Sveriges lantbruksuniversitet
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Clinical significance of *Toxoplasma gondii* in immunocompetent cats

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CONTENT

SAMMANFATTNING

Toxoplasma gondii är en intracellulär parasit som har kattdjur som sin slutgiltiga värd och alla varmblodiga däggdjur och fåglar som mellanvärd. De flesta infekterade värdarna visar inga symptom, speciellt katter som kan utveckla en långvarig immunitet efter den första infektionen. Klinisk toxoplasmos är sällsynt och förekommer vanligt hos patienter som är immunnedsatta. Detta faktum kan göra att man är mindre uppmärksam på hur *T. gondii* kan påverka katterna kliniskt generellt, särskilt för de som är immunokompetenta. Klinisk toxoplasmos är en allvarlig sjukdom som kan vara dödlig för katter som inte får adekvat behandling i tid då det kan utvecklas aggressivt. Symtomen varierar och är vanligtvis ospecifika; några kliniska fall sammanfattas i denna uppsats. De flesta tester ger inte avgörande resultat för att ställa en slutgiltig diagnos, och veterinärer misstänker inte sjukdomen tillräckligt tidigt för att testa för parasiten. Dessa faktorer utgör en stor utmaning för kliniker att göra en tidig och korrekt diagnos som är nyckeln till behandling.

Nyckelord: *Toxoplasma gondii*, toxoplasmos, katt, kattdjur, immunokompetent

SUMMARY

Toxoplasma gondii is an obligate intracellular parasite, with felines as its definitive host and all warm-blooded mammals and birds as intermediate hosts. It is known that most infected hosts are asymptotic, especially cats are supposed to be able to develop a long-term immunity after first time infection. Clinical toxoplasmosis is rare and occurs in patients that are usually immunosuppressed, this fact can make one be less alert about the significance of how *T. gondii* can potentially affect cats clinically in general, especially for those that are immunocompetent. Clinical toxoplasmosis is a serious disease that can be fatal to cats that do not receive adequate treatment in time as it can progress aggressively. The symptoms vary and are usually nonspecific; a few cases will be summarized in this paper. Most tests, when conducted alone, do not give conclusive information to make a definitive diagnosis. As toxoplasmosis is usually not included in the list of differential diagnosis when it comes to acute cases, because it occurs so rarely, so the running of diagnostic tests is often delayed. These factors pose a great challenge for clinicians to make an early and correct diagnosis which is the key for treatment.

Keyword: *Toxoplasma gondii*, toxoplasmosis, cat, feline, immunocompetent

INTRODUCTION

Toxoplasm gondii, a zoonotic intracellular protozoan, can infect all mammals including human or birds (Taylor *et al.*, 2007). The definitive hosts are cats and other feline mammals in which they reproduce sexually and produce oocysts which are excreted via faeces to the environment. Intermediate hosts are those in which the parasites develop to tissue cysts and await to complete the life cycle when the infected tissue is ingested by their definitive host. Individuals can be infected by ingesting either infectious oocyst or tissue cyst in intermediate host. Infection by oocyst takes only as low as a few sporulated oocysts (Deplazes *et al.*, 2016). Cat can develop immunity for up to 6 years after the oocyst-shedding phase (Cenci-Goga *et al*, 2011).

T. gondii is an interesting parasite because of its ability to infect a wide range of species and it can be found worldwide, affecting 1/3 of the human population (Deplazes *et al.*, 2016), both indicate superior survival ability and ease of transmission.

Even though infection is common in cats (Hartmann *et al*., 2013) it is believed that clinical toxoplasmosis is rare, and it occurs most likely in young or immunosuppressed individuals (Deplazes *et al.* 2016). In this case the affected organs are usually the central nervous system (CNS), muscles, lungs and eyes (Hartmann *et al*., 2013). Rare occurrence of clinical toxoplasmosis on final and intermediate host is another survival advantage of the parasite as sick individual are less likely to help the parasite to complete its life cycle. However, can *T. gondii* have any clinical significance on immunocompetent cats? If so, how are they affected?

The above-mentioned questions will be discussed and the clinical significance of toxoplasmosis in immunocompetent cats will be addressed below, and some cases of clinical toxoplasmosis will also be presented.

MATERIAL AND METHOD

The literature search was mainly done on Primo, the library search tool of the Swedish University of Agricultural Sciences. Key words like “cat”, “toxoplasma gondii”, “toxoplasmosis”, “feline”, “case report”, “vaccine”, “diagnostic methods” were used during article search and preferably if they were included in the titles of the articles. As the number of articles generated by the search is very large so only those with higher relevance were selected. Articles that are peer-reviewed and within 10 years were preferred but if an older article is highly relevant it would still be used and cited. Textbooks from previous immunology, parasitology and virology courses were also cited in this paper.

LITERATURE REVIEW

Background of the parasite

T. gondii has an indirect life cycle, which consists of two parts, the enteroepithelial life cycle and the extraintestinal life cycle (Hartmann *et al.*, 2013).

The enteroepithelial life cycle only happens in their definitive host – felines. When a cat ingests tissue cysts from an intermediate host (e.g. rat), the cysts wall is broken down by the enzyme in the stomach and bradyzoites are released (Weiss & Kim, 2014). The bradyzoites are resistant to gastric juice (Weiss & Kim, 2014) and are able to reach the small intestine where they enter the epithelial cells of the intestine. They then go through merogony and gamogony to form oocyst (Hartmann *et al*., 2013). The oocysts are excreted to the environment via faeces. After being exposed to air and moisture the oocysts sporulate and become infectious (Hartmann *et al*., 2013). Each sporulated oocyst contains two sporocysts and each sporocyst contain four sporozoites (Taylor *et al.,* 2007). Sporulated oocysts can survive in moist soil for 1-1.5 years and cool fresh water for 4.5 years (Deplazes *et al.,* 2016, p.104). "Shedding of oocyst is more common in the latter half of the year in the northern hemisphere" (Hartmann *et al*., 2013, p.632). Cats can shed a large number of oocysts without showing any clinical sign (Dubey & Powell, 2013). As mentioned above, cats develop long-term immunity after the shedding oocysts. Prepatent period lasts for 3-10 days if bradyzoites are ingested while 18 days or more if tachyzoites or sporozoites are ingested (Weiss & Kim, 2014).

Extraintestinal life cycle takes place in all the susceptible species, all mammals and birds, even in felines (Hartmann *et al.,* 2013). When oocysts are ingested, they reach the small intestine and hatches so the sporozoites are released (Hartmann *et al.,* 2013). Sporozoites penetrate and invade intestinal cell and even macrophages (Tizard, 2012), they go asexual reproduction called endodyogeny and transform into tachyzoites. Tachyzoites can multiply in almost any cell and their rapid intracellular replication can cause necrosis of the infected tissues, it can also disseminate via blood and lymph during active infection and invade other cells (Cohen *et al.*, 2016). Eventually they transform into bradyzoites and modifies the membrane of the parasitophorous vacuole to a cyst wall and change the metabolism. Bradyzoites multiply slowly, compared to tachyzoites, by endodyogeny and they encyst. Cyst wall and matrix develop as the tissue cysts mature and they are important for protecting the bradyzoites from the environment and the host immune response by serving as a barrier (Weiss & Kim, 2014). Tissue cysts are most often found in visceral organs, musculature and neural tissue and they can persist in the host for years (Deplazes *et al.,* 2016). When the intermediate host is consumed by the definitive or another intermediate host, the tissue cyst wall will be broken down in the stomach and bradyzoites are released to infect the intestine (Hartmann *et al.,* 2013). In definitive hosts the bradyzoites will then replication sexually as mentioned above in the enteroepithelial cycle and their life cycle is completed. Even cats can be infected by sporulated oocysts and the infection proceed as extraintestinal life cycle, they are more susceptible to tissue cyst (bradyzoites) when they consume an infected prey (Turner *et al.,* 2013).

Immune response to *T. gondii* infection

Upon infection, both innate and adaptive immunity respond to *T. gondii* (Deplazes *et al.,* 2016).

The innate immune response starts when pathogen-associated molecular pattern (PAMPs), in this case, profilin on the protozoa induce effector cell reactions (macrophages, neutrophils and others) by binding to Toll-like receptors (TLR) (Deplazes *et al.*, 2016). Interleukin-12 (IL-12) and interferon-γ (IFN-γ) are the main cytokines involved in the innate response, both cytokines activated NK cells and macrophage to control the parasite. In an activated macrophage the intracellular parasites can be killed by lysosome-vacuole fusion (Tizard, 2012).

According to Tizard (2012), the adaptive immune system responds to protozoa infection with both antibody- and cell-mediated immune response; antibody response targets mainly control parasite in blood and tissue fluid while cell-mediated response works against intracellular parasites. Antibodies can potentially opsonize, agglutinate, immobilize and even inhibit parasite division but they have no impact over the intracellular form of *T. gondii* and therefore the cell-mediated immune response is the more effective response to combat this protozoon (Tizard, 2012). *T. gondii* penetrate cells with a system called "gliding" and they reside in a parasitophorous vacuole, this cell invasion does not trigger normal phagosome formation or maturation, and therefore the parasitophorous vacuole does not fuse with lysosome which makes it possible for *T. gondii* to survive in the cell and replicate without antibodies attacking them (Tizard, 2012). However, cytotoxic T cell can kill tachyzoites and *T. gondii* infected cells (Tizard, 2012).

T. gondii is found to be able to actively manipulate the biology of the hosts cell, especially the intracellular signalling of the immune system. For example, it can interfere with the apoptosis in infected macrophages and it can even block the signalling of IFN-γ and TLR (Weiss & Kim, 2014), which is important for the activation of the cell-mediated immune response as mentioned above. According to Rang *et al.* (2016, p. 658) *"T. gondii* has a highly virulent replicating stages and to ensure its hosts' survival it can stimulate the production of IFN-γ and modulate the cell-mediated response to promote encystment of the parasite in the tissues". Additionally, the immune surveillance in CNS, where the tissue cysts are usually formed, is different from the one present in other tissues (Fabry *et al.* 1994 see Kim & Boothroyd 2005), which is another strategy for the parasite to persist in the hosts for life. Furthermore, bradyzoite-specific surface antigens "appear to be poorly or not at all immunogenic in infection" (Kim & Boothroyd, 2005, p.8039) which allows bradyzoites to persist in their immunocompetent hosts for a long time as they can evade the immune system.

Toxoplasmosis

Toxoplasmosis is the disease caused by *T. gondii.* In a study published by Dubey and Carpenter (1993), clinical data of 100 cats that had histologically verified toxoplasmosis were examined and they found that fever, dyspnoea, polypnea and abdominal discomfort were the most common clinical signs. The age of the cats included in their study ranged from 2 weeks old to 16 years old (mean age 4 years old).

There are some stress conditions that can induce bradyzoite development from tachyzoites and they are temperature stress, pH stress, and certain chemical stress (e.g. IFN-γ and NO)

(Weiss & Kim, 2014). Conversely, the conversion of bradyzoites to tachyzoites can be induced *in vitro* by the removal of stress factor (Deplazes *et al.*, 2016). This finding can lead to the speculation that the absence of stress factor could be contributing to the reactivation of the chronic infection which is dominated by bradyzoites residing in tissue cysts into an active infection which is dominated by tachyzoites (Weiss & Kim, 2014). Immunosuppression is said to be able to induce tissue cyst rupture and thereby the multiplication and dissemination of tachyzoites (Barrs *et al.* 2006). As immune response from the hosts can put the parasite under stress so for those immunodeficient hosts the bradyzoites can probably be more likely to convert to tachyzoites compared to an immunocompetent individual, which leads to active toxoplasmosis. In a study done by Barrs *et al.* (2006), two cases of clinical toxoplasmosis in cats were presented and both cats had been treated with therapeutic doses of cyclosporine (an immunosuppressant medication) prior to the disease. "Reactivation of latent *Toxoplasma gondii* infection secondary to cyclosporine-induced immunosuppression was considered likely in both cases" (Barrs *et al.*, 2006, p. 30).

One can suspect that FIP can increase the risk of clinical toxoplasmosis as FIP virus infection cause T-helper cells depletion due to the virus cytopathic effect and replication in T-helper lymphocytes which in turn causes deterioration of cell-mediated immunity, the main defence again *T. gondii* (Quinn, 2011). However, according to Dubey and Prowell (2013), there are only a few cases of clinical toxoplasmosis in cats with FIV infection.

As mention above in the introduction, cats usually can develop a firm immunity against the parasite and it should be enough to control re-infection and oocyst excretion (Deplazes *et al.*, 2016).

Clinical toxoplasmosis is rare and the reason behind it is not fully understood (Dubey & Prowell, 2013) and "it is unknown whether the severity of toxoplasmosis in immunocompetent hosts is due to the parasite strain, host variability, or to other factors" (Dubey & Prowell, 2013, p.158). Future research will be required to have a deeper understanding of the disease.

Diagnostic methods

There are different kinds of diagnostic methods available for *T. gondii*, including direct (faecal microscopy, molecular based approaches and DNA based approaches; Bajwa *et al.*, 2014) and indirect methods (serology). Faecal microscopy is based on the detection of oocysts in faecal samples of cats; this method is a common practice as it is cheap and does not require advanced equipment but since oocysts of similar size can be detected in cat faeces (i.e. Hammondia) (Daplaze *et al.*, 2016), a molecular typing is required to confirm the diagnosis. Moreover, the oocyst-shedding phase is quite short, and some infected animals could be missed if using only faecal analyses. Examples for DNA based approaches are conventional polymerase chain reaction (PCR) and real time-PCR (Bajwa *et al.*, 2014). "Tissue stages may be detected histologically, by means of immunohistology or DNA analyses in tissues samples taken post mortem" (Deplazes *et al.*, 2016, p.107). Common diagnostic tools are represented by serology which can detect the presence of certain antibodies (e.g. Enzyme-linked

immunosorbent assay and indirect fluorescent antibody test) and stage specific immunoglobulins.

Treatment

According to the Swedish National Veterinary Institute (SVA) (2017) in Sweden, the recommendation of treatment for clinical toxoplasmosis in cat is clindamycin. The second choice of treatment would be a combination of sulphonamide and trimethoprim, but this combination is used when clindamycin gives side effects or insufficient effects or when CNS symptom is observed. The recommended dose of clindamycin is 12.5-2.5mg/kg body weight per oral every 12 hours for 2-4 weeks (Deplazes *et al.*, 2016). However, bradyzoites in the tissue cysts are not affected by the chemotherapeutic agents used for treatment (Weiss & Kim, 2014).

Cases of clinical toxoplasmosis in immunocompetent cats

Case 1 by Lindsay et al. (2010):

The patient was a 10-year-old spayed Cornish Rex, it was vaccinated against feline panleukopenia virus, calicivirus, herpesvirus and feline immunodeficiency virus (FIV) within the past 12 months. It showed neurological symptoms mostly on the right side and they indicate problems with spinal cord segment C6-T2. *T. gondii* titres shows no indication of active toxoplasmosis but previous exposure. At post-mortem examination multifocal areas of necrosis and malacia were found at the cervical intumescence of the spinal cord. Immunohistochemical (IHC) staining confirmed the diagnosis of reactivated toxoplasmosis and it also showed tachyzoites free in the neutral parenchyma. A diagnose of marked segmental non-suppurative myelitis was made. Judging from the result of the examinations, the authors assumed that the CNS inflammation was caused by the reactivation of latent *T. gondii* infection rather than an acute one. In this case the cat had no known underlying cause of immunodeficiency or immunosuppression and it was vaccinated recently before the symptoms showed. It had been previously infected by *T. gondii* some time in her life and it has encyst in the CNS tissue. It has not affected the health of the cat until the reactivation of the infection, and even with intensive treatment no improvement was detected and therefore the cat was euthanised. As mentioned above, the reactivation of infection probably requires the absence of stress factor (e.g. immunity against *T. gondii)* but this case shows that it is possible for an immunocompetent individual to suffer from *T. gondii* infection reactivation.

Case 2 by Nagel et al. (2013)

A 10-year-old spayed, and apparently immunocompetent cat (good body condition and no history of immunosuppressing chemicals) was admitted due to sudden anorexia and polydipsia. She had severe abdominal pain and leukopenia. She was tested FIV, FeLV and FIP (at post-mortem examination) negative. Haematology and serum biochemistry implied severe hepatic injury or hepatitis with sepsis. Even with treatment the cat died 72 hours after admission. Necropsy confirmed severe hepatitis, pneumonia, subacute multifocal pancreatitis, multifocal encephalitis, multifocal myocarditis and mild nephritis with small fluid-filled cysts.

IHC staining confirmed the diagnosis of disseminated toxoplasmosis that bradyzoite-containing cysts and free tachyzoites were found in most tissue. This case shows that not only can *T. gondii* cause clinical diseases in immunocompetent cats, it does not only limit to certain organs (CNS, muscle and lung as mentioned above) but disseminate to other organs and cause inflammations at the same time. The patient was treated with broad spectrum antibiotics amoxycillin-clavulanic acid against infectious hepatitis which was not the most appropriate choice of treatment in this case, this led to the speculation that if the correct diagnosis was made and the right antibiotics was given to the patient it would probably have increased the chance of survival.

Case 3 by Cohen et al. (2016)

22-month-old neutered cat with symptoms of lethargy, vomiting and reduced appetite, FIV and FeLV negative, was admitted. An irregular hyperechoic mass in the abdomen was detected with ultrasound. Although being treated symptomatically and with antibiotics the cat's condition deteriorated and was euthanized. Enlarged mesenteric lymph nodes were seen at the necropsy. Histopathology examination revealed extensive necrosis at the liver and the mesenteric lymph nodes. IHC confirmed the presence of *T. gondii* in the liver so a diagnosis of disseminated extraintestinal toxoplasmosis was made. The patient in this case is significantly younger than the two previous cases presented, it showed common signs of clinical toxoplasmosis, but they are very nonspecific, which makes it difficult to diagnose correctly.

The above presented cases demonstrated that the infected cats are all apparently immunocompetent and their profile varies. Symptom-wise their health was affected in different ways but severely even though they suffered from the same disease and it was fatal. These cases showed the clinical significance of toxoplasmosis in the cat population; and demonstrate the difficulties of making correct diagnosis and giving the appropriate treatment.

DISCUSSION

Clinical toxoplasmosis is rare in cats, let alone it happens on the immunocompetent ones, but when it occurs it can adversely affect the health of the cats and can even be fatal. As *T. gondii* is so widely spread, the clinical importance of the parasite should be addressed which is also the aim of this study.

Challenges of ante mortem diagnosis and clinical significance

According to Cohen *et al.* (2016) ante mortem diagnosis of clinical toxoplasmosis is challenging as clinical signs are usually nonspecific and the disseminated form of the disease can be rapidly progressive which does not allow the clinicians much time. A definitive diagnosis can be achieved by demonstration of *T. gondii* in the tissue associating with the inflammation, however it is usually done by necropsy (Lappin, 1999). It can be achieved ante mortem if bradyzoites or tachyzoite are found in tissues or effusion (Lappin, 1999).

Serological tests could be used to diagnose toxoplasmosis, but antibodies can be found in both healthy and sick cats (Cohen *et al.*, 2016), therefore it is not possible to confirm a diagnosis solely base on these tests (Lappin, 1999). However, if a high IgM antibody titer or a 4-fold change in IgG titer is demonstrated it can lead to a presumptive diagnosis, combining with the presentation of clinical signs a more reliable diagnosis can be made (Cohen *et al.*, 2016, p.485).

Faecal analysis of oocyst is not very accurate as *T. gondii* oocysts have similar appearance as other coccidian species (Cohen *et al.*, 2016). Pneumonia can be an indicator for feline toxoplasmosis as "it was documented in all fatal cases observed in a previous case series" (Cohen *et al.*, 2016, p.483).

The challenge is not only about the inconclusive result yielded from available tests but also the awareness of the disease from the clinicians. As clinical toxoplasmosis is so rare, and the symptoms are so non-specific, it is understandable that a veterinarian would not suspect this disease when a generally affected cat is admitted, and hence the delayed treatment. Clinicians must bear in mind that there is a possibility that toxoplasmosis can be the cause for a generally affected cat when the cat does not respond to treatments given for another disease. Regardless of the immunity competency of the cat, appropriate tests should be conducted as soon as possible for an early diagnosis and appropriate treatment in order to increase the chance of survival.

Prevention and control

As *T. gondii* has a wide range of susceptible hosts it is important to minimize the risk of infection and transmission of the parasite. As cats can shed a large number of oocysts when infected primarily and oocysts can survive in the environment for a long time, it is important for focus on minimizing the infection of cats as to reduce the contamination of the environment by oocysts. Domestic cats should be fed only with cooked or frozen meat, and one should try to prevent them from eating small mammals, which could be difficult if the cats are allowed to roam free outdoor (Deplazes *et al.*, 2016). Cat owner should remove cat faeces from the litter box daily as oocysts require one day to sporulate and become infectious. "None of the commonly employed disinfectants kill *T. gondii* oocyst" (Dubey & Prowell, 2013, p. 159) but they can be killed by being exposed to higher than 70 degrees Celsius. Persons including cat owners, clinicians or anyone who attend to infected cats should take appropriate hygiene measure and be aware of the consequences of this disease (Dubey & Prowell, 2013).

According to Verma and Khanna (2013) the process of developing vaccine against feline shedding of oocyst is still ongoing and live vaccines are mostly used, a few vaccines mentioned in their studies function by either inhibiting the sexual development of *T. gondii* in cats or by making the parasite only develop partially in the intestines of cats. However, these vaccines target on minimizing the oocyst-shedding instead of preventing toxoplasmosis in cats. In a recent study done by Liu *et al.* (2017), they were testing a DNA vaccine with the gene TgSOD from *T. gondii*, and the vaccine triggered a stronger humoral and cellular

immune response in the treatment group compared to the control group. This development could become the foundation of a future vaccine that can prevent toxoplasmosis in cats.

Future studies

Future studies in this subject should focus on the underlying cause of clinical toxoplasmosis, exploring the possibility of a more virulent strain of *T. gondii* by genotyping those that are found on patient died from the disease, and how the immunity competency play a role in the development of diseases in healthy individuals. Easier, faster and more accurate tests and diagnostic method should also be developed to allow clinicians to make correct and early diagnosis and give appropriate treatment. An effective and economic vaccine targeting infection in cats should also be developed.

Conclusion

Toxoplasmosis is a serious disease for any mammals and cats and it is not only limited to immunodeficient individuals. Cats, being the definitive host of the parasite, are regarded as low risk individuals for the disease as they are supposed to be able to develop a long-term immunity against the parasite. However, they are just as susceptible as any other potential hosts. This study has addressed the clinical significance of toxoplasmosis in immunocompetent cats and the difficulties of diagnosis. Hopefully this will raise awareness and encourage future research on this subject.

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