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Neosporosis in Farm Animals

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ABSTRACT

Neospora caninum was thought to be the parasitic agent causing bovine abortion and newborn mortality in Norway's dogs as well as other countries. Cyst-producing protozoan parasite; *Neospora caninum,* that have two reproductive stages during its life cycle include asexual stage occurs in a broad intermediate host range includes (cattle, buffalo, sheep and goat) and a sexual stage which occurs in different Canids species is the cause of the disease. It is transferred from the definitive host to the intermediate hosts directly by direct contact with dogs or indirectly through contamination of their feed by dogfeces. Other animals may share in the complete life cycle like birds and rodents. In farm animal, it can spread vertically via transplacental transfer from the dam to the fetus or horizontally by consumption of oocysts that the hosts disseminated with their feces. It causes storm of abortions, stillbirths, infertility, and loss of milk production, which lead to great economic losses. In addition, the *Neospora caninum* antibodies in dogs were detected all over the world. Additionaly *Neospora caninum*is a possible cause of canine neuromuscular paralysis. *N. caninum* in seropositive animals could be detected by numerous techniques including, indirect fluorescent antibody technique, enzyme-linked immunosorbent assay(ELISA), the direct agglutination technique and immunoblotting. Many restrictions are undergone to control neosporosis wide distribution among animals and consequently its economic loses. It has been concluded that the main methods useful for control of *Neospora caninum* include good farm biosecurity, therapy and vaccination. The current review focused on a broad overview of *Neospora caninum* in farm animals.

Keywords: Egypt, Farm Animals and *Neospora caninum.*

INTRODUCTION

Worldwide, neosporosis has become a significant condition affecting cattle and dogs. Clinical cases of neosporosis have also been documented in small ruminants, rhinoceros, horses and deers. *N. caninum* was detected from foxes, felids, camels, and water buffalo sera (Dubey, 2003). Ruminants are susceptible to the intracellular *Neospora caninum*, which can result in stillbirth and abortion (Nie *et al.*, 2018), uncertainty exists over the financial state of neosporosis in small ruminants. Neosporosis has occasionally been cited as the cause of reproductive failures for these animals, including newborn mortality and abortion (Gharekhani *et al.*, 2020).

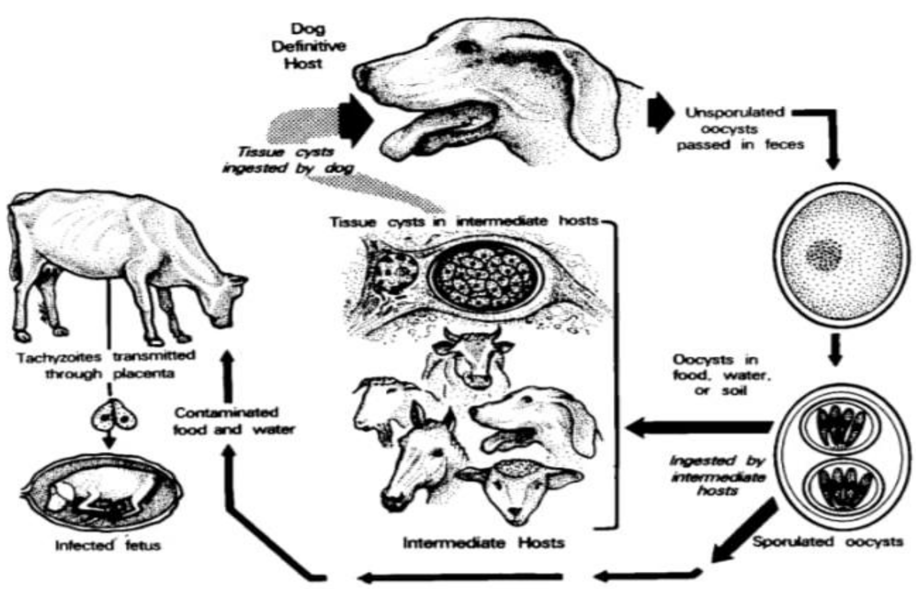
Etiology:

One of the main etiologies why cows and other small ruminants have abortions is neosporosis, which is brought on by infection with the protozoan parasite *N. caninum*. This has an adverse effect on the health of the animals and increases the cost of production (Zhao *et al.*, 2022). Recent research highlighted the significance of *Neospora caninum* as an abortion inducer in sheep and goats, despite that it was one of the primary causes of cattle abortion (Arranz-Solís *et al.*, 2015).

N. caninum lives inside cells that was found in Norway in 1984 (Bjerkas *et al.*, 1984) in forms of encephalomyelitis and myositis, in a Norwegian litter of six congenitally diseased puppies, resulted from an unknown parasite. *N. caninum* was detected from hundreds of tissue samples from deceased pets who died from a *Toxoplasma*-like disease at the Angell Memorial Animal Hospital in Boston, United States, between 1952 and 1987, which showed no immunohistochemical action with anti-*Toxoplasma gondii* antibodies (Dubey *et al.*, 1988).

A polysystemic parasite that has a global prevalence, is *N. caninum* (Protozoa: Apicomplexa). In various mammalian species, *N. caninum* was identified as the etiologies of the frail newborns, stillbirth and abortions (Gharekhani *et al.*, 2020). *N. caninum* has recently been implicated as a significant abortifacient in small ruminants (Moreno *et al.*, 2012) and is considered a common parasitic cause of cattle abortion (Dubey and Schares, 2011). This parasite is a coccidian protozoan (Dubey *et al.*, 2007).

Life cycle:



Tachyzoite, tissue cyst, and oocyst are the three infectious phases of *N. caninum's* life cycle, which mostly affects ruminants and other ungulates serving as intermediate hosts and canines as a definitive host (Dubey *et al.*, 2002).

The heteroxenous life cycle of *N. Caninum* includes two different reproductive forms: asexual reproduction that occurred in intermediate hosts like cattle, small ruminants, and sexual reproduction, that only occurs in canids, the final hosts that infected from eating contaminated meats. The duration of oocysts' persistence in the environment is unknown. They are expelled with canine feces. Intermediate hosts become infected through consuming polluted grass or tissues cysts (postnatal or horizontal transmission). Despite not excreting oocysts, intermediate hosts could still spread the organism via the placenta (vertical transmission) (Manca *et al.*, 2022).

The intermediate host, that can include a variety of domestic and wild animals contracts the parasite either by ingesting infected oocysts along with food and water (horizontal transmission) or by transplacental route (vertical transmission). Oocysts then change into motile, rapidly dividing tachyzoites, which then change into bradyzoites. The life cycle of *Neospora*is ended when the definitive host consumes infected tissue cysts and forms oocysts in its intestine (Dubey *et al.*, 2005 and 2007).

A wide variety of hosts are used by *N. caninum*, and its heteroxenous life cycle includes two different reproductive forms: ① asexual reproduction, thatoccurs in an intermediate host ② sexual reproduction, that takes place in a specific host-like dogs (McAllister *et al.*, 1998). The stages of *N. caninum* development include gametogenic and schizogonic stages. The unsporulated diploid oocyst, which can withstand freezing and drying in its natural habitat, is the sole sexual stage of *N. caninum* that is now understood. Infected canids excrete unsporulated oocysts, which then in the environment, go through meiosis to produce sporozoites (Dubey *et al.*, 2017). After the intermediate hosts consume the sporulated oocysts, the oocysts are ruptured, releasing the sporozoites and develop into the quickly developing stage known as tachyzoites. Tachyzoites then enter host cells and repeatedly endodyogenize within a parasitophorous vacuole to reproduce asexually. Parasites exit the host cell following numerous rounds of endodyogeny (Ojo *et al.*, 2014) for the re-infection of other new cell hosts, subsequently producing innate and adaptive immune responses. Regarding the immunological response of the host, tachyzoites change into bradyzoites that are seen in cystic tissue and are slowly growing inactive stage. Particularly in immunocompromized hosts, cystic tissue can remain in a host cell for a lengthy time and transform into tachyzoites to produce a latent disease (Dubey, 1999).

Only dogs, coyotes, grey wolves and Australian dingoes are now thought to be the parasite's only known final hosts (McAllister *et al.*, 1998 and Gondim *et al.*, 2004).

Epidemiology:

1. ***Distribution all over the world:***

N. caninum caused abortion in does and high mortality rate in newly borns, which were originally documented in the USA (Barr *et al.*, 1992). Neosporosis has also been linked to stillbirth and abortion in dairy does in Costa Rica (Dubey *et al.*, 1996), Brazil (Mesquita *et al.*, 2013), Italy (Eleni *et al.*, 2004). Congenital neosporosis results in neurologic symptoms such as ataxia, vision disturbance, opisthotonos, and weakness in does kids (Varaschin *et al.*, 2012).

For the first time, antibodies against *Toxoplasma gondii* and *N. caninum* were found in the Swiss South American camelids (SAC) in Switzerland (Basso *et al.,* 2020),

Sheep herding has gained popularity in the Mediterranean region because of its elevated production level. Recently, they are threatening because of poor feeding and management practices, stressful circumstances, and decreased resistance to opportunistic diseases like *N. caninum* (Tamponi *et al.*, 2015). Despite the fact that *N. Caninum* has been identified in sheep and goats all around the world(Dahourou *et al.*, 2019 and Villagra-Blanco *et al.*, 2017).

N. caninum positivity rates in Khuzestan province were 10.8% and 32.4% for sheep and goats flocks, respectively. While, the mixed infection of *N. caninum and T. gondii* was 5.4%. (Gharekhani *et al.,* 2018a and b).

Inaresearch study by Nasir *et al.*(2011) in Pakistan, the results indicated the presence of *N. caninum*in buffaloes. Because most animals are in the middle of gestation, which could affect by immune system modifications and parasite transmission, especially during the summer. Neospora-linked with buffalo abortion was initially noted in India, where immunohistochemistry results from tissues from two fetuses were positive (Mahajan *et al.,* 2020).

In central China,the seroprevalence of canines *N. caninum* revealed overall 15% (172/1176) (Wang *et al.*, 2016).

2. Distribution in Egypt:

In Egypt, ovine neosporosis' epidemiological status investigations are rare. Only two studies have been recorded there, and it is still unclear what the present situation is (Selim *et al.,* 2021).

The few information on Egyptian *N. Caninum* revealed thatit is moderately endemic; 3.6% in camels (Hilali *et al.*, 1998), 20.43% in cattle (Dubey *et al.*, 1998), 8.9% in cattle in southern Egypt; the authors noted that cows neosporosis was much more prevalent in Sohag than they were in Qena (Fereig *et al.,* 2016), 68% in buffaloes and 14.75% in chickens in Kafr-elsheikh (Ibrahim, 2013).

Dubey *et al.* (1998) reported that the prevalence of *N. caninum* in the Egyptian delta region is high. *N. Caninum* antibodies were detected in cattle and buffalo at 68% and 20.43%, respectively, in addition, El-Mohamady *et al.* (2022) found that the detected antibodies for *N. caninum*was30.17% from the116investigatedsera of cattle. As a result, among cows with higher abortion rate, they revealed higher seroprevalence rate for *N. caninum* that showed the underutilized part of *N. Caninum* infection as the cause of abortion in Egyptian dairy herds (Gaber *et al.*, 2021).

In a recent study of (Selim *et al.*, 2021), sheep from various regions were tested for *N.caninum* antibodies presence where 8.6% of the samples tested positive that increased noticeably with increasing animal age.

3. Source of infection:

Cattle infection results from consuming water or food contaminated with oocytes deposited in dog feces (Pan *et al.,* 2004 and Olum *et al.,* 2020).

Cattle which were bought with *N. caninum* parasite in postnatal period through the consumption of oocysts secreted by dogs and other canids, as well as transplacentally from the pregnant dam to the fetus following reactivation of a chronic infection during gestation. Fetuses may pass away in the womb following transplacental infection, or they may be born healthy but with congenital infections or clinical neuromuscular symptoms (Dubey *et al.,* 2011).

Neosporosis seroprevalence in dairy cows has been linked to a number of risk factors, the main are the presence and quantity of dogs (Collantes-Fernández *et al.,* 2008 and Ribeiro *et al.,* 2019).

In Egypt, *N. caninum* infection prevalence was (27.6%) as a higher percentage, among stray dogs (El-Ghayash *et al.,* 2003) contributing a significant part in the spread of infection between animals horizontally (Wang *et al.,* 2018) or by a simple route because there is a higher chance of coming into contact with *N. caninum* sporulated oocysts.

Due to the frequent presence of dogs, particularly stray dogs living on farms, it has been established that dogs can put their feces in food that poses serious danger for disease because oocysts of *N. Caninum* spread through dog excrement (Lindsay *et al.,* 1999; Dijkstra *et al.,* 2001b and De Souza *et al.,* 2002).The main channels for horizontal transmission of *N. caninum* are water and oocyst-contaminated food, and they are in charge of allowing infection into dairy cows free of neosporosis (Dubey *et al.,* 2007).

The fact that exposure to dogs was linked to infection in Pakistani buffalo raises the possibility that dogs are responsible for tainted buffalo feed and subsequent horizontal transmission (Nasir *et al.,* 2011).

A potential cause of buffalo and cattle neosporosis was determined to be the presence of rabbits, foxes, rats, poultry and cats (Olmo, 2019). Additionally, feeding on degraded food suppresses the immune system and has been linked to the seroprevalence of neosporosis (Bartels *et al.,* 1999). Additionally, there are other risk factors as handling of miscarried cows and the addition of fresh livestock to the herd (Darío *et al.,* 2013 and Llano *et al.,* 2018).

Farms with poor hygiene practices, aborted cows, and related materials including abortion placentas, aborted fetuses, and uterine secretions enhance the risk of *Neospora* infection because these materials are the main sources of infection, particularly for dogs who consume them. Additionally, keeping the aborted cows on the farms raises the chance of calves testing positive for *N. caninum* due to transplacental infection (Williams *et al.,* 2009, Trees, and Williams, 2005).

4. <u>Susceptible animals:</u>

For the past 30 years, *N. caninum* has gained recognition on a global scale, initially as a disease affecting dogs and later as a significant factor in cattle abortion (Reichel *et al.,* 2020). Neosporosis also causes nervous symptoms in dogs and reproductive failure in cattle, horses and small ruminants (Zhou *et al.,* 2016)

A variety of mammals and birds can contract the obligatory intracellular protozoan known as *Neospora caninum* (Basso, 2014). Cattle, sheep, buffalo and birds are its intermediate hosts, and it only infects dogs as its primary hosts (Dubey and Schares, 2011).

According to breed and sex, there are no appreciable differences in the seroprevalence of *N. caninum* in dogs. The *N. caninum* infection rate in the rural dogs (18%) was greater than in the urban dogs (11%). This intriguing discovery may be explained by feeding habits (such as consuming raw meat that contains parasite cysts).

Farm dogs that had frequent contact with animals that were seropositive and their secretions were at a greater risk of infection. Most reports indicate that stray dogs older than two years old have a significant infection rate. They are more likely to be exposed to *N. caninum* (Hossininejad and Hosseini, 2011).

N. Caninum can be dangerous to cats and is spread transplacentally and postnatally. The presence of cats on farms poses a problem since they play a crucial role in the spread of the disease by consuming diseased rodents (Dubey and Schares, 2011).

Regardingthe results of Fereig *et al.* (2016), there was higher rate of *N. caninum* in small cattle herds (50.2%) with compared to large farms (37.8).

Rodrigues *et al.* (2004) showed that buffalo are *N. caninum* native intermediate hosts. This research represents the first result for the parasite's isolation in buffalo. Additionally, the greater seroprevalence for *N. caninum* was more prevalent in adults compared to calves and young buffalo (Ba˘rbura *et al.,* 2019), which might be explained by the longevity of exposure to oocysts in adults. It was confirmed that elder buffalo (those older than 10) had a higher prevalence, indicating that horizontal transfer affects buffalo more frequently than vertical one (Kengradomkij *et al.,* 2015). Alternatively, previous studies from Brazil (Gennari *et al.,* 2005), Thailand (Nam *et al.,* 2012) and India (Sengupta *et al.,* 2012) could not show any statistical differences among age-related groups.

By analyzing the buffaloes' gender, differences, in investigations from Argentina, *N. caninum* were not found in males or females (Campero *et al.,* 2007), Pakistan (Nasir *et al.,* 2011 and Nasir *et al.,* 2014) and Thailand (Kengradomkij *et al.,* 2015); however, Iranian serological surveys (Hajikolaei *et al.,* 2007) and surveys in Romania (Ba˘rbura *et al.,* 2019) revealed that there was a statistically significant variations for gender in female buffalo with higher prevalence.

Sheep and goats have a high incidence of neosporosis, especially when the animals frequently come to stray dogs. Similar to toxoplasmosis, the condition has occasionally been implicated as a cause of reproductive failures in these animals, including abortion and newborn mortality (Dubey and Schares 2011; Razmi and Naseri 2017)

Regarding the analysis of the sex factor's impact on the seropositive rate, it was evident that it greatly increased in ewes compared to rams (Selim *et al.,* 2021 and Wang *et al.,* 2018) that was possibly linked to various hormone concentrations between ewes and rams (Dubey 2003) and stressful conditions associated with gestation and lactation stages.

One of the intermediary hosts for *N. caninum* is rodents that are important for maintaining the parasite life cycle (Dubey *et al.,* 2007). Rodents are a risk factor for neosporosis in herds and their presence on dairy farms had a positive link with the seroprevalence rate in cows (Gharekhani and Yakhchali 2019). Mexican rock squirrels, house mice and brown rats showed *N. caninum* infection rates of 71%, 77%, and 50%, respectively (Medina-Esparza *et al.,* 2013).

Different species of birds had *Neospora caninum* antibodies, but no active parasites had been proven or isolated by bioassays (Barros *et al.,* 2018).

5. Mode of transmission:

N. caninum spreads both horizontally and vertically in cattle herds. A fetus's placental infection may lead to stillbirth, abortion, or the delivery of an animal with an infection that is not symptomatic (Dubey *et al.,* 2006). Additionally, congenital transmission is crucial to *N. caninum*induced abortion because this parasite can live for years in farms (Dubey, 2003)

The frequency of neosporosis is higher in dogs and cattle than in horses, small ruminants, thus, the livestock must be segregated from dogs because they are key players in the disease's transmission and spread (Zhou *et al.,* 2016). This happens as a result of Egypt's animal husbandry practices, as most farms are placed close to rural settlements where *N.caninum* can thrive, as a result of the large number of dogs that roam around freely and enter the cattle farms and the lack of veterinary care (Gaber *et al.,* 2021)

Ruminants are susceptible to *N. Caninum* which can spread vertically or horizontally or via the pregnant dam to their fetuses through transplacental transmission (Dubey 2010 & Dubey *et al.*, 2017).

There are two ways that cattle might become infected. The first is through ingesting oocysts from infected dogs' faeces, which is thought to be the main method that *N. caninum* enters cattle bodies (Gondim *et al.*, 2002). Transplacental infection of *N. caninum* is a second mode of infection, the pathway through which the parasite spreads from pregnant dams to their fetuses through the placenta, is thought to be the most crucial for maintaining the infection by congenital infection throughout subsequent pregnancies (De Aquino Diniz *et al.*, 2019 and Japa *et al*., 2019).

Heifers born from miscarried cows revealed increased prevalence rate to *N. caninum* and the seropositive rate was at 28% (Gaber *et al.*, 2021). Also, a higher prevalence of congenital transfer of *N. caninum* in seropositive cattle was at 94% (French *et al.*, 1999), 67.53% (Andreotti *et al.*, 2010), 36.8% (Vianna *et al.*, 2008), and 55.5% (Lagomarsino *et al.*, 2019).

Omnivorous and carnivorous can become infected through eating tissues from diseased hosts that include cyst stages (Devine and Dikeman2014).In addition, the parasite can pass across generations of dogs by transplacental transmission (Dubey *et al.*, 1990)

Vertical transmission in buffalo has also been reported, but further research is needed to fully understand its significance for the disease's epidemiology in this species. Additionally, it is necessary to clarify the reproductive significance of neosporosis in buffalo, however it appears to be less significant than in cattle (Reichel *et al.*, 2015).

Cats have been identified as the best intermediate hosts for *N. caninum* and could have a considerable impact on the parasite's epidemiology (Dubey and Schares 2011). Cats on the farm could be infected via eating rat cysts and oocysts from the surroundings (Donahoe *et al.*, 2015).

The function of birds in *N. caninum* life cycle is unknown, however they might aid in the transmission of parasites during sylvatic cycles such as mechanical vectors or intermediate hosts. Bartels *et al.*, (1999) stated that there is a strong correlation between the presence and number of poultry and the rise in seroprevalence *N. caninum* related abortion storms in the farms.

6. ***Economic Importance:***

Enormous financial losses caused by *N. caninum* could be detected in a number of wild and domestic animals. In addition to indirect losses like the cost of reduced milk production, fetal death, body weight retardation, culling and veterinarian costs (Nam *et al.*, 2012a).

Iranian dairy herds suffer enormous financial losses as a result of abortion, culling, and reproductive problems brought on by *N. caninum* infection (Gharekhani *et al.*, 2020).

Neosporosis causes a reproductive failure in sheep, which has a significant economic impact (Moreno, 2012). In addition, it causes encephalitis in dogs, which is accompanied by muscle atrophy and subsequently food regurgitation.

Regarding to Fereig *et al.*, (2016), neosporosis might cause abortion and a high rate of culling due to its ability to produce persistent infections and the ability to cause reproductive losses (Chernick *et al.*, 2018).

Reproductive issues caused by neosporosis in cattle, such as stillbirths, abortions, reduced milk production, early culling and infertility result in enormous economic losses (Reichel *et al.,* 2013).

Neosporosis is a key contributor to dairy herd abortions, which makes it difficult to advance livestock productivity, which is a vital income source for low income nations like Egypt (Semango *et al.*, 2019)

Neosporosis could be a cause of higher economic loss because of the higher services number for seropositive cows per conception (Hall *et al.,* 2005). Additionally, the seropositive cows in that study showed a tendency to have longer days open than their negative counterparts. A positive heifer has 1.8 times more chances of not getting pregnant than a negative one (Munoz-Zanzi *et al.,* 2004).

7. Clinical signs:

One of the most common etiologies of abortion in livestock is *N. caninum*, including cows, water buffaloes and camels (Ciuca *et al.,* 2020). *N. caninum* is of the most important ruminant abortifacients (Selim *et al.,* 2021). *N. Caninum* infection results in infertility in cattle, small ruminants, and horses as well as nervous symptoms in dogs (Zhou *et al.*, 2016)

N. Caninum is a significant contributor to canine nervous symptoms and the abortion or neonatal mortality of other animal species, such as small ruminants, deers and horses (Asadpour *et al.,* 2012)

Congenital neosporosis results in neurologic symptoms such as ataxia, vision disturbance, opisthotonos, and weakness in does kids (Varaschin *et al.,* 2012).

At days 40, 90, or 120 of gestation, 106 tachyzoites of the *N. caninum-Spain*7 strain were intravenously administered to pregnant ewes. All fetuses whom infected during the first term died between days 19 and 21 after infection; their organs primarily had necrotic lesions in the liver and had the highest levels of parasite DNA in the placenta and other internal organs (Arranz-Solís *et al.,* 2015). Fetal death was also discovered in all ewes after infection at day 90, the lesions were primarily inflammatory (34–48 days after infection). The least frequent lesions, as well as the least number of parasites found, were found in fetal livers. All infected ewes at day 120 gave birth to live lambs, 3 of the 9 lambs displayed recumbency and frailty. All lamb except one had *Neospora* DNA (Arranz-Solís *et al.,* 2015)

Does intravenously infected with 1,000,000 tachyzoites of the *N. caninum Spain*7 isolate at 40 (G1), 90 (G2), and 120 (G3) days of gestation were used in an experiment to test the pathogenic effects of *Neospora caninum* infection. All infected does were seropositive when they gave birth or experienced an abortion between 5- and 9-dayspost inoculation (dpi). Between 10 and 21 dpi in G1 (n = 7) and 27 to 35 dpi in G2 (n = 4), there were fetal deaths. Seropositive stillbirths (n = 1) and healthy offspring (n = 2) also occurred in does in group G2. G3 goats (n = 3) produced three weak, three healthy, and three seropositive offspring (Porto *et al.*, 2016).

N. caninum might be a real; cause of abortion in sheep because some Iranian studies reported DNA of *N. caninum* in 9.9% and 8.5% from brain of aborted fetuses (Razmi and Naseri 2017).

Abortions can occur in cattle as early as the third month of pregnancy due to *N. caninum*, but they are most prevalent during the fifth and sixth months (Dubey and Schares 2006).

Neospora caninum's infection results in abortion and loss of fetuses by causing placental destruction or by releasing maternal prostaglandins, which then trigger luteolysis (Dubey *et al.,* 2007 and Olum *et al.*, 2020).

However, little is known about the etiology of the disease in buffalo. *N.*

caninum is one of the leading causes of reproductive failures in cattle. However, only one study has so far documented neosporosis-caused spontaneous abortion in buffalo (Auriemma *et al.,* 2014).

Neosporosis was reported to be related to retained fetal membranes and abortion in buffalo (Ciuca *et al.,* 2020).

Dogs with congenitally infected neosporosis are more likely to show severe neuromuscular symptoms such ascending paralysis and hyper extended hind limbs (Langoni *et al.,* 2012).

Young dogs are most frequently affected by neosporosis caused by transplacental transfer of the organism, and clinically characterized generally by a polymyositis-polyradiculoneuritis syndrome that usually appears between 4 weeks and 6 months of age. Meningoencephalitis typically affects adult dogs, and the reactivation of a subclinical infection over time is most likely what causes the clinical condition. It is also possible to acquire the organism horizontally (Dubey and Lappin, 2012)

Neosporosis is typically thought of as a neuromuscular condition, although it can also affect the myocardial, lungs, liver, eyes and pancreas. This disseminated infection is most common in older dogs and is frequently accompanied by immunosuppression. Skin neosporosis has been classified as a distinct clinical entity and is a more commonly understood form of *N. caninum* infection in both immunocompetent and impaired dogs (Decome *et al.,* 2019). Mann *et al.* (2016) had been hypothesized that age-related immunosuppression might be the cause of the cutaneous type of neosporosis in older dogs.

In naturally infected animals, the clinical features of Neospora infection in cats remain uncertain (Dubey *et al.,* 2007). Immune-competent cats have been reported to have an experimental infection (Hamidinejat *et al.,* 2011a).

*8. **P.M. lesion:***

The hepatic parenchyma of liver lesions contained several foci of coagulative necrosis that were scattered randomly, while placenta from animals infected with *N. caninum* demonstrated multifocal non-purulent necrotic placentitis. In addition, glial foci that are randomly dispersed are seen in multifocal non-purulent necrotizing encephalitis (Arranz-Solís *et al.,* 2015)

In an Italian farm with a history of abortion, the histological examination of the brains of the fetuses of two buffalo revealed *Neospora*-like cysts (Guarino *et al.,* 2000).

Histopathological results of the intravenously injected female buffalo with *N. caninum* after being slaughtered revealed that nine out of ten *N. caninum* tachyzoites exhibit the development of non-suppurative placentitis. Additionally, gliosis and non-suppurative inflammatory infiltration at the pia mater with numerous necrotizing foci at the central nervous system were seen in fetuses with meningoencephalitis. In addition, immunohistochemistry revealed intra-lesional tachyzoites in two fetuses that were linked to focal necrosis and gliosis in the central nervous system in various fetal tissues, such as the lungs, kidneys, heart, liver and striated muscle (Konrad *et al.,* 2012).

Two pregnant buffalo that had been infected with NC-1 tachyzoites showed mild cerebral satellitosis. These animals also had non-suppurative hepatitis with centrilobular macrophage infiltration. Additionally, the brain tissues of these pregnant buffalo'sfetuses showed multifocal necrosis and gliosis linked to *N. caninum* infection, and thefetuses had pericarditis, myocarditis, hepatitis, and nephritis (Chryssafidis *et al.,* 2015).

Severe suppurative meningoencephalitis was associated with neosporosis-affected small ruminants (Sasani *et al.,* 2013). The authors evaluated the glial reactivity in aborted fetal brain tissues with focal or diffuse gliosis in addition to various cellular and vascular lesions.

Diagnosis:

Neosporosis is challenging to detect since there are few parasites present in the infected tissues and the initial clinical signs and symptoms are unclear (Ellis *et al.,* 1999). Additionally, the pathological abnormalities and clinical symptoms are similar to the abnormalities resulted from *T. gondii*. In some studies, *N. caninum* was possible incorrectly recognized as *T. gondii* (González-Warleta *et al.,* 2014). Furthermore, the neosporosis diagnosis is difficult and expensive (Ahmed *et al.,* 2017).

Today, a wide variety of commercially available diagnostic procedures using both established and emerging approaches are available for the identification of ovine neosporosis. Recently, the primary method utilized to identify an ovine abortion caused on by protozoa was the histological examination of fetal tissues (Shaapan, 2016), 13.73% of 51 placenta samples from aborted ewes tested by PCR showed positive results for *N. caninum* DNA (Al-Shaeli *et al.,* 2020).

The primary methods used for the serological diagnosis of neosporosis in buffalo were indirect IFAT and ELISA; however, a *Neospora* agglutination test (NAT) and competitive-inhibition ELISA (cELISA) were also applied. The NAT assay was nearly similar to MAT assay for detecting toxoplasmosis due to its high sensitivity and specificity and lack of need for an anti-IgG conjugate (Romand *et al.,* 1997). Indirect ELISA techniques were developed according to the fragments of NcGRA7 recombinant for the detection of neosporosis in buffalo (Hamidinejat *et al.,* 2015). They established an excellent correlation with a commercial ELISA, showing 98.6% sensitivity and 86.5% specificity using buffalo sera, and it appears to be a useful tool for the serological screening of neosporosis in buffalo.

A milk ELISA has recently been tested as an alternate approach to detect *N. caninum* antibodies in lactating buffalo (Nasir *et al.,* 2018). Compared to competitive ELISA using serum samples, the milk ELISA detected significantly fewer positive milk samples; nonetheless, a moderate degree of agreement between the two methods was seen, suggesting that this milk ELISA could be applied in lactating dairy herds with a higher prevalence of *N. caninum* (Nasir *et al.,* 2018).

Serological diagnosis for the antibodies of anti-*neosporosis* in sera from cows in forty different farms and the results obtained on the sera through using ELISA (ID Screen® *Neospora caninum* competition ELISA kits) for the anti-*neosporosis* antibodies, the collected information demonstrated that neosporosis is a significant contributor to both abortion and financial losses (Manca*et al.,* 2022)

The majority of papers were seroepidemiological investigations utilizing ELISA to identify *Neospora* infections in Iranian cow populations (Gharekhani *et al.,* 2020). Neosporosis diagnosis was detected by using ELISA and PCR, 70 aborted fetuses and pregnant dams from farms of dairy ewes in the northwest of Iran (Asadpour *et al.,* 2012).

Several studies In Egypt demonstrate the detection of antibodies in serum samples to *N. caninum* and *T. gondii* in numerous hosts (Fereig *et al.,* 2016; Rouatbi *et al.,* 2019; Abbas *et al.,* 2020; Selim *et al.,* 2021). However, another study in Egypt had detected antibodies Form milk samples (Gerges *et al.,* 2018). However, Milk samples can represent a valuable, cheap, and non-invasive tool in monitoring *N. caninum* and *T. gondii* prevalence as alternatives to serum samples (Schares *et al.,* 2004) and the risk factors for antibodies in milk and bulk milk samples testing need to evaluate.

An enzyme-linked immunosorbent assay (ELISA) to detect antibodies to *Neospora* species in cattle was developed. The specificity and sensitivity of the ELISA were 96 % and 95 %, respectively, when compared with the IFAT (Williams *et al.,* 1997).

The standard diagnosis is based on indirect immunofluorescence (IFI); however, cross-reactivity with other protozoa proteins is common. Aiming a more specific diagnosis, recombinant antigens have been tested in several immunoassays; of these, NcSAG1 (surface antigen-1) and NcSRS2 (SAG1-related sequence 2) were the most promising. In this context, we developed an indirect ELISA with recombinant NcSRS2 (ELISA- rNcSRS2) and NcSAG1 (ELISA-rNcSAG1) proteins alone and in association (ELISA-rNcSRS2/rNcSAG1) for the diagnosis of cattle and ovine neosporosis. This indicated that indirect ELISA using the rNcSRS2 and rNcSAG1 proteins are a highly sensitive and specific method, especially when used in association, for detecting antibodies in cattle and ovine populations infected with *N. caninum* (Alves Sinnott, et al., 2020).

Moreover, Previous studies have established surface antigen 1 (SAG1), SAG1-related sequence (SRS2), and dense granule protein 6 (GRA6) or GRA7 to be the most frequently used antigens for diagnosis of *N. caninum* infection, in either cattle or dogs (Sinnott *et al.,* 2017, Dubey *et al.,* 2006). In addition, anti-NcSAG1 antibodies have been reported in both acute and chronic *N. caninum* infection, whereas anti-NcGRA7 antibodies have been widely accepted as markers for acute *N. caninum* infection (Hiasa *et al.,* 2012)

N. caninum was isolated from the brain tissues of six seropositive buffalo by IFAT using cell culture, bioassay in dogs, and gerbils. After being fed buffalo brain, four dogs were observed to lose oocysts through their feces. The highest number of oocysts shed was 820,655, and the longest period of oocyst shedding was 26 days. These findings prompt the authors to diagnose based on cell cultures. Two buffalo brains from which *N. caninum* was extracted were used, and three buffalo brains from which the parasite was isolated using a gerbil bioassay. Furthermore, the status of the isolates, designated NCBrBuf-1 through NCBrBuf4, was confirmed. Targeting the Nc5 and ITS-1 regions using PCR and DNA sequencing revealed little genetic diversity compared to other (Rodrigues *et al.*, 2004).

A 3-month-old fetus that tested negative for *N. caninum* at IFAT but tested positive for the infection in brain tissue by PCR analysis that focused on the ITS1 and Nc5 regions,that may have been the first instance of congenital transmission in buffalo. These contradictory findings may be related to the fetus's immunocompetency (Chryssafidis *et al.*, 2011).

DNA of *N. caninum* using PCR assay was detected only at the diaphragm from 6 animals (8.1%, 6/74) of various ages; 3 of them showed negative results by serology (Ba˘rbura *et al.,* 2019),these findings showed that the diaphragm might be a suitable tissue for the detection of *N. caninum* in buffalo.

Prevention and Control:

The most effective methods for the reduction of *N. caninum* are proper prevention and control measures due to the economic significance of neosporosis and the unavailability of therapy and vaccine (Selim *et al.,* 2021).

To effectively control neosporosis, serological studies for gathering seroprevalence data are crucial (Fereig *et al.,* 2016).

Neosporosis can be prevented and controlled by lowering the danger of vertical or horizontal transmission. Numerous strategies have been suggested, including as "testing and culling" reproductive management, chemotherapy, good farm biosecurity and vaccine (Nam *et al.,* 2012b)

Breaking the parasite's life cycle aids in its control by preventing the horizontal transmission between final and intermediate hosts through dog quarantines, as dogs are crucial in the spread of the disease and its transmission (Zhou *et al.*, 2016). Additionally, final hosts should not have access to the placenta from cows and calves, or aborted fetuses. Cattle should only be given covered food and water to avoid oocyst infection. An eradication program should be applied for all rodents, including mice, rats, and rabbits to prevent their role in disease transmission (Hughes *et al.,* 2008). Due to the possibility that chickens and pigeons could act as the parasite's intermediate hosts, a similar strategy should be implemented for poultry (Costa *et al.,* 2008; Mineo *et al.*, 2009).

There are currently no effective vaccines available, making it difficult to protect animals from neosporosis through vaccination (Nam *et al.,* 2012).

The effective control programs should be include the cost-benefit calculation compared to the expenses of testing and control measures with the benefit of reduced economic losses due to *N. caninum* infection or abortion (Hasler et al., 2006). Since, neosporosis is not considered a zoonotic disease, no special measures are recommended at this stage from a public health. A general strategy to control neosporosis worldwide is not applicable because of regional differences in the epidemiology of bovine neosporosis, and it is prudent to thoroughly study regional epidemiology of neosporosis before embarking on a control program (Dubey et al., 2007).From the economic point of view, in a few situations, the application of test and cull strategy includes the following points: (i) test and cull seropositive dams or seropositive aborting dams; (ii) test and inseminate the progeny of seropositive dams with beef bull semen only; and (iii) test and cull the progeny of seropositive dams from breeding. (Hall, et al., 2005). Treatment of cattle seems to be uneconomic for effective preventive measure and hence must be long period, likely producing unfit milk or meat residues or withdrawal periods (Reichel and Ellis.2002). An effect of toltrazuril and its derivative ponazuril on tachyzoites of *N. caninum* has been shown in vitro (Darius et al., 2004) and in vivo in calves (Haerdi, et al., 2006). In calves treated with ponazuril, the parasite was no longer detectable in the brain and other organs (Haerdi, et al., 2006). In experimentally infected mice, evidence that treatment with toltrazuril may be able to block transplacental transmission of the infection was obtained (Gottstein et al., 2005).

***Future prospects**:*

Studies on the genotyping and molecular diagnostics of *N. Caninum* strains are required; that will aid in the creation of effective vaccines and reveal the link between domestic and wild disease cycles. It is advised to conduct research studies on additional hosts, particularly on wild and unusual species, to determine the actual prevalence of neosporosis. Moreover, educational programsabout the risk conditionslinkedtoneosporosis arerequired for farmers and rural public.

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