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TOXOPLASMOSIS IN PUBLIC HEALTH

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INTRODUCTION

Toxoplasmosis is categorized as a parasitic disease, caused by *Toxoplasma gond*ii (*T. gondii*); it infects humans all over the world. Toxoplasmosis spreads through multiple intermediate hosts including, rodents, marine mammals, cattle, birds, goats, sheep, pigs and humans by ingestion of speculated oocytes. Due to the emergence of different ongoing, as well as previously reported, pandemics (human deficiency syndrome virus) which severely damaged the immune system, the risk of *T. gondii* increases in humans and it became a worldwide public health concern (Hooshyar et al. 2007; Gonzalez et al. 2007; Rafique 2017). *T. gondii* remains asymptomatic in healthy persons but can be very severe in immunocompromised population. *T. gondii* is most common zoonotic pathogen/ parasite that can be transferred from animals to humans and cause disease (Hill et al. 2005). *T. gondii* is excreted in cat feces, which had been transferred from other animals in the form of toxoplasma oocysts. *T. gondii* causes huge economic losses in livestock industry through neonatal losses, abortions and stillbirth (Buxton 2000). The global distribution of toxoplasmosis in humans depends on the geographical distribution. *T. gondii* causes the primary infection in pigs, sheep and goats during their pregnancy period and causes abortions, stillbirths or infertility according to the stage of pregnancy. In the case of abortion, a doe or ewe in mid gestation period gives birth to stillborn lamb before the predicted end of pregnancy period.

History

T. gondii was discovered first time in a rodent "Gundi", a small rodent lived in hilly area and mountains in north of African sub-continent. Similar discovery was reported in Sao Paolo, Brazil in rabbits (Splendore 1908). In New York, *T. gondii* was isolated from the tissue of congenitally infected infant's tissue in 1939. Moreover, Sabin and Feldman (1948) created serological test based on the patient's antibodies to alter the staining of Toxoplasma. This test proved more significant in sheep abortion storms during 1957. In 1970s, bone marrow transplant and immune suppressant treatment were on peak, AIDS pandemic in 1980s gave more importance to Toxoplasma (Ferguson 2009).

Toxoplasma Genotypes/Strains

Various strains of Toxoplasma have been reported in literature with passage of time (Keymer 1981; Levine 1985). In the current era with the aid of molecular techniques, Toxoplasma has been separated from protozoan on the basis of morphology and physical characteristics. Overall, it is accepted worldwide that Toxoplasma has only one species, *T. gondii*. Differences can be observed on the basis of pathogenicity in variety of hosts (Dubey 2010). Before the evolution of genetic markers, *T. gondii* was grouped on the basis of virulence in outbred mice. In 1980s-1990s, discoveries in genetics made easier to identify the genetic differences between *T. gondii* isolated from humans and animals (Pfefferkorn and Pfefferkorn 1980; Darde et al. 1988; Tibayrenc et al. 1991; Sibley et al. 1992; Howe and Sibley 1995; Darde 2008). Howe and Sibley (1995) classified the organism on the basis of DNA RFLP (restriction fragment length polymorphisms) into three types (I, II, III) and related them with the virulence in mice. The result showed that type I was 100% virulent and lethal, while type II and type III proved avirulent strains in mice (Howe et al. 1996). The type I and type III *T. gondii* are more frequently prevalent in clinical Toxoplasmosis than type II. The limited molecular and genetic characterization of clinical isolates of Toxoplasmosis shows the suggested results. As concerned to general human population, the genetic analysis and diversity of *T. gondii* is not much reported, with diminutive literature is available about humans. The recent revolutionary change adopted by *T. gondii* is direct oral transmission through recombination among other parasites and discrete clonal lines of parasites. The direct transmission of *T. gondii* aids in very quick and global distribution (Montoya and Liesenfeld 2004).

Epidemiology

Toxoplasmosis is a worldwide disease that is prevalent in both animals and humans. The disease is usually found in the asymptomatic to mild states, but its spread and prevalence depends on the age and lifestyle of the host. Globally, half a billion humans have the *T. gondii* antibodies. The frequency of disease may vary within a country in humans and animals. The variation factors of Toxoplasmosis in a country are still unknown. The severity and transmission of the disease may be depending on factors such as different animal species, physical and environmental factors and cultural habits. According to the CDC (2013), nearly 22.5% population aged ≥12 year in USA has been infected with Toxoplasma. The rate of Toxoplasmosis increased in Europe from 50 to 75%, while

in Asia, Africa and South America the disease rate is high (up to 90%), probably due to the awareness about the disease and improved diagnostic facilities. The rural areas and farms with poor hygiene conditions have Toxoplasmosis as an endemic disease. A seroprevalence based study in USA shows the 10.8 and 11.0% Toxoplasma prevalence in 6-49 years and 15-44 years old persons, respectively. In immunocompetent individuals, Toxoplasmosis infection causes latent chronic infection that is efficiently handled by immune system, while there is high risk of active disease in the babies and immunocompromised or immunosuppressive persons. The immunosuppressive category includes AIDS patients, organ or bone marrow transplant individuals and hematologic malignancy patients (Hodgkin's disease) (Frenkel et al. 1975). The prevalence of Toxoplasmosis manifestation is unusual, with >200 CD_4 cells/ul in patients. However, 30% of AIDS patients without getting any Toxoplasma prophylaxis or antiviral therapy (HAART) showed Toxoplasma encephalitis with <100 CD_4 cells/ul. By implying the prophylaxis measures against Toxoplasmosis, HAART against viral infections and through proper awareness, the occurrence of Toxoplasma and Toxoplasma related encephalitis mortality of Toxoplasmosis was significantly decreased in USA.

Toxoplasmosis can be transmitted in humans and animals through ingestion of oocysts from uncooked food, contaminated hands and infected animals (cats etc.). The oocysts in spores form may be present in soil and can be transmitted to humans while handling cats, gardening, contaminated vegetables, fruit and water (Bahia-Oliveira et al. 2003). Undeniably, in rural areas during pregnancy, impure or contaminated water has been reported as main source of infection (Andiappan et al. 2014). Most of the farm animals including goats, sheep, pigs, lambs, game animals and chickens have been reported as major source of Toxoplasma cysts in USA (Dubey et al. 2005; Dehkordi et al. 2013).

Causative Agent

The causative agent of Toxoplasma is *T. gondii*, which belongs to the protozoan parasite subclass Coccidasina. Usually, the coccidia have complex life cycle. The life cyle of *T. gondii* consists of three stages; tachyzoites, bradyzoites and sporozoites (Fig. 1). The size and shape are similar to red blood cells and crescent shape respectively in tachyzoites stage. The posterior and anterior ends are round and pointed. Various organelles are enclosed in outer covering called pellicle. The bradyzoites are slightly different in shape compared to tachyzoites, as the nucleus in tachyzoites is situated in the center of the cells, while in bradyzoites nucleus is located slightly towards the posterior end. The bradyzoites are more slender than tachyzoites and less affected by proteolytic enzymes. The cysts present inside the tissue in the form of bradyzoites possibly are not virulent and can persist for the long period in the host body (Dubey 2010).

Life cycle

The development of rapidly multiplying tachyzoites and slowly multiplication of bradyzoites occurs in asexual cycle. During acute infection, penetration of tachyzoites causes rupturing of host cells, leading to exposure of tachyzoites into the blood stream. As immunity is developed against tachyzoites, they are retained in tissue and grow into bradyzoites with very slow multiplication rate and maintain the infection in the host. The microscopic cysts can be observed frequently in the skeletal muscles and brain tissues of the host during quiescent stage. Parasites keep their size and shape, but multiplication rate decreases and parasites undergo quiescent phase (Ajioka et al. 2001). The cysts present in meat (muscles) are main cause of infection in humans.

Those animals which fail to survive in acute infection of tachyzoites can be demonstrated in tissue sections of various affected organs, acetic fluid and through lung impression. The sexual cycle of *T. gondii* befall in interepithelial cells of definitive host (feline) that results in production of Toxoplasma oocysts. In cats, primary infection is followed by shedding of oocysts in feces for days. Then the oocysts spread in environment in the form of spores for the next 1-5 days. The sporulation time depends on the environmental factors including humidity, temperature and aeration. These spores (oocysts) are very resistant and remain infective for long time. The size of oocysts (spores) is 11 ×13 um (diameter) and each two sporocysts contain four sporozoites (Dubey and Beattie 1988). Sporulated oocysts penetrates the intestinal lining after the ingestion by the susceptible host (animal), followed by change into tachyzoites and cause infection. These cysts can persist for life time in the body of humans, sheep, goats and pigs after exposure (Dubey & Beattie 1988). Toxoplasma usually does not cause the clinical symptoms in deer, camelids and cattle but causes severe disease in marsupials, new world monkeys and other animals including hares (*Lepus europaeus*; *L. timidus*) (Gustafsson and Uggla, 1994), the Pallas cat (Brown et al. 2005), the arctic fox (Sørensen et al. 2005), some birds and marine mammals (Dubey 2010).

Oral transmission

The causal agent of Toxoplasmosis, *T. gondii,* usually is transmitted through oral rout when tissue cysts or oocysts are eaten accidently (Weiss and Dubey 2009). People living in the countries where undercooked meat-eating habits are common are more prone to Toxoplasmosis because this disease is related to ingestion of raw or under-cooked contaminated meat like pork, poultry, lamb etc. After raw material, contaminated hands with tissue cysts, is the potential factor in the transmission of the causal agent. Contact with contaminated things like knives, utensils or cutting board with raw meat leads to transmission of causal agent from hand to mouth. A study in Europe revealed that 60% transmission of Toxoplasma infection is through meat consumption and only 20% is through contact with contaminated soil. It means that

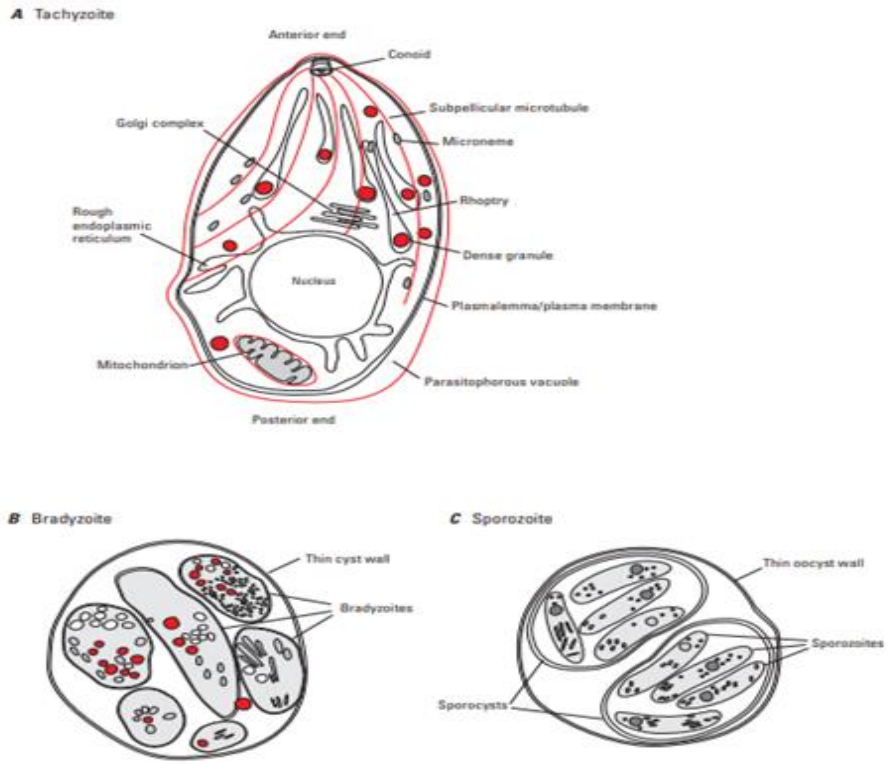

Fig. 1: Infectious stages of *T. gondii*: A, tachyzoite, B, bradyzoite, and C, sporozoite (Ajioka et al. 2001).

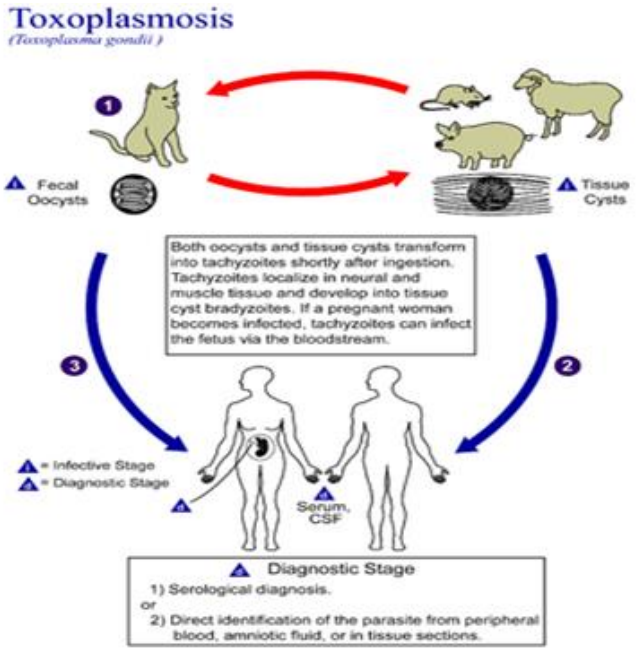

Fig. 2: Modes of transmission of Toxoplasmosis.

gardening related activities are also responsible for *T. gondii* transmission (Cook et al. 2000). Other transmission factors include raw vegetables and fruit contaminated with cat feces (Jones and Dubey 2012). As *T. gondii* is only secreted in cat feces, that's why contamination with cat feces is the only way of transmission. After work in garden or cleaning cat litter box, unwashed hand contact to mouth, can be a potential risk factor for infection. Potential causal agent can survive for months in environment, so direct contact to cat feces or children's sand pits also ultimately leads to oral transmission of *T. gondii* (Dubey 2017). Not only solid food stuff, liquid untreated food stuff is also responsible for the transmission of *T. gondii* like consumption of foods prepared through untreated water, directly drinking unfiltered water, unpasteurized milk and milk products. Eating birds and rodents can cause disease in cats and causal agent is secreted in their feces, which remains for months after disease development. After ingestion of contaminated food by cats, the pathogen shedding starts after third day of infection and may continue for months. Without sporulation, the excreted oocysts are not infectious. After sporulation process, these oocysts becomes potential infectious. Besides mammals and birds, human beings also serve as intermediate host in Toxoplasmosis and are actively involved in the transmission of infection. Transmission and pathogenicity of *T. gondii* is also dependent upon the type of species involved (Assadi-Rad et al. 1995).

Through organ transplantation

Organ transplantation always requires immune suppression of the organ recipient. If the donor of an organ is serum positive for Toxoplasmosis or recently infected, it is quite possible that the disease may be transmitted through transplantation and the recipient is at risk of developing the disease; without screening for Toxoplasmosis the recipient may the develop disease. Similarly, hematogenous stem cells transplantation requires longer period of immuno-suppression, so there are high chances of developing Toxoplasmosis in the recipient without screening.

Risk of Toxoplasmosis is also high in lungs and heart transplantation because of immunosuppression and

striated muscles active response in heart making process. As these muscles contain cysts, so there is high risk of transmission to other organs, cells and tissues (Coster 2013). This risk of disease development can be reduced by screening both donor and recipient for Toxoplasmosis prior to organ transplantation.

Congenital transmission

Toxoplasmosis also has character of vertical transmission from mother to fetus. Mothers infected with this disease during pregnancy can transmit causal agent to their fetuses through placenta. In acute cases, clinical signs may include neural signs, hepatomegaly, lymphadenopathy and interstitial pneuminia. After postmortem examination, enlarged liver, lymph nodes and spleen, are observed and later pale foci have been observed (Coster 2013).

Environmental Factors

Environmental factors also affect the survival, occurrence and transmission of *T. gondii*. Occurrence of *T. gondii* has also been observed in Canadian act that is too extreme low temperature for its survival. As the temperature increases, its survival rate also increases. As precipitation or snow melting process increases, presence of *T. gondii* oocyst also increases. Oocyst migration among birds, insects and rodents can have a huge impact on *T. gondii* distribution, as these animals can serve as vectors and reservoirs. Degradation of natural environment by urbanization also affects the distribution patterns and increases the *T. gondii* transmission.

Clinical signs

The incubation period of Toxoplasma infection is 5 to 23 days. Symptoms includes elevated liver enzyme, lymphocytosis, flue, prolonged fever, lymphadenopathy and weakness. Immunocompetent patients are more prone to this disease and with above symptoms, disseminated disease or chorioretinitis can also occur. Severe illness like pneumonitis, and fatal encephalitis can also develop in immunocompromised patients. Infants with congenital Toxoplasmosis are often asymptomatic, but systematic symptoms, neurological disorder and eye disease may develop. At later stage of life visual impairment, learning disabilities, and cognitive deficits may also develop (Montoya and Liesenfeld 2004). Child hearing problems may also develop in congenital Toxoplasmosis and intellectual disability with sensor neural hearing loss are also reported in 30% newborn children. Fig. 2 illustrates modes of transmission of Toxoplasmosis.

Clinical diagnosis

Human Toxoplasmosis can be diagnosed by serological, molecular or histological methods. These methods are sometimes used in combination to enhance the efficiency of diagnosis (Dubey 2002). As Toxoplasmosis mimics its clinical signs with many other infections; its signs are closely related to central nervous system lymphoma. Diagnosis of Toxoplasmosis cannot be made on the basis of clinical signs and symptoms, because these signs depict symptoms of many other infections. As a result, trial and therapy technique can be used. Folic acid, sulfadiazine and pyrimethamine are used as therapeutic agents.

The causal agent may also be detected in cerebrospinal fluid, amniotic fluid and blood through polymerase chain reaction (Switaj et al. 2005). Sometimes the potential agent hides in the host body and cannot be detected through these tests.

Serological testing

Through serological testing, antibodies against *T. gondii* can be detected in the blood stream. Different methods can be used for antibodies detection like indirect hemagglutination assay, direct hemagglutination assay, Sabin Feldman dye test (DT), indirect immune fluorescent assay (IFA), latex agglutination test (LAT), immunosorbent agglutination assay and enzyme linked immunosorbent assay (ELISA) (Dubey 2002).

In IFA and dye test, color change of tachyzoite is observed under microscope. Agglutination based serological test depends on the agglutination; and agglutination of red blood cells, latext particles and Toxoplasma tachyzoites is observed in indirect hemagglutination test, latex agglutination test and direct agglutination test, respectively. ELISA based diagnosis depends on the change in color of substrates with the degree of availability of antibodies. After infection with Toxoplasmosis, antibodies are developed within one to two week, peak within two months and decline with various rates (Montoya 2002). As IgG antibodies against Toxoplasma generally persist for longer period, so their occurrence always remain persistent in blood stream (Jones et al. 2014). The DT, IFA test, DAT and ELISA are outlined below and the IFA test is given in more detail.

The dye test (Sabin and Feldman 1948) is also called the gold standard test for diagnosis of Toxoplasmosis in humans by detecting antibodies in serum. In this test, the patient's serum is incubated with live Toxoplasma tachyzoites and necessary complement like accessary factors at 37°C for 1 hour. After incubation, methylene blue is added. If antibodies are present in the patient serum, these antibodies enhance the permeability of parasite membrane, the cytoplasm leaks out and tachyzoites do not incorporate the dye, test appears colorless which indicates Toxoplasmosis positive. If the antibodies are not present in the serum, parasite membrane permeability remains intact, and tachyzoites incorporate the dye color. So, the test color appears, showing that the result is Toxoplasmosis negative. This test is only applicable for Toxoplasmosis diagnosis in humans, and not in any other species. This test procedure is potentially hazardous because live pathogen is used. So, high level of care, and technical staff is required to perform this test. It should be noted that for the

production of tachyzoites laboratory cannot be used due to welfare and ethical reasons. Animal cell lines can be used for this purpose.

The IFA test (Munday and Corbould 1971) is used to detect the presence of antibodies against *T. gondii* in the patient's serum. This test is quite simple and widely used for the diagnosis of Toxoplasmosis. This test is performed by mixing killed Toxoplasma tachyzoites with fluorescent antiserum antibodies and diluted test serum, and incubated. After incubation, results are seen under fluorescent microscope. This test is not too expensive, as fluorescent antibodies are available commercially for all animals. Test kits are easily available and inexpensive. Test method is also simple and results can be seen by eyes but availability of fluorescent microscope is necessary. As results can be read by eyes, so variation in results may occur. Sometimes cross reactivity may occur with other factors like rheumatoid factors, so there is difficult to find specific conjugation.

The DAT (Desmonts and Remington 1980) is both specific and sensitive. The process of this test is simple. A U shaped microtiter plate is used for this test. Formalinized Toxoplasma tachyzoites are added to this titer plate and then diluted test sera is applied. Positive test sample will produce agglutination in the well, while negative test shows button formation of precipitated tachyzoites at the bottom of the well. This test is simple and easy to perform but requires high amount of antigen. Test kits are easily available commercially. Sera treatment with mercaptoethanol is compulsory to avoid non-specific antibody false positive result. Dubey and Desmonts (1987) modified the DAT test and named it as modified agglutination test (MAT). This test is very important and extensively used for the diagnosis of *T. gondii* in all animal species. Disadvantage of this test is that it can give false positive result in the early stage of infection or if performed on canine sera. Latex agglutination test is also simple and kits are easily available but this test has low sensitivity as compared to IFA and MAT.

ELISA based serological test is also useful in the diagnosis of Toxoplasmosis infection. The original method of ELISA (Voller et al. 1976) uses antigen prepared from Toxoplasma RH strain tachyzoites, embedded inside the bottom of microtiter plate. Test can be performed by adding test sera in titer well, followed by anti-specie antibodies linked with enzymes like horseradish peroxidase-labelled anti-ovine-IgG. This system changes its color after adding their substrates if conjugates are attached with antibodies. If there are antibodies in the serum, antigen-antibody complex formation occurs, that provides attachment opportunity to conjugate enzymes that ultimately leads to change in substrate color. This color change can be detected through spectrophotometer at the absorbance specific for the substrate used. This test is simple, easy to perform and can be used for large number of samples. ELISA kits are commercially available for Toxoplasmosis parasite diagnosis. But spectrophotometer requirement is mandatory for this test. This test design has advantage that it can be applied for large number of samples.

ELISA modified test named "kinetics ELISA (KELA)" has been developed (Werre et al. 2002). This modified test actually measures the reaction rate of bounded enzymes and substrate that results in change of substrate color. After 45 seconds intervals, three different optical densities are measured and graphically represented in the form of slope. Both tests KELA system and ELISA have high correlation between them and these tests are most important as diagnostic tool.

ELISA affinity and specificity for Toxoplasma has been improved by using recombinant antigen (Johnson and Illana 1991). Specific antigen of Toxoplasma parasite has been developed in sheep (Lekutis et al. 2001) but these tests are not in routine use (Sager et al. 2003; Tenter et al. 1992).

There is an urgent need to distinguish a current or new infection (acute infection) from long term (chronic) infection. Conventional ELISA test may permit the detection and discrimination between chronic and acute infections by identifying Toxoplasma specific IgG, IgM and IgA antibodies. In an assay avidity of IgG antibody for *T. gondii* P30 antigen has been developed in sheep. It was found that avidity increased over the period of time post infection (Sager et al. 2003). IgM antibodies cannot be used for the chronic infection. These antibodies can be used for the detection of chronic infection (Jones et al. 2014).

Congenital diagnosis

Congenital diagnosis of Toxoplasmosis is crucial and can be performed at every developmental stage. In prenatal developmental stage, diagnosis of Toxoplasmosis can be made through ultrasonic examination and amniotic fluid testing. Diagnosis methods at neonatal developmental stage include molecular testing of cord blood and placenta, clinical examination and comparative mother-child serological test at birth. At early childhood, diagnosis based on ophthalmic and neurological examination is better and same is true for serology survey during early years of life (Sterkers et al. 2011). Serological diagnosis at three-week intervals is necessary during pregnancy (Sensini 2006).

Molecular Methods

Diagnosis of Toxoplasmosis can also be made by using different clinical specimens including blood, tissue biopsy, amniotic fluid and cerebrospinal fluid, through using molecular methods. PCR based diagnosis methods have been developed and most affective technique is nested-PCR, followed by PCR product hybridization technique (Lin et al. 2000). The major limitations of these techniques are that they do not provide quantitative data and they are also time-consuming (Lin et al. 2000).

Methods involved in detection of pathogen by gene expression, gene regulation, use real time PCR. In this technique, Taq DNA polymerase activity to cleave 5' nucleotides is used (Lin et al. 2000). During the extension phase of PCR assay, fluorescent labeled probes are cleaved and a second dye 6-carboxy-tetramethyl-rhodamine

quenches the intact probe fluorescence (Lin et al. 2000). During PCR assay, hybridized probe cleavage activity increases the fluorescence proportional to PCR product formed, which can be monitored by a detector (Lin et al. 2000).
Immunostaining can also be used for detection of Toxoplasmosis. Lymph nodes affected by Toxoplasma have characteristic changes, including scattered epithelioid histiocytes, poorly demarcated reactive germinal centers and clusters of monocytoid B cells.

Treatment

Most widely used drugs for the treatment of Toxoplasmosis are pyrimethamine and Sulfadiazine (Chirgwin et al. 2002). These drugs are affective in the acute stage of disease, when organism is multiplying rapidly, but do not eradicate the organism completely from the body. In subclinical cases, these drugs show little affect, but in mice sulfonamides have shown complete eradication of tissue cyst. Other drugs useful in Toxoplasmosis include spiramycine, pyrimethamine, diclazuil, atovaquone and clindamycin.

Vaccines

There is a dire need for an effective vaccine to protect humans and animals from cyst production, especially in cats at different stage of development. It is unfortune that there is no effective and safe vaccine available against Toxoplasma infection. Only live-attenuated Toxoplasma (Toxovax®) vaccine is available for limited use in veterinary with little success (Buxton and Innes, 1995). This vaccine has protected Toxoplasma infected sheep from abortion but is not always safe and effective. Various DNAs form proteins, a virulence factor of parasite, are strong vaccine candidates but have shown no protection or limited protection yet. One-week pregnant rats were given irradiated tachyzoites orally following challenge with organisms, but there was no protection in dams and pups. However, immunization protected the birth rate and litter size (Camossi et al. 2014).

Prevention measures

The causal agent of Toxoplasmosis, *T. gondii,* present in meat, can be killed by soap and water (Dubey and Beattie 1988; Lopez et al. 2000). Washing hands contaminated with *T. gondii* after handling of raw meat is essential to prevent Toxoplasmosis infection. Washing all things that come in contact with raw meat like knives, sink top, cutting boards with soap and water will kill the parasite and prevent Toxoplasmosis.
Extreme heat and cold can also be used to kill organisms in meat. Heating meat at 67°C for 4 minutes kills the tissue cyst in meat (Dubey 2001). Cooling meat to -13°C for 3 days also kills *T. gondii* (Kotula et al. 1991). Exposure to gamma rays to 400 grays is also helpful in killing the *T. gondii* (Dubey and Thayer 1994). It is recommended that heating any animal meat to 67°C will kill the *T. gondii* and tasting should be avoided while cocking. As *T. gondii* presence is associated with contaminated soil, raw meat and cats, so pregnant women should avoid possible exposure to these objects, which will decrease the potential risk of Toxoplasmosis.
Cats play a key role in the transmission of *T. gondii*. Most effective and efficient method to minimize its transmission is adoption of good hygiene practices. Cooked food or dry canned food-based diet will prevent the infection of pet cats and control oocyst secretion. Cleaning cat box and emptying litter box decreases the oocyst load and pregnant women should avoid doing all these functions.
Gardeners should wear gardening gloves that will prevent from direct hand contact with cat faeces buried in soil. Fruit and vegetables washing with clean water will also decrease the risk of Toxoplasmosis.
Awareness development among pregnant women through education will protect them from harmful effects of Toxoplasmosis (Foulon et al. 1994, 2000). There is no environmental monitoring method available that can detect *T. gondii* oocysts in environment. To control the transmission of Toxoplasmosis in animals, preventive measure should be adopted to avoid direct contact of cats with other animals.
On farm, preventive measures include cat population controlling through spying programs. Affective rearing practices for domestic animals such as pigs, like confinement rearing to avoid their contact with cats, will limit the transmission risk among animals. In the same way, pregnant goats and sheep should be confined to limit their contact with cats. *T. gondii* infection in zoo animals can be prevented by housing all the animals separately, especial marsupials, New World monkeys and cats. Spread of infection through captive felid and domestic cats can be controlled by controlling their uncooked meat feeds.
Cleaning and disinfection of cages of cats will decrease the risk of oocyst transmission. Control of feral cats within zoos is also important.
In domestic poultry operations, risk of oocyst prevalence can be controlled by limiting presence of the potential source of infective tachyzoites like rodents, cats and coprophagic arthropods in the vicinity of rearing facility. For disinfection of the rearing facilities, ammonia is used, followed by drying at 55°C (Springer 1991). The combination of disinfection and other preventive measures will also limit the risks like use of serological testing and postmortem lesions analysis to determine the cause of bird death. Avoiding overcrowding is also helpful in limiting the disease transmission (Sanger 1971). *T. gondii* oocysts can resist the harsh environmental conditions and survive for long periods, even years (Dabritz et al. 2006, 2007). Over and uncontrolled population of feral cats in the United States makes the elimination of oocysts from the environment impossible.

REFERENCES

Ajioka JW et al., 2001. *Toxoplasma gondii* genomics: Shedding light on pathogenesis and chemotherapy.

Cambridge University Press, Expert Reviews in Molecular Medicine 3: 1-19.
Andiappan H et al., 2014. Toxoplasma infection in pregnant women: Current status in Songklanagarind hospital, Southern Thailand. Parasites and Vectors 7: 1-7.
Assadi-Rad AM et al., 1995. Risk factors associated with transmission of *Toxoplasma gondii* to sows kept in different management systems in Tennessee. Veterinary Parasitology 57: 289-297.
Bahia-Oliveira LM et al., 2003. Highly endemic, waterborne Toxoplasmosis in north Rio de Janeiro state Brazil. Emerging Infectious Diseases 9: 55-62.
Brown M et al., 2005. Exploring the ecological basis for extreme susceptibility of Pallas' cats (*Otocolobus manul*) to fatal Toxoplasmosis. Journal of Wildlife Diseases 41: 691-700.
Buxton D and Innes EA, 1995. A commercial vaccine for ovine Toxoplasmosis. Parasitology 110: 11-16.
Buxton D, 2000. Toxoplasmosis and neosporosis. In: Diseases of Sheep, Martin W.B. & Aitken I.D. (editors). Blackwell Science, Oxford, UK, pp: 86-94.
Camossi LG et al., 2014. Immunization of Wistar female rats with 255-Gy-irradiated *Toxoplasma gondii*: Preventing parasite load and maternofoetal transmission. Experimental Parasitology 145: 157-163.
Chirgwin K et al., 2002. Randomized phase II trial of atovaquone with pyrimethamine or sulfadiazine for treatment of Toxoplasmic encephalitis in patients with acquired immunodeficiency syndrome: ACTG 237/ANRS 039 Study. Clinical Infectious Diseases 34: 1243-1250.
Cook AJC et al., 2000, Sources of Toxoplasma infection in pregnant women: European multicentre case control study. British Medical Journal 321: 142-147.
Coster LO, 2013. Parasitic infections in solid organ transplant recipients. Infectious Disease Clinics of North America 27: 395-427.
Dabritz HA et al., 2006. Outdoor fecal deposition by free-roaming cats and attitudes of cat owners and nonowners toward stray pets, wildlife, and water pollution. Journal of American Veterinary Medical Association 229: 74-81.
Dabritz HA et al., 2007. Detection of *Toxoplasma gondii*-like oocysts in cat feces and estimates of the environmental oocyst burden. Journal of American Veterinary Medical Association 231: 1676-1684.
Darde ML, 2008. *Toxoplasma gondii*: "New" genotypes and virulence. Parasite—Journal de la Societe Francaise de Parasitologie 15: 366-371.
Darde ML et al., 1988. Isoenzymic characterization of seven strains of *Toxoplasma gondii* by isoelectrofocusing in polyacrylamide gels. American Journal of Tropical and Medical Hygiene 39: 551-558.
Dehkordi FS et al., 2013. Detection of *Toxoplasma gondii* in raw caprine, ovine, buffalo, bovine, and camel milk using cell cultivation, cat bioassay, capture ELISA, and PCR methods in Iran. Foodborne Pathogens Diseases 10: 120-125.
Desmonts G and Remington JS, 1980. Direct agglutination test for diagnosis of Toxoplasma infection: Method for increasing sensitivity and specificity. Journal of Clinical Microbiology 11: 562-568.
Dubey JP and Beattie CP, 1988. Toxoplasmosis of Animals and Man. CRC Press, Boca Raton, Florida, USA.
Dubey JP and Desmonts G, 1987. Serological responses of equids fed *Toxoplasma gondii* oocysts. Equine Veterinary Journal 19: 337-339.
Dubey JP and Thayer DW, 1994. Killing of different strains of *Toxoplasma gondii* tissue cysts by irradiation under defined conditions. Journal of Parasitology 80: 764-767.
Dubey JP, 2001. Oocyst shedding by cats fed isolated bradyzoites and comparison of infectivity of bradyzoites of the VEG strain *Toxoplasma gondii* to cats and mice. Journal of Parasitology 87: 215-219.
Dubey JP, 2002. A review of Toxoplasmosis in wild birds. Veterinary Parasitology 106: 121-153.
Dubey JP, 2010. *Toxoplasma gondii* infections in chickens (*Gallus domesticus*) clinical disease diagnosis and public health significance. Zoonoses and Public Health 57: 60-73.
Dubey JP, 2017. Swine Toxoplasmosis. Veterinary Division, Animal Health Programs. West Edenton Street, Raleigh, NC pp:3-22.
Dubey JP, et al., 2005. Prevalence of viable *Toxoplasma gondii* in beef, chicken, and pork from retail meat stores in the United States: Risk assessment to consumers. Journal of Parasitology 91: 1082-1093.
Ferguson DJ, 2009. *Toxoplasma gondii*: 1908–2008, homage to Nicolle, Manceaux and Splendore". Memórias do Instituto Oswaldo Cruz 104: 133-148.
Foulon W et al., 1994. Evaluation of the possibilities for preventing congenital Toxoplasmosis. American Journal of Perinatology 11: 57-62.
Foulon W et al., 2000. Prevention of congenital Toxoplasmosis: Journal of Perinatal Medicine 28: 337-345.
Frenkel JK et al., 1975. Immunosuppression and Toxoplasmic encephalitis: Clinical and experimental aspects. Human Pathology 6: 97-111.
Gonzalez LE et al., 2007. *Toxoplasma gondii* infection lower anxiety as measured in the plus-maze and social interaction tests in rats: A behavioral analysis. Behavioural Brain Research 177: 70-79.
Gustafsson K and Uggla A, 1994. Serologic survey for *Toxoplasma gondii* infection in the brown hare (*Lepus europaeus* P.) in Sweden. Journal of Wildlife Diseases 30: 201-204.
Hill DE et al., 2005. Biology and epidemiology of *Toxoplasma gondii* in man and animals. Animal Health Research Reviews 6: 41-61.
Hooshyar D et al., 2007. Trends in perimortal conditions and mortality rates among HIV-infected patients. AIDS 21: 2093-2100.
Howe DK and Sibley D, 1995. *Toxoplasma gondii* comprises three clonal lineages: Correlation of parasite genotype with human disease. Journal of Infectious Diseases 172: 1561-1566.

Howe DK et al., 1996. Acute virulence in mice is associated with markers on chromosome VIII in *Toxoplasma gondii*. Infection and Immunity 64: 5193-5198.

Jones JL and Dubey JP, 2012. Foodborne Toxoplasmosis. Clinical Infectious Diseases 55: 845-851.

Jones JL et al., 2014. Neglected parasitic infections in the United States: Toxoplasmosis. American Journal of Tropical Medicine and Hygiene 90: 794-799.

Keymer IF, 1981. Protozoa. In: Cooper JE and Jackson OF (editors), Diseases of the Reptilia (Volume I). Academic Press, London, UK; pp: 235-290.

Kotula AW et al., 1991. Effect of freezing on infectivity of *Toxoplasma gondii* tissue cysts in pork. Journal of Food Protection 54: 687-690.

Lekutis C et al., 2001. Surface antigens of *Toxoplasma gondii*: Variations on a theme. International Journal of Parasitology 112: 1-10.

Levine ND, 1985. Veterinary Protozoology. Iowa State University Press, Ames, Iowa, USA; pp: 411.

Lin MH et al., 2000. Real-Time PCR for quantitative detection of *Toxoplasma gondii*. Journal of Clinical Microbiology 38: 4121-4125.

Lopez A et al., 2000. Preventing congenital Toxoplasmosis. Morbidity and Mortality Weekly Report 49: 59-75.

Montoya JG and Liesenfeld O, 2004. Toxoplasmosis. The Lancet 363: 1965-1976.

Montoya JG, 2002. Laboratory diagnosis of *Toxoplasma gondii* infections and Toxoplasmosis. The Journal of Infectious Diseases 185: S73-82.

Munday BL and Corbould A, 1971. The application of the Toxoplasma indirect fluorescent-antibody test to sheep sera. Australian Journal of Medical Technology 2: 3-6.

Pfefferkorn LC and Pfefferkorn ER, 1980. *Toxoplasma gondii*; Genetic recombination between drug resistant mutants. Experimental Parasitology 50: 305-316.

Rafique A et al., 2017. Seroprevalence of *Toxoplasma gondii* and its effect of hematological picture in commensal rodents in Faisalabad Pakistan. Pakistan Journal Agricultural Sciences 54: 195-199.

Sabin AB and Feldman HA, 1948. Dyes as microchemical indicators of a new immunity phenomenon affecting a protozoon parasite (Toxoplasma). Science 108: 660-663.

Sager H et al., 2003. Immunodiagnosis of primary *Toxoplasma gondii* infection in sheep by the use of a P30 IgG avidity ELISA. Parasitology Research 91: 171-174.

Sanger VL, 1971. Toxoplasmosis. In: Davis JW, Anderson RC, Karstad L and Trainer DO (editors), Infectious and Parasitic Diseases of Wild Birds. Iowa State University Press, Ames, Iowa, USA; pp: 313-316.

Sensini A, 2006. *Toxoplasma gondii* infection in pregnancy: Opportunities and pitfalls of serological diagnosis. Clinical Microbiology and Infection 12: 504-512.

Sibley LD et al., 1992. Generation of a restriction fragment length polymorphism linkage map for *Toxoplasma gondii*. Genetics 132: 1003-1015.

Sørensen KK, et al. 2005. Acute Toxoplasmosis in three wild arctic foxes (*Alopex alopex*) from Svalbard; one with co-infections of *Salmonella enteritidis* PT1 and *Yersinia pseudotuberculosis* serotype 2b. Research in Veterinary Science 78: 161-167.

Springer WT, 1991. Other blood and tissue protozoa. In: Calnak, B.W. (editor), Diseases of Poultry, 9th Edition). Iowa State University Press, Ames, Iowa, USA; pp: 821-824.

Sterkers Y et al., 2011. Diagnosis of congenital Toxoplasmosis by polymerase chain reaction on neonatal peripheral blood. Diagnostic Microbiology and Infectious Disease 71(2): 174-176.

Switaj K et al., 2005. Recent trends in molecular diagnostics for *Toxoplasma gondii* infections. Clinical Microbiology and Infection 11(3): 170-176.

Tenter AM et al., 1992. Development of ELISAs based on recombinant antigens for the detection of *Toxoplasma gondii*-specific antibodies in sheep and cats. Veterinary Parasitology 43: 189-201.

Tibayrenc M et al., 1991. Are eukaryotic microorganisms clonal or sexual? A population genetics vantage. Proceedings of the National Academy of Sciences of the United States of America 88: 5129-5133.

Voller A et al., 1976. A microplate enzymeimmunoassay for Toxoplasma antibody. Journal of Clinical Pathology 29: 150-153.

Weiss LM and Dubey JP, 2009. Toxoplasmosis: A history of clinical observation. International Journal of Parasitology 39: 895-901.

Werre SR et al., 2002. Evaluation of kinetics and single-read enzyme-linked immunoassays for detection of *Toxoplasma gondii* antibodies in sheep. Journal of Veterinary Diagnosis and Investigation 14: 225-230.