The prevalence of *Neospora caninum* antibodies in bulk milk of dairy herds in the Czech Republic: a case report

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ABSTRACT: *Neospora caninum* is a protozoan parasite causing bovine abortion all over the world. The aim of this study was to investigate how common *N. caninum* infection is among dairy herds in the Czech Republic. Bulk milk samples were collected from 495 dairy herds and analysed for the presence of specific antibodies by a commercial *N. caninum* iscom ELISA. Five out of 495 dairy herds (1.01%) had percentage positive values ≥20 and were considered positive. In the positive herds, blood samples were collected from cows and pregnant heifers and analysed by the ELISA test. The within-herd seroprevalence ranged from 2.5 to 50%. The bulk milk ELISA could be a useful and inexpensive method for rapid screening of *Neospora caninum* infection in dairy herds in large areas.

Keywords: cattle; iscom ELISA; bulk milk ELISA

Neospora caninum (Apicomplexa: Toxoplasmatinae) is a worldwide-distributed pathogen which causes abortions in cows leading to economic losses in the cattle industry. Numerous studies addressed the prevalence of *N. caninum* in European countries, but a direct comparison of reported seroprevalences is difficult because different study populations, serological tests and cut-off values are used (Dubey, 2003). The seroprevalence varies among dairy and beef cattle as well as in non-aborting and aborting populations of cattle, e.g. 2% dairy cows and 7% aborting cows were seropositive in Sweden (Bjorkman et al., 2000); 3% dairy cattle, 10% beef cattle and 17% dairy and 19% beef cattle in aborting herds were seropositive in Belgium (De Meerschman et al., 2000); 39% dairy cattle from herds with abortions had *N. caninum* antibodies in the Netherlands (Dijkstra et al., 2001) and 36% dairy and 18% beef cattle were seropositive in Spain (Quintanilla-Gozalo et al., 1999).

There are 573 000 cows out of 1 430 000 head of cattle in the Czech Republic (Czech Statistical Office, http://www.czso.cz/). A previous serological survey of 463 aborting cows showed that 3.9% of them were *N. caninum* infected (Vaclavek et al., 2003). The aim of the present study was to get a rapid overview of *N. caninum*-infection among dairy herds in the Czech Republic. Moreover, sera of positive cows and heifers were tested by avidity ELISA method to distinguish between acute and chronic *N. caninum*-infection in the herds (Bjorkman et al., 1999, 2003).

MATERIAL AND METHODS

We obtained 495 samples of bulk milk from the State Veterinary Institutes (SVI): 240 from SVI Jihlava, 112 from SVI Prague, 73 from SVI Hradec Kralove and 70 from SVI Brno. A single bulk milk

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sample was collected from each examined dairy herd. Only herds up to 200 lactating cows were examined.

In each herd that was found *Neospora caninum*-positive in the bulk milk, individual blood samples were collected from all cows and pregnant heifers by vacutainer puncture of the coccygeal vein. The milk and blood samples were centrifuged at 1 000 × g for 10 min and skimmed milk and sera were collected and stored at –20°C until analysis. The samples were analysed using a commercial *N. caninum* iscom ELISA kit (Svanova, Sweden) according to the manufacturer's instructions. The skimmed milk and sera were diluted 1 : 2 and 1 : 100 in PBS-Tween before analysis. The optical density (OD) was measured on an automatic reader (Labsystem Multiscan RC) at 450 nm and the values were correlated to a positive control serum with mean OD of 1.0 to obtain a percentage positive value (PP). A sample was considered positive when the PP value was ≥20 (cut-off value 0.200). Blood sera of positive animals were tested also by immunofluorescent antibody test (IFAT) for validation, using the method previously described Vaclavek et al. (2003). The sera of positive cows and heifers from herds No. 1, 2 and 4 were sent for the examination by avidity ELISA method to SWEPAR laboratory (Uppsala, Sweden), where IgG avidities (functional affinities) were determined for a decision on acute or chronic *N. caninum* infection in the herds (Bjorkman et al., 1999, 2003).

RESULTS

Five out of the 495 (1.01%) examined bulk milk samples gave positive results with PP values 22–89 (Table 1). Three positive samples obtained from State Veterinary Institute in Jihlava originated from two farms in the Plzeň region and one farm from the Pardubice region. Two positive samples obtained from State Veterinary Institute in Prague originated from Central Bohemian region and Vysocina region.

To establish within-herd prevalence, blood sera from cows and pregnant heifers from three *N. caninum*-positive herds (No. 1, 2, 4) were individually tested. In the fourth herd (No. 3), only milk samples could be individually tested to establish within-herd prevalence. The seroprevalence in herds 1–4 ranged between 2.5 and 50% (Table 1). The individual PP values of the 16 positive cows and heifers (examined by iscom ELISA) ranged from 34 to 93 (Table 2). The presence of *N. caninum* antibodies in the sera was confirmed by IFAT, the antibody titres ranged from 1 : 200 to 1 : 5 120 (Table 2).

The values of the IgG avidity of 16 positive sera tested by avidity ELISA ranged from 61 to 88, only one cow showed the value 26 (Table 2). These values indicated chronic infection in the three herds.

DISCUSSION

The samples of bulk milk can be easily collected and tested for antibodies of infectious diseases in dairy herds by ELISA method (Pritchard, 2001). According to pertinent legislative regulations, bulk milk testing is routinely used to detect bovine leucosis and brucellosis antibodies in dairy cattle in the Czech Republic. Testing of bulk milk is a useful way for a rapid finding of the presence of *Neospora caninum* in a large area. In Germany, Schares et al. (2003) reported 7.9% prevalence in 3 260 examined herds using bulk milk ELISA method, when a maximum of 50 cows contributed to bulk milk sam-

Table 1. *Neospora caninum* antibody status in bulk milk and within-herd prevalence in 5 dairy herds with diagnosed *N. caninum* infection, as examined by *N. caninum* iscom ELISA

Herd number	Percentage positivity of bulk milk	Number of lactating cows	Number of sampled cows and heifers	Seroprevalence in % (positive/total)
1	89	7	10	50.0 (5/10)
2	69	19	23	26.1 (6/23)
3	23	35	40	2.5 (1/40)
4	29	38	48	10.4 (5/48)
5	24	98	0	ND

ND = not determined

Table 2. *Neospora caninum* antibody status in individual serum samples from cows and heifers of 3 dairy herds as measured by *N. caninum* iscom ELISA, immunofluorescent antibody test (IFAT) and avidity ELISA

Herd number	Cow/heifer number	Iscom ELISA (PP)	IFAT (titres)	Avidity ELISA (avidity index)
1	1	65	1 : 200	64
	2	56	1 : 1 280	77
	3	93	1 : 5 120	81
	4	49	1 : 1 280	73
	5	87	1 : 5 120	69
2	6	80	1 : 2 560	79
	7	34	1 : 2 560	65
	8	90	1 : 1 280	83
	9	70	1 : 640	66
	10	73	1 : 640	72
	11	54	1 : 640	26
4	12	78	1 : 320	88
	13	68	1 : 200	80
	14	75	1 : 640	74
	15	48	1 : 200	61
	16	58	1 : 200	80

PP = percentage positivity

ples and 0.15 cut-off value was used. In Thailand, 220 herds were analysed by bulk milk ELISA and 46% herds were found positive (Chanlun et al., 2002). In both cases the laboratories used their own ELISAs. A commercial ELISA (IDEXX Laboratories Inc., Westbrook, USA) was tested for the detection of *N. caninum* antibodies in bovine milk (Schares et al., 2004) and gave good results using 1 : 2 dilutions of milk and cut-off value 0.261.

Iscom ELISA for the detection of *Neospora caninum* antibodies in blood serum and milk was developed to decrease the cross-reactivity (Bjorkman et al., 1997; Bjorkman and Lunden, 1998) and a commercial iscom ELISA kit (Svanova, Sweden) was designed for diagnostics of bovine *Neospora*-specific antibodies in blood serum. There is no doubt that the commercial kit can be used for milk samples, considering the same components and conditions proceed from previous studies (Bjorkman et al., 1997; Bjorkman and Lunden, 1998; Chanlun et al., 2002).

Establishing the appropriate cut-off value is a key point in all ELISA methods (Chanlun et al., 2002; Frossling et al., 2004; Schares et al., 2004). A relatively high cut-off value (0.200) was used in our study. Currently, but after finishing our study, data on testing the iscom ELISA for the examination of bulk milk samples appeared addressing the relationship between the bulk milk OD and within-herd prevalence (Frossling et al., 2004). High cut-off (0.200) was recommended to rule in infection and lowering the cut-off was suggested for general screening. Regardless of this fact, our data demonstrate generally low prevalence of *N. caninum* in cattle in the Czech Republic that corresponds with 3.9% prevalence in aborting cows (Vaclavek et al., 2003).

Diagnosing the *N. caninum* in cattle in the Czech Republic started just recently and it is difficult to speculate about reasons for the low prevalence. Limited occurrence of this pathogen in the country can be tentatively explained by indoor housing that prevailed in the Czech cattle industry in the last four decades. Moreover, limited contacts of cattle and dogs and extremely low prevalence of *N. caninum* in dogs (Slapeta et al., 2002) can be attributed to low prevalence in cattle as well.

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