NOTE ***Parasitology***

An Abortion Storm in Cattle Associated with Neosporosis in Taiwan

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ABSTRACT. An abortion storm associated with acute neosporosis involving 18 cattle was observed in a dairy farm in Taiwan. Aborted fetus age ranged from 3 to 8 months. Of the 38 cattle in that farm examined during the abortion storm, 52.6% (20/38), 13.2% (5/38) and 10.5% (4/38) contained both IgG and IgM, only IgG and only IgM antibodies to *Neospora caninum*, respectively. No antibody to *N. caninum* was detected prior to the abortion storm. Follow-up study conducted a year later showed that 23 out of 28 cattle had seroconverted. Since some cattle were positive to either only IgG or IgM, we suggest that both IgG and IgM should be tested for diagnosing neosporosis. Neosporosis surveillance of naive cattle herd is recommended.

KEY WORDS: abortion storm, neosporosis,Taiwan.

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Neosporosis, caused by the protozoan parasite, *Neospora caninum*, has been recognized as a major cause of abortion in cattle worldwide. It produces great economic loss because the aborted cow failed to produce milk if the abortion occurred in heifer [6]. It may also bring about the death of the infected newborn calf after parturition [8]. Seropositive cows have three times greater risks of abortion than seronegative cows [12]. Mainar-Jaime *et al.* [16] reported that 38.7 % of the abortions in northern Spain, while Kim *et al.* [15] confirmed that 21.1% of the cattle abortion in Korea, were attributed to *N. caninum*. Nevertheless, the difficulty in diagnosing *N. caninum*-associated reproductive failure is that many of the aborted fetuses were not available to the veterinary diagnostic laboratory, and thus, diagnosis of *N. caninum* as the causative agent of abortion is often based solely on assaying the cow sera for the presence of antibodies. Abortion storm in cattle probably due to neosporosis had also been reported in Germany [11]. We reported herein an abortion storm in a dairy farm in Taiwan that was probably free of *N. caninum* infection before the episode and discussed the various possible factors that might have contributed to the event.

Between May 30 to June 11, 2002, a dairy farm with 250 Freisien-Holstein cattle in Chang-Hua, central Taiwan, reported experiencing 18 cattle abortions. The farm had no record of abortion for the past year. Serum sample of cattle were collected during (June 7 and 11, 2002) and after the abortion storm (June 9, 2003) from the caudal vein. A total of eight additional cattle also aborted between June 11, 2002 to June 9, 2003. Screening for antibodies against *N. caninum* in the bovine sera was carried out by Immuno-fluorescent antibody test (IFAT) using *N. caninum* tachyzoite antigen commercially obtained from Kyoto Biken, Japan. Cattle sera were screened at 1:50 and 1:200 dilutions for detecting the IgM and IgG antibodies to *N. caninum*, respectively. For secondary antibody, either FITC-conjugated affinity purified goat anti-bovine IgM or IgG, obtained from Jackson ImmunoResearch Laboratories, U.S.A., was used. The tachyzoites were observed under ultraviolet light fluorescence microscope at 400 × magnification. Diffused or peripheral staining of the tachyzoites was considered as positive, but tachyzoites that were stained only at the polar ends were considered as negative.

The age of the aborted fetuses during the period of abortion storm ranged from 3 to 8 months as shown in Fig. 1. The abortion storm affected mainly the cattle in mid-term pregnancy. Antibody profile of the cattle previous to and after the abortion storm is shown in Table 1. Serological examination of a total of 38 cattle in that farm carried out on June 7 and 11, 2002, showed 52.6% (20/38) having both IgG and IgM, 13.2% (5/38) only IgG and 10.5% (4/38) only IgM antibodies to *N. caninum*, respectively. Moreover, the 16 sera sample collected on June 7, 2002 were also tested for *Toxoplasma gondii* antibodies at 1:100 serum dilution using IFAT with antigen from VMRD, U.S.A. and *Chlamydia psittaci* using ELISA test (ImmunoComb®) with antigen from Biagal Co., Israel. All the 16 sera tested were negative

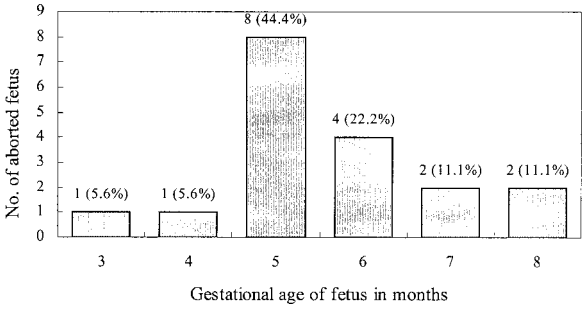

Fig. 1. Age and number of aborted fetus during the abortion storm, from May 30 to June 11, 2002.

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Table 1. Antibody profile of the cattle before and after the abortion storm

Date of sera sample (month/year)	Antibodies to *N. caninum*			Total	
	IgG+ IgM+	IgG+ IgM–	IgG– IgM+	Positive	Negative
03/2002[a)]	0/30	0/30	0/30	0	30
06/2002[b)]	20/38 (13/20)[d)]	5/38 (2/5)[d)]	4/38 (1/4)[d)]	29 (16)[e)]	9 (2)[f)]
06/2003[c)]	13/28 (8/13)[d)]	3/28 (2/3)[d)]	7/28 (3/7)[d)]	23 (13)[e)]	5 (0)[f)]

a) Date was before the period of the abortion storm, which was from May 30 ~ June 11.
b) Date was during the period of the abortion storm.
c) Date was after the abortion storm one year later.
d) Parenthesis shows cattle aborted / sero-positive cattle.
e) Parenthesis shows no. of cattle aborted from among sero-positive cattle.
f) Parenthesis shows no. of cattle aborted from among sero-negative cattle.

for *T. gondii*. However, the antibody titers for *C. psittaci* were 1:32 for 8 cattle, <1:32 for 3 cattle and 5 were completely negative. Positive control serum showed a 1:64 antibody titer.

Retrospective serological examination of thirty cattle whose blood were drawn on March 19, 2002, which was prior to the abortion storm, showed no antibodies to *N. caninum*. Of the 38 cattle that were serologically examined during the abortion storm, 7 were the same cattle whose blood had been drawn before the episode. Of these 7, four of them had sero-converted in both IgG and IgM from negative to positive during the abortion storm. In a follow-up study, one year later, 28 cattle from that farm were examined for antibodies to *N. caninum*. All cattle were negative for antibodies to *N. caninum* when their bloods were drawn on March 19, 2002. Of those 28 cattle, 23(82%) had sero-converted in at least either for IgG or IgM or both from negative to positive. Among the 23 cattle that were found to have sero-converted a year later, 5 of those cattle were antibody-negative when tested during the abortion storm but were sero-converted later. All the cattle that aborted did not show any other conspicuous clinical sign whatsoever, before or after the abortion. Only one aborted fetus was examined for *N. caninum* DNA by PCR [22] but with negative result. Samples used in the PCR included the brain, heart, lung, liver, spleen, kidney and vaginal swabs. No other abortion causing pathogen such as IBR virus (infectious bovine rhinotracheitis; using the specific primers, IBR260F: 5'-GCACCTCTGTGAACTGCATC and IBR555R: 5'-GCACACGTTYTTGCGCTTGG), *Chlamydia* (using the specific primers, OMP1142F: 5'-GCAGCAGCTAATTACAAAAC and 860R: 5'-AGTATCAGCTGTAGCTTCTC), *T. gondii* (using the specific primers, T30p890F: 5'-CGTAGCATACGATCGAGTC and T301050R: 5'-ATCCTCCATAGCAGCTGATC) and *N. caninum* (using the primers, NP4F: 5'-CCTCCCAATGCGAACGAAA and NP7R: 5'-GGGTGAACCGAGGGAGTTG; Np6: 5'- CAGTCAACCTACGTCTTCT and Np21: 5'- GTGCGTCCAATCCTGTAAC) were detected by PCR, nor Akabane disease virus (using the primers, Aka75F: 5'-GGGTATGTGGCATTTATCAG and Aka605R: 5'-GTCCAACTTAGATGTCATCC), Chung-Shan disease virus (using the primers, ChulF: 5'-GGCTGCATCGTACGCTAC and Chu587R: 5'-ATCGTACCGATCGACTCC), BVD virus (Bovine Viral Diarrhea; using the primers, BVD220F: 5'-CTAGTATTCGTACTAGGCGC and BVD442R: 5'-CGTAAAGCTTGCAGCCTACTG) were detected by RT-PCR. We set up the aforementioned primers with reference to the gene bank. They always produced the specific bands when tested against respective pathogens. These PCR products had been verified for their base pair sequences and found to be reliable. Virus isolation using BHK and vero cells were also carried out. However, all of the aforementioned DNA tests showed negative result. Since other aborted fetuses were discarded by the farmer, we could not perform the confirmatory tests of detecting the protozoan.

In our serological examination after the abortion storm, the cattle sera were found to contain antibodies to *N. caninum*, but not to *T. gondii* and also lower antibodies titer than positive control to *C. psittaci*. If the cattle were acutely infected with *C. psittaci*, the antibody titer of some of the cattle should be higher than 1:32. However all cattle sera tested showed antibody titers to *C. psittaci* that were much lower than the positive control. This lends support to the cattle being infected with *N. caninum* in this particular farm. Also, the mean gestational age of the 18 aborted fetuses was 5.6 months and this is similar to the reported bovine abortion associated with *N. caninum* (5.7 months) [9]. Romero and others' 2002-questionnaire survey demonstrated that the common age of the aborting cattle were 3–6 years old [18]. In our study, the ages of the aborting cow were between 3–8 years and the mean age was 5.2 years.

In the present study, we showed that cattle of the affected farm were sero-negative for *N. caninum* antibodies before the abortion storm but most of the cattle tested were sero-positive after the episode. This is the first record of a high rate of seroconversion for *N. caninum* infection in cattle in Taiwan. Since many of the sero-positive cattle aborted, it is implicated that *N. caninum* might be a major cause of bovine abortion in this case. To date, most of the serological survey on neosporosis carried out in cattle involved only the detection of IgG [20]. Since we observed that some cattle were positive to either only IgG or IgM, we suggest that both the presence of IgG and IgM should be tested in a seroepidemiological survey of neosporosis in cattle. This suggestion concurred with that proposed by González and

others [9] in that both IgG and IgM need to be tested. Results of serologic testing for IgM and IgG antibodies to *N. caninum* before during and after the abortion storm were compared (Table 1). It has been reported that IgM were detected earlier in the course of infection than IgG in canine hepatozoonosis [1]. Similar findings have been reported by experimental inoculations with tachyzoites of *N. caninum* [3] and natural infection in the aborted fetus [14]. Moreover, in some cases, the presence of IgM to *N. caninum* in the absence of IgG titer may indicate a more recent infection. In our study, since IgM were continuously found even one year after the abortion storm, it might indicate that reinfection of *N. caninum* had been occurring in the cattle farm.

Abortion storm in cattle had been attributed to the 'point source' exposure to *N. caninum* oocyst [19]. The only animal known to be able to excrete *N. caninum* oocyst is the dog [2], which had been reported to serve as the definitive host of the parasite [13]. In our study, the dairy farmer changed some sources of cattle feed in April 2002, in that he planted grass on a plot of land nearby his farm. After harvesting the fresh grass, he mixed them thoroughly with other cattle feed in a TMR (Total mixed ration) machine and fed them to the cattle. Since we have observed many stray dogs roaming around the farm, it might be probable that they might shed *N. caninum* oocysts on the grassplot and thus contaminated the grass used as cattle feed. We postulated that the abortion storm of this magnitude might not have occurred if the farmer did not use the TMR machine to thoroughly cut the grass into small uniform pieces. Furthermore, the cattle would have avoided eating the intact dog feces because of the foul smell. Moreover, even if the cattle had accidentally ingested the dog feces, only very few cattle might have been affected instead of more than a dozen.

We believe that acute infection of *N. caninum* in a naive cattle herd can be manifested as an abortion storm as seen in our study. The incubation period from infection to the outbreak of the abortion storm might probably be less than 2 months because we observed that the cattle in the farm were probably not yet infected in March 2002, and the farmer only started to give his cattle the TMR feed only in April 2002. The abortion storm began at the end of May 2002. This is perhaps the first report to suggest an approximate incubation period for the abortion storm that is probably caused by neosporosis.

A comparatively high seroprevalence of *N. caninum* among dogs in Turkey [4] and in Japan [21] had been reported. In our previous serological survey for antibodies against *N. caninum* in Taiwan, we reported that 23% (3/13) of the farm dogs in Taiwan were sero-positive [17]. Thus, control of dogs in cattle farm should be considered as a measure to prevent neosporosis-associated abortion in the farm, especially preventing the dogs from eating the aborted fetus [5, 7, 10]. Furthermore, we also recommended that neosporosis sero-negative dairy farm should be identified and the cattle serologically tested at a regular interval to prevent suspected neosporosis-associated abortion storm.

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