Veterinary Immunology and Immunopathology 208 (2019) 53–57

Contents lists available at ScienceDirect

Veterinary Immunology and Immunopathology

journal homepage: www.elsevier.com/locate/vetimm

Short communication

Evidence of reduced vertical transmission of *Neospora caninum* associated with higher IgG1 than IgG2 serum levels and presence of IFN-γ in non-aborting chronically infected cattle under natural condition

Rodrigo Pereyra[a,b], Florencia Celeste Mansilla[c], Marcos Ivan Petersen[b,c], Victor Suarez[a], Alejandra Victoria Capozzo[b,c,*]

[a] Área de Investigación en Sanidad Animal IIACS-CIAP- Estación experimental Agropecuaria EEA, INTA Cerrillos, Salta, Argentina
[b] Consejo Nacional de Investigaciones Científicas y Tecnológicas, CONICET, Argentina
[c] Instituto de Virología. Centro de Investigaciones en Ciencias Veterinarias y Agronómicas, Instituto Nacional de Tecnología Agropecuaria (INTA), Buenos Aires, Argentina

ARTICLE INFO

Keywords:
Neospora caninum
Transplacental transmission
Chronic infection
Immune response
IgG subtypes

ABSTRACT

Neospora caninum infection of cattle can be vertically transmitted, resulting in abortion or birth of infected calves. Vertical transmission occurs both in acutely or chronically infected cattle. There is little information on the immune response needed to prevent endogenous transplacental transmission, particularly from chronically infected cattle to their offspring in a natural environment. In this study, *N. caninum* seropositive pregnant cattle from three different farms with high avidity antibodies and low IgM titers were selected and their newborn colostrum-deprived calves were tested for anti-*N. caninum* antibodies. Based on these results, dams were grouped according to their congenital transmission status. The analysis of the immune profile of the chronically-infected pregnant cattle revealed that higher ratio between IgG1 and IgG2 anti-*N. caninum* serum titers and higher levels of systemic IFN-γ were associated with diminished vertical transmission rates, compared to dams with the opposite profile. Our results evidenced an association between the immune profile and vertical transmission in non-aborting chronically infected dams, and confirm that vertical transmission, even when not leading to abortion, is related to a defined immune profile. This is important information to accomplish successful vaccine development efforts.

1. Introduction

Infection with the parasite *Neospora caninum* is a major cause of reproductive failure in cattle worldwide. Bovine neosporosis has a pronounced economic impact in meat and dairy industries, related to abortion, premature culling and reduced milk yields (Dubey and Schares, 2011; Innes et al., 2005). The parasite may be transmitted through ingestion of oocysts shed by canids, the definitive host of the parasite, or vertically from mother to fetus, sometimes over several generations (Wouda et al., 1998). Both, endogenous and exogenous infections can be the cause of abortions. Depending on the stage of the gestation in which the intra-utero infection occurs, bovine neosporosis can be associated to abortions, birth of weak calves with nervous symptoms or the birth of clinically healthy, but chronically infected calves (Innes et al., 2005). Therefore, targeting control measures to reduce disease and levels of vertical transmission in cattle would be highly desirable.

Type 1 immune responses are known to play an important role in protection against intracellular pathogens. This type of response is usually down-regulated in some phases during pregnancy, and this issue has been related to an increase the in frequency of vertical transmission (Williams et al., 2000). In murine neosporosis the induction of maternal type 1 responses against *N. caninum* prevented vertical transmission and modulation of type 2 cytokines reduced the frequency of vertical transmission of *N. caninum* (Long and Baszler, 2000). Vertical transmission studies are usually based on experimental infections, so most of the available information on immune responses and prevention of congenital infection are related to acute infections. In natural condition, chronically infected cattle play a key role in maintaining the parasite in the herd.

Analyzing the immune response of cattle in the field is not easy and should rely on reproducible and simple methods using samples that can

* Corresponding author at: INTA- Instituto de Virología, Centro de Investigaciones en Ciencias Veterinarias y Agronómicas (CICVyA), N. Repetto y De los Reseros de S/N, Hurlingham, 1686, Buenos Aires, Argentina.
E-mail address: capozzo.alejandra@inta.gob.ar (A.V. Capozzo).

https://doi.org/10.1016/j.vetimm.2019.01.001
Received 27 September 2018; Received in revised form 11 December 2018; Accepted 7 January 2019

be freeze-stored for long periods of time, enabling transportation and high-throughput testing. For this purpose, ELISAs based on serum samples are frequently used. ELISAs can be applied to titrate a specific antibody subclass, for instance, IgG1/IgG2 ratio estimated from ELISA titers is used as a simple marker to establish whether type 1 or type 2 responses predominate following immunization or infection (Lavoria et al., 2012; Spellberg and Edwards, 2001). IFN-γ measurements are also used, as there are many commercial ELISAs available, and this cytokine is stable even during long-term storage (Weynants et al., 1995). The use of these simple methods can help in assisting the evaluation of efficacy of new vaccines; provided a protection-related immune profile has been defined.

Almeria et al. (2009) studied isotype responses in pregnant chronically-infected heifers that aborted in a natural environment. They found that in the presence of systemic IFN-γ, a predominance of IgG2 antibodies might protect against *N. caninum* abortion in chronically infected cattle, while in absence of IFN-γ, predominance of IgG2 did not increase protection (Almeria et al., 2009). In a previous study, Guy et al. reported that the transplacental infection was associated with a rise in anti-*N.caninum* maternal IgG2 titers (Guy et al., 2001). In this study, analyzed the immune profile of non-aborting chronically infected cattle in natural condition, to discern whether the anti-*N. caninum* immune profile was related to an increased risk of congenital infection.

2. Materials and methods

2.1. Animals and sampling

Pregnant animals (n = 75, Holstein) were sampled for this study. They belonged to three different dairy farms: farm A (n = 16), B (n = 39) and C (n = 20). These farms have a mean prevalence of 35%, and most of the animals were chronically infected. Annual abortion rate was about 7% for the three farms. All these animals were multi-vaccinated (at least three times) against foot-and-mouth disease virus (FMDV) and the last vaccination was applied ˜6 month before calving. Newborn calves were separated from their dams and sampled right after birth, before colostrum intake. After sampling, they were returned to their dams. Serum samples (5 ml) were obtained from the jugular vein using Vacutainer® (BD, Franklin Lakes, NJ) tubes and needles (21 G). Dams and newborns were monitored by a veterinarian on daily bases for five days following the procedure. Animal handling and sample collection were performed by trained personnel under the supervision of two veterinarians in accordance with national animal welfare regulations and following institutional guidelines (INTA's animal care committee "CICUAE INTA", protocol 02/2010).

2.2. Experimental design

Three dairy farms located in Salta, Argentina, with history positive *N. caninum* serology and no recent diagnosed infections (up to 10 months before sampling) were chosen for this study. No new animals were introduced to the farm during the study. A total of 75 pregnant cows and heifers (equally represented) and their newborns were sampled and tested for anti *N. caninum* total antibodies. Positive samples were run for avidity of specific antibodies. Sera were taken during the last trimester of pregnancy, while newborns were sampled right after birth, before ingesting colostrum. The absence of colostrum intake was confirmed by measuring serum levels of γ-glutamyl transferase (GGT Activity Colorimetric Assay Kit, Sigma) and antibodies against A-24 Cruzeiro foot-and-mouth disease virus (FMDV) vaccine strain using a liquid-phase blocking ELISA (data not shown) as previously described (Mansilla et al., 2015).

Dams were tested for endemic viral diseases known to modulate the immune response profile against a third-party antigen. Antibodies against Bovine Leukemia Virus (BLV) were assessed using an in-house ELISA "Leukofast" (Trono et al., 2001). Antibodies against bovine viral diarrhea virus (BVDV) were determined using a commercial kit (Priocheck BVDV-Ab, ThermoFisher) following the manufacturer´s instructions. Analysis of BVDV RNA in serum samples was performed by RT-nested PCR following standard procedures (Malacari et al., 2018)

2.3. Total antibody and avidity ELISAs

The presence of serum anti-*N. caninum* IgG was analyzed using in an *in-house* ELISA validated against a commercial kit. Briefly, single 1:50 dilutions of each serum sample were incubated in ELISA plates precoated with a soluble lysate of *N. caninum* tachyzoites (10 μg/ml) for 1 h at 37 °C. A positive control obtained from pooled samples of vaccinated and infected animals, and pre-immune sera of these animals were used as positive and negative controls, respectively (Mansilla et al., 2016.) Presence of specific antibodies is revealed using anti-bovine HPRT conjugate (Jackson ImmunoResearch Laboratories, Inc. PA, USA). Results are expressed as S/P values.

Avidity of anti-*N. caninum* IgG antibodies was measured using the same protocol detailed above, except that samples were run in duplicates and one of the replicates is was to a 6 M urea washing step to removing low-avidity binders. The Avidity Index (AI) was calculated as the percentage of residual activity of the serum sample after urea washing, relative to that of the untreated sample: AI% = (OD sample with urea /OD sample without urea) X 100.

2.4. Isotype ELISAs

The same ELISA platform described above was used to titer the different Ig isotypes, by revealing with the corresponding conjugate: anti-bovine IgM 1:1750, IgG1 1:1000 and IgG2 1:2500 (AbD Serotec, BioRad). Serum samples were run by two-fold serial dilutions starting at 1:50. Titters were expressed as Log_2 dilution^{-1} reaching the cut-off value (OD = 0.2 that correspond to the mean OD values of 50 negative serum samples + 2 SD).

2.5. IFN-γ ELISA

IFN-γ levels were quantified in individual serum samples using a commercial ELISA kit (ID Screen® Ruminant IFN-γ–IDVet, Montpellier, France), following the manufacturer's instructions. Concentration of IFN-γ was estimated from a calibration curve performed with a recombinant cytokine provided with the kit.

2.6. Data analysis

Animals were grouped according to their transmission status, measured as congenital infection of their offspring as described above. Groups were named "VT" for "vertical transmission", and "NT" refers to those dams that did not transmit. Differences between groups were computed by Man Whitney's test (distribution of values was not normal according to Shapiro-Wilk normality test). The odds ratio was used, and significance was estimated by applying the Fisher's Exact Probability test to analyze whether the proportions of dams that transmitted the parasite to their offspring or not was related to either an immunological parameter, gender, number of pregnancies, or infections with BLV or BVDV. The confidence interval used was 95%. Statistical analyses were carried out using GraphPad Prism v5.0 (GraphPad Software).

3. Results and discussion

A group of 75 pregnant animals of different categories belonging to three different dairy farms (A, B, C; n = 16, 39 and 20, respectively) with no recent history of neosporosis were sampled during the third trimester of gestation and tested for serum anti *N. caninum* antibodies. They were also tested for BVDV and BLV, both viral infections known to

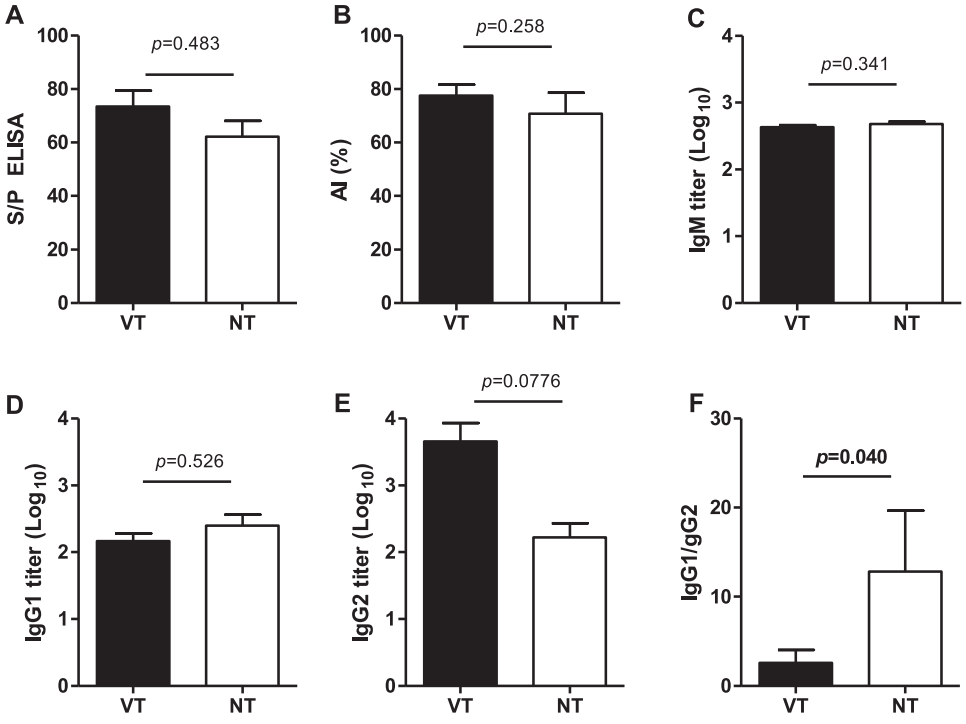

Fig. 1. Serological assays performed on dams´ serum samples. Dams were grouped according their vertical transmission status as VT or NT, as explained above. Serum samples from each group were individually tested by different ELISA assays. (A) Presence of anti-*N. caninum* IgG. Samples were run in an *in-house* ELISA. Results are expressed as S/P values. (B) Avidity of anti-*N. caninum* IgG antibodies. The protocol is identical to the one to measure total antibodies, except that samples are run in duplicates and one well is washed with 6 M urea to remove low avidity binders. (C) IgM ELISA. Anti-*N. caninum* IgM was revealed using an ant-bovine IgM peroxidase conjugate as described before. IgG subtypes titration was performed using the same ELISA platform. Samples were titrated in serial dilutions starting 1:50 revealing with an (E) anti-bovine IgG1 or (F) IgG2 peroxidase conjugate (AbD Serotec, diluted IgG1 1:1000 and igG2 1:2500). The ratio between IgG1 and IgG2 titers in both groups is shown in figure (G). Data were analyzed using Man Whitney's tests. *p* values are shown in each graph.

affect the profile of the immune response against a third-party antigen (Chase, 2013; Puentes et al., 2016). The seroprevalence of BLV in terms of anti-BLV (whole virus) antibodies was extremely high in all the farms, ranging from 80 to 90%. Seropositivity for BVDV anti NS3-antibodies was about 50% for all the farms. The three farms had history of vaccination against this BVDV with inactivated whole-virus commercial vaccines.

All the dams included in this study had high avidity specific antibodies (above 60%) and low IgM levels (Fig. 1C), both indicators of chronic disease (Bjorkman et al., 1999) meaning they had been infected before pregnancy (Table 1). Newborn calves were bled right after birth and absence of colostrum intake was confirmed by measuring serum levels of γ-glutamyl transferase and anti-FMDV antibodies (Mansilla et al., 2015). FMDV antibodies served as an excellent marker of passive antibody transfer, as dams had been vaccinated at least four times against foot-and-mouth disease using a commercial tetravalent vaccine and the last vaccination was performed during the pregnancy period, a condition that ensures high levels of passively transferred antibodies (Bucafusco et al., 2014). These simple serological tools allowed us to classify dam-calf pairs according to their transmission status. Although we cannot discard the event of horizontal re-infection, it is most probable that dams under examination had experienced an endogenous

Table 1
N. caninum seropositive dams, of different categories and farms, were tested for antibodies (Abs) against bovine leukemia virus ("BLV" whole-virus antigen) and Bovine viral diarrhea virus ("BVDV", NS3 antigen). BVDV genome was detected by RT- nested PCR in serum samples. All the animals had high avidity anti-*N. caninum* antibodies (expressed as avidity index in percentage, "AI"). Gender, *N. caninum* status and AI of newborn calves are also shown.

N. caninum seropositive dams							Newborn calves		
ID	Category	Farm	BLV- Abs	BVDV serology (Abs anti-NS3)	BVDV (RT-PCR)	N. caninum Abs – AI	Gender	Calves *N. caninum* status	N. caninum Abs - AI
A 72	Heifer	A	+	+	–	39	Male	+	46
A 73	cow	A	+	–	–	83	Male	+	85
A 74	Heifer	A	+	+	–	90	Female	+	52
B 61	Heifer	B	+	–	+	63	Male	+	52
B 62	Heifer	B	+	–	+	80	Female	+	72
B 63	Heifer	B	+	–	–	73	Male	+	70
B 64	cow	B	+	+	–	89	Female	+	59
B 65	Heifer	B	+	–	+	87	Male	+	27
B 67	cow	B	+	–	–	94	Male	+	77
B 66	Heifer	B	+	–	+	91	Female	+	52
B 68	Heifer	B	+	+	–	79	Male	+	92
B 69	cow	B	+	–	+	72	Female	+	76
B 70	Heifer	B	+	+	–	57	Male	+	100
B 71	Heifer	B	+	–	–	74	Female	+	72
C 75	Heifer	C	+	+	–	70	Female	+	49
A 37	cow	A	+	+	–	46	Male	–	–
A 43	cow	A	+	+	–	90	Male	–	–
B 24	Heifer	B	–	+	–	93	Female	–	–
C 45	cow	C	+	+	–	82	Female	–	–
C 55	Heifer	C	–	+	–	44	Female	–	–
C 56	cow	C	+	+	–	81	Female	–	–

infection due to the absence of circulating parasite in the herd, supported by the lack of acutely-infected animals in the seronegative dams that did not seroconvert by the end of the experiment and served as sentinels for each farm (data not shown).

By confirming the absence of colostrum intake, we attributed the presence of anti-*N. caninum* antibodies in the calves' sera to the occurrence of vertical transmission. Considering dams with positive serology against *N. caninum* and including only those calves bled before colostrum intake, 22 pairs dam/calf were selected and grouped regarding their vertical-transmission status.

No association was found between the transmission rate and the number of pregnancies (first: heifers, multiple: cows), BVDV infection or the calves´ gender ($p > 0.05$, Fisher´s exact test). Due to the high prevalence of BLV-seropositive animals, it was not possible to compute differences between groups. Some animals were positive for BVDV due to an acute episode in Farm B, all of them tested negative for BVDV genome and positive to NS3 antibodies after calving (Table 1). Most of the BVDV-infected dams were part of the VT-group. In the non-transmission group none of the animals were infected with BVDV at the time of the study (only one heifer, B 24 belonged to farm B within the NT group). It is known that BVDV induces an immune-suppression that can be related to a decreased immune response (Chase, 2013) which may in turn increase the risk of transmission. A conclusive result on this regard cannot derivate from our data, mostly due to the limited number of BVDV-infected animals. A fit for purpose study will be needed to verify if these two diseases are related.

Total IgG levels, IgG-subtype (IgG1 and IgG2) titers as well as avidity of specific IgG and IFN-γ levels were assessed in chronically-infected pregnant cattle to study the possible association between a particular immune profile and congenital transmission of *N. caninum.*

Vertical transmission was not related to the amount of total anti-*N. caninum* antibodies, as no significant differences were found between NT and VT groups (Fig. 1A). Avidity was equally high in the two groups of (Fig. 1B). IgM levels (Fig. 1C) were similar between both groups and OD values were low compared to those associated with a primary immune response (Mansilla et al., 2015). Specific IgG1 serum titers were also similar between both groups (Fig. 1D) while IgG2 titers were lower for the NT group (Fig. 1E) though not statistically significant, probably due to the limited number of animals. However, and even considering the small number of animals tested, there was a significant difference ($p < 0.05$) between both groups in the IgG1/IgG2 ratio (Fig. 1F), meaning that the dams that transmitted the parasite to their offspring had higher IgG2 than IgG1 serum titers. In order to analyze if a low IgG1/IgG2 ratio was related to a higher chance of transmitting *N. caninum* to the offspring, we examined the significance of the association (contingency) between the two conditions using the Fisher's exact test. The results show that 80% of the dams that transmitted the parasite had IgG1/IgG2 values below 1, meaning more IgG2 than IgG1; while 90% of the non-transmitters had a ratio over 1 (Fig. 2A). These differences were statistically significant ($p < 0.01$). We tested for systemic IFN- γ by ELISA and confirmed that the presence of IFN-γ and an IgG1/IgG2 ratio over 1 was associated to the lower transmission rate, (Fig. 2B).

Our study included infected dams and congenitally infected, full-term calves, and found an association between higher anti-*N. caninum* IgG1 than IgG2 serum titers and presence of IFN-γ with protection against congenital infection, while the opposite profile (higher IgG2 than IgG1 in the absence of IFN-γ) was related to vertical transmission. Our results are in concordance with those of Guy et al. who reported high IgG2 titers cows which delivered congenitally infected calves in a natural environment (Guy et al., 2001), Almeria et al. (Almeria et al., 2009) analyzed if IgG1, IgG2 and/or IFN-γ systemic levels were associated with abortion in chronically infected heifers, and concluded that aborting cattle had higher IgG2 than IgG1 titers and low systemic IFN-γ levels. In that study, the authors discussed the variations in antibody subtypes and their relationship with the presence or absence of IFN-γ, which still remains unclear for cattle.

Bovine IgG1 shares some functional characteristics with IgG2, as binding to neutrophils and mediating opsonofagocytosis, but also has unique attributes: it is the main Ig-subclass in colostrum and can be released as secretory immunoglobulin on the mucosa (Saini et al., 2007), being then particularly important for protection. Both IgG1 and IgG2 are considered important contributors to protection against intracellular and extracellular pathogens (Welsh et al., 2005). The IgG1/IgG2 ratio has emerged as correlate of protection for several bovine diseases, more than the absolute titer (Brito et al., 2014; Heriazon et al., 2011; Lavoria et al., 2012). For foot-and-mouth disease virus, protection has been related to higher IgG1 than IgG2 titers (Capozzo et al., 1997; Lavoria et al., 2012) and associated with the presence of IFN-γ (Bucafusco et al., 2015). In previous studies we showed that a vaccine formulation containing a soluble extract of tachyzoites and a soy-based adjuvant with TLR agonists elicited high levels of high-avidity specific antibodies and IFN-γ in vaccinated cattle (Mansilla et al., 2013). The immune profile elicited by this vaccine was similar to that described for controlling acute *N. caninum* infection (Maley et al., 2001; Rosbottom et al., 2007; Williams et al., 2000). Moreover, IFN-γ production during pregnancy prevented abortion in naturally infected cattle (Almeria et al., 2009; Lopez-Gatius et al., 2007).

Altogether, our results suggest that an immune profile combining anti-*N.caninum* IgG1/IgG2 > 1 with high systemic IFN-© levels is associated with a diminished risk of transplacental infection in chronically infected heifers and cows. It is important to note that these immunological parameters can be easily assessed by running ELISAs using field samples; this is paramount when working in vaccine field trials. Vaccine efforts should focus on eliciting this particular immune profile to reduce the risk of congenital transmission in chronically-infected dams.

Acknowledgements

This study was financed by INTA through PNSA 1115053. RP and MIP are PhD fellows of CONICET. AVC is researcher of CONICET. The authors would like to thank Ms. Marcela Martinez, Mr. Roque Chavez, Mr. Ramón Miraval and Mr. Antonio Chocobar, for their help with field work and animal care.

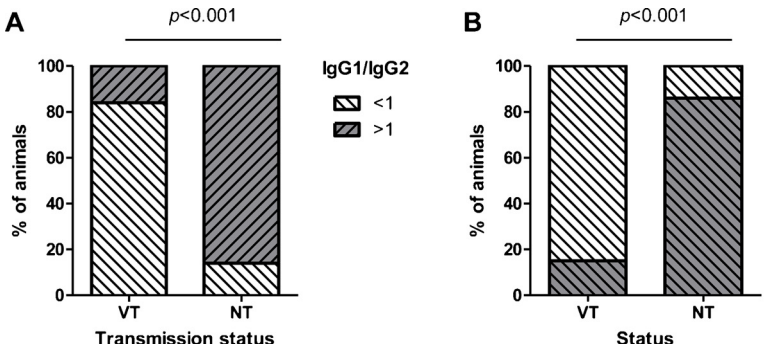

Fig. 2. Association between immune-profile and transmission rate. Fisher's exact test was applied to analyze whether the proportions of animals that transmitted or not the parasite to their offspring is related with (A) the IgG1 to IgG2 serum titer ratio or with (B) the levels of systemic IFN-γ. *p* values were below 0.01 as indicated over each graph.

References

Almeria, S., Nogareda, C., Santolaria, P., Garcia-Ispierto, I., Yaniz, J.L., Lopez-Gatius, F., 2009. Specific anti-Neospora caninum IgG1 and IgG2 antibody responses during gestation in naturally infected cattle and their relationship with gamma interferon production. Vet. Immunol. Immunopathol. 130, 35–42.

Bjorkman, C., Naslund, K., Stenlund, S., Maley, S.W., Buxton, D., Uggla, A., 1999. An IgG avidity ELISA to discriminate between recent and chronic Neospora caninum infection. J. Vet. Diagn. Invest. 11, 41–44.

Brito, B.P., Perez, A.M., Capozzo, A.V., 2014. Accuracy of traditional and novel serology tests for predicting cross-protection in foot-and-mouth disease vaccinated cattle. Vaccine 32 (January (4)), 433–436. https://doi.org/10.1016/j.vaccine.2013.12.007. Epub 2013 Dec 14.

Bucafusco, D., Di Giacomo, S., Pega, J., Juncos, M.S., Schammas, J.M., Perez-Filgueira, M., Capozzo, A.V., 2014. Influence of antibodies transferred by colostrum in the immune responses of calves to current foot-and-mouth disease vaccines. Vaccine.

Bucafusco, D., Di Giacomo, S., Pega, J., Schammas, J.M., Cardoso, N., Capozzo, A.V., Perez-Filgueira, M., 2015. Foot-and-mouth disease vaccination induces cross-reactive IFN-gamma responses in cattle that are dependent on the integrity of the 140S particles. Virology 476, 11–18.

Capozzo, A.V., Periolo, O.H., Robiolo, B., Seki, C., La Torre, J.L., Grigera, P.R., 1997. Total and isotype humoral responses in cattle vaccinated with foot and mouth disease virus (FMDV) immunogen produced either in bovine tongue tissue or in BHK-21 cell suspension cultures. Vaccine 15, 624–630.

Chase, C.C., 2013. The impact of BVDV infection on adaptive immunity. Biologicals 41, 52–60.

Dubey, J.P., Schares, G., 2011. Neosporosis in animals–the last five years. Vet. Parasitol. 180, 90–108.

Guy, C.S., Williams, D.J.L., Kelly, D.F., McGarry, J.W., Guy, F., Bjorkman, C., Smith, R.F., Trees, A.J., 2001. Neospora caninum in persistently infected, pregnant cows: spontaneous transplacental infection is associated with an acute increase in maternal antibody. Vet. Rec. 149, 443–449.

Heriazon, A., Hamilton, K., Huffman, J., Wilkie, B.N., Sears, W., Quinton, M., Mallard, B.A., 2011. Immunoglobulin isotypes of lactating Holstein cows classified as high, average, and low type-1 or -2 immune responders. Vet. Immunol. Immunopathol. 144 (December (3-4)), 259–269. https://doi.org/10.1016/j.vetimm.2011.08.023. Epub 2011 Sep 3.

Innes, E.A., Wright, S., Bartley, P., Maley, S., Macaldowie, C., Esteban-Redondo, I., Buxton, D., 2005. The host-parasite relationship in bovine neosporosis. Vet. Immunol. Immunopathol. 108, 29–36.

Lavoria, M.A., Di-Giacomo, S., Bucafusco, D., Franco-Mahecha, O.L., Perez-Filgueira, D.M., Capozzo, A.V., 2012. Avidity and subtyping of specific antibodies applied to the indirect assessment of heterologous protection against Foot-and-Mouth Disease Virus in cattle. Vaccine 30, 6845–6850.

Long, M.T., Baszler, T.V., 2000. Neutralization of maternal IL-4 modulates congenital protozoal transmission: comparison of innate versus acquired immune responses. J. Immunol. 164, 4768–4774.

Lopez-Gatius, F., Almeria, S., Donofrio, G., Nogareda, C., Garcia-Ispierto, I., Bech-Sabat, G., Santolaria, P., Yaniz, J.L., Pabon, M., de Sousa, N.M., Beckers, J.F., 2007. Protection against abortion linked to gamma interferon production in pregnant dairy cows naturally infected with Neospora caninum. Theriogenology 68, 1067–1073.

Malacari, D.A., Pecora, A., Perez Aguirreburualde, M.S., Cardoso, N.P., Odeon, A.C., Capozzo, A.V., 2018. In vitro and in vivo characterization of a typical and a high pathogenic bovine viral diarrhea virus type II strains. Front. Vet. Sci. 5, 75.

Maley, S.W., Buxton, D., Thomson, K.M., Schriefer, C.E., Innes, E.A., 2001. Serological analysis of calves experimentally infected with Neospora caninum: a 1-year study. Vet. Parasitol. 96, 1–9.

Mansilla, F.C., Czepluch, W., Malacari, D.A., Hecker, Y.P., Bucafusco, D., Franco-Mahecha, O.L., Moore, D.P., Capozzo, A.V., 2013. Dose-dependent immunogenicity of a soluble Neospora caninum tachyzoite-extract vaccine formulated with a soy lecithin/beta-glucan adjuvant in cattle. Vet. Parasitol. 197, 13–21.

Mansilla, F.C., Moore, D.P., Quintana, M.E., Cardoso, N., Hecker, Y.P., Gual, I., Czepluch, W., Odeon, A.C., Capozzo, A.V., 2015. Safety and immunogenicity of a soluble native Neospora caninum tachyzoite-extract vaccine formulated with a soy lecithin/beta-glucan adjuvant in pregnant cattle. Vet. Immunol. Immunopathol. 165, 75–80.

Mansilla, F.G.I., Pérez-Aguirreburualde, M.S., Cardoso, N.P., Quintana, M.E., Capozzo, A.V., 2016. Desarrollo y validación de un kit de ELISA para el diagnóstico de la neosporosis bovina. XXI Reunión Científico-técnica de la Asociación Argentina de Veterinarios de Laboratorios de Diagnóstico., Jujuy, Argentina.

Puentes, R., De Brun, L., Algorta, A., Da Silva, V., Mansilla, F., Sacco, G., Llambi, S., Capozzo, A.V., 2016. Evaluation of serological response to foot-and-mouth disease vaccination in BLV infected cows. BMC Vet. Res. 12, 119.

Rosbottom, A., Guy, C.S., Gibney, E.H., Smith, R.F., Valarcher, J.F., Taylor, G., Williams, D.J., 2007. Peripheral immune responses in pregnant cattle following Neospora caninum infection. Parasite Immunol. 29, 219–228.

Saini, S.S., Farrugia, W., Muthusamy, N., Ramsland, P.A., Kaushik, A.K., 2007. Structural evidence for a new IgG1 antibody sequence allele of cattle. Scand. J. Immunol. 65, 32–38.

Spellberg, B., Edwards Jr, J.E., 2001. Type 1/Type 2 immunity in infectious diseases. Clin. Infect. Dis. 32, 76–102.

Trono, K.G., Perez-Filgueira, D.M., Duffy, S., Borca, M.V., Carrillo, C., 2001. Seroprevalence of bovine leukemia virus in dairy cattle in Argentina: comparison of sensitivity and specificity of different detection methods. Vet. Microbiol. 83, 235–248.

Welsh, M.D., Cunningham, R.T., Corbett, D.M., Girvin, R.M., McNair, J., Skuce, R.A., Bryson, D.G., Pollock, J.M., 2005. Influence of pathological progression on the balance between cellular and humoral immune responses in bovine tuberculosis. Immunology 114 (January (1)), 101–111.

Weynants, V., Godfroid, J., Limbourg, B., Saegerman, C., Letesson, J.J., 1995. Specific bovine brucellosis diagnosis based on in vitro antigen-specific gamma interferon production. J. Clin. Microbiol. 33, 706–712.

Williams, D.J., Guy, C.S., McGarry, J.W., Guy, F., Tasker, L., Smith, R.F., MacEachern, K., Cripps, P.J., Kelly, D.F., Trees, A.J., 2000. Neospora caninum-associated abortion in cattle: the time of experimentally-induced parasitaemia during gestation determines foetal survival. Parasitology 121 (Pt 4), 347–358.

Wouda, W., Moen, A.R., Schukken, Y.H., 1998. Abortion risk in progeny of cows after a Neospora caninum epidemic. Theriogenology 49, 1311–1316.