those sampled in summer (P < 0.01). ELISA values using protein G and protein A conjugates were significantly correlated (Spearman’s rho = 0.797; n = 185; P < 0.001).

In order to improve serodiagnostic methods for the diagnosis of acute toxoplasmosis during pregnancy, Pfrepper et al. (2005) developed a recombinant antigen based new test system. Five recombinant Toxoplasma gondii antigens (ROP1, MAG1, SAG1, GRA7, and GRA8) were cloned in Escherichia coli, purified, and applied directly onto nitrocellulose membranes in a line assay (recomLine Toxoplasma). A panel of 102 sera from 25 pregnant women with supposed recent toxoplasmosis and from two symptomatic children was compared to a panel of 71 sera from individuals with past infection. Both panels were analyzed using a recombinant line assay for immunoglobulin G (IgG), IgM, and IgA antibodies and a reference enzyme-linked immunosorbent assay. Within the IgM-positive samples, antibodies against ROP1 were predominant regardless of the infection state. In IgG analysis a characteristic antibody pattern was found for very recent infections. This pattern changed to a different one during the time course of infection: antibodies against GRA7 and GRA8 were characteristic for very early IgG, whereas antibodies against SAG1 and MAG1 appeared significantly later. These results were further confirmed by determination of the IgG antibody avidity for every single recombinant antigen. In the time course of infection, IgG antibodies against the early recognized antigens matured significantly earlier than those directed against the later antigens did. The IgA patterns did not give reliable information about the infection time points. The data revealed that the
recombinant line assay provides valuable information on the actual state of infection, especially during the early infection time points.

Lu et al. (2006) sequenced the complete P35 surface antigen of *Toxoplasma gondii* (GenBank AF310261). The P35-GST protein was used as antigen to detect 125 sera samples by double-sandwich ELISA. P35-IgM positive rate in a chronic infected group, a persistent IgM positive chronic group, a recently seroconvered group and an acute infected group were 4% (1 out of 25), 16% (4 out of 25), 88% (22 out of 25), and 100% (25 out of 25), respectively. The sensitivity and specificity of the recombinant full-length P35 antigen were 100 and 96%, respectively. The detailed expression patterns of P35 antigen were studied in 36 IgM and IgG positive sequential samples from 10 recently seroconvered patients. Results showed that the P35-IgM positive rate decreased as the time after the first seroconversion increased. P35-IgM positive samples in the first, second, third, fourth, and fifth month after the first seroconversion test were 90, 78, 57, 50, and 33%, respectively. P35-IgG positive samples in the first, second, third, fourth, fifth, sixth, and seventh month after the first seroconversion test were 70, 100, 100, 100, 67, 100, and 100%, respectively. All samples were P35-IgM negative after the fifth month, and P35-IgG negative after the seventh month from seroconversion. P35-IgM existed the shortest time and was a more specific marker for *T. gondii* acute infection than P35-IgG, IgM, and IgG to whole tachyzoites antigens.

Laboratory diagnostics of toxoplasmosis depends primarily on serological methods detecting specific antibodies. Since these methods do not always enable specific and sensitive recognition of the infection and phase of toxoplasmosis, the search for new diagnostic tools continues. Recombinant antigens promise a new alternative in diagnostics of *Toxoplasma gondii* infections. Gatkowska et al. (2006) evaluated the usefulness of six recombinant *T. gondii* antigens, viz. GRA1, GRA6, GRA7, p35, SAG1, and SAG2 for the detection of primary murine toxoplasmosis. Sera obtained from infected mice differing in their natural susceptibility to *T. gondii* infection, BALB/c (relatively
resistant) and C57BL/6 (relatively susceptible), were tested using ELISA. During acute infection high response to GRA7, GRA6, and p35 antigens was noticed, whereas a strong reactivity with surface antigens SAG1 and SAG2 was characteristic for chronic toxoplasmosis. The results showed that the recombinant antigens were useful in distinguishing between acute and chronic toxoplasmosis regardless of the genetically determined susceptibility of the host.

Carvalho et al. (2008) evaluated the performance of three monoclonal antibodies (MAbs) in reverse enzyme-linked immunosorbent assays (ELISAs) for detecting immunoglobulin G (IgG), IgM and IgA antibodies against *Toxoplasma gondii* in 175 serum samples from patients at different stages of *T. gondii* infection, as defined by both serological and clinical criteria. The results were compared with those obtained by indirect ELISA using soluble *Toxoplasma* total antigen (STAg). Our data demonstrated that MAb A3A4 recognizes a conformational epitope in SAG1-related-sequence (SRS) antigens, while A4D12 and 1B8 recognize linear epitopes defined as SAG2A surface antigen and p97 cytoplasmatic antigen, respectively. Reverse ELISA for IgG with A3A4 or A4D12 MAbs was highly correlated with indirect ELISA for anti-STAg IgG, whereas only A4D12 reverse ELISA showed high correlation with indirect ELISA for IgM and IgA isotypes. It was the first report analyzing the performance of a reverse ELISA for simultaneous detection of IgG, IgM, and IgA isotypes active toward native SAG2A, SRS, and p97 molecules from STAg, using a panel of human sera from patients with recent and chronic toxoplasmosis. Thus, reverse ELISA based on the capture of native SAG2A and SRS antigens of STAg by MAbs could be an additional approach for strengthening the helpfulness of serological tests assessing the stage of infection, particularly in combination with highly sensitive and specific assays that are frequently used nowadays for diagnosis of toxoplasmosis during pregnancy or congenital infection in newborns.

Hosseininejad et al. (2009) use D affinity purified 30 KDa *Toxoplasma gondii* surface antigen (SAG1) in an indirect ELISA and compared it with an existing indirect fluorescent antibody test.
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(IFAT) using 245 serum samples collected from dogs from three adjacent Iranian provinces. IFAT examination revealed positive results in 73 dogs, with titers ranging from 1:16 to 1:1024. A suitable ELISA cut-off was determined by ROC analysis in comparison with IFAT. Results showed a relative sensitivity and specificity of 94.52% and 93.60%, respectively. No cross-reactivity was detected between antibodies against *T. gondii* and a closely related protozoan parasite, *Neospora caninum*, using this newly developed ELISA test.

Wua *et al.* (2009) expressed a truncated SAG1 gene in *Escherichia coli*. An ELISA kit based on the purified recombinant truncated SAG1 (rtSAG1) was developed which was used to detect antibodies against *T. gondii* in human sera. The results showed that the infection of *T. gondii* could be detected sensitively and specifically by this serologic method. The positive concordance between rtSAG1-ELISA and Western blot, the gold standard, was 93.9% (31/33). However, the positive concordance between the commercial available ELISA Kit 1 (Haitai, Zhuhai, China) and ELISA Kit 2 (DiaSorin ETI-TOXOK-M reverse Plus, Italy) with Western blot was 79.5% (31/39) and 91.2% (31/34), respectively. Comparatively, the positive concordance of ELISA Kit 1 and 2 with Western blot was lower than rtSAG1-ELISA, in particular, the ELISA Kit 1 (*P* < 0.01), which indicated that the rtSAG1 protein could be used as the diagnostic antigen for human toxoplasmosis.

### 3.3 Indirect fluorescent antibody test:

Van der Puije *et al.* (2000) used the enzyme-linked immunosorbent assay (ELISA) to detect anti-*Toxoplasma gondii* antibodies in 1258 small ruminants (732 sheep and 526 goats) sampled from 28 different locations in the three ecological zones of Ghana. The animals sampled had an overall seroprevalence of 30.5% (384 of the total). Sheep had a higher overall prevalence (33.2%) compared to the goats (26.8%). Animals sampled from the Coastal Savannah and the Forest zones had prevalence of 39.4% and 39.1%, respectively, which were significantly higher (*P*<0.01) than the prevalence recorded for the drier Guinea
Savannah zone (20%). Prevalence of antibodies in female animals (35.8%) was significantly higher ($ P<0.01$) than that for males (21.1%). Significant differences were also observed between breeds and age groups. The ELISA was found to be both highly sensitive (92%) and specific (91%) when compared to the IFAT, which was used as a reference test.

Figueiredo et al. (2001) a comparative study of the indirect haemagglutination (IHA), immunofluorescence (IFAT) and immunoenzymatic (ELISA) tests was carried out to determine the prevalence of *Toxoplasma gondii* antibodies in goats. One hundred seventy-four serum samples were obtained from four goat herds from the region of Uberlândia, State of Minas Gerais. The distribution of the animals, according to their origin, was as follow: 71 from herd I; 39 from herd II; 37 from herd III; and 27 from herd IV. Serum samples were analyzed by IHA, IFAT and ELISA, considering the reactivity of the serum samples at dilution $\geq 1:64$ as cut off titer for the three tests. A global seroprevalence of 18.4% was observed, with significantly higher positivity rate in the herd II (66.7%) and older animals ($> 36$ months). A high and significant positive correlation was found between the titers obtained by the IHA versus IFAT, IHA versus ELISA, and ELISA versus IFAT. Therefore, it can be concluded that the three analyzed tests have shown to be highly concordant and appropriate for epidemiological surveys of *Toxoplasma* infection in goats. Although the seroprevalence of *T. gondii* infection in goats is relatively low in this region as compared to other regions of the country, adequate management might be useful and essential to control the infection in the goat herds.

Bartova et al. (2006) collected sera from hunter-killed wild boars (*Sus scrofa*) during 1999–2005 from seven different regions of the Czech Republic and assayed for antibodies to *Toxoplasma gondii* by indirect fluorescence antibody test (IFAT). Antibodies to *T. gondii* were detected in 148 (26.2%) of 565 wild boars with serum dilutions of 1:40 in 40, 1:80 in 40, 1:160 in 27, 1:320 in 19, 1:640 in 18 and 1:1280 in 4 wild boars.
Garcia et al. (2006) reported Indirect ELISA and IFAT as more sensitive and specific than agglutination tests. However, MAT is cheaper, easier than the others and does not need special equipment. The purpose of this study was to compare an enzyme linked immunosorbent assay using crude rhoptries of *Toxoplasma gondii* as coating wells (r-ELISA) with indirect fluorescence antibody test (IFAT) and modified agglutination test (MAT) to detect anti-*T. gondii* antibodies in sera of experimentally infected pigs. Ten mixed breed pigs between 6.5 and 7.5 weeks old were used. All pigs were negative for the presence of *T. gondii* antibodies by IFAT (titre < 16), r-ELISA (OD < 0.295) and MAT (titre < 16). Animals received $7 \times 10^7$ viable tachyzoites of the RH strain by intramuscular (IM) route at day 0. Serum samples were collected at day − 6, 0, 7, 14, 21, 28, 35, 42, 50, and 57. IFAT detected anti-*T. gondii* antibodies earlier than r-ELISA and MAT. The average of antibody levels was higher at day 35 in IFAT ($\log_{10} = 2.9$) and in MAT ($\log_{10} = 3.5$), and at day 42 in r-ELISA (OD = 0.797). The antibody levels remained high through the 57th day after inoculation in MAT, and there was a decrease tendency in r-ELISA and IFAT. IFAT was used as “gold standard” and r-ELISA demonstrated a higher prevalence (73.3%), sensitivity (94.3%), negative predictive value (83.3%), and accuracy (95.6%) than MAT. Kappa agreements among tests were calculated, and the best results were shown by r-ELISA × IFAT ($\kappa = 0.88$, $p < 0.001$). Cross-reaction with *Sarcocystis miescheriana* was investigated in r-ELISA and OD mean was $0.163 \pm 0.035$ ($n = 65$). Additionally, none of the animals inoculated with *Sarcocystis* reacted positively in r-ELISA. Our results indicate that r-ELISA could be a good method for serological detection of *T. gondii* infection in pigs.

In a comparative serological examination of 300 serum samples from sheep slaughtered in the main abattoir in Cairo, Egypt, Shaapan et al. (2008) revealed a higher prevalence of toxoplasmosis (43.7%) with the modified agglutination test (MAT), followed by the enzyme linked immune-sorbant assay (ELISA) (41.7%) and the indirect fluorescent antibody test (IFAT) (37%), while the lowest prevalence was detected with
the dye test (DT) (34%). When the data from the first three serological tests were compared with that of the DT test, which was used as a reference test for toxoplasmosis, MAT had the highest sensitivity (96%), followed by ELISA (90.1%) and IFAT, which demonstrated the lowest sensitivity (80.4%). Conversely, IFAT had the highest specificity (91.4%), followed by MAT (88.9%) and ELISA (85.9).

3.4 Characterization of native whole tachyzoites lysate antigen

SDS-PAGE and western blot

Toxoplasma gondii tachyzoites were surface radio-iodinated by the lactoperoxidase technique and the solubilized membrane proteins were analyzed by two-dimensional polyacrylamide gel electrophoresis (Handman et al., 1980). Four major labeled proteins with apparent molecular weight of 43,000, 35,000, 27,000, and 14,000 were detected.

A soluble and an insoluble fraction of ultrasonicated Toxoplasma gondii (RH strain) tachyzoites were prepared by ultracentrifugation. The insoluble parasite fraction was solubilized by incubation in 1% sodium deoxycholate (DOC). The molecular weights (m.w.) of the polypeptides in the fractions were determined by SDS-PAGE (Johnson et al., 1981). After comparison with the molecular weight of the polypeptides of appropriate controls the soluble Toxoplasma fraction contained at least 9 parasite polypeptides with molecular weight of 20K, 22K, 23K, 29K, 66K, 69K, 90K, 98K and 133K and the insoluble parasite fraction contained at least 8 Toxoplasma derived polypeptides with molecular weight of 28K, 41K, 43K, 49K, 53K, 61K, 70K and 80K. The presence of glycosylated polypeptides was detected by incubation of sliced gels with $^{125}$I Concanavalin A (Con A). The soluble Toxoplasma fraction contained no major glycopeptide bands while the insoluble parasite fraction contained at least 3 glycosylated polypeptides of Toxoplasma origin with molecular weight of 29K, 53K and 123K.

Partanen et al. (1983) separated proteins of Toxoplasma gondii by SDS-polyacrylamide gel electrophoresis with subsequent transfer to a nitrocellulose sheet by electrophoretic blotting. Immunologically reactive
polypeptides were detected by human sera with previously known *Toxoplasma* antibody levels. Heavy chain-specific, peroxidase-conjugated anti-human immunoglobulins were used as the indicator antibodies for the separate identification of IgG and IgM reactive polypeptides. IgG toxoplasma antibodies reacted with several antigens of $M_r \approx 27000–67000$, while toxoplasma-specific IgM seemed to detect only a few polypeptides. The $M_r$ of 35000 for the dominating IgM reactive polypeptide was observed.

Sharma *et al.* (1983) shown that three major antigens of apparent molecular weight of 32,000, 22,000 and 6000 respectively were recognized both IgM and IgG antibodies *Toxoplasma gondii* infected human sera. In addition, IgG antibodies recognized at least 17 other antigenic component.

Huskinson *et al.* (1989) studied the immunoglobulin G (IgG) subclass response to *Toxoplasma gondii* antigens during the acute and chronic stages of *T. gondii* infection by immunoblots with reduced antigen (RA) and Non reduced antigen(NRA) preparations. In all groups, an antigen with molecular weight (MW) of 30,000 was the most intensely stained and frequently recognized by IgG1 antibodies in NRA preparations. In RA preparations, antigens of MW 35,000 and 30,000 were the most intensely stained and frequently recognized by IgG1 antibodies. An antigen with a MW of 22,000 was intensely stained in the IgG immunoblots of the NRA preparation and to lesser extent in the RA preparation. In contrast to an immunoblots with IgG1 antibodies, very few antigens in the RA and NRA preparation were detected by IgG2 and IgG3 antibodies; IgG4 antibodies were rarely detected by any antigens. Of interest was that IgG2 antibodies detected antigens distributed over the entire MW range, whereas those detected by IgG3 antibodies were located mostly below the 35,000 MW marker.

Chardes *et al.* (1990) studied the *Toxoplasma gondii*-specific antibody responses in serum, intestinal secretions, and milk were identified with an enzyme-linked immunosorbent assay following a single oral infection of mice with strain 76K cysts of *T. gondii*. They reported...
that immunoglobulin A production began during week 2 of infection in serum and milk and during week 3 of infection in intestinal secretions and persisted in all three throughout the experiment (17 weeks). IgG but not IgM antibodies were detected in intestinal secretions later in the infection. Serum and milk IgG and IgM production began at the same time after infection as did the IgA response. In Western blotting (immunoblotting), intestinal IgA antibodies were shown to react with antigens co-migrating with the *T. gondii* proteins p22, p23, p30, and p43, the 28-kilodalton antigen, and the 55- and 60-kilodalton rhoptry proteins, as recognized by specific monoclonal antibodies. Milk IgA antibodies reacted with antigens co-migrating with p30 and p43. Most of the antigens recognized by IgA antibodies were also detected by IgG antibodies. IgA antibodies from all three biological samples detected the same major *T. gondii* antigens, therefore the authors concluded that there was apparently no specific antibody production unique to one locality.

A selective *Toxoplasma gondii* antigen preparation consisting of extracted *T. gondii* tachyzoite cell membrane proteins arranged into submicroscopical particles (iscoms), was used by Lovgren et al., (1987) as coating antigen in an ELISA (iscom ELISA). SDS-PAGE revealed that the *Toxoplasma* iscoms were found to consist of 4 major proteins with estimated molecular weights of 50 kD, 44 kD, 32 kD and 20 kD. By immunoblot analysis using *T. gondii* positive sheep sera, 3 major antigenic components with estimated molecular weights of 50 kD, 44 kD and 32 kD were detected. 85 sheep sera submitted for routine *T. gondii* serology were used to compare the results in the iscom ELISA, the *Toxoplasma* IFAT (indirect fluorescent antibody test) and an ELISA (FT-S ELISA) employing a soluble (cytoplasmatic) *T. gondii* antigen. The iscoms ELISA was found to be as sensitive as the other tests in detecting low levels of *T. gondii* antibodies and in discriminating positive and negative sera. In strongly positive sera, most likely also containing antibodies to intracellular components, the FT-S ELISA gave considerably higher titres than did the iscom ELISA and the IFAT. Since positive FT-S ELISA
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results have been recorded during recent mono infection with *Sarcocystis cruzi* in calves, whereas no such cross-reaction was observed in the iscom ELISA of IFAT (Uggl et al., 1987), it was concluded that the iscom ELISA combines the specificity of the IFAT with the objectivity and simplicity of the ELISA.

Potasman *et al.* (1987) used The Sabin-Feldman dye test, the immunoglobulin M (IgM) immunosorbent agglutination assay, and the immunoblot technique to study the evolution of the antibody response to *Toxoplasma gondii* and to examine antigens of the organism recognized by antibodies in the sera of 12 congenitally infected infants and 7 mothers. In the sera of eight infants, a significant rise was noted in the dye test titers, while the serum of only one infant demonstrated a late increase in the IgM immunosorbent agglutination assay titer. In each infant and mother, antigens with approximate masses of 35,000 and 115,000 daltons were strongly recognized by IgG antibodies. An antigen(s) with an approximate mass of 4,000 daltons was recognized by IgM antibodies in the sera of each of the mothers but was recognized by antibodies in the sera of only two of the infants.

Yamamoto *et al.* (1997) performed the immunoblotting by taking both absorbed and non-absorbed serum samples from patients with chronic and acute toxoplasmosis were taken into account for analysis. Finally he found the antigenic bands of *Toxoplasma gondii* recognized by IgG antibodies from chronic toxoplasmosis patients before and after absorption was 93, 82, 76, 63, 58, 54, 50, 45, 43, 39, 35, 30, 27, 24, 22, 20, 14KDa and 63, 58, 54, 50, 43, 35, 30, 27, 24 KDa respectively whereas in case of acute cases for the detection of IgM antibodies 91, 84, 74, 69, 64, 60, 54, 50, 47, 35, 32, 30, 18 KDa and 69, 60, 54, 35, 32, 30, 18 KDa respectively with the G-toxo-HA reagent, in the immunoblot assay.

Harkins *et al.* (1998) analysed IgG antibody responses of sheep, goats and cattle inoculated subcutaneously with live *Neospora caninum* tachyzoites of the Ncl isolate by Western blotting. These sera were also used to probe blots of *Toxoplasma gondii* antigen and, while a number of protein bands were recognized, there was no consistency
within or between animal species. The IgG antibody responses of sheep, goats and cattle orally infected with *T. gondii* oocysts of the M3 isolate were analysed by the same methods. Antibodies were detected to a range of S48 *Toxoplasma* tachyzoite antigens (11 to 83 kDa). The dominant antibody responses were directed against proteins at 11, 16–17, 21·5, 22·5–23·5, 26–28·5, 32–35, 49·5, 50·5, 53, 54·5, 60·5 and 61 kDa, with sera from all three species.

On analysis of western blotting, Saavedra and Ortega (2004) found that three bands of 30-32, 26, and 4-6 KDa were frequently observed in addition to other bands, such as 22-24, 15-17, and 10 KDa. The band of 30-32 KDa was the most predominant antigen that corresponds to the 30 KDa membrane glycoprotein, i.e. surface antigen P30 (SAG1).
3.1 **Sera samples:**

3.1 a. **Sheep sera:**

A total number of 444 sera samples were collected from sheep of three different breeds *viz.* Shahabadi, Muzaffarnagri and Chotanagpuri. The collection was made from two government farms of Chatra and Ranchi district of Jharkhand as well as from some selected villages of Ranchi.

3.1 b. **Goat sera:**

A total number of 445 sera samples were collected from goats of two different breeds *viz.* Black Bengal and Beetal. The sera samples from sheep and goats were collected from two different government farms located in Chatra and Ranchi districts of Jharkhand as well as from some selected villages of Ranchi.

3.1 c. **Pig sera:**

A total number of 240 sera samples were collected from pigs of four different breeds *i.e.* Hampshire, Tamworth, crossbreds of Tamworth and local indescriptive breed (T&D) as well as local breed from an organized farm located in Ranchi as well as from slaughter house (Namkum, Satrangi, Shalimar).

The sera samples were collected randomly during the months of July to November, 2010 from the different species of animals mentioned above differing in age, sex and body weight. However, sheep and goat of less than 6 months of age and pigs less than 3 months of age were excluded from sampling to avoid measuring antibodies passively transferred.

3.1 d. **Reference sera:**

The reference positive sera and negative sera of the sheep, goat and pigs were procured from the *Toxoplasma* laboratory, Division of
Parasitology, IVRI.

The blood samples (2-3 ml) were collected aseptically from the jugular vein of sheep, goats and pigs using sterile syringes without anticoagulant for separation of sera in wide mouth test tubes (Borosil). The bigger pig ear vein had also been selected for collection of blood. From slaughterhouse, blood samples were collected during slaughter of the animals. The blood was allowed to clot in normal temperature and then transported to the laboratory on ice. Following isolation all the sera samples were labeled properly and preserved in – 20°C till use. The sera samples were tested for detection of \textit{T. gondii} specific antibodies in the Division of Parasitology, Indian Veterinary Research Institute, Izatnagar.

\subsection*{3.2.1 \textit{Toxoplasma gondii}, RH strain:}
A mouse adopted RH strain was used in the present study. The strain was obtained from Division of Parasitology, IVRI, Izatnagar.

\subsection*{3.2.2 Mice:}
Adult Swiss albino mice of either sex weighing 25- 30 g were used in the present study for routine passage of tachyzoites required for native whole cell lysate \textit{Toxoplama} antigen preparation.

\subsection*{3.2.3 Maintenance of \textit{T. gondii} infection in mice:}
Experimental mice were inoculated with $1 \times 10^3$ mouse adopted RH strain of \textit{T. gondii} intraperitoneally for setting up of infection. The infected mice were examined daily for the development of peritonitis. Peritoneal fluid containing the tachyzoites was collected aseptically for serial passage in outbred Swiss albino mice.

The infected mice were euthanized following development of peritonitis using chloroform anaesthesia. About 5 ml of PBS (pH7.2) was injected in the peritoneal cavity using a 10 ml glass syringe (Dispovan) fitted with 21 gauze needle. The peritoneal lavage was aspirated into the same syringe avoiding rupture to other abdominal organs. The process was repeated twice or thrice till the peritoneal lavage become clear. The
3.4.5 Purification of tachyzoites:

The peritoneal lavage containing free tachyzoites, as well as, infected macrophages was collected in PBS (pH, 7.4) and sedimented by centrifugation at 5000 rpm for 10 min. The pellet was re-suspended in small quantity of PBS (pH, 7.4). The intracellular tachyzoites were rendered cell-free by rupturing the intact parasitized macrophages mechanically and passing the contents through a 27 G needle fitted in a 10 ml sterile syringe. The resultant tachyzoites suspension was washed again by re-suspending in 20 ml PBS. The supernatant was collected carefully and passed through a wet polycarbonate membrane filter of 3 \( \mu \)m pore size (Millipore) slowly at the rate of 1ml per 2-3 min (Gross et al., 1991). The filtered suspension was further centrifuged at 3000 rpm for 10 min to sediment the tachyzoites. The supernatant was completely removed and the sedimanted tachyzoites were used for various experimental purposes.

3.4.6 Enumeration of tachyzoites:

The tachyzoite suspension was thoroughly pipette to break down the clumps and two dilutions \( i.e. \) 10 and 100 fold were made to facilitate the cell counting. The first dilution was made by adding 10 \( \mu l \) of Trypan blue (0.4%) solution (10 fold dilution). The second dilution (100 fold dilution) was prepared by adding 10 \( \mu l \) of Trypan blue to 1 \( \mu l \) of the first solution. Ten microliters of each of these dilutions was charged into the two different chambers of a Neubauer’s haemocytometer. After allowing the tachyzoites to settle, the unstained (viable) tachyzoites were counted in all four corner of squares (WBC chamber) using 40x objective of the microscope. The total number of tachyzoites in the suspension was calculated using the following formula:

\[
\text{Tachyzoites per milliliter} = \text{Average number of tachyzoites (from contents were washed thrice in PBS (pH-7.2) and the number of live tachyzoites was counted. The tachyzoites were passaged in successive groups of mice following the same technique.}
four corners squares) x dilution factor x $10^3$

Values were considered only if those of the two dilutions were concordant.

**3.4.7 Preparation of whole tachyzoite lysate antigen:**

The purified tachyzoite suspension in PBS (pH, 7.4) was subjected to rapid freezing and thawing for five times in liquid nitrogen, followed by 10 cycles of ultrasonification (soniprep) at 15 amplitude, each for 30 sec, on ice. An interval of 30 sec was given between the cycles. The disruption of tachyzoites was monitored microscopically at each step. After confirming the total disruption, the sonicate was centrifuged at 13,000 rpm for 30 min at 4°C. The supernatant was carefully aspirated and the protein concentration was estimated following the method of Lowry et al. (1951).

**3.5 Enzyme linked immunosorbent assay (ELISA):**

The test was performed following the method of Lin et al. (1992) with minor modifications. The dilution of the reagents for ELISA was optimized by checkerboard titration. Polystyrene plate (Maxisorp, Nunc) were coated with 100 µl of either TLA antigen (2µg/ml) or recombinant SAG1 protein (2µg/ml) diluted in carbonate-bicarbonate buffer (pH, 9.6) per well. The plates were coated by incubation at 37°C for 1h and then at 4°C for overnight. Excess unbound antigen was removed by washing the plates thrice in PBS (pH 7.2) containing 0.05% Tween -20. The free binding sites in the wells of the ELISA plate were blocked with 5% fat-free milk powder (Ameresco) prepared in PBS by incubating at 37°C for 2h. Following washing 100 µl of test serum sample (Sheep/ Goats/ Pigs) diluted 1:100 in 1% non fat milk powder was added to the individual wells and incubated at 37°C for 1h. Excess unbound antibodies were removed by washing as mentioned above and 100 µl of HRPO- labeled antispecies conjugate, diluted 1:40000 in 1% non-fat milk powder, was added to the individual wells. The plates were incubated at 37°C for 1h. Following incubation the plates were given final wash with PBS-T thrice.
and 100 µl of OPD substrate dissolved in citrate-phosphate buffer (pH 5.0) containing H$_2$O$_2$ was added to each individual well. The reaction was allowed to progress for 10 min at dark and subsequently stopped by adding 50 µl of 3N HCl. Absorbance was read at 492 nm in an ELISA reader (Microscan).

### 3.6 Preparation of antigen slide for IFAT:

Antigen slides were prepared, as per the procedure outlined in the United State Department of Health, Education and Welfare (USDHEW) manual (1976) with minor modification. Circles of 5 mm diameter were marked on the opposite side of clean, grease free glass slides using a glass marking diamond pencil. The purified *T. gondii* (RH strain) tachyzoites were suspended in PBS (pH 7.4) and the number was adjusted to 100-200 tachyzoites per high power field. Approximately, 10-15 µl of the tachyzoite suspension was directly charged in a circle of the glass slide and sucked back immediately with the help of micropipette to facilitate preparation of a thin smear. Once the spot had dried up, the slides were placed in coupling jar containing chilled acetone and incubated at 4°C over night. The antigen coated slides were then air-dried, wrapped in aluminium foil and stored at -20°C for future use in IFAT.

#### 3.6.1 Indirect Fluorescent antibody test (IFAT)

The indirect Fluorescent antibody test (IFAT) was performed following the method described in USHDEW manual (1976) with minor modification. Antigen slides were thawed at room temperature for 5 min, rinsed with distilled water and air dried. The circles on the slide were marked with wax pencil to prevent capillary movement of the well contents. Experimental neat sera samples were double diluted up to 1:4096 and were loaded in antigen wells. The positive and negative reference control sera were diluted upto 1:16 and 1:8, respectively and each were loaded in duplicate wells of all slides. Slides were incubated at 37°C for 30 min in a humid chamber. Subsequently, the slides were rinsed once with distill water and thrice in PBS (pH 7.6). Each washing
was given for 5 min with a final rinse in distill water. Following air-
drying ten micro liters of rabbit anti-goat IgG-FITC conjugate, diluted 1:80 in 1% Evan blue (PBS pH 7.6), was loaded in each well. The slides were incubated, washed and air dried as before. The slides were mounted immediately using a drop of buffered glycerol under 40X magnification of a fluorescent microscope for detection of specific fluorescence.

3.2.1 Determination of sensitivity and specificity of diagnostic ELISA using rSAG1 and WTLA antigen against standard IFAT.

The diagnostic sensitivity and specificity of the ELISA using rSAG1 and WTLA as diagnostic antigen was determined using the formulae (Ruiz-Tiben et al., 1979; Martin et al., 1987):

\[
\text{Sensitivity} = \frac{TP}{TP + FN} \times 100
\]

Where, TP is true positive i.e. serum samples positive both with IFAT and ELISA. FN is false negative i.e. serum samples positive with IFAT but negative with ELISA.

\[
\text{Specificity} = \frac{TN}{TN + FP} \times 100
\]

Where, TN true negative i.e. serum samples negative both with IFAT and ELISA. FP is false positive i.e. serum samples negative with IFAT but positive with ELISA.

The sensitivity and specificity of the ELISA was determined for the individual target species, viz. sheep, goat and pigs separately.

3.8 Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) of soluble T. gondii tachyzoite lysate proteins

SDS-PAGE analysis of the soluble T. gondii proteins was carried out following the method of Laemmli (1970) with minor modification. A
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6-15% gradient polyacrylamide gel was prepared for electrophoretic separation of the constituent proteins of *T. gondii* tachyzoite using a mini gel electrophoresis apparatus (Atto). The whole tachyzoite lysate protein was boiled for 3 min in sample buffer and 40µl of the sample was charged into individual wells of a precasted gel. The samples were run against a molecular marker (Fermentas) at 150 volt for 3 hr. Following electrophoresis, the gel was stained with 0.1% Coomassie brilliant blue-R-250 for 1h and destained for visualization and documentation of the polypeptides, separated on the basis of molecular mass, using syngene software in a gel documentation system.

3.9 Western blotting

Immunoblotting of *T.gondii* RH strain soluble tachyzoites antigen was carried out following the method described by Lin (1997). The separated polypeptides were electrophoretically transferred to PVDF membrane using transfer blot apparatus (BIO-RAD) following the method of Towbin *et al.*, (1979). Electrophoresis was carried out using constant voltage of 2mA/cm² gel for 3 h. The transfer efficiency was checked by staining the membrane with 0.1% Ponceau-S stain. The PVDF membrane was incubated in 5% skimed milk prepared in PBS at 4°C for O/N to block the free sites. The PVDF membrane was washed 3 times (15 min each) with PBS-T and was marked with pencil and incubated at 25°C for 90 min with pooled field sera diluted 1:100 in PBS-T containing 5% skimmed milk. The membrane was washed thrice in PBS-T, each for 15 min, and then incubated with rabbit anti-species IgG HRPO conjugate diluted 1:500 in PBS-T containing 3% skimmed milk at 25°C for 1 h. Following washing, the membrane was incubated with diamino benzidine substrate at 25°C in a dark chamber. The reaction was stopped after optimum development of colour.
4.1 Experimental *T. gondii* infection in mice

The symptoms of illness appeared on day 3 post infection (PI) in the experimentally infected mice. Marked peritonitis with tachypnoea was noticed on day 4 PI. In the terminal stage i.e. 4-5 hr prior to death the mice were unable to move on flat surface due to ascites.

The peritoneal fluid was collected from the experimentally infected mice on development of peritonitis. The microscopic examination revealed the presence of large number of tachyzoites with few macrophages or RBC contamination. On an average, an adult mouse yielded about of $3.6 \times 10^6$ tachyzoites/ml. (Fig. 1)

4.2 Purification of tachyzoites

The purification of tachyzoites through polycarbonate membrane filter (3.0 µm pore size) did not affect the viability or virulence of the organisms. However, the count of tachyzoites was affected due to membrane filtration resulting in 40% recovery. (Fig. 2)

4.3 Soluble whole tachyzoite lysate antigen

The purified tachyzoites, suspended in appropriate quantity of PBS (pH 7.4), were subjected to repeated freezing and thawing for five times and 10 cycles of sonication at 15 amplitude, each for 30 sec with an interval of 30 sec between the cycles. After ensuring total disruption of sonicated tachyzoites microscopically, the suspension was centrifuged at 10,000 rpm for 40 min and the supernatant was collected as soluble whole tachyzoites lysate antigen. The protein concentration was determined as 0.61 mg/ml.
4.4 Immunodiagnostic test

4.4.1 Indirect fluorescent antibody test (IFAT)

The antigen slides with predetermined cell count of 100–200 tachyzoites per high power field was prepared for application in IFAT.

The optimal dilutions of reference positive and negative control sera used in the test were standardized by serial 2-fold dilutions of the neat sera in PBS (pH 7.6). No reactivity, whatsoever, with negative control serum at 1:16 dilution was noted and accordingly used as cut off value for determining positive and negative sera samples.

The specific conjugates against the three target species i.e., rabbit anti-goat IgG FITC, rabbit anti-sheep IgG FITC, and rabbit anti-pig IgG FITC showed fluorescence against known positive sera upto a dilution of 1:80. A positive reaction showed bright yellowish green fluorescence around the entire periphery of the organism, whereas in negative reaction either fluorescence was absent or a faint reddish fluorescence observed either on the surface or at the polar region. Test sera diluted 1:16 in PBS (pH- 7.2) for studying their sero-reactivity by IFAT.

In case of pig serum samples, out of 240 samples collected from an organized government farm and slaughter houses, 106 samples (44.16%) were detected positive with a cut value 1: 16.

In case of sheep, out of 444 samples collected from organized government farm and villages, 170 samples (38.29%) were found positive considering cut off value as 1:16.

In goat samples, out of 445 samples, 177 samples (39.77%) were found positive following cut off value as 1:16.

4.4.2 Enzyme linked immune sorbent assay (ELISA)

A comparative analysis on the serodetection potential of the crude whole tachyzoite lysate antigen and the rSAG1 was done by ELISA for detection of T. gondii specific antibodies in the sera samples of sheep, goat and pigs collected either from organized farms or from field. The optimum concentration of the reagents employed in ELISA was determined by Chequer board titrations. For objective comparison, a
common concentration of 2µg/ml of antigen was used for both the antigens for coating the plates. The sera samples were diluted 1:100 and the species specific conjugates were diluted 1:40000. The dilution factor for positive and negative control sera for all three species was kept same i.e. 1:100.

Diagnostic evaluation of the assay was made on the basis of critical analysis of absorbance (OD<sub>492nm</sub>) values of positive and negative controls of each ELISA plate. The operational cut off value of the assays was determined as sum of mean absorbance values of all the plates of the negative control sera plus three standard deviations.

### 4.5 Seroprevalence of toxoplasmosis in pig

A total number of 240 pig sera samples were collected from organized farm as well as from the slaughter house. The recombinant surface antigen1 (rSAG1) based ELISA could detect *T. gondii* specific antibodies in 44.58 % of the samples (n=107) (Fig.3), whereas, whole tachyzoite lysate protein as detection antigen could detect *Toxoplasma* specific antibodies in 40% (n=96) of the same sera samples by indirect ELISA (Fig. 4). The OD<sub>492nm</sub> cut off value for the rSAG1 antigen was 0.698, whereas, the same for the whole tachyzoite lysate antigen was 0.349. The individual OD<sub>492nm</sub> values of the sera samples have been presented in the scattered chart (Fig. 3 and 4). Several factors related to the prevalence of *T. gondii* are presented in Table 1, 2, 3 and 4. A total number of 86 (53.75%) samples collected from slaughter house was detected positive by rSAG1 ELISA, whereas 77 (48.12%) samples were detected positive by the crude whole tachyzoite lysate antigen (Table no. 3 & 4). So far as the samples collected from the organized farm is concerned, 21 (26.25%) and 19 (23.75%) samples were positive against the rSAG1 and WTLA ELISA respectively (Table no. 1 & 2).
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4.5 a. Seroprevalence studies involving samples collected from organized farm

The sera samples collected from an organized farm were segregated based on age, sex and breed of the animals for drawing correlation with seroprevalence of toxoplasmosis (Table - 1 & 2). For the study of age-wise prevalence the animals were divided into three different groups, i.e. 3 months age group, 4-8 months age group and 9 months or above age group. The different age groups showed statistically significant difference. In the 3 months age group, 3 samples out of 29 samples (9.37%) studied were detected positive by both the antigens i.e. rSAG1 antigens and WTLA ELISA. In the 4-8 months age group, 5 samples out of 24 samples collected (20.83%) were detected positive by rSAG1, whereas 4 samples (16.67%) could be detected positive by WTLA ELISA. In the 9 months and beyond age group, 13 samples out of 24 (54.17%) were detected positive by rSAG1 and 12(50%) samples by WTLA. Statistically significant difference was found in both rSAG1 and WTLA based ELISA.

The sex-wise prevalence study revealed a higher number of samples collected from females were positive than to that of male. The variation in the detection ability of the two diagnostic antigens was also conspicuous. Significant differences were found in case WTLA ELISA but not in case of rSAG1 based ELISA. A total number of 7 sera samples out of 33 males (21.21%) were detected positive using rSAG1, whereas, 5 samples (15.15%) were detected positive with WTLA ELISA. So far as the samples procured from female is concerned, 14 (29.79%) out of 47 samples were detected positive by both rSAG1 and WTLAELISA.

The breed-wise analysis did not reveal any significant difference so far as seroprevalence of toxoplasmosis is concerned. The details of the sex wise, age wise and breed wise prevalence rate studied using rSAG1 and WTLA in indirect ELISA, has been presented in Fig. 5 and 6 respectively.
4.5 b. Seroprevalence studies involving samples collected from slaughter houses

From the slaughter houses a total number of 160 samples were collected from both Deshi and cross of Tamworth and Deshi. Breed and sex of the pigs were considered as measurable parameters for the seroprevalence study. Out of 160 samples tested, 86 (53.75%) and 77 (48.12%) samples were seropositive against rSAG1 and WTLA ELISA, respectively. The sex and breed wise prevalence of toxoplasmosis has been presented in (Tab. No- 3 & 4 and Fig.7 & 8) respectively against the rSAG1 and WTLA ELISA. Since the data on age was not available, the parameter could not be incorporated for the correlation of age with toxoplasmosis.

The breed wise prevalence study did not reveal a significant difference between the two breeds studied. The observation established that both the Deshi and the cross with Tamworth and Deshi were equally susceptible for toxoplasmosis. A total number of 59 sera samples were collected from T&D (C.B) out of which 33 samples (55.93%) were detected positive with rSAG1 and 29 samples (49.15%) were detected positive with WTLA ELISA. Serum samples collected from 101 Deshi breed of pigs revealed 53 samples (52.48%) and 48 samples (47.52%) samples were positive with rSAG1 and WTLA ELISA, respectively.

No significant difference was observed between the sexes. A total of 98 number of male and 62 number of female serum samples were collected. Out of 98 male samples, 48 samples (48.98%) and 46 samples (46.94%) were detected positive using rSAG1 and WTLA as antigen in ELISA. In case of female out 62 samples, 38 samples (61.29%) and 31 samples (50%) were found positive by rSAG1 and WTLA as antigen in ELISA respectively.
4.5 Sensitivity and specificity of ELISA using Whole tachyzoite lysate and rSAG1 as diagnostic antigen in comparison to IFAT in swine

For analysis of sensitivity and specificity of ELISA by using WTLA and rSAG1 as antigens, IFAT had been performed considering it as gold standard. In case of rSAG1 based ELISA, 87.73% sensitivity and 89.55% specificity were determined with a positive predictive value of 86.91% and negative predictive value of 90.22%. With WTLA, 76.41% sensitivity and 88.80% of specificity were determined with Positive predictive value 84.38% and negative predictive value of 82.63% (Table – 5 & 6).

4.6 Seroprevalence of toxoplasmosis in sheep:

A total number of 444 sera samples were collected from sheep, of which 184 samples were collected from Chatra farm which includes two breeds, viz. Muzaffarnagar (78 samples) and Shahabadi (109 samples), 148 sample from Instructional Farm of Small Ruminants (IFSR), Ranchi having Chotangpuri breed and 112 samples from villages rearing Chotangpuri breed. ELISA was performed to know the seroprevalence of Toxoplasma gondii by using whole tachyzoites lysate antigen and rSAG1 as antigen resource. The operational cut off value was made by taking the sum of mean absorbance values of the negative control sera of all the plates plus three standard deviations. The final cut off value for assay using rSAG1 as antigens was 0.687 and for whole tachyzoite lysate antigen was 0.321. The scattered chart between O.D values verses serial number of samples taken during study were shown in fig. 9 and 10. Out of 444 samples 188 samples (42.34%) were found positive by rSAG1, whereas 131 samples (29.5%) by whole tachyzoites lysate antigen (WTLA) ELISA. Some important factors like age, sex, breed, weight and the site from which the samples were taken into consideration to the analysis.

The serum samples collected from a Chatra farm were segregated based on sex and breed of the animals for drawing correlation with seroprevalence of toxoplasmosis (Table - 7 & 8; Fig.- 11 & Fig-12).
In sex wise prevalence study, a total number of 32 samples (42.67%) out of 75 males were detected positive using rSAG1, whereas, 22 samples (29.33%) were detected positive against WTLA ELISA. So far as the samples procured from female sheep are concerned, 48 samples (44.03%) out of 109 samples were detected positive with rSAG1, whereas 37 samples (33.94%) were detected positive with WTLA ELISA. No significant difference was found in a sex wise study.

In a breed wise analysis, two breed viz. Muzaffarnagari and Shahabadi were studied for Seroprevalence of toxoplasmosis. Statistically no significant difference was found. In Muzaffarnagari breed, 35 samples (42.45%) out of 78 samples were found positive by rSAG1, whereas 22 samples (29.33%) were detected positive by WTLA ELISA. In Shahabadi breed, 45 samples (44.87%) out of 106 samples studies were found positive by rSAG1, whereas 34 samples (32.08%) detected positive by WTLA ELISA.

In the Instructional Farm of Small Ruminants (IFSR), Ranchi, two factors viz. sex and weight were taken into account for analysis (Table - 9 & 10; Fig.-13 & 14). In a sex wise analysis a total number of 5 samples (33.3%) out of 15 samples collected from male were found positive by rSAG1 whereas 3 samples (20%) were detected positive by WTLA ELISA. So far as the samples procured from female sheep are concerned, 59 samples (44.36%) out of 133 samples studied were detected positive with rSAG1 whereas 37 samples (27.82%) were detected positive with WTLA ELISA. No significant difference was, however found in a sex wise study.

For the study of weight wise prevalence, the animals were divided into three groups i.e. 6.1-12.0 kg, 12.1-19 kg and 19.0 kg or more. The difference in different weight groups showed statistically significant difference (p-value< 0.0001 by rSAG1 & 0.0006 by WTLA). In the 6.1-12.0 kg group 3 samples (13.04%) out of 23 samples studied were found positive using rSAG1, whereas 2 samples (8.67%) were detected positive by WTLA. In the 12.1-19 kg group, 56 samples (49.55%) out of 113 samples studied were detected positive using rSAG1, whereas 34
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54 samples (30.09%) were detected positive by WTLA ELISA. In the 19.0 kg body weight group and beyond, 5 samples (41.66%) out of 12 samples studied were detected positive by rSAG1 and 4 samples (33.3%) by WTLAEELISA.

For analysis of the samples collected from village condition, two factors viz. sex and age were taken into consideration for analysis of prevalence of toxoplasmosis (Table - 11 & 12 & Fig.- 15 &16).

In a sex wise analysis a total a total number of 14 samples (31.81%) of male out of 44 samples were found positive by rSAG1 whereas 11 samples (25%) were detected positive by WTLA ELISA. So far as the samples procured from female are concerned, 30 samples (44.11%) out of 68 samples were detected positive with rSAG1 whereas 21 samples (30.88%) were detected positive against WTLA ELISA. No significant differences were found in a sex wise study.

For the study of age wise prevalence, the animals were divided into three groups i.e. 6-12 months, 13-24 months and 24 months or more. The difference in age groups showed statistically significant difference (p-value-0.0012 by rSAG1 & 0.0007 by WTLA). In the 6-12 months age group, 2 samples (16.67%) out of 12 samples were detected positive with rSAG1, whereas 1 sample (8.33%) was found positive using WTLA ELISA. In the 13-24 months age group, 16 samples (37.2%) out of 43 samples studied were detected positive using rSAG1, whereas 13 samples (30.23%) were detected positive by WTLA ELISA. In the 24 months and beyond age group, 26 samples (45.61%) out of 57 samples studied were detected positive by rSAG1 and 18 samples (31.57%) by WTLA ELISA.

In an overall analysis, sex wise, breed wise, farm wise, farm/villages and altitude wise study were also made (Table13 &14; Fig-17 &18).None of these factors showed any significant difference.

In a sex wise analysis a total number of 51 samples (38.05%) out of 134 samples studied from male, were found positive by rSAG1, whereas 36 samples (26.87%) were detected positive by WTLA ELISA. So far as the samples procured from females were concerned, 137 samples
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(44.09%) out of 310 samples studied were detected positive with rSAG1, whereas 95 samples (30.65%) were detected positive against WTLA ELISA.

For a breed wise analysis, three breeds, Muzaffarnagari, Shahabadi, and Chotanagpuri were studied. Out of 106 samples of Shahabadi breed studied, 45 samples (42.45%) were found positive by using rSAG1 and 34 samples (32.08%) by using WTLA ELISA. In Muzaffarnagari breed, out of 78 samples studied, 35 samples (44.87%) were found positive using rSAG1 and 25 samples (32.05%) were positive with WTLA ELISA. In a Chotanagpuri breed, out of 260 samples analyzed 108 samples (41.53%) were found positive using rSAG1 and 72 samples (27.69%) by using WTLA ELISA.

For a farm wise analysis, two farms viz. Chatra farm and Ranchi farm were studied. From Chatra farm, 80 samples (43.48%) out of 184 samples tested was found positive using rSAG1 and 59 samples (32.06%) were positive with WTLA ELISA. From Ranchi farms, 64 samples (43.24%) out of 148 samples studied were found positive using rSAG1 whereas, 40 samples (27.07%) were detected positive by WTLA.

The comparative analysis of samples collected from farm and villages, out of 332 samples collected from farms, 144 samples (43.37%) were found positive using rSAG1 and 99 samples (29.82%) were positive with WTLA ELISA. The samples procured from villages, 44 samples (39.29%) out of 112 samples were positive using rSAG1 ELISA, whereas 32 samples (28.57%) detected positive by WTLA ELISA.

In an altitude wise analysis, Chatra district (24° 12’N, 84° 51’E) is located in the Hazaribagh plateau at the height of 1400 ft above the mean sea level whereas Ranchi district (22° 45’- 23 45’ N, 84° 45’- 84°- 50’) lies in the southern part of the Jharkhand state at the height of 2140 ft above the mean sea level. From Chatra, 184 samples were collected, out of which 80 samples (43.48%) were found positive with rSAG1, whereas 59 samples (32.06%) were detected positive with WTLA ELISA. A total number of 260 samples were collected from Ranchi, of
which 108 samples (41.53%) were found positive using rSAG1 and 72 samples (27.69%) were detected positive with WTLA ELISA.

4.6 a. **Sensitivity and specificity of ELISA using Whole tachyzoite lysate and rSAG1 as diagnostic antigen in comparison to IFAT in Sheep**

For analysis of sensitivity and specificity of ELISA by using WTLA and rSAG1 as antigens, IFAT had been performed. In case of rSAG1 based ELISA, 87.78% sensitivity and 88.63% specificity were determined with a positive predictive value of 84.04% and negative predictive value of 91.40%. With WTLA, 71.1% sensitivity and 92.72% of specificity were determined with Positive predictive value of 97.70% and negative predictive value of 83.39%. (Table- 15 & 16).

5.1 **Seroprevalence of Toxoplasmosis in Goats:**

A total number of 445 serum samples of goat were collected, out of which 120 samples were collected from Chatra farm maintaining Beetal and 65 samples were collected from Instructional Farm of Small Ruminants (IFSR), Ranchi, maintaining Black Bengal and a total number of 260 samples from villages rearing Black Bengal breed of goats. ELISA was performed to know the seroprevalence of *Toxoplasma gondii* by whole tachyzoites antigen and rSAG1 ELISA. The operational cut off value were determined by taking the sum of mean absorbance values of the negative control sera of all the plates taken during analytical study plus three standard deviations. The final cut off value for assays using rSAG1 antigens was 0.577 and for whole tachyzoites lysate antigen was 0.240. The scattered chart between O.D values verses serial number of samples taken during study were shown in (Fig.19 and Fig. 20). Out of 445 samples 189 samples (42.47%) were found positive by rSAG1 and 131 samples (29.44%) by whole tachyzoite lysate (WTLA) indirect ELISA.
The sera samples collected from Chatra farm were segregated based on sex and age of the animals for drawing correlation with seroprevalence of toxoplasmosis (Table no. 17 & 18; Fig- 21 & 22).

The sex wise study, revealed a total number of 4 samples (33.33%) positive out of 12 male samples studied with rSAG1, whereas, 3 samples (25%) were detected positive by WTLA ELISA. So far as the samples procured from female goats are concerned, 49 samples (45.37%) out of 108 samples studied were detected positive with rSAG1, whereas 35 samples (32.40%) were detected positive against WTLA ELISA. No significant difference was found in a sex wise study.

For the study of age wise prevalence, the animals were divided into three groups i.e. 9-12 months, 13-24 months and 24 months or above age group. The difference in age groups showed statistically significant difference (p-value-0.05 for rSAG1 & <0.0001 for WTLA). In the 9-12 months age group, 8 samples (30.76%) out of 26 samples studied were detected positive with rSAG1, whereas, 3 samples (11.53%) were found positive using WTLA ELISA. In the 13-24 months age group, 25 samples (42.37%) out of 59 samples were detected positive using rSAG1, whereas 17 samples (28.81%) were detected positive by WTLA ELISA. In the 24 months and beyond age group, 20 samples (57.14%) out of 35 samples studied were detected positive by rSAG1 and 18 samples (51.42%) by WTLA.

For the samples collected from Instructional farm of small ruminants, Ranchi, two factors viz. sex and weight of the animals were taken into account for determining the correlation with the prevalence of toxoplasmosis. (Table -19 & 20; Fig.- 23 & 24). In a sex wise analysis a total number of 3 samples (33.33%) of male out of 9 samples studied were found positive by rSAG1 whereas 2 samples (22.22%) were detected positive by WTLA ELISA. So far as the samples procured from female goats are concerned, 25 samples (44.64%) out of 56 samples were detected positive with rSAG1 whereas 14 samples (25%) were detected positive against WTLA ELISA. No significant difference was found in a sex wise study.
In order to correlate the body weight of the animals with the prevalence of toxoplasmosis, the animals were divided into three groups i.e., 7.5-12.5 kg, 12.6-17.5 kg and 17.5kg or more. The difference in different body weight groups showed statistically significant difference (p-value-0.0409 by rSAG1 & 0.012 by WTLA). In the 7.5-12.5 kg group, 5 samples and no one was found positive by rSAG1 ELISA, whereas 1 sample (20%) was detected positive by WTLA ELISA. In the 12.6-17.5 kg group, 8 samples (34.78 %) out of 23 samples studied were detected positive with rSAG1, whereas 3 samples (13.04%) were detected positive by WTLA ELISA. In the 17.5 kg and beyond body weight group, 20 samples (54.05%) were positive out of 37 by rSAG1 and 12 samples (32.43%) were detected positive by WTLA ELISA.

In the Village samples, two factors viz. sex and age were taken into consideration for determining the correlation between the prevalence of toxoplasmosis. (Table-21 & 22; Fig.- 25 & 26). In a sex wise analysis a total number of 45 samples (34.88%) of male out of 129 samples were found positive by rSAG1 whereas 26 samples (20.16%) were detected positive by WTLA ELISA. So far as the samples procured from female goats are concerned, 63 samples (48.09%) out of 131 samples were detected positive with rSAG1, whereas 51 samples (38.93%) were detected positive against WTLA ELISA. Significant difference was found between sexes in case of WTLA based ELISA whereas no significant difference were found in rSAG1 ELISA (P value-0.0146 for WTLA ELISA and 0.1470 for rSAG1 ELISA).

For the study of age wise prevalence, the animals were divided into three groups i.e. 9-12 months, 13-24 months and 24 months or more. The ELISA performed using rSAG1 showed significant difference in age group (p value- 0.0409), whereas in WTLA ELISA (P value- 0.1870), it did not show any significant difference. In the 9-12 months age group, 25 samples (37.31%) out of 90 samples were detected positive with rSAG1, whereas 17 samples (22.97%) were found positive using WTLA ELISA. In the 13-24 months age group, 59 samples (45%) out of 131 samples were detected positive using rSAG1, whereas 46 samples (35.11%) were
detected positive by WTLA. In the 24 months and beyond age group, 24 samples (61.54%) out of 39 samples were detected positive by rSAG1 and 14 samples (35.9%) by WTLA ELISA.

In an overall analysis involving sex wise, breed wise, farm wise, farm/villages and altitude wise study, no significant difference was observed in the prevalence of toxoplasmosis. (Table- 21 & 22; Fig- 27 & 28).

In a sex wise analysis a total number of 52 samples (34.66%) of male studied found positive out of 150 samples by rSAG1, whereas 31 samples (20.67%) were detected positive by WTLA ELISA. So far as the samples procured from females were concerned, 137 samples (46.44%) out of 295 samples studied were detected positive with rSAG1, whereas 100 samples (33.90%) were detected positive with WTLA ELISA.

The breed wise analysis revealed a total number of 93 samples (28.61%) of Black Bengal were positive out of 325 samples studied by using WTLA and 136 (41.84%) by using rSAG1 ELISA. In Beetal breed, out of 120 samples studied, 38 samples (31.67%) were found positive with WTLA ELISA and 53 samples (44.17%) using rSAG1 ELISA.

The farm wise analysis, two farms viz. Chatra farm and IFSR farm were taken. From Chatra farm, 38 samples (31.67%) out of 120 samples were found positive using WTLA and 53 samples (44.17%) using rSAG1 ELISA. From IFSR farm, 16 samples (24.61%) out of 65 samples were found positive using WTLA whereas, 28 samples (43.08%) detected positive by rSAG1.

In a farm verses village, out of 185 samples collected from organized government farms, 54 samples (29.19%) were found positive with WTLA and 81 samples (43.78%) were positive with rSAG1 ELISA. A total of 108 samples (41.53%) out of 260 samples procured from village with rSAG1 ELISA, whereas 77 samples (29.61%) were detected positive with WTLA ELISA.

In an altitude wise analysis, Chatra district (24° 12’N, 84° 51’E) which is located in the Hazaribagh plateau at the height of 1400 ft above the mean sea level whereas Ranchi district (22° 45’- 23 45’ N, 84° 45’- 84°.50’) lies in the southern part of the Jharkhand state at the height of
2140 ft above the mean sea level. From chatra, 120 samples were collected, out of which 38 samples (31.67%) were found positive by WTLA and 53 samples were positive by rSAG1 ELISA. A total of 325 samples were collected from Ranchi out of which 93 samples (28.61%) were found positive with WTLA and 136 samples (41.84%) were positive with rSAG1 ELISA. No significant difference was found in altitude basis.

5.2 Sensitivity and specificity of ELISA using Whole tachyzoite lysate and rSAG1 as diagnostic antigen in comparison to IFAT in goats

For analysis of sensitivity and specificity of ELISA by using WTLA and rSAG1 as antigens, IFAT was considered as standard. In case of rSAG1 based ELISA, 92.66% sensitivity and 90.67% specificity were determined with a positive predictive value of 84.04% and negative predictive value 91.40 %. With WTLA, 66.67% sensitivity and 95.14% of specificity were determined with Positive predictive value 90.07% and 81.21% of negative predictive value (Table – 25 & 26).

5.3 Characterization of soluble tachyzoite lysate proteins of T. gondii, RH strain

The T. gondii specific polypeptides were resolved in the molecular range of 73.0 to 11 KDa in a 6-15 % gradient SDS-PAGE under denaturing condition. The major polypeptides were in the Molecular range of 73, 64, 55, 49, 40, 38, 37, 30, 25, 14 and 11 KDa.

5.4 Western blot analysis:

To identify immunodominant protein in whole tachyzoites antigen lysate of T. gondii western blotting was performed. Among these 73, 64, 49, 40, 37, 30, and 25 KDa band had shown a good immunoreactivity against goat field serum, whereas 55, 49, 37, 25 KDa band shown a good immunoreactivity against sheep field serum.
Toxoplasma gondii, an intracellular apicomplexan protozoan parasite, is prevalent worldwide. The organism is considered as one of the most successful parasitic protozoa because of its ability to infect almost any type of cell within an exceptionally broad host range— including humans, livestock, companion animals and wildlife. All the stages of lifecycle of T. gondii, viz. tachyzoites, bradyzoites and sporulated oocysts are potentially infective. Unlike many other coccidian parasites, oocysts of T. gondii are less infective and less pathogenic to definitive host (cats), in comparison to the intermediate hosts, viz. mice, pigs, humans etc. Nevertheless, bradyzoite induced cycle is found to be the most efficient for setting up infection in cats because nearly all cats fed tissue cysts shed oocysts, whereas less than 30% of cats fed tachyzoites or oocysts shed oocyst. On the other hand, oocyst induced infection is more severe in humans and animals than tissue cyst induced infection irrespective of the number of organisms affected and has a great epidemiological relevance (Dubey and Beattie, 1988). Toxoplasma gondii is responsible for approximately 20% of all deaths attributed to food borne pathogens in the US, and the Centers for Disease Control estimated that 50% of all human exposures to T. gondii are food borne (Mead et al., 1999). The worldwide seroprevalence of toxoplasmosis is estimated in cattle 9%, sheep 30% and goats 15% (Duby, 2004). Information on prevalence of toxoplasmosis in animals and man is scanty from India. According to available literature, seroprevalence of T. gondii ranges from 7.9% to 80% in various species of domesticated animals studied with highest incidence in goats.

Diagnosis of toxoplasmosis in animals is based on the demonstration of the parasites in situ or in biological materials in acute cases and by application of serological tools for indirect detection of chronic or latent infections. Although several serological tests have been
described in literature, in the absence of standardized reagent or methodologies suitable for a reference laboratory and field use, sero-monitoring of toxoplasmosis using the improved techniques has not been widely employed or practiced. The serological methods are time consuming and often subject to the limitations of sensitivity and specificity. The commonly used agglutination tests, *viz.* modified agglutination test and dye test require handling of live tachyzoites and there is a potential risk of infection to human handlers. Difficulties associated with harvesting native antigenic proteins by mass production and isolation of the parasites from peritoneal fluid through experimental infection in mice or from tissue culture, as well as their variable degree of sensitivity or specificity for use in diagnostic assays constitute the major drawbacks with the commercially available native protein based diagnostic tests. Therefore, development of recombinant protein based diagnostic methods and their application for generating reliable epidemiological data on the disease has been emphasized.

Since there is no information available on the prevalence of toxoplasmosis among the economically important farm animals from the Jharkhand state, the present study was undertaken with the objective of studying the seroprevalence of *T. gondii* in small ruminants and pigs. In order to accomplish the objective, ELISA was laboratory standardized using standard reagents. A native protein based antigen was prepared from the purified tachyzoites of *T. gondii*, whole tachyzoite lysate antigen (WTLA) and was used in the indirect ELISA for screening the *Toxoplasma*-specific IgG molecules present in the sera. The serodetection potential of the native WTLA based ELISA was further compared with a recombinant surface antigen 1(SAG1) protein based ELISA. The serum samples were collected randomly from the target animals maintained either as free range management system in rural house holds as well as from the organized Government farms of Ranchi and Chatra districts of Jharkhand state. While collecting the samples, the age, sex and breed of the animals were recorded for drawing correlations with the prevalence of toxoplasmosis, if existing.
Chatra district (24° 12'N, 84° 51'E) is located in the Hazaribagh plateau at the height of 1400 ft above the mean sea level. The district is flanked by Gaya (Bihar state) district in the north, Palamu district in the west and Latehar in the South and Koderma and Hazaribagh district in the east. It has an area of 3706 sq. km. Since the district consists of part of upper Hazaribagh Plateau and lower Hazaribagh Plateau and Northern scrap, it presents diverse physiographic features. The district receives an annual rainfall of 1250 mm. During the winter season the area receives an average of 1 to 2 mm rain fall. Although the mean annual temperature remains around 25°C, during summer it reaches upto 46°C and during winter it falls down to 2 to 3°C. The soil pH ranges from 4.7 to 8.1. The majority of the area is acidic (66.2% of TGA).

Ranchi district (22° 45'- 23° 45' N, 84° 45'- 84° 50') lies in the southern part of the Jharkhand state at the height of 2140 ft above the mean sea level. The district is bounded on the north by Hazribag and Chatra district, on the south by west Singhbhum, on the west by Gumla and Lohardagga district and on the east by East Singhbhum and Purulia district of West Bengal. The district has the total area of 7698 sq. km. Though tropic of capricorn passes over it, the district experiences pleasant climatic condition due to its higher elevation. Since relative humidity remains low, the summer season is not much harsh. December is the coldest month with minimum temperature around 10.3°C and May being the hottest month with maximum temperature around 37.2°C. Average annual rainfall of the district is 1375 mm and more than 80 percent precipitation received during monsoon months. The soil pH ranges from 4.2 to 7.6. The majority of the area is acidic (94% of TGA).

**Seroprevalence of toxoplasmososis in pigs**

Pig is one of the most important domestic stocks maintained mostly in the backyards by the tribal population of this region and pork is popular among the people of this region. Therefore, rearing of pigs in the backyard as small house hold stocks or as commercial farm is contributing significantly to the economy. *Toxoplasma gondii* infection of
pigs is very common and infected pork is a major source of human infection (Dubey, 1986; Dubey et al., 1991, 1992, 1996). A reliable method of identification of the infected animals in the herds would facilitate adoption of measures towards reducing the prevalence of infection in swine. The commercially available indirect haemagglutination test (IHA) and latex agglutination tests (LAT) are not only too expensive for routine testing in the swine herds, they also suffers from low calculated sensitivity of 46% for LAT and 29% for IHA. On the contrary, ELISA is simple and applicable in a herd basis with large throughput value suitable for routine application in the herds.

There are no programs for the slaughter inspection of pigs for *T. gondii* because it is not possible to detect the microscopic tissue cysts by visual inspection. Methods for testing pigs, including serology and bioassay, are either not suitable or not sufficiently reliable for purposes of meat inspection. However, serology testing is a useful method for epidemiological purposes and for estimating infection rates by farm, herd or region. Assessment of infection rates, coupled with efforts to reduce infection by improved management on the farm, is the best way to reduce potential exposure of consumers to *T. gondii* in pork.

Based on this premise, the diagnostic performance of a native tachyzoite antigen based ELISA was compared with that of a recombinant tachyzoite stage specific surface antigen, rSAG1, based ELISA to study the seroprevalence of toxoplasmosis in pigs. A total number of 240 pig serum samples were collected from organized farm and slaughter houses. The recombinant surface antigen1 based ELISA was superior in detection of the *T. gondii* specific IgG antibodies in 44.58% of the serum samples tested over the crude whole tachyzoites lysate antigen (WTLA) which could detect the same in 40% of the samples. The prevalence of the disease was found more in the samples collected from slaughter house *i.e.*, 53.75% and 23.75% than that of farm *i.e.*, 48.12% and 26.25% by rSAG1 and WTLA based ELISA, respectively. A recombinant surface antigen 1 MBP 30 could recognize the antibody response in the market age, *i.e.*, 6 months, of pigs. The recombinant
antigen could recognize sera from infected pigs through 51 weeks of infection and only a slight decrease in antibody reactivity was reported in the later part of infection (Gamble et al., 2000). However, the native protein, used for ELISA based serodetection of toxoplasmosis, could also detect the specific antibodies throughout the 51 week infection period.

For study of the seroprevalence of toxoplasmosis in pigs reared in a farm, three factors, viz. age, sex and breed of the animals were taken into consideration. The seroprevalence of toxoplasmosis in different age groups of the animals was statistically significant indicating the age factor had a positive correlation with the sero-positivity of the animals. In an age group of 3 months, 9.37% samples were positive both by rSAG1 and WTLA based ELISA, whereas 20.83% and 16.67% samples were positive by rSAG1 and WTLA based ELISA, respectively in the pigs of 4-8 months of age. Maximum number of serum samples was tested positive in the pigs belonging to the age of 9 months and above. As high as 54.17% and 50% of the samples were detected positive for *Toxoplasma* specific antibodies by rSAG1 and WTLA based ELISA, respectively. More number of females were found positive (29.79%) than that of the males ranging from 15.15% to 21.21% with the rSAG1 and WTLA. The variation was statistically significant by WTLA based ELISA but was insignificant by rSAG1 based ELISA. The breed wise analysis revealed no significant difference leading to the conclusion that both the breeds studied were equally susceptible to toxoplasmosis. The findings are in agreement with that of Damriyasa et al., (2004) who detected antibodies specific to *T. gondii* by ELISA in 19% of the sows in Germany. The farm based study revealed sixty-nine percent of the farms had at least one seropositive sow and a within-farm sero-prevalence of 50% was observed in 14% of all farms. Further a positively correlation was found between the prevalence of anti-*T. gondii* antibodies with the age of sows. The within-herd seroprevalence was significantly higher in farms with reproductive disorders than in those without such problems. An ELISA based study from Ghana reported antibodies against *Toxoplasma gondii* in pig serum with a sensitivity and specificity of 90.2% and
92.3%, respectively when compared with IFAT. The age of the animal, the breed, the environmental conditions and the management practices were shown to be the major determinants of prevalence of antibodies against *T. gondii*. The *T. gondii* specific antibodies were detected more in the crossbreed pigs which had a positive correlation with age (Arko-Mensah *et al.*, 2000). A commercial enzyme-linked immunosorbent assay (Institut Pourquier, France) based study revealed the presence of *T. gondii*-specific antibodies in 16.3% of Sicilian pigs. The lowest seroprevalence of 7% was found in the age group 5-7 months (market pig) and the highest seroprevalence of 19% was found in the age group of greater than 24 months. The study revealed that pigs aged 11-24 months compared to younger were less susceptible (OR 5.62; CI 1.52-20.8). The incidence was less in farrow-to-finish farming type (OR 6.85; CI 1.87-25.01) compared to finishing and farrow-to-breed type; hard size (less than 50 pigs on the farm OR 6.8; CI 1.76-26.2); no use of rodenticides (OR 2.71; CI 1.10-6.64) and use of water coming from private sources (especially wells; OR 2.8; CI 1.03-7.72). Pigs raised in Sicily showed a high prevalence of antibodies against *T. gondii* (Vesco *et al.*, 2007). An enzyme-linked immunosorbent assay (ELISA) based serological survey revealed specific antibodies against *Toxoplasma gondii* in 11.9% cattle, 8.9% swine and 3.2% goats in four Mexican states (Garcia-Vazquez *et al.*, 1993). Van der Giessen *et al.*, (2007) reported the prevalence of toxoplasmosis using ELISA as higher in outdoor farming systems than in indoor farming systems in the Netherlands. The overall seroprevalence of *Toxoplasma* in the total number of 845 serum samples tested was 2.6%, ranging from 0.38% in intensively raised pigs to 5.62% in free-range pigs. Of the housing systems tested, 4% (intensive farms) to 33% (free-range farms) was infected with *Toxoplasma gondii*. The prevalence of *Toxoplasma* specific antibodies in the serum samples collected from the animals maintained in free-range system was statistically higher (almost 16 times higher) in comparison to the same from an intensive farm and the risk of seropositivity was twice as high as in free-range compared with organic
farms. The prevalence of *Toxoplasma gondii* was investigated on a poorly managed pig farm in Maryland by Dubey *et al.*, (2008) using both ELISA and the modified agglutination test (MAT). Under an on-farm serological sampling component for monitoring seroprevalence of various pathogens, including *Toxoplasma gondii*, under the National Animal Health Monitoring System (NAHMS) in 1983, sera and data on management practices were collected from 185 grower/finisher swine production sites located in 16 states accounting for greater than 90% of US swine production (Arkansas, Colorado, Iowa, Illinois, Indiana, Kansas, Michigan, Minnesota, Missouri, Nebraska, North Carolina, Ohio, Pennsylvania, South Dakota, Texas and Wisconsin). A commercial ELISA based seroprevalence study revealed 2.6% serum samples positive out of a total of 6238 sera screened for *T. gondii* antibodies with a herd prevalence of 21.6% and a mean within-herd prevalence of 2.7%. Analysis of swine management practices indicated that rodent control methods and carcass disposal methods were associated with differences in the number of *T. gondii* positive samples on farm (Hill *et al.*, 2010).

In order to study the seroprevalence of toxoplasmosis in pigs based on sera collected from slaughter house data on breeds and sexes of the pigs were collected and correlated. However, the age wise prevalence could not be studied due to unavailability of data. A total of 160 samples were collected from slaughter houses which included Deshi and crosses of Tameworth and Deshi breeds (T&D). The rSAG1 and WTLA based ELISA could detect 53.75% and 48.12% sera samples, respectively, as positive. The difference of seroprevalence between the breeds was statistically insignificant which led to conclusion that both of the breeds were equally susceptible to *T. gondii* infection. Among the crosses of T&D, the rSAG1 and WTLA antigen based ELISA revealed 55.93% and 49.15% serum samples positive, whereas 52.48% and 47.52% serum samples were positive with rSAG1 and WTLA, respectively among the Deshi breed of pigs. The rSAG1 based ELISA revealed 48.98% of male and 61.29% female sera samples positive, whereas 46.94% male and 50% of female samples were positive with WTLA antigen based
ELISA. No significant difference was found so far as sex of the animals was concerned and both the sexes were found equally susceptible. Based on the study of samples collected from abattoirs, Suaréz-Aranda et al., (2000) reported the seroprevalence of toxoplasmosis as higher in 5-month old pigs from Peru (32.3%) in comparison to Brazil (9.6%) as detected by ELISA and Western blot.

Although toxoplasmosis in pigs is not a production problem, pork is one of the most important sources for food borne infection to humans (Evans, 1992). The data on prevalence of toxoplasmosis in generated in the present study indicates the existence of a high percentage of seropositive pigs in the Jharkhand region irrespective of sex, breed and practice of husbandry. Therefore, adoption of sufficient sanitary measures during slaughter of the pigs as well as thorough cooking of pork for human consumption is needed to prevent transmission of infection to humans.

Sensitivity and specificity of the indirect ELISA performed using two separate antigen viz. WTLA and rSAG1 were evaluated by performing indirect fluorescent antibody test considering it as gold standard for the same serum samples. In case of rSAG1 based ELISA, 87.73 % sensitivity and 89.55% specificity were determined with a positive predictive value of 86.91% and negative predictive value of 90.22%. With WTLA, 76.41% sensitivity and 88.88 % of specificity were determined with Positive predictive value and 84.38 % of negative predictive value.

**Seroprevalence of toxoplasmosis in Sheep**

Sheep are one of the most important species of domestic animals largely maintained in the temperate zones of India; nonetheless the sheep population in rural areas is not less. The sheep are mostly reared by the small and marginal farmers for production of meat, milk and wool. The animals are largely maintained as free range system which involves minimal rearing and maintenance costs, *Toxoplasma gondii* infection is responsible for significant reproductive and economic losses. Ovine toxoplasmosis was first described by Hartley *et al.* (1954) and
Hartley and Marshall (1957) and thereafter it was recognized in many other countries. Infection of ewes by *Toxoplasma gondii* may induce abortions. Although the infection in sheep is often asymptomatic, abortion storms in sheep have also been described worldwide (Dubey and Towle, 1986). Besides the production losses associated with the sheep husbandry, ingestion of undercooked mutton constitutes an important source of infection for toxoplasmosis in humans (Skjerve *et al.*, 1998). While the exact loss attributable to ovine toxoplasmosis is difficult to assess, Blewett and Trees (1987) estimated it between 1 and 2% of neonatal losses per annum in the UK and if the incidence were to be similar in sheep rearing areas of the EU then in 2003 over 1.25 million lambs would have been lost as per their assessment. Ingestion of as few as 200 sporulated oocysts may cause a primary infection leading to development of clinical ovine toxoplasmosis in a pregnant sheep (McColgan *et al.*, 1988). The sporozoites are released in the small intestine following excystment of the oocysts ingested and by the fourth day *Toxoplasma* tachyzoites can be found multiplying in the mesenteric lymph nodes (Dubey, 1984). During this stage the sheep develops a fever that may last until the 10th day after infection and during period the parasite may be found circulating in the blood (Dubey and Sharma, 1980; Reid *et al.*, 1982; Wastling *et al.*, 1993).

Among the different serological assays used, various forms of agglutination tests and the enzyme-linked immunosorbent assay (ELISA) are important. One test that has been reported to be both sensitive and specific is the modified agglutination test (MAT) using preserved whole tachyzoites (Desmonts and Remington, 1980; Dubey *et al.*, 1995). This test, however, is not suitable for use in the slaughterhouse or in the field due to the length of time required to obtain a result. The availability of an ELISA that is both sensitive and specific would allow wider use of serologic testing.

The epidemiology of toxoplasmosis in sheep in the Jharkhand region has not been studied so far and therefore, the prevalence of toxoplasmosis in the sheep population at this region is unknown. Sera
samples were collected from Muzaffarnagari, Shahabadi and Chotanagpuri breeds of sheep from Chatra and Ranchi districts of Jharkhand. The rSAG1 showed diagnostic superiority over the native WTLA. Out of 444 samples analysed, 188 samples (42.34%) were found positive by rSAG1 ELISA, whereas 131 samples (29.5%) were positive with whole tachyzoite lysate ELISA. The sex wise prevalence among the samples collected from Chatra farm revealed 32 samples (42.67%) of male and 48 samples of female (44.03%) as positive with rSAG1, whereas 22 samples of male (29.33%) and 37 samples of female (33.94%) were positive with WTLA ELISA, respectively. However, the difference was statistically insignificant (p> 0.883 by rSAG1 and p> 0.562 for WTLA) which was suggestive of that both the sexes are equally susceptible to *T. gondii* infection. Similarly, no significant difference was observed in the susceptibility for toxoplasmosis in the different breeds of sheep studied and both the Muzaffarnagari and Shahabadi breeds were equally susceptible to toxoplasmosis. A total of 35 samples (44.87%) of Muzaffarnagari and 45 samples (42.45%) of Shahabadi were positive with the rSAG1, whereas the WTLA could detect 25 samples (32.05%) of Muzaffarnagari and 34 samples (32.08%) of Shahabadi as positive.

Out of 148 serum samples of Chotanagpuri breed collected from the Instructional Farm of Small Ruminants, Ranchi, 64 samples were seropositive. The sex wise survey revealed 5 males (33.3%) and 59 females (44.36%) were positive with rSAG1 ELISA, whereas 3 males (20%) and 37 females (27.82%) were positive with the WTLA ELISA. The difference was statistically insignificant (P>0.2109 for rSAG1 and 0.2581 for WTLA). A weight wise survey for toxoplasmosis was also done since the age of the animals was not maintained in the farm. 3 samples (13.04%) in the first group of body weight below 12Kg, 56 samples (49.55%) in the second group, 12 to 19 Kg body weight group and 5 samples (41.66%) in the third group of body weight greater than 19 Kg were found positive through rSAG1 ELISA, whereas 2 (8.67%), 34 (30.09%) and 4(33.3%) samples from the three different weight groups were found positive by WTLA ELISA. The difference was statistically
significant for both the antigens used in ELISA (P<0.0001 for rSAG1 and 0.0006 for WTLA) which indicated a positive correlation between infection and body weight or in other words with age of the animals. The finding was in agreement with that of Clementino et al., (2007) and Caballero-Ortega et al. (2008).

Out of the 112 samples collected from village conditions revealed that 44 samples (39.28%) were positive with rSAG1 while 32 samples (28.57%) were positive by WTLA ELISA. Among the samples collected from villages, the sex wise study revealed 14 samples of male (31.81%) and 30 samples of female (44.11%) were positive with rSAG1 ELISA, whereas 11 samples of male (25%) and 21 samples of female (30.88%) were positive with WTLA ELISA. The difference was statistically insignificant (P>0.1581 in rSAG1 and 0.4313 by WTLA). The age wise study revealed 2 samples (16.67%) and 1 sample (8.33%) in an age group of 6-12 months, 16 (37.2%) and 30 samples (30.23 %) in a age group of 13-24 months and 26 samples (45.61%) and 18 samples (31.57%) in the age group of greater than 24 months as positive using rSAG1 and WTLA antigen, respectively. The difference was statistically significant for both the antigens used in ELISA (P value - 0.0012 for rSAG1 and 0.0007 for WTLA) which indicated a positive correlation between infection and age of the animals.

The overall sex wise, breed wise, farm wise, farm/villages and altitude wise study were also made. In sex wise prevalence, a total number of 134 samples of male were collected out of which 36 samples (26.87%) were found positive using WTLA and 51 samples (38.05%) were found positive using rSAG1. In a female 310 samples were collected out of which 95 samples (30.65 %) were found positive using WTLA and 137 samples (44.09%) were found positive using rSAG1.

So far as prevalence of toxoplasmosis in different breeds of sheep is concerned, the difference was statistically insignificant. Out of 106 samples of Shahabadi breeds of sheep tested, 34 samples (32.08%) were positive by WTLA and 45 samples (42.45%) were positive by rSAG1 ELISA. Out of 78 samples tested from Muzaffarnagari breed of sheep, 25
samples (32.05%) were positive WTLA and 35 samples (44.87%) were positive by rSAG1 ELISA. In a Chotanagpuri breed out of 260 samples analysed, 72 samples (27.69%) were found positive using WTLA ELISA and 108 samples (41.53%) by using rSAG1 ELISA.

The farm wise analysis of data revealed, out of 184 samples collected from Chatra farm, 80 samples (43.48%) were positive by rSAG1 ELISA and 59 samples (32.06%) were positive by rSAG1 ELISA. No significant difference was observed in prevalence of toxoplasmosis between the farms studied.

No significant difference was found between the samples collected from farm and village conditions indicating that prevalence of the parasite was ubiquitous in that region. The comparative analysis of samples collected from farm and villages, out of 332 samples collected from farms, 144 samples (43.37%) were found positive using rSAG1 and 99 samples (29.82%) were positive with WTLA ELISA. The samples procured from villages, 44 samples (39.29%) out of 112 samples were positive using rSAG1 ELISA, whereas 32 samples (28.57%) detected positive by WTLA ELISA.

Although the altitude of Ranchi and Chatra districts are different as Chatra district (24° 12'N, 84° 51'E) is located in the Hazaribagh plateau at the height of 1400 ft above the mean sea level, whereas Ranchi district (22° 45’- 23 45’ N, 84° 45’- 84°-50’) is located in the southern part of the Jharkhand state at the height of 2140 ft above the mean sea level, there was no significant difference observed in the seroprevalence of toxoplasmosis in different animal species studied. From Chatra, 184 samples were collected, out of which 80 samples (43.48%) were found positive with rSAG1, whereas 59 samples (32.06%) were detected positive with WTLA ELISA. A total number of 260 samples were collected from Ranchi, of which 108 samples (41.53%) were found positive using rSAG1 and 72 samples (27.69%) were detected positive with WTLA ELISA.

Clementino et al., (2007) detected 30 (29.41% n=102) sera samples were positive for IgG by Toxoplasma-ELISA test from Lajes, Brazil. The
Discussion

Discussion revealed that there was positive correlation between the age of the animals and seropositivity for toxoplasmosis. The number of seropositive animals was greater with advancing age. Antibodies to *Toxoplasma gondii* were found in 31.00% (62/200) of sheep, 17.00% (34/200) of goat and 11.00% (22/200) of cattle from the State of São Paulo, Brazil (Meireles *et al.*, 2003). Antibodies against *T. gondii* were found in 325 sheep (59%) with prevalence ranging from 11% to 96% in different farms of Czech Republic (Bártová *et al.*, 2009). The authors also reported the presence of antibodies against *N. caninum* in 12% sheep with prevalence ranging from 4% to 21% in different farms and mixed infections was reported in 10% of the sheep studied. An ELISA used detection of anti-*T. gondii* antibodies using 1258 small ruminants (732 sheep and 526 goats) samples from 28 different locations in the 3 ecological zones of Ghana revealed an overall seroprevalence of toxoplasmosis 30.5% (n=384). Sheep had a higher overall prevalence (33.2%) in comparison to the goats (26.8%) and the prevalence of antibodies in female animals (35.8%) was significantly higher (*P*<0.01) than that for males (21.1%). Significant differences were also reported between the breeds and age groups. The ELISA was found to be both highly sensitive (92%) and specific (91%) when compared to the IFAT (Van der Puije *et al.*, 2000), Sera from 186 sheep, 83 cattle, and 103 water buffaloes from Punjab, India, were evaluated for antibodies to *Toxoplasma gondii* using a commercial ELISA kit. Antibodies to *T. gondii* were found in 7 of 186 sheep, 2 of 83 cattle, and 3 of 103 buffaloes which was indicative of a low prevalence of *T. gondii* in ruminants tested from the state of Punjab (Sharma *et al.*, 2000). Vesco *et al.*, (2007) reported the overall seroprevalence of *Toxoplasma*-specific IgG-antibodies as 49.9% (937/1876) by ELISA in sheep in Sicily, southern Italy. The study was based on sera collected from 1961 sheep just before slaughtering from 62 farms located in 8 out of 9 Sicilian administrative districts. The presence of cats on the farm, farm size and using surface water as drinking water for the animals were risk factors for infection in sheep. Caballero-Ortega *et al*. (2008) studied the frequency of specific
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antibodies in sheep from the coast, a mountain, and a hill of Colima, Mexico. Serum samples from 351 sheep were tested by a previously standardized indirect ELISA. The frequency of antibodies depended on the altitude, being higher at sea level than at 1200 meters above sea level (OR=3.77, 95% CI=1.79-7.94, P<0.0001), and the size of the flock, being higher in the large ones (OR=2.23, 95% CI=1.35-3.71, P=0.002). Older animals were more frequently positive and with a stronger response than young ones (OR=1.77, 95% CI=1.07-2.93, P=0.016). No differences were observed between male and female sheep.

Skjerve et al. (1998) screened lambs from 194 sheep flocks from different areas of Norway of which 44.3% of the flocks were found seropositive against Toxoplasma gondii using an ELISA test with antibodies found in 16.2% of the 1940 individual animals. The risk factors for the occurrence of antibodies to Toxoplasma gondii found in the multivariate logistic regression were daily presence of a young cat in the sheep house (Odds ratio, OR=4.11, 95% CI=1.01–19.7); ‘atypical’ grazing of lambs (OR=6.35, CI=2.36–17.11); use of mouse poison in the sheep house (OR=2.26, CI=1.02–5.00) and farm situated at an altitude greater than 100 meters above sea level (101–250 m: OR=1.20, CI=0.49–2.92; 251–500 m: OR=4.97; CI=2.04–12.0; >500 m: OR=3.66, CI=1.33–10.1). A lower risk was found for flocks with perforated metal floors in the sheep house (OR=0.47, CI=0.23–0.96) and timber construction of the sheep house (OR=0.34, CI=0.15–0.80). Based on these findings it was recommended that farmers should avoid keeping young cats in the sheep houses, close-to-farm grazing should be avoided or kept to a minimum and that perforated metal floors be used in the sheep houses.

Sensitivity and specificity of the indirect ELISA performed using two separate antigen viz. WTLA and rSAG1 were evaluated by performing indirect fluorescent antibody test considering it as gold standard for the same serum samples. In case of rSAG1 based ELISA, 87.78 % sensitivity and 88.63% specificity were determined with a positive predictive value of 84.04% and negative predictive value of 91.40%. With WTLA, 71.1%
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sensitivity and 92.72% of specificity were determined with Positive predictive value of 97.70% and 83.39% of negative predictive value.

Seroprevalence of toxoplasmosis in goats

The seroprevalence of toxoplasmosis was studied in the Beetal and Black Bengal breeds of goats reared in the organized farm of RVC, Ranchi and Chatra as well as from the village conditions of Chatra and Ranchi districts of Jharkhand. A total number of 445 serum samples were collected of which 120 samples were collected from Chatra farm maintaining Beetal breed of goats and 65 samples were collected from Instructional farm of small ruminants, Ranchi maintaining Black Bengal breed of goats. Rest 260 samples were collected from Black Bengal goats reared at village conditions. The rSAG1 ELISA was superior in detection the Toxoplasma specific IgG molecules and could detect 42.47% of the total serum samples as positive, whereas whole tachyzoite lysate ELISA could detect 29.44% of the samples as positive.

So far as prevalence in the different sexes is concerned from Chatra farm, no significant difference in the infectivity between male and female (p> 00.174 by rSAG1 and p> 0.329 for WTLA) could be established which was indicative of that both male and female goats were equally susceptible to T. gondii infection. The rSAG1 ELISA could detect 33.33% (n=4) of the male samples as positive and 45.37% (n=49) female samples as positive at Chatra farm. The WTLA ELISA revealed 25% (n=3) samples of male and 32.4% (n=35) samples of female were positive.

So far as the distribution of toxoplasmosis in the animals of different age groups is concerned, Toxoplasma specific antibodies were detected most in the animals older than 24 months of age. The rSAG1 ELISA detected 30.76% of the sera samples as positive in the age group of 9-12 months, 42.37% samples positive in the age group of 13-24 months and 57.14% samples as positive in the animals of more than 24 months of age. The WTLA ELISA was positive for 11.53%, 28.81% and 51.42% samples positive for T. gondii specific IgG from different age
The rSAG1 could detect more number of samples as seropositive as its native counterpart.

The sex wise prevalence of toxoplasmosis in the Instructional farm of small ruminants, Ranchi, revealed 3 samples of males (33.33%) and 25 samples of females (44.64%) were positive with rSAG1 ELISA, whereas 2 samples of male (22.22%) and 14 samples of female (25%) were positive by WTLA ELISA. Statistically no significant difference was found in sex wise study.

Further in the absence of data on the age of the goats, prevalence of toxoplasmosis was studied by dividing the animals into three different groups based on their body weight. The *Toxoplasma* specific antibodies were detected most in the animals of higher body weight. No sample in group of 7.5 to 12.5 kg was detected positive, whereas 8 samples (34.78%) in 12.6-17.5 kg group and 20 samples (54.05%) in greater than 19 kg body weight groups were found positive by rSAG1 in ELISA. On the contrary, 1 sample (20%) in 7.5-12 kg group, 3 samples (13.04%) in 12.6-17.5 kg group and 12 samples (32.43%) in greater than 17.5 kg group were found positive by WTLA ELISA. The difference between the two different antigens based ELISA was statistically significant (P<0.0409 for rSAG1 and 0.0120 for WTLA).

Among the samples collected from villages, the sex wise study revealed 45 samples of male (34.88%) and 63 samples of female (48.09%) were positive with rSAG1 ELISA, whereas 26 samples of male (20.16%) and 51 samples of female (38.93%) were positive with WTLA ELISA. Here also the test performed by WTLA ELISA showed statistically significant difference but not in case of rSAG1 ELISA (P value- 0.0146 for WTLA and 0.1470 for rSAG1).

The age wise study revealed 25 (37.31%) and 17 samples (22.97%) in an age group of 9-12 months, 59 (45%) and 46 samples (35.11%) in a age group of 13-24 months and 24 samples (61.54%) and 14 samples (35.9%) in the age group of greater than 24 months as positive using rSAG1 and WTLA antigen, respectively. Statistically no significant differences were found in case of rSAG1 whereas WTLA based ELISA
showed a significant differences (P value- 0.01870 for rSAG1 and 0.0409 for WTLA).

The overall sex wise, breed wise, farm wise, farm/villages and altitude wise study were also made. In sex wise prevalence, a total number of 150 samples of male were collected out of which 31 samples (20.67%) were found positive using WTLA and 52 samples (34.66%) were found positive using rSAG1. In a female 295 samples were collected out of which 100 samples (33.90%) were found positive using WTLA and 137 samples (46.44%) were found positive using rSAG1. Statistically no significant difference was found.

So far as prevalence of toxoplasmosis in different breeds of goats is concerned, the difference was statistically insignificant. Out of 325 samples of Black Bengal goats tested, 93 samples (28.61%) were positive by WTLA and 136 (41.84%) were positive by rSAG1 ELISA. Out of 120 samples tested from Beetal breed of goats, 38 samples (31.67%) were positive WTLA and 53 samples (44.17%) were positive by rSAG1 ELISA.

The farm wise analysis of data revealed, out of 120 samples tested from Beetal breed of goats from Chatra farm, 38 samples (31.67%) were positive by WTLA and 53 samples (44.17%) were positive by rSAG1 ELISA. From Ranchi farm, 16 samples (24.61%) out of 65 samples were found positive using WTLA ELISA whereas, 28 samples (43.08%) detected positive by rSAG1 ELISA. Again no significant difference was found.

In a farm verses village, out of 185 samples collected from organized government farms, 54 samples (29.19%) were found positive with WTLA and 81 samples (43.78%) were positive with rSAG1 ELISA. A total of 108 samples (41.53%) out of 260 samples procured from village with rSAG1 ELISA, whereas 77 samples (29.61%) were detected positive with WTLA ELISA. No significant difference was found between the samples collected from farm and village conditions indicating that prevalence of the parasite was ubiquitous in that region.

Although the altitude of Ranchi and Chatra districts are different as Chatra district (24° 12’N, 84° 51’E) is located in the Hazaribagh plateau at the height of 1400 ft above the mean sea level, whereas
Ranchi district (22° 45'- 23 45' N, 84° 45'- 84°-50') is located in the southern part of the Jharkhand state at the height of 2140 ft above the mean sea level, there was no significant difference observed in the seroprevalence of toxoplasmosis in different animal species studied. From Chatra, 120 samples were collected out of which 38 samples were positive by WTLA and 53 samples (44.17%) were positive by rSAG1. Out of 325 samples collected from Ranchi, 93 samples (28.61%) were positive by WTLA and 136 samples (41.84%) were positive by rSAG1 ELISA.

Rodríguez-Ponce et al. (1995) recorded prevalence of *Toxoplasma gondii* specific IgG antibodies in 63.31% of Canary goats from the island of Grand Canary based on analysis of 1052 sera samples by indirect ELISA. A significant difference in the seroprevalence was recorded by the authors between the four climatic zones of the island. The highest seroprevalence was recorded in animals from low altitude zones with very little rainfall, high temperatures and a predominance of trade winds. Meireles et al. (2003) in a serological survey of antibodies (IgG) to *Toxoplasma gondii* in goats from São Paulo state, Brazil, 17% (34/200) were found positive by Enzyme Linked Immunosorbent Assay. Van der Puije et al. (2000) in a seroprevalence study of *T. gondii* in Ghana, out of 526 samples collected from 28 different locations in the three different ecological zones, 26.8% were detected positive by Indirect ELISA. Significant differences were also observed between breeds and age groups. Garcia-Vazque et al., (1993) collected a total number of 707 goat serum samples from nine farms of four Mexican state for serological survey for antibody activity against *Toxoplasma gondii* by the enzyme-linked immunosorbent assay (ELISA) test. Five out of the nine goat farms were found to have *T. gondii* seropositive goats and the average seroprevalence was 3.2%.

Sensitivity and specificity of the indirect ELISA performed using two separate antigen *viz.* WTLA and rSAG1 were evaluated by performing indirect fluorescent antibody test considering it as gold standard for the same serum samples. In case of rSAG1 based ELISA, 92.66 % sensitivity and 90.67% specificity were determined with a positive predictive value.
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of 84.04% and negative predictive value 91.40%. With WTLA, 66.67% sensitivity and 95.14% of specificity were determined with Positive predictive value 90.07% and 81.21% of negative predictive value.

Characterization of soluble tachyzoites lysate proteins of T. gondii, RH strain

The Toxoplasma gondii specific polypeptides were resolved in the molecular range of 73.0 to 11 KDa in a 6-15% gradient SDS-PAGE under denaturing condition. The major polypeptides detected were in the molecular range of 73, 64, 55, 49, 40, 38, 37, 30, 25, 14 and 11 KDa.

Western blot analysis:

To identify immunodominant protein in whole tachyzoite antigen lysate of T. gondii western blotting was performed. The proteins in the molecular range of 73, 64, 49, 40, 37, 30 and 25 KDa showed immunoreactivity against goat serum collected from field, whereas 55, 49, 37, 25 KDa band shown a good immunoreactivity against sheep serum collected from field.

The ELISA based detection method applied in the present study involving pig, sheep and goat population reared either as free range husbandry system or in organized farms generated important data on seroprevalence of toxoplasmosis at Jharkhand region. The major advantage of ELISA based serodiagnosis was that the assay produced a quantifiable and reliable result. In the present study, a recombinant protein, viz. SAG1 was used as diagnostic antigen and the reactivity pattern was further compared with a native tachyzoite lysate protein. The performance rSAG1 ELISA was consistent and was characterized by higher OD in comparison to its native counterpart. Further, the diagnostic sensitivity and specificity of the ELISA was determined against indirect fluorescent antibody test which is considered as a standard test for detection of Toxoplasma specific antibodies.

Several studies have shown that antigen recognition varies with the stage of infection (Lappin et al., 1994; Tomavo et al., 1994), animal
species investigated and the stage of haplotypes (Denkers et al., 1994) involved. Variation in the immune response has also been demonstrated with different strains of *T. gondii* (Bohne et al., 1993). Variation in the response may also occur depending on the stage of infection, *viz.* chronic stage of infection caused by bradyzoite stage of the parasite (Torpier et al., 1993) or tachyzoite induced acute stage of infection (Dubey et al., 1995). The use of recombinant proteins in the ELISA has several advantages including large scale production of the specific protein using heterologous expression system and thereby reduced cost, reproducibility of result and increased diagnostic specificity. The important desirable attribute of the recombinant protein based diagnosis is that there is no need for dependence on animals for propagating the tachyzoites for production of antigen.
Toxoplasma gondii, an intracellular apicomplexan protozoan parasite, is prevalent worldwide. All the stages of lifecycle of *T. gondii*, viz. tachyzoites, bradyzoites and sporulated oocysts are potentially infective. Information on prevalence of toxoplasmosis in animals and man is scanty from India. Since there is no information available on the prevalence of toxoplasmosis among the economically important farm animals from the Jharkhand state, the present study was undertaken with the objective of studying the seroprevalence of *T. gondii* in small ruminants and pigs. In order to accomplish the objective, ELISA was laboratory standardized using standard reagents. A native protein based antigen was prepared from the purified tachyzoites of *T. gondii*, whole tachyzoite lysate antigen (WTLA) and was used in the indirect ELISA for screening the *Toxoplasma*-specific IgG molecules present in the sera. The serodetection potential of the native WTLA based ELISA was further compared with a recombinant surface antigen 1 (SAG1) protein based ELISA. The serum samples were collected randomly from the target animals maintained either as free range management system in rural house holds as well as from the organized Government farms of Ranchi and Chatra districts of Jharkhand state. While collecting the samples, the age, sex and breed of the animals were recorded for drawing correlations with the prevalence of toxoplasmosis, if existing.

A total number of 240 pig serum samples were collected from organized farm and slaughter house. The recombinant surface antigen1 based ELISA was superior in detection of the *T. gondii* specific IgG antibodies in 44.58% of the serum samples tested over the crude whole tachyzoite lysate antigen (WTLA) which could detect the same in 40% of the samples. The prevalence of the disease was found more in the samples collected from slaughter houses *i.e.*, 53.75% and 23.75% than
that of farm *i.e.*, 26.25% and 48.12% by rSAG1 and WTLA based ELISA, respectively.

From an Organized farm, seroprevalence of toxoplasmosis in different age groups of the animals was statistically significant indicating the age factor had a positive correlation with the sero-positivity of the animals. In an age group of 3 months, 9.37% samples were positive both by rSAG1 and WTLA based ELISA, whereas 20.83% and 16.67% samples were positive by rSAG1 and WTLA based ELISA, respectively in the pigs of 4-8 months of age. Maximum number of serum samples was tested positive in the pigs belonging to the age of 9 months and above. As high as 54.17% and 50% of the samples were detected positive for *Toxoplasma* specific antibodies by rSAG1 and WTLA based ELISA, respectively. More number of females were found positive (29.79%) than that of the males ranging from 15.15% to 21.21% with the rSAG1 and WTLA. The variation was statistically significant by WTLA based ELISA but was insignificant by rSAG1based ELISA. The breed wise analysis revealed no significant difference leading to the conclusion that both the breeds studied were equally susceptible to toxoplasmosis.

A total of 160 samples were collected from slaughter houses which included Deshi and crosses of Tameworth and Deshi breeds (T&D). The rSAG1 and WTLA based ELISA could detect 53.75% and 48.12% sera samples, respectively, as positive. The difference of seroprevalence between the breeds was statistically insignificant which led to conclusion that both of the breeds were equally susceptible to *T. gondii* infection. Among the crosses of T&D, the rSAG1 and WTLA antigen based ELISA revealed 55.93% and 49.15% serum samples positive, whereas 52.48% and 47.52% serum samples were positive with rSAG1 and WTLA, respectively among the Deshi breed of pigs. The rSAG1 based ELISA revealed 48.98% of male and 61.29% female sera samples positive, whereas 46.94% male and 50% of female samples were positive with WTLA antigen based ELISA. No significant difference was found so far as sex of the animals was concerned and both the sexes were found equally susceptible.
Sera samples were collected from Muzaffarnagari, Shahabadi and Chotangpuri breeds of sheep from Chatra and Ranchi districts of Jharkhand. The rSAG1 showed diagnostic superiority over the native WTLA. Out of 444 samples analysed, 188 samples (42.34%) were found positive by rSAG1 ELISA, whereas 131 samples (29.5%) were positive with whole tachyzoites lysate ELISA. The sex wise prevalence among the samples collected from Chatra farm revealed 32 samples (42.67%) of male and 48 samples of female (44.03%) as positive with rSAG1, whereas 22 samples of male (29.33%) and 37 samples of female (33.94%) were positive with WTLA ELISA, respectively. However, the difference was statistically insignificant which was suggestive of that both the sexes are equally susceptible to *T. gondii* infection. Similarly, no significant difference was observed in the susceptibility for toxoplasmosis in the different breeds of sheep studied and both the Muzaffarnagari and Shahabadi breeds were equally susceptible to toxoplasmosis. A total of 35 samples (42.45%) of Muzaffarnagari and 45 samples (44.87%) of Shahabadi were positive with the rSAG1, whereas the WTLA could detect 25 samples (32.05%) of Muzaffarnagari and 34 samples (32.08%) of Shahabadi as positive.

Out of 148 serum samples of Chotanagpuri breed collected from Instructional farm of small ruminants, Ranchi, 64 samples (43.24%) with rSAG1 while 40 samples (27.03%) were positive with WTLA ELISA. The sex wise survey revealed 5 males (33.3%) and 59 females (44.36%) were positive with rSAG1 ELISA, whereas 3 males (20%) and 37 females (27.82%) were positive with the WTLA ELISA. The difference was statistically insignificant. A weight wise survey for toxoplasmosis was also done since the age of the animals was not maintained in the farm. 3 samples (13.04%) in the first group of body weight below 12Kg, 56 samples (49.55%) in the second group, 12 to 19 Kg body weight group and 5 samples (41.66%) in the third group of body weight greater than 19 Kg were found positive through rSAG1 ELISA, whereas 2 (8.67%), 34 (30.09%) and 4(33.3%) samples from the three different weight groups were found positive by WTLA ELISA. The difference was statistically
significant for both the antigens used in ELISA which indicated a positive correlation between infection and body weight or in other words with age of the animals. The finding was in agreement with that of Clementino et al. (2007) and Caballero-Ortega et al. (2008).

Out of the 112 samples collected from village conditions revealed that 44 samples (39.28%) were positive with rSAG1 while 32 samples (28.57%) were positive by WTLA ELISA. The sex wise difference was statistically insignificant for both types of antigens used for serodetection in ELISA. A total a total number of 14 samples (31.81%) of male out of 44 samples were found positive by rSAG1 whereas 11 samples (25%) were detected positive by WTLA ELISA. So far as the samples procured from female are concerned, 30 samples (44.11%) out of 68 samples were detected positive with rSAG1 whereas 21 samples (30.88%) were detected positive against WTLA ELISA.

The difference in age groups showed statistically significant difference. In the 6-12 months age group, 2 samples (16.67%) out of 12 samples were detected positive with rSAG1, whereas 1 sample (8.33%) was found positive using WTLA ELISA. In the 13-24 months age group, 16 samples (37.2%) out of 43 samples studied were detected positive using rSAG1, whereas 13 samples (30.23%) were detected positive by WTLA ELISA. In the 24 months and beyond age group, 26 samples (45.61%) out of 57 samples studied were detected positive by rSAG1 and 18 samples (31.57%) by WTLA ELISA.

In an overall analysis, sex wise, breed wise, farm wise, farm/villages and altitude wise study, none of these factors showed any significant difference.

In a sex wise analysis a total number of 51 samples (38.05%) out of 134 samples studied from male, were found positive by rSAG1, whereas 36 samples (26.87%) were detected positive by WTLA ELISA. So far as the samples procured from females were concerned, 137 samples (44.09%) out of 310 samples studied were detected positive with rSAG1, whereas 95 samples (30.65%) were detected positive against WTLA ELISA.
For a breed wise analysis, out of 106 samples of Shahabadi breed studied, 45 samples (42.45%) were found positive by using rSAG1 and 34 samples (32.08%) by using WTLA ELISA. In Muzaffarnagari breed, out of 78 samples studied, 35 samples (44.87%) were found positive using rSAG1 and 25 samples (32.05%) were positive with WTLA ELISA. In a Chotanagpuri breed, out of 260 samples analyzed 108 samples (41.53%) were found positive using rSAG1 and 72 samples (27.69%) by using WTLA ELISA.

For a farm wise analysis, two farms viz. Chatra farm and Ranchi farm were studied. From Chatra farm, 80 samples (43.48%) out of 184 samples tested was found positive using rSAG1 and 59 samples (32.06%) were positive with WTLA ELISA. From Ranchi farms, 64 samples (43.24%) out of 148 samples studied were found positive using rSAG1 whereas, 40 samples (27.07%) were detected positive by WTLA.

The comparative analysis of samples collected from farm and villages, out of 332 samples collected from farms, 144 samples (43.37%) were found positive using rSAG1 and 99 samples (29.82%) were positive with WTLA ELISA. The samples procured from villages, 44 samples (39.29%) out of 112 samples were positive using rSAG1 ELISA, whereas 32 samples (28.57%) detected positive by WTLA ELISA.

In an altitude wise analysis, Chatra district (24° 12'N, 84° 51'E) is located in the Hazaribagh plateau at the height of 1400 ft above the mean sea level whereas Ranchi district (22° 45'- 23 45' N, 84° 45'- 84°-50') lies in the southern part of the Jharkhand state at the height of 2140 ft above the mean sea level. From chatra, 184 samples were collected, out of which 80 samples (43.48%) were found positive with rSAG1, whereas 59 samples (32.06%) were detected positive with WTLA ELISA. A total number of 260 samples were collected from Ranchi, of which 108 samples (41.53%) were found positive using rSAG1 and 72 samples (27.69%) were detected positive with WTLA ELISA.

The seroprevalence of toxoplasmosis was studied in the Beetal and Black Bengal breeds of goats reared in the organized farm of RVC, Ranchi and Chatra as well as from the village conditions of Chatra and
Ranchi districts of Uttarakhand. A total number of 445 serum samples were collected of which 120 samples were collected from Chatra farm maintaining Beetal breed of goats and 65 samples were collected from Instructional farm of small ruminants, Ranchi maintaining Black Bengal breed of goats. Rest 260 samples were collected from Black Bengal goats reared at village conditions. The rSAG1 ELISA was superior in detection the toxoplasma specific IgG molecules and could detect 42.47% of the total serum samples as positive, whereas whole tachyzoites lysate ELISA could detect 29.44% of the samples as positive.

So far as prevalence in the different sexes is concerned from Chatra farm, no significant difference in the infectivity between male and female could be established which was indicative of that both male and female goats were equally susceptible to *T. gondi* infection. The rSAG1 ELISA could detect 33.33% (n=4) of the male samples as positive and 45.37% (n=49) female samples as positive at Chatra farm. The WTLA ELISA revealed 25% (n=3) samples of male and 32.4% (n=35) samples of female were positive.

So far as the distribution of toxoplasmosis in the animals of different age groups is concerned, *Toxoplasma* specific antibodies were detected most in the animals older than 24 months of age. The rSAG1 ELISA detected 30.76% of the sera samples as positive in the age group of 9-12 months, 42.37% samples positive in the age group of 13-24 months and 57.14% samples as positive in the animals of more than 24 months of age. The WTLA ELISA was positive for 11.53%, 28.81% and 51.42% samples positive for *T. gondii* specific IgG from different age groups mentioned above. The rSAG1 could detect more number of samples as seropositive as its native counterpart.

No significant difference in the predisposition of infection between the sexes was observed from Instructional farm of small ruminants. The sex wise prevalence of toxoplasmosis revealed 3 samples of males (33.33%) and 25 samples of females (44.64%) were positive with rSAG1 ELISA, 2 samples of male (22.22%) and 14 samples of female (25%) were positive by WTLA ELISA. Further in the absence of data on the age of the
goats, prevalence of toxoplasmosis was studied by dividing the animals into three different groups based on their body weight. The *Toxoplasma* specific antibodies were detected most in the animals of higher body weight. No sample in group of 7.5 to 12.5 kg was detected positive, whereas 8 samples (34.78%) in 12.6-17.5 kg group and 20 samples (54.05%) in greater than 19 kg body weight groups were found positive by rSAG1 in ELISA. On the contrary, 1 sample (20%) in 7.5-12 kg group-3 samples (13.04%) in 12.6-17.5 kg group and 12 samples (32.43%) in greater than 17.5 kg group were found positive by WTLA ELISA. The difference between the two different antigens based ELISA was statistically significant.

Among the samples collected from villages, the sex wise study revealed 45 samples of male (34.88%) and 63 samples of female (48.09%) were positive with rSAG1 ELISA, whereas 26 samples of male (20.16%) and 51 samples of female (38.93%) were positive with WTLA ELISA. Here the test performed by WTLA ELISA showed statistically significant difference but not in rSAG1 ELISA. The age wise study revealed 25 (37.31%) and 17 samples (22.97%) in an age group of 9-12 months, 59 (45%) and 46 samples (35.11%) in a age group of 13-24 months and 24 samples (61.54%) in the age group of greater than 24 months as positive using rSAG1 and WTLA antigen, respectively.

The overall sex wise, breed wise, farm wise, farm/villages and altitude wise study were also made. In sex wise prevalence, a total number of 150 samples of male were collected out of which 31 samples (20.67%) were found positive using WTLA and 52 samples (34.66%) were found positive using rSAG1.In a female 295 samples were collected out of which 100 samples (33.90%) were found positive using WTLA and 137 samples (46.44%) were found positive using rSAG1. Statistically no significant difference was found in sex wise study.

So far as prevalence of toxoplasmosis in different breeds of goats is concerned, the difference was statistically insignificant. Out of 325 samples of Black Bengal goats tested, 93 samples (28.61%) were positive by WTLA and 136 (41.84%) were positive by rSAG1 ELISA. Out of 120
samples tested from Beetal breed of goats, 38 samples (31.67%) were positive WTLA and 53 samples (44.17%) were positive by rSAG1 ELISA.

The farm wise analysis of data revealed, out of 120 samples tested from Beetal breed of goats from Chatra farm, 38 samples (31.67%) were positive by WTLA and 53 samples (44.17%) were positive by rSAG1 ELISA. From Ranchi farm, 16 samples (24.61%) out of 65 samples were found positive using WTLA whereas 28 samples (43.08%) detected positive by rSAG1 ELISA.

No significant difference was found between the samples collected from farm and village conditions indicating that prevalence of the parasite was ubiquitous in that region. Out of 185 samples collected from the organized farms, 54 samples were positive by WTLA and 81 samples were positive by rSAG1 ELISA.

Although the altitude of Ranchi and Chatra districts are different as Chatra district (24° 12'N, 84° 51'E) is located in the Hazaribagh plateau at the height of 1400 ft above the mean sea level, whereas Ranchi district (22° 45’- 23 45’ N, 84° 45'- 84°-50) is located in the southern part of the Jharkhand state at the height of 2140 ft above the mean sea level, there was no significant difference observed in the seroprevalence of toxoplasmosis in different animal species studied. From Chatra, 120 samples were collected out of which 38 samples (31.67%) were positive by WTLA and 53 samples (44.17%) were positive by rSAG1. Out of 325 samples collected from Ranchi, 93 samples (28.61%) were positive by WTLA and 136 samples (41.84%) were positive by rSAG1 ELISA.

The Toxoplasma gondii specific polypeptides were resolved in the molecular range of 73.0 to 11 KDa in a 6-15% gradient SDS-PAGE under denaturing condition. The major polypeptides detected were in the molecular range of 73, 64, 55, 49, 40, 38, 37, 30, 25, 14 and 11 KDa. To identify immunodominant protein in whole tachyzoite antigen lysate of T. gondii western blotting was performed. The proteins in the molecular range of 73, 64, 49, 40, 37, 30 and 25 KDa showed immunoreactivity against goat serum collected from field, whereas 55, 49, 37, 25 KDa
band shown a good immunoreactivity against sheep serum collected from field.

The ELISA based detection method applied in the present study involving pig, sheep and goat population reared either as free range husbandry system or in organized farms generated important data on seroprevalence of toxoplasmosis at Jharkhand region. A high seroprevalence of toxoplasmosis among several food animals warrants for adoption of control measures to prevent transmission to susceptible humans.
Conclusions:

In seroprevalence study of pigs, out of 240 samples 107 samples (44.58%) were found positive through rSAG1 based ELISA whereas 96 samples (40%) were found positive with WTLA based ELISA.

In case of sheep, out of 444 sera samples 188 samples (42.34%) were found positive with rSAG1 based ELISA whereas 131 samples (29.5%) were found positive WTLA based ELISA.

In case of goats, out of 445 samples 189 samples (42.47%) were found positive with rSAG1 based ELISA whereas 131 samples (29.44%) were found positive with WTLA based ELISA.

The major advantage of ELISA based serodiagnosis was that the assay produced a quantifiable and reliable result.

The important desirable attribute of the recombinant protein based diagnosis is that there is no need for dependence on animals for propagating the tachyzoites for production of antigen.

In the immunocharacterization study, the major polypeptides were detected in the molecular range of 73, 64, 55, 49, 40, 38, 37, 30, 25, 14 and 11 KDa in a 6-15% gradient SDS- PAGE, out of which 73, 73, 64, 49, 40, 37, 30 and 25 KDa showed good immunoreactivity against a goat serum collected from the field whereas 55, 49, 37, 25 KDa showed a good immunoreactivity against sheep serum collected from field.


Kniel, K. E., Lindsay, D. S., Sumner, S. S., Hackney, C. R., Pierson, M. D. and Dubey, J. P. (2002). Examination of attachment and
survival of *Toxoplasma gondii* oocysts on raspberries and blueberries. *J. Parasitol.*, **88**: 790–793.


Bibliography…


General buffers and reagents

**10X Phosphate buffered saline (PBS) pH 7.2**

- Sodium chloride: 80.0 g
- Potassium chloride: 2.0 g
- Disodium hydrogen phosphate: 11.5 g
- Potassium dihydrogen phosphate: 2.0 g
- Distilled water: 1000 ml

At this point pH is not 7.2

**PBS (pH- 7.2) working solution**

- Stock solution of PBS: 100 ml
- Distilled water to: 1000 ml

Adjust the pH either with 0.1N NaOH or 0.1N HCl to 7.2.

**PMSF stock (100mM)**

- Phenyl methyl sulfonyl fluoride: 17.4 mg
- Isopropanol: 1 ml

Store at – 20°C

**Giemsa stain**

- Giemsa powder: 3.8 g
- Methyl alcohol (Acetone free): 250 ml
- Warm glycerine: 250 ml

The stain was diluted 1:20 in PBS (pH 7.2)

**Trypan blue (0.4% solution)**

- Trypan blue powder: 40 mg
- Distilled water: 1 ml
Reagents for Enzyme Immuno Assays

Enzyme linked Immunosorbent Assay (ELISA)

Coating buffer
Sodium carbonate (Na$_2$CO$_3$) 159 mg
Sodium bicarbonate (NaHCO$_3$) 293 mg
Dissolve in 100 ml of distilled water and adjust the pH to 9.6. Autoclave and store at 4ºC.

ELISA wash buffer
0.05% of Tween-20 was added to sufficient amount of PBS (pH 7.2).

ELISA Blocking Buffer
5% skimmed milk powder solution prepared in PBS (pH 7.2)

Substrate Buffer- Phosphate Citrate Buffer (0.05 M, pH 5.0)

Solution A
Citric Acid 2.1 g
Distilled water to 100 ml

Solution B
Disodium Hydrogen Phosphate 3.56 g
(Na$_2$HPO$_4$.2 H$_2$O)
Distilled water to 100 ml
Mix 6.425 ml of solution A and 6.075 ml of solution B before use and make the volume upto 25 ml with distilled water, add 10 mg OPD and 10µl of H$_2$O$_2$ just before use. Use within 1 h after preparation.
Keep in dark.

Stopping solution (3N HCl)
Conc. HCl 25 ml
Distilled water 75 ml
Immunofluorescence antibody technique (IFAT)

10X Phosphate buffer saline (PBS) pH 7.6

Sodium Chloride 85.0 g
Disodium hydrogen phosphate (Anhydrous) 12.36 g
Disodium hydrogen phosphate 1.80 g
Add the distilled water to a final volume of 1000 ml

At this point pH is not 7.6

PBS (pH 7.6) working solution

Stock solution of PBS (pH 7.6) 100 ml
Distilled water to a final volume of 1 L. Adjust the pH either with 0.1 N NaOH or 0.1N HCl to 7.6

Buffered glycerol (pH 9.0)

Phosphate buffer 1 vol.
\[(Na_2HPO_4) \ 0.284 \text{ g} / 10 \text{ ml of water, pH 9.0}\]
Glycerol 9 vol.

Check pH with indicator paper

Evan’s blue (1% stock solution)

Evan’s blue dye powder 0.5 g
PBS (pH 7.6) 50 ml
### Reagents and solution for SDS-PAGE

#### 30% Acrylamide stock solutions

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acrylamide</td>
<td>29.2 g</td>
</tr>
<tr>
<td>Bisacrylamide</td>
<td>0.8 g</td>
</tr>
</tbody>
</table>

Dissolve to make a final volume of 100 ml. Filter and store at 4°C in dark bottle.

#### Separating Gel Buffer (1.5 M Tris-HCl, pH 8.8)

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tris base</td>
<td>18.18 g</td>
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<tr>
<td>Distilled water</td>
<td>75 ml</td>
</tr>
</tbody>
</table>

Dissolve Tris and adjust the pH to 8.8 using conc. HCl. Make up the volume to 100 ml with distilled water.

#### Stacking gel buffer (0.5 M Tris-HCl, pH 6.8)

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tris base</td>
<td>6.0 g</td>
</tr>
<tr>
<td>Distilled water</td>
<td>75 ml</td>
</tr>
</tbody>
</table>

Dissolve Tris and adjust the pH to 6.8 using conc. HCl. Make up the volume to 100 ml with distilled water.

#### Sodium Dodecyl Sulphate (10% w/v)

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>SDS</td>
<td>1 g</td>
</tr>
</tbody>
</table>

| Distilled water          | 10 ml         |

#### Ammonium per sulphate (10% w/v)

<table>
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<tr>
<th>Reagent</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ammonium per sulphate</td>
<td>100 mg</td>
</tr>
</tbody>
</table>

| Distilled water          | 1 ml          |

#### 5X SDS-PAGE Electrophoresis Buffer (pH 8.3)

<table>
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<tr>
<th>Reagent</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tris base</td>
<td>15.0 g</td>
</tr>
<tr>
<td>Glycine</td>
<td>72.0 g</td>
</tr>
<tr>
<td>SDS</td>
<td>5.0 g</td>
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</tbody>
</table>

| Distilled water to       | 1000 ml       |

#### Sample buffer (2X)

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycerol(20%)</td>
<td>2 ml</td>
</tr>
<tr>
<td>ß – Mercaptophenol (2%)</td>
<td>0.2 ml</td>
</tr>
<tr>
<td>10% SDS</td>
<td>4.0 ml</td>
</tr>
<tr>
<td>1M Tris pH 6.8</td>
<td>1.25 ml</td>
</tr>
</tbody>
</table>
Bromophenol blue 0.02 mg
Adjust the volume to 10 ml with distilled water. Store at 4°C.

**CBB Staining solution**
Coomasie brilliant blue R-250(CBB) 200 mg
Methanol 40 ml
Acetic acid 10 ml
First dissolve the powder with methanol then add acetic acid.
Distilled water adds to make up to 100 ml.

**Destaining solution**
Methanol 40 ml
Acetic acid 10 ml
Distilled water add to make up to 100 ml.

**Recipe for 6% separating gel (5 ml)**
Distilled water 2.6 ml
30% Acrylamide mix 1.0 ml
1.5 M Tris (pH- 8.8) 1.3 ml
10% SDS 50 µl
10% Ammonium persulphate 50 µl
TEMED 4 µl

**Recipe for 15% separating gel (5 ml)**
Distilled water 1.1 ml
30% Acrylamide mix 2.5 ml
1.5 M Tris (pH- 8.8) 1.3 ml
10% SDS 50 µl
10% Ammonium persulphate 50 µl
TEMED 2 µl

**5% Stacking gel (4 ml)**
Distilled water 2.7 ml
30% Acrylamide mix 0.67 ml
1.5 M Tris (pH- 6.8) 0.5 ml
10% SDS 40 µl
10% Ammonium persulphate 40 µl
TEMED 4 µl
Reagents for western blotting

Electrode (running) buffer

Tris base 3 g
Glycine 14.4 g
Distilled water 1000 ml

Just before use add 200 ml of cold methanol and 10 ml of 10% SDS.

Blocking buffer (5% skimmed milk PBS)

Skimmed milk powder 5 g
PBS (pH 7.4) 100 ml

Buffer for serum dilution

Skimmed milk powder 5 g
Tween-20 50 µl
Triton- X 100 1 ml

Buffer for conjugate dilution

Skimmed milk powder 3 g
PBS-T 100 ml

Substrate buffer

Tris (2M) pH- 7.35 1.25 ml
DAB 20 mg
H₂O₂ 7.5 µl
8% Nickel Chloride 150 µl
Distilled water 25 ml
Fig. 1:  Tachyzoites of *T. gondii* from peritoneal fluid of experimental mouse (unstained, X 400)

Fig. 2:  Purified cell free tachyzoites of *T. gondii* from peritoneal fluid of experimental mouse (Giemsa stained, oil immersion, X 1000)
Fig. 3: Scatter chart showing the OD$_{492}$ values of pig samples collected from farm and slaughter house by indirect ELISA using recombinant SAG1 as detection antigen.

Fig. 4: Scatter chart showing the OD$_{492}$ values of pig samples collected from farm and slaughter house by indirect ELISA using whole tachyzoite lysate as detection antigen.
Table 1: The sex, age and breed wise variation in the seroprevalence of *Toxoplasma gondii* with recombinant SAG1 antigen based indirect ELISA in pigs of Instructional Pig Farm Unit (IPFU), Ranchi

<table>
<thead>
<tr>
<th>Factors</th>
<th>Category</th>
<th>No. of samples examined</th>
<th>No. of samples Positive</th>
<th>% Positive</th>
<th>Chi-square</th>
<th>Degree of freedom</th>
<th>P value</th>
</tr>
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<tr>
<td>Sex</td>
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<td>14</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
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<td>3</td>
<td>9.37</td>
<td>38.75</td>
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<td>&lt;.0001</td>
</tr>
<tr>
<td></td>
<td>4-8 month</td>
<td>24</td>
<td>5</td>
<td>20.83</td>
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<td></td>
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<td></td>
<td>≥9 month</td>
<td>24</td>
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<td>54.17</td>
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</tr>
<tr>
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<td>T &amp; D (CB)</td>
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<td>13</td>
<td>27.08</td>
<td>0.103</td>
<td>2</td>
<td>0.9494</td>
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<tr>
<td></td>
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<td>25.0</td>
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<tr>
<td></td>
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<td>12</td>
<td>3</td>
<td>25.0</td>
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Table 2: The sex, age and breed wise variation in the seroprevalence of *Toxoplasma gondii* with whole tachyzoite lysate antigen based indirect ELISA in pigs of Instructional Pig Farm Unit (IPFU), Ranchi

<table>
<thead>
<tr>
<th>Factors</th>
<th>Category</th>
<th>No. of samples examined</th>
<th>No. of samples Positive</th>
<th>% positive</th>
<th>Chi-square</th>
<th>Degree of freedom</th>
<th>P value</th>
</tr>
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<tbody>
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<td>0.029</td>
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<tr>
<td></td>
<td>Female</td>
<td>47</td>
<td>14</td>
<td>29.79</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>3 month</td>
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<td>3</td>
<td>9.37</td>
<td>37.002</td>
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<td></td>
<td>4-8 month</td>
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<td>≥9 month</td>
<td>24</td>
<td>12</td>
<td>50</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Breed</td>
<td>T &amp; D(C.B)</td>
<td>48</td>
<td>12</td>
<td>25</td>
<td>0.7143</td>
<td>2</td>
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<tr>
<td></td>
<td>Hampshire</td>
<td>20</td>
<td>4</td>
<td>20</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>Tamworth</td>
<td>12</td>
<td>3</td>
<td>25</td>
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</table>
Fig. 5: Graph showing the sex, age and breed wise variation in the seroprevalence of *Toxoplasma gondii* based on recombinant SAG1 antigen based indirect ELISA in pigs of Instructional Pig Farm unit (IPFU), Ranchi.

Fig. 6: Graph showing the sex, age and breed wise variation in the seroprevalence of *Toxoplasma gondii* based on whole tachyzoite lysate antigen based indirect ELISA in pigs of Instructional Pig Farm unit (IPFU), Ranchi.
Table 3: The sex and breed wise variation in the seroprevalence of *Toxoplasma gondii* with recombinant SAG1 antigen based indirect ELISA in pigs of slaughter house in Ranchi.

<table>
<thead>
<tr>
<th>Factors</th>
<th>Category</th>
<th>No. of samples examined</th>
<th>No. of samples Positive</th>
<th>% positive</th>
<th>Chi-square</th>
<th>Degree of freedom DF</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
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<td>48</td>
<td>48.98</td>
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<tr>
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<td>Female</td>
<td>62</td>
<td>38</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Breed</td>
<td>T &amp;D(C.B)</td>
<td>59</td>
<td>33</td>
<td>55.93</td>
<td>52.48</td>
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<td>Deshi</td>
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<td>53</td>
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</table>

Table 4: The sex and breed wise variation in the seroprevalence of *Toxoplasma gondii* with whole tachyzoite lysate antigen based indirect ELISA in pigs of slaughter house in Ranchi.

<table>
<thead>
<tr>
<th>Factors</th>
<th>Category</th>
<th>No. of examined</th>
<th>No. of samples Positive</th>
<th>% positive</th>
<th>Chi-square</th>
<th>Degree of freedom DF</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>Male</td>
<td>98</td>
<td>46</td>
<td>46.94</td>
<td>50</td>
<td>0.0967</td>
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</tr>
<tr>
<td></td>
<td>Female</td>
<td>62</td>
<td>31</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Breed</td>
<td>T &amp;D(C.B)</td>
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<td>29</td>
<td>49.15</td>
<td>47.52</td>
<td>0.0274</td>
<td>0.8685</td>
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<td>Deshi</td>
<td>101</td>
<td>48</td>
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</table>
Fig. 7:  Graph showing the sex and breed wise variation in the seroprevalence of *Toxoplasma gondii* in pig sera samples collected from slaughter house based on recombinant SAG1 antigen based indirect ELISA.

Fig. 8:  Graph showing the sex and breed wise variation in the seroprevalence of *Toxoplasma gondii* in pig sera samples collected from slaughter house based on whole tachyzoite lysate antigen (WTLA) based indirect ELISA.
Table: 5  The sensitivity and specificity of rSAG1 ELISA in comparison to Indirect Fluorescent Antibody Test for the serodetection of Toxoplasmosis in swine.

<table>
<thead>
<tr>
<th>ELISA with rSAG1</th>
<th>IFAT</th>
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<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
<td>Positive Predictive Value</td>
<td>86.91%</td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>93</td>
<td>14</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>13</td>
<td>120</td>
<td>Negative Predictive value</td>
<td>90.22%</td>
<td></td>
</tr>
</tbody>
</table>

Sensitivity: 87.73%
Specificity: 89.55%

Table: 6  The sensitivity and specificity of WTLA ELISA in comparison to Indirect Fluorescent Antibody Test for the serodetection of Toxoplasmosis in swine

<table>
<thead>
<tr>
<th>ELISA with WTLA</th>
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</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
<td>Positive Predictive Value</td>
<td>84.38 %</td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>81</td>
<td>15</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>25</td>
<td>119</td>
<td>Negative Predictive value</td>
<td>82.63 %</td>
<td></td>
</tr>
</tbody>
</table>

Sensitivity: 76.41%
Specificity: 88.80 %
Fig. 9: Scatter chart showing the OD$_{492}$ values of sheep samples collected from farm and villages by indirect ELISA using recombinant SAG1 as detection antigen

Fig. 10: Scatter chart showing the OD$_{492}$ values of sheep samples collected from farm and slaughter house by indirect ELISA using recombinant whole tachyzoite lysate as detection antigen
Table: 7  The sex and breed wise variation in the seroprevalence of *Toxoplasma gondii* with recombinant SAG1 antigen based indirect ELISA in sheep of Chatra Farm.

<table>
<thead>
<tr>
<th>Factors</th>
<th>Category</th>
<th>No. of samples examined</th>
<th>No. of samples positive</th>
<th>% positive</th>
<th>Chi-square</th>
<th>Degree of freedom DF</th>
<th>P value</th>
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</thead>
<tbody>
<tr>
<td>Sex</td>
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<td>75</td>
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<td>42.67</td>
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<td>0.883</td>
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<tr>
<td></td>
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<td>109</td>
<td>48</td>
<td>44.03</td>
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<tr>
<td>Breed</td>
<td>Muzaffarnagari</td>
<td>78</td>
<td>35</td>
<td>42.45</td>
<td>0.0670</td>
<td>1</td>
<td>0.7957</td>
</tr>
<tr>
<td></td>
<td>Shahabadi</td>
<td>106</td>
<td>45</td>
<td>44.87</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table: 8  The sex and breed wise variation in the seroprevalence of *Toxoplasma gondii* with whole tachyzoite lysate antigen based indirect ELISA in sheep of Chatra Farm

<table>
<thead>
<tr>
<th>Factors</th>
<th>Category</th>
<th>No. of samples examined</th>
<th>No. of samples positive</th>
<th>% positive</th>
<th>Chi-square</th>
<th>Degree of freedom DF</th>
<th>P value</th>
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<tbody>
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<td>Male</td>
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<td>0.336</td>
<td>1</td>
<td>0.5621</td>
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<td>Female</td>
<td>109</td>
<td>37</td>
<td>33.94</td>
<td></td>
<td></td>
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<tr>
<td>Breed</td>
<td>Muzaffarnagari</td>
<td>78</td>
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</table>
Fig. 11: Graph showing the sex and breed wise variation in the seroprevalence of *Toxoplasma gondii* based on recombinant SAG1 based indirect ELISA in sheep of Chatra Farm

Fig. 12: Graph showing the sex and breed wise variation in the seroprevalence of *Toxoplasma gondii* based on whole tachyzoite lysate antigen antigen based indirect ELISA in sheeps of Chatra Farm
The sex and weight wise variation in the seroprevalence of *Toxoplasma gondii* with rSAG1 antigen based indirect ELISA in sheep of Instructional Farm of Small Ruminants, Ranchi

<table>
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<th>No. of samples positive</th>
<th>% positive</th>
<th>Chi-square</th>
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The sex and weight wise variation in the seroprevalence of *Toxoplasma gondii* with whole tachyzoite lysate antigen based indirect ELISA in sheep of Instructional Farm of Small Ruminants, Ranchi

<table>
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<th>Factors</th>
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<th>Chi-square</th>
<th>Degree of freedom DF</th>
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<td></td>
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<td>37</td>
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Fig. 13: Graph showing the sex and weight wise variation in the seroprevalence of *Toxoplasma gondii* based on recombinant SAG1 antigen based indirect ELISA in sheep of Instructional farm of Small Ruminants, Ranchi

Fig. 14: Graph showing the sex and weight wise variation in the seroprevalence of *Toxoplasma gondii* based on whole tachyzoite lysate based indirect ELISA in sheep of Instructional farm of small ruminants, Ranchi
Table 11: The sex and age wise variation in the seroprevalence of \textit{Toxoplasma gondii} with rSAG1 antigen based indirect ELISA in sheep of villages

<table>
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<th>Factors</th>
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<th>No. of samples examined</th>
<th>No. of samples positive</th>
<th>% positive</th>
<th>Chi-square</th>
<th>Degree of freedom DF</th>
<th>P value</th>
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<tbody>
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<td>Age</td>
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<td>16.67</td>
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<td>0.0001</td>
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<tr>
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<td>43</td>
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<td></td>
<td>&gt;24 months</td>
<td>57</td>
<td>26</td>
<td>45.61</td>
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Table 12: The sex and age wise variation in the seroprevalence of \textit{Toxoplasma gondii} with whole tachyzoite lysate antigen based indirect ELISA in sheep of villages

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<th>Factors</th>
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<th>Chi-square</th>
<th>Degree of freedom DF</th>
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<td>68</td>
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<tr>
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<td>8.33</td>
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<td>13-24 month</td>
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<td>13</td>
<td>30.23</td>
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<td></td>
<td></td>
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<tr>
<td></td>
<td>&gt;24 months</td>
<td>57</td>
<td>18</td>
<td>31.57</td>
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Fig. 15: Graph showing the sex and age variation in the seroprevalence of *Toxoplasma gondii* based on recombinant SAG1 antigen based indirect ELISA in sheep of villages.

Fig. 16: Graph showing the sex, age wise variation in the seroprevalence of *Toxoplasma gondii* based on whole tachyzoites lysate antigen based indirect ELISA in sheep of villages.
Table 13: The sex, breed, farm, farm/village and altitude wise variation in the seroprevalence of *Toxoplasma gondii* with recombinant SAG1 antigen based indirect ELISA in sheep

<table>
<thead>
<tr>
<th>Factors</th>
<th>Category</th>
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<th>No. of samples positive</th>
<th>% positive</th>
<th>Chi-square</th>
<th>Degree of freedom</th>
<th>P value</th>
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<tr>
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<td>Chotanagpuri</td>
<td>260</td>
<td>108</td>
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<td>Villages</td>
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<td>44</td>
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<td>Altitude</td>
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<td>Ranchi</td>
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Table 14: The sex, breed, farm, farm/villages and altitude wise variation in the seroprevalence of *Toxoplasma gondii* with whole tachyzoite lysate antigen based indirect ELISA in sheep

<table>
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<tr>
<th>Factors</th>
<th>Category</th>
<th>No. of samples examined</th>
<th>No. of samples Positive</th>
<th>% positive</th>
<th>Chi-square</th>
<th>Degree of freedom</th>
<th>P value</th>
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<td>Chotanagpuri</td>
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<td>34</td>
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<td>Ranchi</td>
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</table>
Fig. 17: Graph showing the overall sex, breed, farm, farm/village, altitude wise variation in the seroprevalence of *Toxoplasma gondii* based on recombinant SAG1 antigen based indirect ELISA in sheep.

Fig. 18: Graph showing the overall sex, breed, farm, farm/village, altitude wise variation in the seroprevalence of *Toxoplasma gondii* based on whole tachyzoite lysate antigen based indirect ELISA in sheep.
Table: 15  The sensitivity and specificity of rSAG1 ELISA in comparison to Indirect Fluorescent Antibody Test for the serodetection of Toxoplasmosis in sheep

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<thead>
<tr>
<th>IFAT</th>
<th>Positive</th>
<th>Negative</th>
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</thead>
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<tr>
<td></td>
<td>Negative</td>
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<td></td>
<td>Sensitivity</td>
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</table>

Table: 16  The sensitivity and specificity of WTLA ELISA in comparison to Indirect Fluorescent Antibody Test for the serodetection of Toxoplasmosis in sheep

<table>
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</thead>
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<td>ELISA with WTLA</td>
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</tr>
<tr>
<td></td>
<td>Negative</td>
<td>52</td>
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<tr>
<td></td>
<td>Sensitivity</td>
<td>71.1 %</td>
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Fig. 19: Scatter chart showing the $OD_{492}$ values of goat samples collected from farm and villages by indirect ELISA using recombinant SAG1 as detection antigen

Fig. 20: Scatter chart showing the $OD_{492}$ values of goat samples collected from farm and villages by indirect ELISA using whole tachyzoite lysate antigen as detection antigen
Table 17: The sex and age wise variation in the seroprevalence of *Toxoplasma gondii* with rSAG1 antigen based indirect ELISA in goats of Chatra farm

<table>
<thead>
<tr>
<th>Factors</th>
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<th>No. of Positive samples</th>
<th>% positive</th>
<th>Chi-square</th>
<th>Degree of freedom</th>
<th>P value</th>
</tr>
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<tr>
<td>Sex</td>
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<td>0.1747</td>
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<tr>
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<td>Female</td>
<td>108</td>
<td>49</td>
<td>45.37</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age wise</td>
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<td>34.61</td>
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<td>0.05</td>
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<td>13-24 month</td>
<td>59</td>
<td>25</td>
<td>42.37</td>
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<tr>
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<td>35</td>
<td>20</td>
<td>57.14</td>
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Table 18: The sex and age wise variation in the seroprevalence of *Toxoplasma gondii* with whole tachyzoites lysate antigen based indirect ELISA in goats of Chatra farm

<table>
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<tr>
<th>Factors</th>
<th>Category</th>
<th>No. of samples examined</th>
<th>No. of Positive samples</th>
<th>% positive</th>
<th>Chi-square</th>
<th>Degree of freedom</th>
<th>P value</th>
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<td>3</td>
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<td>13-24 month</td>
<td>59</td>
<td>17</td>
<td>28.81</td>
<td></td>
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</tr>
<tr>
<td></td>
<td>&gt;24 months</td>
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<td>18</td>
<td>51.42</td>
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Fig. 21:  Graph showing the sex and age wise variation in the seroprevalence of *Toxoplasma gondii* based on rSAG1 antigen based indirect ELISA in goat of Chatra Farm

Fig. 22:  Graph showing the sex and age seroprevalence of *Toxoplasma gondii* based on whole tachyzoite lysate antigen based indirect ELISA in goats of Chatra farm
Table 19: The sex and weight wise variation in the seroprevalence of *Toxoplasma gondii* with rSAG1 antigen based indirect ELISA in goats of Instructional Farm of Small Ruminants, Ranchi

<table>
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<th>Factors</th>
<th>Category</th>
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<th>No. of samples Positive</th>
<th>% positive</th>
<th>Chi-square</th>
<th>Degree of freedom DF</th>
<th>P value</th>
</tr>
</thead>
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<td>33.33</td>
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<td>0.1161</td>
</tr>
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<td>Female</td>
<td>56</td>
<td>25</td>
<td>44.64</td>
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<td>0.0409</td>
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<td>34.78</td>
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<td>&gt;17.5</td>
<td>37</td>
<td>20</td>
<td>54.05</td>
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Table 20: The sex and weight wise variation in the seroprevalence of *Toxoplasma gondii* with whole tachyzoite lysate antigen based indirect ELISA in goats of Instructional Farm of Small Ruminants, Ranchi

<table>
<thead>
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<th>Factors</th>
<th>Category</th>
<th>No. of samples examined</th>
<th>No. of samples Positive</th>
<th>% Positive</th>
<th>Chi-square</th>
<th>Degree of freedom DF</th>
<th>P value</th>
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<td>Female</td>
<td>56</td>
<td>14</td>
<td>0</td>
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<td>Weight wise (in Kg)</td>
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<td>1</td>
<td>20</td>
<td>8.8425</td>
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<td>12</td>
<td>32.43</td>
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</table>
Fig. 23: Graph showing the sex and weight wise variation in the seroprevalence of *Toxoplasma gondii* based on rSAG1 antigen based indirect ELISA in goats of Instructional farm of small ruminants, Ranchi.

Fig. 24: Graph showing the sex and weight wise variation in the seroprevalence of *Toxoplasma gondii* based on whole tachyzoite lysate antigen based indirect ELISA in goats of Instructional farm of small ruminants, Ranchi.
Table 21: The sex and age wise variation in the seroprevalence of *Toxoplasma gondii* with rSAG1 antigen based indirect ELISA in goats of Villages

<table>
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<th>Factors</th>
<th>Category</th>
<th>No. of samples examined</th>
<th>No of samples Positive</th>
<th>% positive</th>
<th>Chi-square</th>
<th>Degree of freedom DF</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
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<td>129</td>
<td>45</td>
<td>34.88</td>
<td>2.1032</td>
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<tr>
<td></td>
<td>Female</td>
<td>131</td>
<td>63</td>
<td>48.09</td>
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<td></td>
</tr>
<tr>
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<td>9-12 month</td>
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<td>37.31</td>
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<td>0.0409</td>
</tr>
<tr>
<td></td>
<td>13-24 month</td>
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<td>59</td>
<td>45.01</td>
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<td>&gt;24 months</td>
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<td>24</td>
<td>61.54</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 22: The sex and age wise variation in the seroprevalence of *Toxoplasma gondii* with whole tachyzoite lysate antigen based indirect ELISA in goats of villages

<table>
<thead>
<tr>
<th>Factors</th>
<th>Category</th>
<th>No. of samples examined</th>
<th>No. of samples Positive</th>
<th>% positive</th>
<th>Chi-square</th>
<th>Degree of freedom DF</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>Male</td>
<td>129</td>
<td>26</td>
<td>20.16</td>
<td>5.9623</td>
<td>1</td>
<td>0.0146</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>131</td>
<td>51</td>
<td>38.93</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age wise</td>
<td>9-12 month</td>
<td>90</td>
<td>17</td>
<td>22.97</td>
<td>3.3538</td>
<td>2</td>
<td>0.1870</td>
</tr>
<tr>
<td></td>
<td>13-24 month</td>
<td>131</td>
<td>46</td>
<td>35.11</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>&gt;24 months</td>
<td>39</td>
<td>14</td>
<td>35.9</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Fig. 25: Graph showing the sex and age wise variation in the seroprevalence of *Toxoplasma gondii* based on rSAG1 antigen based indirect ELISA in goats of villages.

Fig. 26: Graph showing the sex and age wise variation in the seroprevalence of *Toxoplasma gondii* based on whole tachyzoite lysate antigen based indirect ELISA in goats of villages.
Table 23: The sex, breed, farm, farm/village and altitude wise variation in the seroprevalence of *Toxoplasma gondii* with rSAG1 antigen based indirect ELISA in goats

<table>
<thead>
<tr>
<th>Factors</th>
<th>Category</th>
<th>No. of samples examined</th>
<th>No. of samples positive</th>
<th>% positive</th>
<th>Chi-square</th>
<th>Degree of freedom</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sex wise</strong></td>
<td>Male</td>
<td>150</td>
<td>51</td>
<td>34</td>
<td>46.44</td>
<td>1</td>
<td>0.1654</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>295</td>
<td>137</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Breed wise</strong></td>
<td>Black</td>
<td>325</td>
<td>136</td>
<td>41.84</td>
<td>44.17</td>
<td>1</td>
<td>0.8016</td>
</tr>
<tr>
<td></td>
<td>Bengal</td>
<td>120</td>
<td>53</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Beetal</td>
<td>120</td>
<td>136</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Farm wise</strong></td>
<td>Chakra</td>
<td>120</td>
<td>53</td>
<td>44.17</td>
<td>43.08</td>
<td>1</td>
<td>0.8075</td>
</tr>
<tr>
<td></td>
<td>Ranchi</td>
<td>65</td>
<td>28</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Farm/Village</strong></td>
<td>Farms</td>
<td>185</td>
<td>81</td>
<td>43.78</td>
<td>41.53</td>
<td>1</td>
<td>0.8016</td>
</tr>
<tr>
<td></td>
<td>Villages</td>
<td>260</td>
<td>108</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Altitude</strong></td>
<td>Chakra</td>
<td>120</td>
<td>53</td>
<td>44.17</td>
<td>41.84</td>
<td>1</td>
<td>0.8016</td>
</tr>
<tr>
<td></td>
<td>Ranchi</td>
<td>325</td>
<td>136</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 24: The sex, breed, farm, farm/village and altitude wise variation in the seroprevalence of *Toxoplasma gondii* with whole tachyzoite antigen lysate antigen based indirect ELISA in goats

<table>
<thead>
<tr>
<th>Factors</th>
<th>Category</th>
<th>No. of samples examined</th>
<th>No. of samples positive</th>
<th>% positive</th>
<th>Chi-square</th>
<th>Degree of freedom</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sex wise</strong></td>
<td>Male</td>
<td>150</td>
<td>31</td>
<td>20.67</td>
<td>32.20</td>
<td>1</td>
<td>0.1128</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>295</td>
<td>95</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Breed wise</strong></td>
<td>Black</td>
<td>325</td>
<td>93</td>
<td>28.61</td>
<td>31.67</td>
<td>1</td>
<td>0.6935</td>
</tr>
<tr>
<td></td>
<td>Bengal</td>
<td>120</td>
<td>38</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Beetal</td>
<td>120</td>
<td>38</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Farm wise</strong></td>
<td>Chakra</td>
<td>120</td>
<td>38</td>
<td>31.67</td>
<td>24.61</td>
<td>1</td>
<td>0.3467</td>
</tr>
<tr>
<td></td>
<td>Ranchi</td>
<td>65</td>
<td>16</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Farm/Village</strong></td>
<td>Farms</td>
<td>185</td>
<td>54</td>
<td>29.19</td>
<td>29.61</td>
<td>1</td>
<td>0.9563</td>
</tr>
<tr>
<td></td>
<td>Villages</td>
<td>260</td>
<td>77</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Altitude</strong></td>
<td>Chakra</td>
<td>120</td>
<td>38</td>
<td>31.67</td>
<td>28.61</td>
<td>1</td>
<td>0.6935</td>
</tr>
<tr>
<td></td>
<td>Ranchi</td>
<td>325</td>
<td>93</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Fig. 27:  Graph showing the overall sex, breed, farm, farm/village, altitude wise variation in the seroprevalence of *Toxoplasma gondii* based on recombinant SAG1 antigen based indirect ELISA in goats.

Fig. 28:  Graph showing the overall sex, breed, farm, farm/village, altitude wise variation in the seroprevalence of *Toxoplasma gondii* based on whole tachyzoite lysate antigen based indirect ELISA in goats.
Table: 25  The sensitivity and specificity of rSAG1 ELISA in comparison to Indirect Fluorescent Antibody Test for the serodetection of Toxoplasmosis in goats

<table>
<thead>
<tr>
<th>ELISA with rSAG1</th>
<th>IFAT</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>Positive</td>
<td>164</td>
<td>25</td>
</tr>
<tr>
<td>Negative</td>
<td>13</td>
<td>243</td>
</tr>
</tbody>
</table>

Sensitivity: 92.66 %
Specificity: 90.67 %

Table: 26  The sensitivity and specificity of WTLA ELISA in comparison to Indirect Fluorescent Antibody Test for the serodetection of Toxoplasmosis in goats

<table>
<thead>
<tr>
<th>ELISA with WTLA</th>
<th>IFAT</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>Positive</td>
<td>118</td>
<td>13</td>
</tr>
<tr>
<td>Negative</td>
<td>59</td>
<td>255</td>
</tr>
</tbody>
</table>

Sensitivity: 66.67 %
Specificity: 95.14 %
Fig. 29 Polypeptide profile of whole tachyzoites lysate antigen in 5% - 15% gradient SDS-PAGE.
Lane M: Molecular weight marker (250 KDa to 10 KDa)
Lane 1: Resolved polypeptide bands of whole tachyzoites lysate of *T. gondii* (RH strain)

![Polypeptide profile](image)

Fig. 30 & 31 Immunoblot assay of *T. gondii* tachyzoite proteins against sheep and goat serum collected from field respectively.
Lane M: Molecular weight marker (250 KDa to 10 KDa)
Lane 1 & 2: Reaction of antibodies against resolved polypeptide bands of whole tachyzoites lysate of *T. gondii* (RH strain) in sheep.
Lane 3 & 4: Reaction of antibodies against resolved polypeptide bands of whole tachyzoites lysate of *T. gondii* (RH strain) in goat.

![Immunoblot assay](image)