Application of a new therapeutic protocol against *Neospora caninum*-induced abortion in cattle: a field study.

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Short title: Therapy against *Neospora caninum* in cattle

Abstract

A field evaluation of a new therapeutic protocol based on Toltrazuril and/or Sulphadiazine and Trimethoprim was carried out for treatment of *Neospora caninum*-induced abortion in dairy cattle. 936 randomly chosen Friesian bovine belonging to 18 dairy herds of Central and South Italy with history of high number of abortions per year were considered.

Since the high *N. caninum* seroprevalence, Sulphadiazine 200 mg/ml and Trimethoprim 40 mg/ml was given to the cattle at a dosage of 20 mg/kg bw, following different age-depending protocols. Toltrazuril 50 mg/ml was also given at 20 mg/kg bw to newborn cattle and once every three months for one year to the dogs present in the farm; a periodic treatment of environment was carried out with a phenolic disinfectant.

The concomitant treatment of all the animals present in the herd, dogs included, with a Toltrazuril and/or Sulphadiazine and Trimethoprim based protocol was efficacious for treatment of *N. caninum* infection in dairy cows. The efficacy of this protocol was demonstrated by drastic reduction of both abortion (from 188 to 9) and *N. caninum* seroprevalence (from 68.7% to 0%) after one year of treatment.

Keywords:

Neospora caninum, cattle, abortion, chemotherapy

Introduction

Neospora caninum is a protozoan apicomplexan parasite recently isolated from livestock and companion animals (Dubey et al. 1988). After the initial recognition in dogs by Bjerkas (1984), several *Neospora*-like organisms have been also detected in many other domestic animals including cat, cattle, sheep, goat, and horse.

As documented by the high number of publications appeared in the literature, this parasite has attracted increasing attention, primarily as an important causative agent of abortion in cattle in many countries including UK, Ireland, The Netherlands, Scandinavia, New Zealand, Australia, Japan, South Africa and Israel (Dubey and Lindsay 1996, Dubey 2003).

Infected cows of any age may abort from 3 months to term of gestation, although the most *Neospora*-induced abortions occur at 5–6 months. Foetuses may die *in utero*, be reabsorbed, mummified, autolyzed, stillborn, born alive but diseased, or born clinically normal but chronically infected (Dubey 1999). Cows with *N. caninum* antibodies are more likely to abort than seronegative cows and usually the antibodies do not protect against abortion, that can occur again in the next gestation (Thurmond and Hietala 1997, Moen et al. 1998, Wouda et al. 1998).

The major route of *N. caninum* infection in cattle is transplacental transmission from cow to calf; the parasite has been isolated from aborted bovine foetuses and from congenitally infected calves, and the infection may be propagated vertically through successive generations (Bjorkman et al. 1996, Anderson et al. 1997, French et al. 1998, Schares et al. 1998, Kim et al. 2000). Some researchers state that the prevalence of *Neospora*-specific IgG is a good indicator of parasitaemia and foetal infection during pregnancy in naturally infected cattle, although the immunity generated is insufficient to prevent vertical transmission (Hemphill and Gottstein 2000).

Horizontal transmission seems to be necessary to introduce new infections in the herd, but cow to cow horizontal transmission has not been demonstrated, so others animal species could have an important epidemiological rule. (Parè et al. 1996, Parè et al. 1997, Thurmond and Hietala 1997, French et al. 1998, Schares et al. 1998). Postnatal infection of cattle with *N. caninum* and transmission through ingestion of faecal oocysts spread by a definitive host has been hypothesised and recently confirmed (McAllister et al. 1998, Basso et al. 2001). The dog has been identified as the most important definitive host of *N. caninum*, therefore it is plausible that cattle may be infected by ingestion of oocysts shed by dogs. The presence of *N. caninum* antibodies in wild animals has been reported in coyotes, foxes, dingoes, and racoons (Dubey 2003), and coyotes have been reported as definitive hosts (Gondim et al. 2004), so it is possible that also some of these mammals act as natural hosts and have an epidemiological role. In particular, the recent findings that wild brown rats (*Rattus norvegicus*) collected from cattle farms in China resulted positive to *N. caninum* by serology and PCR let suppose that rats may serve as a reservoir for the protozoan in cattle farms (Huang et al. 2004).

Control of bovine neosporosis is difficult and effective vaccines to protect cattle from abortion or vertical parasite transmission are currently not available (Dubey 1999).

Studies on the efficacy of some chemotherapeutic agents were carried out *in vitro* and in experimentally infected mice (Lindsay et al. 1994, Darius et al. 2004). Although a toltrazuril-based protocol has proved to be efficacious in mice and cattle experimentally infected with *N. caninum*, no definitive data are available in dairy herds (Gottstein et al. 2001, Kritzner et al. 2002).

The aim of this field study was to verify the practical efficacy of a new therapeutic protocol based on Sulphadiazine and Trimethoprim (Norodine 24®; Bayer) and/or Toltrazuril (Baycox® 5%; Bayer) in reducing *N. caninum*-induced abortions in dairy herds with history of a high abortion prevalence.

Materials and Methods

Dairy herds

During 2002, 18 dairy herds in Central and South Italy were selected for the presence of a high number of abortions per year. At the beginning of the study (T-0), 1892 Friesian bovine of different age and sex were present in the selected herds, while one year post-treatment (T-1) a total number of 1979 bovine was present in the farms; the distribution of animals per farm is summarised in Table I.

During the previous year, in all the different farms 118 abortion were recorded. The distribution of the abortion into the farms is summarised in Table I.

Animals

936 animals of different age and sex were randomly chosen at T-0, while one year post treatment (T-1) 901 of these animals were still available.

During the whole length of the treatment, the animals were clinically monitored to record the number of abortions and at T-0 and T-1 the sera were tested for the most important bovine abortion agents.

Serology

Sera were tested for antibodies against *N. caninum* with a Competitive Enzyme-Linked Immunosorbent Assay (c-ELISA) (VMRD, Inc., Pullman, WA, USA). Briefly, undiluted serum sample, if containing antibodies against *N. caninum*, inhibits binding of horseradish peroxidase (HRP)-labelled *N. caninum*-specific monoclonal antibodies to *N. caninum* antigen coated on the plastic wells. Positive and negative controls were used for each plate. The results were read with an ELISA-reader system (Labsystem, Multiskan Ascent) at 620 nm.

All sera were besides tested for *Bovine Herpesvirus – 1* (*BoHV-1*), *Bovine Herpesvirus – 4* (*BoHV-4*), *Bovine Viral Diarrhea Virus* (*BVDV*) and for *Chlamydophila abortus*.

For *BoHV-1*, *BoHV-4* and *BVDV* an ELISA test was used (Abortion Elisa kit, Euroclone, Milan, Italy) following the manufacturer instructions; the results were read with an ELISA-reader system (Labsystem, Multiskan Ascent) at 450 nm.

For *Chlamydophila abortus*, an indirect enzyme-linked immunoassay (Civtest Bovis Chlamydia PS, Hipra, S.A. Laboratorios, Amer, Spain) was used following the manufacturer instructions; the results were read with an ELISA-reader system (Labsystem, Multiskan Ascent) at 450 nm.

Therapeutic protocol

The therapeutic protocol applied was based on the contemporary treatment of all animals, cattle and dogs, present in the herd and on a periodic treatment of environment.

Products used

Toltrazuril 50 mg/ml (Baycox® 5%; Bayer)

Sulphadiazine 200 mg/ml and Trimethoprim 40 mg/ml (Norodine 24®; Bayer)

Phenolic disinfectant (p-chloride-m-cresol, 0.1385 g/L; o-phenilphenole 0.562 g/L; p-chloride-o-benzilphenole 0.467 g/L) (Delegol® NF; Bayer).

Cattle

A) Before first week of age

Toltrazuril was given at 20 mg/kg body weight per os /x day for 3 consecutive days. At six months and at one year of age Sulphadiazine and Trimethoprim was given again at 20 mg/kg bw / endovenous / daily for 4 consecutive days.

B) Before one year of age

Sulphadiazine and Trimethoprim at 20 mg/kg bw / endovenous / daily for 4 consecutive days / every three months until the first year of age.

C) After one year of age

Sulphadiazine and Trimethoprim at 20 mg/kg bw / endovenous / daily for 4 consecutive days every year.

Dogs

Toltrazuril was given at 20 mg/kg bw / per os / once every three months. During the therapy the animals were bind to a chain for 24 hours.

Disinfection of farms

A phenolic disinfectant, diluted 1% in hot water, was distributed with a pressure pump one day after each treatment of the animals. Furthermore, a permanent deratization plan was implemented.

Statistical analysis

The statistical analysis of the results was carried out using MedCalc® Program Version 7.4.3.1.

The difference of *Neospora* and the others abortion agents prevalence between pre- and post-treatment was tested by Chi-square with 1 degree of freedom.

Results

Abortion

During the year following the treatment, in all the herds a significant decrease ($p<0.0001$) of abortion was registered (Table I).

Serology

A statistically significant decrease ($p<0.0001$) in *Neospora* infection prevalence after the treatment was observed, while the prevalence of *BoHV-1*, *BVDV*, *BoHV-4* and *Chlamydophila* infection did not changed ($p>0.05$) between T-0 and T-1 (Table II).

Discussion

Infectious abortions in cattle are really feared by farmers because the consistent economic losses and often the insidious diffusion. Among the well known bovine abortion agents it is important to consider also *N. caninum* infection, usually not frequently investigated but able to cause significant economic damages.

The outcome of *N. caninum* infection is largely dependent on the effectiveness of the maternal immune system, and in many cases it is insufficient to prevent vertical transmission to bovine foetus. Effective vaccines for bovine neosporosis are still not available (Dubey 1999), and few chemotherapeutical treatments have been suggested and verified in experimentally infected mice and calves (Gottstein et al. 2001, Kritzner et al. 2002).

In this study, the application of a new therapeutic protocol based on toltrazuril and/or sulphadiazine and trimethoprim resulted in a drastic reduction of abortion (from 188 to 9) and *N. caninum* seroprevalence (from 68.7% to 0%) in bovine farms during the year post-treatment. The success of this therapeutic protocol is probably due also to the concomitant treatment of all the animals present in the herd, inclusive of dogs. Furthermore, the evidence of *N. caninum* in wild brown rat (Huang et al. 2004) suggests the importance of a constant deratization to reduce the potential risk of infection.

The seroprevalence of *N. caninum* infection (68.7%) in the herds investigated was higher than reported in analogous surveys (Otranto et al. 2003), and could be indicative of an increase of the infection in Italy. In the herds included in the study there were several infected but apparently healthy cows that had uncomplicated pregnancies although seropositive. All together, these data confirm the high prevalence of the infection in bovine herds, and the insidious way of *N. caninum* diffusion may contribute to maintain endemic the disease in herds, with consequent periodic abortions if no therapeutic and preventive measures are adopted. The toltrazuril based protocol tested in this preliminary field study demonstrated his utility in reducing abortion and seropositivity, and could be extensively used to cut down the economic losses in bovine herds.

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Table I. – Distribution of animals per farm, number of samples collected and abortion observed pre-(T-0) and post-treatment (T-1).

Farm	Number of samples collected at T-0	Number of animals present in herd T-0	Number of samples collected at T-1	Number of animals present in herd T-1	Abortion registered in the previous year	Abortion registered in the year post treatment
1	37	78	35	85	11	1
2	170	327	168	320	17	1
3	70	123	69	130	8	0
4	40	82	38	80	6	0
5	55	102	50	136	10	0
6	20	47	20	50	7	0
7	50	97	50	97	9	0
8	32	65	30	65	8	0
9	76	134	76	148	18	0
10	35	68	31	60	5	1
11	35	57	35	50	6	0
12	25	56	25	61	7	0
13	43	90	40	90	16	1
14	24	56	23	55	5	0
15	40	87	35	82	19	2
16	94	203	91	239	15	0
17	50	129	46	141	15	3
18	40	91	39	90	6	0
Total	936	1892	901	1979	188	9

Table II – Serological results and numbers of abortion recorded.

	BoHV-1		*BVDV*		*BoHV-4*		*Chlamydophila abortus*		*Neospora caninum*		Abortion
	+	-	+	-	+	-	+	-	+	-	
T-0	78	858	149	787	38	898	37	899	643	293	188
T-1	80	821	169	732	34	867	25	876	0	901	9
P value	p>0.05		p>0.05		p>0.05		p>0.05		p<0.0001		p<0.0001