

Neosporosis in Thailand: Epidemiology, Risk factors and Diagnostic tools

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Abstract

Neosporosis is one of the most common cause serious diseases in cattle and dogs worldwide, especially causes of bovine abortion. The horizontal route of transmission from the definitive host occurs via ingestion of oocysts contaminating the environment and/or tachyzoites and cysts in the carcass, placental tissues of the infected hosts whereas, the vertical transmission happens when pregnant female whose tissue cysts are likely to be activated during gestation. The present review is a comprehensive and up to date the situation of neosporosis occurrence in Thailand, the epidemiology of disease and their associated risk factors. The application of variety laboratory assays used in neosporosis diagnosis is also discussed.

Keywords: Neosporosis, epidemiology, risk factors, diagnostics assays, Thailand

โรคนี้โอสปอโรซิสในประเทศไทย: วิทยาการระบาด ปัจจัยที่เกี่ยวข้องกับการเกิดโรคและการวินิจฉัยโรค

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บทคัดย่อ

โรคนี้โอสปอโรซิสเป็นหนึ่งในโรคที่สำคัญก่อให้เกิดการแท้งลูกในวัวและสุนัขทั่วโลกโดยเฉพาะการแท้งในวัวซึ่งเป็นสัตว์เศรษฐกิจ การติดเชื้อที่ก่อให้เกิดโรคนี้สามารถเกิดขึ้นได้สองแบบคือติดต่อโดยการได้รับอาหารหรือน้ำที่ปนเปื้อนโอโอซิสต์ของเชื้อที่โฮสต์จำเพาะขับถ่ายออกมาปะปนในสิ่งแวดล้อมและ/หรือเกิดจากการกินซากสัตว์หรือรกสัตว์จากโฮสต์ที่ติดเชื้อก่อโรค (horizontal transmission) และอีกทางติดต่อโดยผ่านทางแม่สู่ลูก (vertical transmission) เมื่อแม่มีการติดเชื้อก่อโรค รายงานนี้มีเนื้อหาครอบคลุมสถานการณ์การเกิดโรคนี้โอสปอโรซิสในประเทศไทย ระบาดวิทยา ปัจจัยที่ก่อให้เกิดโรค พร้อมทั้งกล่าวถึงวิธีการตรวจวินิจฉัยต่างๆ ที่สำคัญและสามารถนำมาประยุกต์ใช้ในการตรวจหาโรคนี้

คำสำคัญ: โรคนี้โอสปอโรซิส ระบาดวิทยา ปัจจัยเสี่ยง การตรวจวินิจฉัย ประเทศไทย

Introduction

Neosporosis is the disease caused by the protozoan *Neospora caninum*. The pathogen is first recognized in 1984 from six dogs with encephalomyelitis in Norway (Bjerkås et al., 1984). Neosporosis has emerged as a serious problem disease in cattle and dogs worldwide. Signs of neosporosis are similar to the parasitic disease toxoplasmosis, which is caused by the protozoan parasite *Toxoplasma gondii*. However, toxoplasmosis is a major disease of sheep and humans, whereas neosporosis is a major disease in cattle and there is no evidence for human infection (Dubey, 2003; Innes et al., 2007). Cattle are the principle intermediate hosts of this agent. The infection leads to abortion, stillbirths, the birth of weak calves and reduced milk production (Dubey 2003; Dubey and Schares, 2011). Apart from bovine animals, neosporosis has also been reported in sheep (González-Warleta et al., 2014), goats (Nunes et al., 2017), axis deer (Basso et al., 2014), rhinoceros (Sommanustweechai et al., 2010), and horses (Finno et al., 2007).

In Thailand, the prevalence of *N. caninum* infection was estimated to be 20.58% in the animal hosts (Table 1). Furthermore, the previous studies have been showed that the most prevalent of the infection was found in dairy and beef cattle. In this paper is review of knowledge on *N. caninum* with an emphasis on life cycle, transmission, clinical manifestation, epidemiology and risk factors. In order to detection of neosporosis, the current diagnostic assays are also focused in this review.

Life cycle and transmission

The life cycle of *N. caninum* resembles the cycle of heteroxenous coccidia, circulating between the definitive carnivorous host and the intermediate hosts consumed by carnivorous hosts. The dog and related

canid can be both the definitive and intermediate host of *N. caninum* and have suffer simultaneously from neosporosis (McAllister et al., 1998). The intermediate hosts include several of animals such as cow (Dijkstra et al., 2003; Udonsom et al., 2018), sheep (Koyama et al., 2001), water buffalo (Rodrigues et al., 2004), horses (Hosseini et al., 2011; AL Anazi and Mohamed 2013) and white-tailed deer (Vianna et al., 2005).

The life cycle is characterized by three infectious stages namely tachyzoite, tissue cyst and oocyst (Dubey et al., 2002). The tachyzoite and tissue cyst (asexual cycle) are found in the intermediate hosts and they multiply intracellularly (Dubey et al., 2002). Tachyzoites are oval or globular (measuring 3-7 x 1-5 μm) and can be found in many types of cells such as neurons, macrophages, fibroblasts, vascular endothelial cells and monocyte in many muscle groups (Speer et al., 1999). The tissue cysts primarily occur in the central nervous system (CNS) and the cysts are round or oval in shape (Dubey et al., 1988a; Dubey et al., 1990). The sexual cycle is found in definitive hosts which is gametogony leading to the formation of oocysts in the intestinal lumen. *N. caninum* unsporulated oocysts are spherical to subspherical oocysts and the oocysts measure approximately 10 μm in diameter (Lindsay et al., 1999). Oocysts sporulated within 3 days and contained two sporocysts, each with four sporozoites (McAllister et al., 1998).

The mode of transmission of the parasite is divided into 2 parts, horizontal and vertical transmissions. The horizontal transmission is occurred by the definitive host contaminating the environment of the intermediate hosts (cattle or other mammals) with infective oocysts (Davison et al., 1999). The vertical transmission happens when pregnant female whose tissue cysts are likely to be activated during gestation, releasing tachyzoites, which

Table 1. Prevalence of *N. caninum* infection among animals in Thailand

Provinces	Type of animals	No. tested	No. positive (%)	Test	References
Kanchanaburi	Beef cattle	98	20(8)	iELISA	Udonsom et al., 2018
Ratchaburi		89	11(4.4)		
Nakorn Patom		63	21(8.4)		
Nan, Chantaburi, Chiang Rai, Kanchanaburi, Songkla	Small mammals (Redentia, Erinaceomorpha)	192	43(22)	PCR	Japa et al., 2018
Lopburi, Nakorn Pathom, Ratchaburi, Sa Kaeo	Dogs (blood) (faecal)	396 115	15(3.8) 9(7.8)	LAMP	Mahittikorn et al., 2017
Ubon Ratchathani Si sa ket Surin Buri Ram Roi Et Sakon Nakhon	Buffaloes	138 61 73 70 81 205	23(16.7) 9(14.8) 7(9.6) 6(8.6) 4(4.9) 8(3.9)	IFAT	Kengradomkij et al., 2015
Chiang Rai	Dairy cattle	392	163(41.6) 117(29.8)	ELISA IFAT	Inpankaew et al., 2014
Chiang Mai		150	74(49.3) 55(36.7)	ELISA IFAT	
Lumpang		100	64(64.0) 48(48.0)	ELISA IFAT	
Kanchanaburi Nakhon Pathom Rachaburi	Dairy cattle	300	29(9.7)	cELISA	Arunvipas et al., 2012
Kanchanaburi Nakhon Pathom Rachaburi	Beef cattle	389	23(5.9)	IFAT	Wiengcharoen et al., 2012a
Kanchanaburi	Elephant	115	38(33.0)	cELISA	Wiengcharoen et al., 2012b
Khon Kaen Maha Sarakham Nong Khai Sakhon Nakhon Udon Thani	Swamp buffalo	187 60 31 78 176	12(6.4) 3(5.0) 0(0.0) 4(5.1) 5(2.8)	ELISA	Nam et al., 2012

Table 1. Prevalence of *N. caninum* infection among animals in Thailand (ประเทศไทย)

Provinces	Type of animals	No. tested	No. positive (%)	Test	References
Khao Kheow open zoo	White Rhinoceros	1	1(100)	IHC&PCR	Sommanustweechai et al., 2010
Khon Kaen Udon Thani Sakhon Nakhon	Dairy cattle	233 121 91	30(12.9) 11(9.1) 11(12.0)	cELISA	Jittapalapong et al., 2008
Khon Kaen	Dairy cattle (sera)	385	50(13)	ELISA	Chanlun et al., 2007
Khon Kaen	Dairy cattle (bulk milk)	418	270(64.6)	ELISA	Chanlun et al., 2006
Nakhon Pathom	Dairy cattle Dogs	549 82	30(5.5) 1(1.2)	cELISA	Kyaw et al., 2004
Loei Nong Bua Lamphu	Dairy cattle	43 40	27(62.5) 28(70)	IFAT	Kashiwazaki et al., 2001
Bangkok Lopburi Nakhon Pathom Nakhon Ratchasima Nonthaburi Pathumthani Phetchaburi Ratchaburi Saraburi Singburi Suphanburi	Dairy cattle	182 37 58 32 24 53 14 32 284 24 164	9 (4.9) 1(2.7) 4 (6.9) 1(3.1) 0 2 (3.8) 0 4 (12.5) 18 (6.3) 0 15 (9.1)	IFAT	Suteeraparp et al., 1999

can vertically infect the fetus, in the majority of species, especially cattle (Davison et al., 1999).

Clinical signs

Infection by *N. caninum* can cause clinical disease neosporosis which most notably presents as abortion in cattle throughout the world (Larson et al., 2004). The majority of fetuses in seropositive dams will be infected via transplacental transmission (Barr et al., 1993; Hietala and Thurmond, 1999; Schares et al., 1998). Cattle may abort from 3 months gestation to term (Dubey et al., 2006a, 2007). The most of aborted fetuses are predominately autolyzed (Dubey et al., 2006a). In some cases, neurological changes may observe such as hydrocephalus, cerebellar and medulla hypoplasia, and scattered pale to dark foci in the brain, spinal cord, heart, and skeletal muscle that correlate histologically with areas of necrosis and inflammation (Dubey, 2003; Dubey et al., 2006b; Schlafer and Miller, 2007). In the infected calves, congenital infection may show small size for gestational age, ataxia, inability to stand, hyperextension or flexion of forelimbs and/or hindlimbs (Dubey, 2003)

In dog, *N. caninum* infection predominantly affects in young dogs, the majority are less than 6 months old (Reichel et al., 2007). This transmission occurs by mother to fetuses during successive gestations (Cavalcante et al., 2012). However, not all puppies in a litter are affected and present with the disease (Knowler and Wheeler, 1995). The infected puppies clinically present with progressive paresis of posterior limbs which are often hyperextended and rigid (Crookshanks et al., 2007). This is the most common presentation in the infected dogs. Without treatment, the disease progress with increasing paralysis of thoracic limbs, apparent cervical weakness or cervical rigidity and signs of encephalitis

such as dysphagia, abnormal food prehension, musculoskeletal disorders, resulting in death in the majority cases (Ruehlmann et al., 1995). Some dogs show with signs of septicemia including pneumonia, encephalitis, myocarditis and adenomegaly (Barber and Trees, 1996).

Epidemiology

The prevalence of *N. caninum* infections have been reported worldwide (Dubey et al., 2007). In Thailand, the first report of *N. caninum* infection was found in dairy cattle, which showed a significant associated of bovine abortion (Suteeraparp et al., 1999). Kyaw et al., (2003) also found *N. caninum* in dairy cattle farms from Chonburi and Saraburi Provinces which having high abortion rate and first indicated the presence of *N. caninum* tachyzoites in placenta of aborted cows. During the last decade, serological tests have been carried out on various animals in Thailand. The most epidemiological studies of *N. caninum* infection are focused on dairy cattle and buffalo from North and Northeast Thailand (Table 1). The highest percentage of *N. caninum* infection was found among dairy cattle population in the North and the lowest prevalence was found in Swamp buffalo in the Northeast of Thailand. Abortion associated with *N. caninum* infection is indicated as a health problem for Thai cows, however other domestic animals such as goat and sheep are affected and demonstrated clinically (Dubey et al., 2007). Therefore, further epidemiological studies on the other animals in different areas in Thailand should be undertaken.

Risk factors associated with *N. caninum* infection

Risk factors associated with the *N. caninum* infection have been assessed in the individual animals

or herds in Thailand (Kengradomkij et al., 2015; Arunvipas et al., 2012; Jittapalaphong et al., 2008). Age of animal has been considered as the as a risk of *N. caninum* infection among cattle and buffalo (Dyer et al., 2000; Sanderson et al., 2000). A study of Kengradomkit et al (2015) reported the risk of exposure to *N. caninum* in Thai buffalo appeared to increase with age. This finding was hypothesized that the age effect might be influenced by variations in the probability of horizontal transmission from definitive canine to intermediated buffalo hosts in enzootic regions (Landmann et al., 2011). The presence of dogs in herd was a risk factor for *N. caninum* infection (BedoyaLlano et al., 2018; Wang et al., 2018a). In Thailand, dogs can be found roaming around dairy and beef cattle farms and have the close contact with the animals. This finding was also observed by Inpankaew et al (2014) who reported that the high seroprevalence of *N. caninum* in dairy cattle was associated with the presence of farm dogs in herds. In addition to farm dogs, dogs kept in the neighborhood of farms may pose an infection risk. Other potential risks associated with the infection have been reported such as the presence on the farm of cat, duck and rabbits (Otranto et al., 2003), source of drinking water (Ould-Amrouche et al., 1999), grazing and fodder (Barling et al., 2001). Recently, Wang et al (2018b) have been reported *N. caninum* infection in bat from China. This finding could be implied that bat serve as an intermediate host of the agent.

Diagnostic tools for neosporosis

Clinical signs associated with *N. caninum* infection are often nonspecific. Therefore, sensitive detection is diagnostically important for veterinarians and epidemiologically in defining the prevalence of the infection in animal farms. The diagnosis in the biological

specimens can be performed by histological, biological, serological and molecular methods, or by a combination of these methods to confirm the infection.

Histological methods

Although *N. caninum* infection can cause lesions in several organs, fetal brain is the most consistently affected tissue. The most characteristic lesion is focal encephalitis characterized by necrosis and nonsuppurative inflammation (Dubey, 1999). Other lesions in the brain were often focal to diffuse non-suppurative meningitis, mononuclear cell infiltration around acentral area of necrosis, glial proliferation and calcification (Wouda et al., 1997; Kamali et al., 2014). Diagnosis of the presence of *N. caninum* parasites can be performed by histopathology and immunohistochemistry (IHC) using aborted fetuses, placenta or other suspected hosts tissues (Cabral et al., 2009; Sommanustweechai et al., 2010; Uzêda et al., 2013)

Bioassay

Mouse inoculation and cell culture have been used to obtain viable *N. caninum* infection, however, the isolation in vitro is often unsuccessful. Attempts at isolation of viable *N. caninum* by bioassay in mice or cell culture have been largely unsuccessful because most parasites stages in fetuses die with the host or due to the low numbers and/or viability of parasites and time consuming (Lindsay and Dubey, 1989; Otter et al., 1995; Davison et al., 1999). The factors that can affect the susceptibility of the host to infection with *N. caninum* may include, mouse strain, parasites strain, dose and route of inoculation and immunosuppressive drug treatment (Lindsay et al., 1995; Maley et al., 2003; Macaldowie et al., 2004).

Serology assays

The serological test is the primary diagnostic method used to indicate the *N. caninum* antibodies of infected animals. A variety of the assay for detection of antibodies level including immunofluorescence antibody test (IFAT), enzyme-linked immunosorbent assay (ELISA), agglutination test, immunoblotting or western blot.

Immunofluorescence antibody test (IFAT)

The IFAT was the first serological test used for the demonstration of antibodies to *N. caninum* (Dubey et al., 1988b). It has been regarded as a reference test to which other assays for *N. caninum* antibodies have been compared and calibrated (Björkman and Uggla, 1999). The test is based on affixing of intact *N. caninum* tachyzoites which is incubated with the diluted test serum, then react with the labeling antibodies fluorescent stain, which gives a bright and greenish yellow color of *N. caninum* organism under ultraviolet light. IFAT has been used to detect antibodies from a large number of animal species such as cattle, dogs, sheep and wildlife (Andreotti et al., 2009; Inpankaew et al., 2014; Ghalmi et al., 2014; Waap et al., 2017).

Enzyme-linked immunosorbent assay

The technique based on the principle of the soluble of *N. caninum* antigen is coated on the plastic surface of microtiter plates. After incubation with test sera the antigen antibody reaction, test is enhanced by the addition of secondary enzyme-linked antigen-antibody system, and the reaction can be assessed by quantification of the color that develop. The results are measured the absorbance or optical density by ELISA reader. Recently, the development of *N. caninum* protein targets is useful and

more sensitive and specific such as NcSAG1, NcGRA2 and NcSRS2 (Huang et al., 2007; Dong et al., 2012; Pinheiro et al., 2013), used of recombinant protein indicated the detection of antibodies during acute infection and chronic infection respectively (Aguado-Martinez et al., 2008). ELISA test is very commonly used to measure in many types of animal species, and is now several commercial ELISA tests are available.

Modified agglutination test

This test has been developed and proved as a rapid, highly sensitive, and easy to use for screening a large number of wildlife or domestic animal samples for the presence of *N. caninum* antibodies without requiring special equipment and suitable for clinical or field applications (Packham et al., 1998; Dubey et al., 2002; Ghalmi et al., 2014; Waap et al., 2017). The agglutination tests are based on the principle that intact tachyzoites agglutinate in the presence of specific IgG antibodies, which it does not require species-specific conjugates (Packham et al., 1998; Canada et al., 2004).

Immunoblot

Immunoblot is a method with demonstrated as a high sensitivity and specificity for antigen/antibody detection and is widely used to evaluate serological assays for *N. caninum* infection (Staubli et al., 2006; Hu et al., 2011; Campero et al., 2015). This technique used to detect specific proteins in the given sample based on their ability to bind to specific antibodies or proteins. The method has been evaluated *N. caninum* infection with the cattle, swine, deer and dog's samples (Hu et al., 2011; Anderson et al., 2007; de Azevedo et al., 2010; Ghalmi et al., 2014). The detection of *N. caninum* antibodies has been compared between milk and sera samples using

western blot, milk showed a strongly agreement for detection, easier and less invasive than obtaining bovine sera (Ortega et al., 2006).

Molecular detection

PCR is a very important tool for *N. caninum* detection which provides a high sensitivity and specificity for the detection of target DNA molecule (Tramuta et al., 2011). The principle is based on the amplification of the small amount parasites DNA in biological samples using specific primers. The genomic DNA was extracted from various parts such as aborted fetus tissue, amniotic or cerebrospinal fluid, placenta, blood, feces and milk. Several formats of PCR have been applied to neosporosis diagnosis such as loop-mediated isothermal amplification (LAMP) and real-time PCR (Mahittikorn et al., 2017; Ramos et al., 2017), nested PCR (Wang et al., 2018b), and multiplex PCR (Meseck et al., 2005; Al-Qassab et al., 2010). The most widely used PCR targets for *N. caninum* are Nc-5 repeat sequence gene (Collantes-Fernandez et al., 2002; Ghalmi et al., 2008; Mahittikorn et al., 2017) and the internal transcribed spacer-1 (ITS1) (Holmdahi and Mattsson, 1996; Cabral et al., 2009; Amdouni et al., 2018).

Conclusions

In Thailand, *N. caninum* appears to be prevalent in many species of animals and the most epidemiological studies of *N. caninum* infection are focused on dairy cattle. Seroprevalence of *N. caninum* infection in Thai dairy cattle population is high in the North which the horizontal transmission from definitive canine seems to be the most frequent route of infection. The ability of the detection assays is important for veterinarian when determining the level of infection and exposure to the parasite. Rapidly and accurately diagnostic methods

are required to improve the understanding of the epidemiology of neosporosis in Thailand and will contribute to the design of effective control strategies.

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