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CONSEQUENCES OF NEOSPOROSIS ON EMBRYO TRANSFER IN BUFFALOES: REVIEW

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Abstract

With a long history and a wide distribution across the globe, the population of buffaloes (Bubalus bubalis) increased by 1.3% annually between 2002 and 2017, suggesting rising interest in this species. The results of assisted reproduction technologies in buffaloes are not as fulfilling as in cows; for this purpose, any other possible inconvenience must be removed. Parasitic and infectious diseases represent the major cause that negatively impact biotechnologies, especially in embryo transfer, since, although the relocation of an embryo from a seropositive donor to a seronegative recipient seems to be disease-free, in 25% of cases abortion occurs. Neosporosis is a parasitosis with effects that interest mainly the female reproduction, being one of the most important abortigenic entities among buffaloes, with an average prevalence in Australia and America of approximately 88%, in Africa 68%, in Asia 54.7-66.7%, and in Europe varying from 9.1% (Czech Republic) to 68.5% (Romania). In.order.to.monitor and control neosporosis, it is imperative that all three existing categories involved in the embryo transfer process (donors, recipients, embryos) should be tested and proved to be free.

Key words: *Neospora caninum, buffalo, embryo transfer, abortion.*

INTRODUCTION

One of the primary factors contributing to abortions in cows is thought to be neosporosis. An apicomplexan parasite called *Neospora caninum* (*N. caninum*) causes abortion and can have major negative economic impact on ruminants worldwide (Reichel et al., 2013). Even if the cattle are the main intermediate affected by this protozoan parasite (Reichel et al., 2020), there are studies that certified neosporosis in small ruminants too (Lindsay & Dubey, 2020a). It seems that the buffaloes have a lower occurrence of abortion even though *N. caninum* has a high seroprevalence (Reichel et al., 2015). *N. caninum* was first found in Norway in 1984 in dogs, in the case of early death of some puppies with myositis and encephalomyelitis (Bjerkas et al., 1984). This parasite was at the beginning confused with *Toxoplasma gondii* (*T. gondii*) because both are tissue-dwelling coccidia, but they have different predominant ways of transmission. *N. caninum* is transmitted predominantly vertical, whereas *T. gondii* has mainly a horizontal transmission (Goodswen et al., 2013).

The three distinct infectious phases of *N. caninum's* life cycle include tachyzoites, tissue cysts, and oocysts. The tachyzoites cause tissue destruction, infection in the whole body of the intermediate host, and are passed through the placenta. Oocysts (10-13/10-11 μm) are shed unsporulated, by the definitive host (dog) in faeces and sporulate outside, in the environment (Lindsay & Dubey, 2020a).

CLINICAL SIGNS

In the adult buffalo females, neosporosis occurs most frequently with abortions, in any month of gestation, starting from the 3rd month until almost the end, although most appear in the 5th-6th months. Fetuses can be stillborn, living and displaying symptoms, dead in utero (resorbed, mummified, autolyzed), or alive and clinically healthy but permanently diseased (Lindsay & Dubey, 2020a).

Apparently, despite the increased seroprevalence of this protozoan in buffalo, clinical signs (such abortion) appear to be uncommon overall, which has sparked speculation that buffalo may be naturally immune to the negative clinical effects of *N. caninum* infection. (Reichel et al., 2015).

However, this small number of abortions could also be due to poor (ineffective) reporting because they can appear in less developed economies, where it is hence less probable that abortions will be registered or carefully investigated (Reichel et al., 2015).

Clinical signs were identified only in buffalo calves that are less than two months, including neurological indicators (ataxia, diminished patellar reflex, loss of conscious proprioception), underweight, inability to rise, limb flexion or hyperextension, exophthalmos, hydrocephalus, narrowing of the spinal cord (Lindsay & Dubey, 2020a).

DIAGNOSIS

For the certain and definitive diagnosis in a case of suspected neosporosis abortion, both the examination of blood serum from the mother and the histological examination of the abortus are necessary (Lindsay & Dubey, 2020a).

PATHOLOGICAL LESIONS

In an experimental study, buffaloes were inoculated with *N. caninum*, and one day after the females aborted, they were euthanized and the lesions present were analysed. Therefore, serum leakage was observed between the foetal villi and the maternal caruncle and fibrosis, multifocal necrosis, diffuse lymphoplasmacytic infiltration within the maternal caruncle (Chryssafidis et al., 2015a).

The most common histopathological lesions observed in fetuses from the experimentally inoculated animals associated with this parasite are the nonsuppurative infiltration and multifocal necrosis (Chryssafidis et al., 2015b), such as nonsuppurative placentitis, meningoencephalitis, many mononuclear inflammatory foci in several fetal tissues, including the liver (periportal-hepatitis), kidney (interstitial nephritis), heart (epicarditis and myocarditis), and lungs (peribronchiolar interstitial pneumonia) (Konrad et al., 2012).

Compared to spontaneous abortions, epizootic abortions are more likely to result in hepatitis. Additionally, lesions are identifiable in the umbilical cord, but it is very difficult to find parasites (Lindsay & Dubey, 2020a).

IMMUNOHISTOCHEMESTRY

Immunohistochemistry is necessary since autolysing organs often only contain a small number of parasites that are not seen on a normal hematoxylin eosin stained section (Lindsay & Dubey, 2020a).

SEROLOGICAL AND MOLECULAR APPROACHES

Antibodies can be detected by various serological tests, such as Enzyme Linked Immunosorbent Assays (ELISA), Indirect Fluorescent Antibody Assay (IFA), agglutination test for Neospora. The modified ELISA method, by evaluating the avidity, can be used when it is desired to find the moment of infection. Thus, if the avidity is low, it can be considered a recent infection (Lindsay & Dubey, 2020a).

N. caninum DNA was found in the fetuses and placentas of the buffalos that had received the vaccination (Konrad et al., 2012).

The distribution of the immune cells (natural killer cells, T. cell subsets, and CD79αcy cells) in buffalo placentomes were comparable to those previously described in calves that had been experimentally infected with N. caninum during the early stages of pregnancy (Maley et al.,2006). Because of the milder lesions, there may have been fewer abortions in this species after infection (Cantón-et al., 2014).

Pro-inflammatory cytokines, such as interleukin (IL): IL-2, IL-12, and Interferon-γ (IFN-γ) are useful in preventing the growth of *N. caninum* and are necessary for the production of T helper (Th) 1-type responses, but they have the potential to be harmful and may result in the foetus being rejected or aborted (Raghupathy R, 1997). The production of IL-10 by fetal trophoblast cells overwhelms the mother's immune system and locally produces a Th2 cytokine environmental at the.maternal fetal

interface. It is well known that IL-10 inhibits IFN-γ production, which could promote the development of *N. caninum* throughout pregnancy and change the parasite-host equilibrium in their favour (Entrican, 2002).
A definite diagnosis is when antibodies are found in the serum of the foetus, but if the result is negative, this fact does not automatically imply that the foetus is not infected, since the synthesis of antibodies based on the gestational stage, exposure level, and interval between infection and abortion. While peritoneal fluid is not often used for serologic diagnosis, other fluids such as blood or serum from the fetus can be used for serologic diagnosis, peritoneal fluid is most relevant. In calves, serum collected before the first suckling can provide a conclusive congenital infection diagnosis (Lindsay & Dubey, 2020a). Apparently, serum ELISA is much more conclusive than the milk ELISA (Nasir, 2018). When a high prevalence is suspected, evaluating pooled milk samples might be a better option than testing individual milk samples (Enachescu et al., 2014).

TRANSMISSION

INTERSPECIES AND INTRASPECIES TRANSMISSION

Intermediate host: In buffaloes, vertical (transplacentally) transmission is the best known and most likely mode of transmission of neosporosis (Lindsay & Dubey, 2020a), as in the case of cows (Baillargeon et al.,.2001; de Oliveira et al., 2010; Enachescu et al., 2012). Transmission through milk or between adult intermediate hosts is impossible Since *N. caninum* has been found in semen, it seems improbable to be transmitted via sperm or embryotransfer from donor cows. Furthermore, embryotransfer is the preferred approach to avoid vertical (Lindsay & Dubey, 2020a). However, a preliminary test is necessary, in order not to transfer an embryo to a seropositive buffalo. Horizontal transmission occurs through exposure of buffaloes to food and water contaminated with oocysts (from the faeces of infected canids). Not much data is known on the regularity with which dogs in the wild shed *N. caninum* oocysts or the oocysts' resistance (Lindsay & Dubey, 2020a). The high number of oocytes (average of 290,347) produced by dogs that ingested generated by dogs who consumed the brains of adult buffaloes that were naturally infected may suggest that buffaloes are the natural intermediate hosts for *N. caninum* (Rodrigues et al., 2004).
Definitive host: The mechanism by which canids can become infected is not yet well understood. It is assumed that they become ill by ingesting infected tissues. Placental membranes can be a source, as opposed to abortions (Lindsay & Dubey, 2020a).
The dogs from more studies were clinically healthy, but they started to shed oocysts five days after consumption of bovine infected tissue (Gondim et al., 2002; Rodrigues et al., 2004). Moreover, four months after the initial incident, a dog had another oocyst shedding episode (McGarry et al., 2003).

EXPERIMENTAL TRANSMISSION

In experimental studies, from seropositive buffalo dams, in 74% of the calves, the antibodies persisted for 7 months, exhibiting the acquisition of *N. caninum* infection in neonates (Rodrigues et al., 2005). Unlike buffaloes, cows have a shorter period of time (180 days) in which the antibodies remain in bloodstream (Hietala & Thurmond, 1999).
Experimentally, tachyzoites were inoculated intravenously at 70 or 90 days of pregnancy, and until the time of slaughter (28- or 42-days post inoculation), there were no negative effects or clinical symptoms (such as fever or abortions) noted (Konrad et al., 2012).
Several methods of digestive (per os) infection have been tried. Thus, by adding 107 tachyzoites to colostrum/milk replacer, calves were shown to seroconvert, in contrast to the calves fed with placental tissue or with colostrum from *N. caninum* - infected cows (Davison et al., 2001).

ZOONOTIC RISK

It is impossible to overlook *N. caninum* zoonotic potential. It seems that primates and humans cannot be naturally infected. Nonetheless, transplacental transmission and fetal infection occurred when pregnant non-human primates were experimentally inoculated with *N. caninum* isolate from cattle (Lindsay & Dubey, 2020a).

THE IMPACT OF *N. caninum* ON GENERAL CONDITION AND EMBRYO TRANSFER

It seems that in seropositive buffaloes some blood parameters are modified, probably due to the stress that infection causes to the host. Therefore, lower monocyte count and higher blood glucose levels were observed (Nasir, 2018).

An effectively method of controlling the transmission of various pathogens in bovine is suggested by the International Embryo Transfer Society (IETS) and it involves the protection of the early stage embryos by several washing and trypsin treatments (Wrathall & Sutmöller, 1998).

Calves of seronegative recipients that received the embryos from seronegative or seropositive donors were seronegative, resulting in a 0% vertical transmission rate (Baillargeon et al., 2001). Nevertheless, regardless of the donor's status, the progeny becomes infected when recipients test positive for *N. caninum* (Baillargeon et al., 2001; de Oliveira et al., 2010). It also seems that the recipients' response to the hormonal therapy (synchronization) in order to be prepared for embryo transfer is associated with their serologic status (Diniz et al., 2016).

It seems that the incidence of the vertical transmission is directly proportional to the titers of *N. caninum* antibodies during the period of the recipients' gestation (de Oliveira et al., 2010).

SEROPREVALENCE

The global seroprevalence of *N. caninum* infection in buffalo is almost 48%, which is three to four times greater than the seroprevalence of the global herd (16.1% for dairy cattle and 11.5% for beef cattle) (Reichel et al., 2015). In Romania, the seroprevalence of Neosporosis in buffaloes was 68.5% (Bărburaș et al., 2019), and as regards cows, depending on the area of the country, the seroprevalence varied as follows: 34.6% in the Centre (Gavrea et al., 2011), 40.3% in the South (Mitrea et al., 2012) and 27.7% in the West (Imre et al., 2012). Apart from the species, the seroprevalence is also dependent on the age. Therefore, it is reasonable to assume that older animals are more prone to be exposed to oocysts at least once throughout their lifetime, the probability of being serologically positive to *N. caninum* increase by 3.5% per year in the populations of buffalo (Moore et al., 2014). In some studies (Nasir et al., 2011; Sengupta et al., 2012), the highest prevalence was found in groups of animals that were 3-5 years old, in others (Guarino et al., 2000; Fujii et al., 2001), the most infected animals belonged to the 6-8 years old groups. On the other side, Gennari et al. (2005) found no correlation between the age of the buffalos and the incidence of neosporosis. The seroprevalence all over the world during the last 10 years is presented in Table 1 and Figure 1.

Regarding the definitive hosts (Rodrigues et al., 2004) observed that while the elder dogs did not shed oocysts, the younger dogs did.

Table 1. Seroprevalence of *Neospora caninum* in buffaloes from different countries in the last 10 years (2013-2023) (selected reports)

Continent	Country	Method*	Seropositive (%)	Reference
EUROPE	Romania	ELISA	68.5	(Bărburaș et al., 2019)
	Czech Republic	IFAT/ELISA	40/20	(Bártová et al., 2017)
	Italy	ELISA	20.2	(Ciuca et al., 2020)
		ELISA	51	(Auriemma et al., 2014)
AFRICA	Egypt	IgG/IgM	13.52/6.97	(Ibrahim et al., 2021)
AMERICA	Brazil	IFAT	27.5	(A. A. Rodrigues et al., 2022)
		IFAT	35.4	(P.R.F. de Oliveira et al., 2018)
		IFAT	39	(da Silva et al., 2017)
		IFAT	36.4	(Portella et al., 2016)
		IFAT	19.1	(Brasil et al., 2015)
		ELISA	88.02	(Chryssafidis et al., 2015c)
		IFAT/ELISA	48.8/55.55	(da Silva et al., 2014)
	Argentina	IFAT	42.2	(Konrad et al., 2013)
		IFAT	43.3	(Moore et al., 2014)
	Mexico	ELISA	41.2	(Baltazar-Pérez et al., 2022)
		ELISA	44.8	(Salguero-Romero et al., 2021)
		ELISA	24.3	(Romero-Salas et al., 2017)

ASIA	India	ELISA	21.6	(Mahajan et al., 2020)
	Philippines	ELISA	46	(Mingala et al., 2020)
	Laos	ELISA	78.5	(Olmo et al., 2019)
		ELISA	68.9	(Olmo et al., 2018)
	Iran	ELISA	83	(Rezvan et al., 2019)
		ELISA	17.7	(Yagoob et al., 2017)
		ELISA	62.3	(Hamidinejat et al., 2015)
	Israel	IFAT	72.2	(Mazuz et al., 2018)
	Pakistan	Milk/serum ELISA	61.64/76.6	(Nasir, 2018)
		ELISA	42.8	(Nasir et al., 2014)
	Thailand	IFAT	9.1	(Kengradomkij et al., 2015)
	Iraq	ELISA	20	(Al-Amery et al., 2016)
OCEANIA	Australia	ELISA	88.3	(Neverauskas et al., 2015)

*IFAT (Indirect Fluorescent Antibody Test), NAT (Neospora agglutination test), DAT (direct agglutination test)

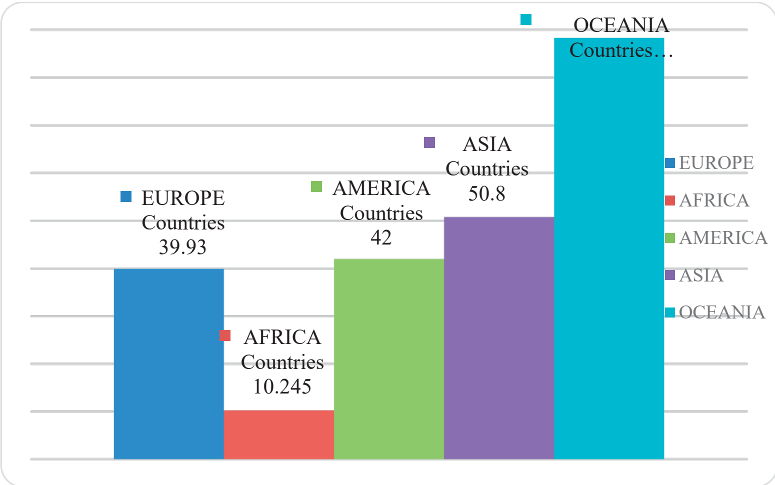

Figure 1. Over the past ten years, the average seroprevalence of *Neospora caninum* in buffaloes from various nations (2013-2023) (selected reports)

METHODS OF CONTROL

Several antimicrobial drugs have been tried *in vitro* (Dubey & Lindsay, 1996; Kim et al., 2002; Lindsay et al., 1997) or *in vivo* in mice (Gottstein et al., 2001). Drugs that eradicate the *N. caninum* bradyzoites found inside tissue cysts do not exist. Although there are no effective ways that can control neosporosis, the most popular approaches to reduce *N. caninum* infection include (Innes et al., 2002; Lindsay & Dubey, 2020b):

(1) reducing/eliminating the possibility of dogs or other possible definitive hosts to contaminate cow feed or water with feces (Mitrea et al., 2013; Paltin et al., 2020);

(2) disposal of dead calves, placentas, and aborted bovine foetuses as soon as possible;

(3) minimizing the number of infected animals that are added to the herd;

(4) culling infected animals (especially the seropositive females and/or calves from seropositive dams in order to reduce the vertical transmission). This is the current best method of prevention, but is impractical if the incidence in a herd is extremely high;

(5) embryo transfer from seropositive buffaloes to seronegative ones.

VACCINE

The creation of suitable attenuated strains that might be used as neosporosis vaccines is of great interest. The advantage of live vaccinations over killed vaccines is that the former are more likely to cause the proper cell-mediated immunity responses in the host animals. On the other hand, there are some disadvantages of the live vaccines, such as cost, limited shelf-life, need for cold storage, and potential for virulence reversal (Innes et al., 2002).

CONCLUSIONS

The recipients' serologic status is related to the risk of the new generation infection. Therefore, it is stated that the most practical manner of producing offspring free of infection with N. caninum at delivery is the transfer of embryos into seronegative recipients, as IETS recommends.

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