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RESEARCH ARTICLE

Abortions in Three Beef Cattle Herds Attributed to Selenium Deficiency

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ABSTRACT

An association between abortion and low selenium (Se) status in cattle has been suspected, but not confirmed previously. Abortion outbreaks in 3 Se-deficient beef cattle herds, between 2010 and 2013, are described. In total, 130 out of the 480 cattle aborted (27.1%): among these, 28/130 (21.5%) abortions were in herd 1, 47/150 (31.3%) in herd 2 and 55/200 (27.5%) in herd 3. Five aborted fetuses were examined grossly and histopathologically. Laboratory examinations for bacterial, viral and parasitic causes of abortions were conducted (fetuses, fetal membranes, cows' blood) and were found negative. Moreover, blood samples from 18 aborting cows were examined for Se, vitamin A and vitamin E concentrations. Blood Se levels in cows belonging to farms that experienced abortions were lower than reference values and significantly lower than 28 control cows from 3 herds without abortions (0.46±0.08 vs. 2.20±0.04 μmol/L; $P<0.0001$). Serum vitamin E and vitamin A concentrations were within reference range and did not differ from controls. Skeletal muscle and myocardial degeneration and atrophy were observed in 5 aborted calves. Abortions were significantly reduced from 26% to 1.1% following the subcutaneous administration of a Se and vitamin E containing commercial preparation to each of the remaining pregnant cows, at a dose 0.1 mg/kg body weight for Se and 8.98 mg/kg body weight for vitamin E. In conclusion, determination of whole blood Se status before late gestation is useful in early diagnosis of Se deficiency and should be included in the diagnostic panel when investigating abortions in cattle.

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INTRODUCTION

Abortions constitute a major problem for the cattle industry, leading to severe financial losses, however, their etiology often remains unidentified (Alves *et al.*, 1996; Khodakaram-Tafti and Ikede, 2005; Radostits *et al.*, 2008). The causative agent is identified in approximately one third (31 to 39%) of such cases and is infectious (primarily bacterial) in the majority of massive abortions, although nutritional deficiencies, particularly of iodine and vitamin A, or toxicities may be implicated less commonly (Kirkbride, 1992; Jamaluddin *et al.*, 1996). Selenium (Se) deficiency is associated with a wide range of health disorders in cattle, including white muscle disease, retained placenta, and mastitis (Surai, 2006). It has been linked with abortions in cattle (Orr and Blakley, 1997), but its direct abortifacient effect has not been proven to date (Waldner and Van de Weyer, 2011; Yuan *et al.*, 2014).

The aim of the present retrospective study was twofold: 1) to report cases of massive abortions in 3 beef cattle herds presumptively attributed to Se deficiency, indicating that Se deficiency can account for a portion of such cases in cattle; and 2) to record that metaphylactic Se administration can eliminate abortions presumptively caused by its deficiency in such herds.

MATERIALS AND METHODS

This study was conducted in 3 beef cattle herds with massive abortions between 2010 and 2013. Analytical data regarding number of animals, breed, feeding regime and conducted vaccinations are shown in Table 1.

History and Diagnostic work up

Herd 1 (130 cows): In 2010, 28 cows aborted on the 7th or 8th month of gestation, while 4 other cows gave birth to stillborn calves. Two aborted fetuses along with their placenta were subjected to necropsy and laboratory examinations for *Brucella* spp., *Listeria* spp., *Escherichia coli*, *Salmonella* spp., *Chlamydia abortus*, *Ureaplasma* spp. and *Trichomonas* spp., according to Quinn *et al.* (2004).

Herd 2 (150 cows): In 2013, 45 cows aborted and 4 gave birth to stillborn calves. One aborted fetus (approximately 7 months old) was subjected to necropsy and laboratory examinations identically to herd 1; additional tests for Bovine viral diarrhea (BVDV), Bovine herpesvirus 1 (BHV-1) and Schmallenberg virus (SBV) were performed.

Herd 3 (200 cows): In 2013, 52 cows aborted between 5^{th} and 7^{th} month of gestation. Moreover, 5 cows gave birth to stillborn calves. Two aborted fetuses were subjected to necropsy and analyses as for herd 2. Retained placentas were not observed in any farm.

Recently aborting cows were sampled for biochemical (Se, vitamin A and vitamin E) and serological (antibodies against BVDV, BHV-1, SBV and *Neospora caninum*) analyses, as follows: a) herd 1: 10 cows for biochemistry and serology, b) herd 2: 10 cows for serology and 5 for biochemistry, and c) herd 3: 3 cows for both analyses. Additionally, 3 samples were obtained from cows that delivered normally in herd 2 for biochemical analyses.

Laboratory analysis: Whole blood Se (Ayannidis and Voulgaropoulos, 1990) and serum vitamin E (Hansen and Warwick, 1969) were determined fluorometrically. Serum vitamin A was determined colorimetrically (Roels and Traut, 1972).

Serology was performed using enzyme-linked immunosorbent assay (ELISA) kits. Competitive ELISA was applied for anti-p80-125 (anti-NSP2-3) antibodies detection for BVDV (ID Screen® BVD p80 antibody competition) and anti-BHV-1 gE antibodies for BHV-1 (ID Screen® IBR gE competition). Indirect ELISA kits were used to detect antibodies against SBV nucleoprotein (ID Screen® Schmallenberg virus indirect multispecies confirmation test) and *Neospora caninum* (ID Screen® *Neospora caninum* indirect multi-species). All immunoassays were performed according to the manufacturer's instructions (ID.vet, Montpellier, France).

Treatment: After Se-deficiency determination in aborting cows, the remaining pregnant animals were metaphylactically injected once (0.1 mg Se /kg BW and 8.98 mg vitamin E /kg BW) with a commercial Se and vitamin E preparation, containing 1.67 mg sodium selenite and 150 mg tocopherol acetate per mL. Blood was drawn from 5 cows in each herd 45 days post-injection to measure Se concentrations.

Control cows: Blood Se, vitamin E and A concentrations from 28 pregnant beef cows that calved normally, from 3 neighboring farms without abortion storms, were used as controls. Sampling and analysis of this dataset was performed at our Laboratory in 2012.

Statistical analysis: SAS software (version 9.3, Cary, North Carolina) was used for calculations. Data normality was tested with Shapiro-Wilk test; because data had a normal distribution, means and standard deviations are given. Comparisons were performed with two sample t-test for independent observations (PROC TTEST) for continuous variables and Chi-square analysis (PROC FREQ) for binary variables. $P<0.05$ was considered significant.

RESULTS AND DISCUSSION

Laboratory examinations were negative for all the infectious agents tested in the 3 examined herds. Necropsy of all the examined fetuses revealed multi-focal severe pallor of several skeletal muscles and the myocardium. Histology showed marked multifocal atrophy and degeneration of the skeletal muscles, and to a somewhat lesser degree of the myocardium, and mild hepatic congestion. All these lesions were compatible with white muscle disease. Brain histopathology of the aborted fetuses was unremarkable.

Whole blood Se concentration was lower than the normal values cited in the literature in all the 3 herds, while serum vitamin E and vitamin A values were normal (Table 2). Whole blood Se concentrations lower than 0.63 μmol/L indicate a deficient state, while values of 0.63-0.95 μmol/L are considered marginal (Surai, 2006). In farm 2, cows that aborted had lower Se levels compared to the 3 cows that did not abort (0.48±0.04 vs. 0.60±0.06 μmol/L, respectively; P=0.02); however, all these cows were Se deficient. There was a highly significant difference regarding Se levels between the farms that experienced abortion storms and those that did not (0.46±0.08 vs. 2.20±0.04 μmol/L, respectively; P<0.0001).

Table 1: Information concerning herd size, nutrition and vaccinations in the 3 beef cattle herds with the abortion outbreaks

	Animals	Feeding regime	Vaccinations
Herd 1	130 Limousin cows	Grazing, concentrates (corn, barley, soybean) no vitamin-trace elements added	Clostridiosis, enterotoxinogenic colibacillosis
Herd 2	150 Simmental cows	Grazing, alfalfa hay, wheat straw, corn, no vitamin-trace elements added	Clostridiosis
Herd 3	200 Brown Swiss x Simmental cows	Grazing, alfalfa hay, wheat straw, corn, no vitamin-trace elements added	Enterotoxinogenic colibacillosis

Table 2: Blood selenium (Se), vitamin E and vitamin A concentrations of aborting and control cows, as well as reference values of the elements

Herd	Whole blood Se (μmol/L)	Serum vitamin E (μmol/L)	Serum vitamin A (μmol/L)
Herd 1	0.44±0.10[a]	2.77±0.46	1.57±0.24
Herd 2	0.48±0.06[a]	2.32±0.17	1.08±0.23
Herd 3	0.38±0.09[a]	2.40±0.07	1.54±0.05
Control cows[1]	2.20±0.09[b]	2.50±0.10	1.30±0.15
Reference values	2.66-15.24[2]	2.31-4.62[3]	0.70-1.70[4]

a,b=different superscripts within the same column denote significant difference (P<0.05); [1]Twenty-eight (28) beef cows from 3 neighboring herds without abortion outbreaks; [2]Surai (2006); [3]Radostits *et al.* (2008); [4]McDowell (2000).

Table 3: Effect of metaphylactic selenium (Se) administration on abortion cases at the 3 investigated beef cattle herds

Herd No.	Total abortion cases	Abortions before Se administration	Abortions after Se administration	P value
Herd 1 (n=130)	28 (21.5)	28 (21.5)[a]	0 (0.0)[b]	<0.0001
Herd 2 (n=150)	47 (31.3)	45 (30.0)[a]	2 (1.3)[b]	<0.0001
Herd 3 (n=200)	55 (27.5)	52 (26.0)[a]	3 (1.5)[b]	<0.0001
All herds (n=480)	130 (27.1)	125 (26.0)	5 (1.1)	<0.0001

Values in parenthesis indicate percentage. a,b=different superscripts within the same row denote significant difference (P<0.05).

Selenium deficiency resulted in an almost 50-fold increase (95% C.I. 21-121) in the probability for a cow to abort compared to the status after Se supplementation. Metaphylactic Se treatment was effective for all the 3 herds, as abortions significantly reduced after some days and for the next 6 months, when the herds were closely monitored. In total, 130 out of the 480 cows aborted (27.1%); 125 cows (26%) aborted before Se injection and 5 (1.1%) after Se injection (Table 3). Treatment raised the Se levels 45 days after the injection, with those values being within the normal range cited in the literature.

This seems to be the first report indicating that selenium administration can be efficiently used as a metaphylactic treatment against abortion in cattle in cases in which selenium deficiency is the presumptive etiologic agent of the abortion. An association between abortion and low selenium status in cattle has been suspected but not confirmed previously (Orr and Blakley, 1997; Enjalber *et al.*, 2006).

Fetal heart failure seems to be the mechanism most likely to explain the abortifacient effect of Se deficiency among the many proposed (Awadeh *et al.*, 1998; Underwood and Suttle, 2004). Orr and Blakley (1997) suggested that signs of heart failure including cardiac ventricular dilation, myocardial necrosis and mineralization, ascites and liver congestion, could represent a fetal form of white muscle disease attributed to Se deficiency. In the same study (Orr and Blakley, 1997) infectious agents that can cause abortion were detected in placenta and fetal tissues of most of the cases, possibly indicating that Se deficiency can create favorable circumstances for an abortifacient infectious agent to act.

In our study, Se deficiency was presumptively the sole cause of abortion based on the negative laboratory testing for a range of other common etiologies, the absence of pathology indicative of the involvement of such agents, the cardiac and skeletal muscle lesions that were compatible with white muscle disease, the lower than reference range selenium levels, the vitamin A and E levels being within reference range, and the clinical outcome (no or few abortions) following metaphylactic Se administration to the pregnant cows that had not aborted.

Another speculation for the abortifacient effect of Se deficiency might be the fact that sufficient progesterone concentration is necessary to maintain pregnancy in late gestation and Se supplementation contributes to adequate progesterone production by the pregnant cows (Kamada *et al.*, 2014). These authors found significantly increased progesterone concentrations in blood serum of pregnant Se-supplemented Holstein heifers after the 7th month of gestation, when compared to the non Se-supplemented ones. However, since progesterone concentrations were not evaluated in the present study, we cannot attribute our cases of abortions in Se-deficient cows to progesterone deficiency. Also, in the study by Kamada *et al.* (2014) Se concentrations were measured in blood serum, as opposed to whole blood in our study, making comparisons difficult.

In conclusion, Se injection can be effectively given as a metaphylactic treatment in cases of massive abortions attributed to Se-deficiency in grazing beef cattle herds. Determination of whole blood Se status before late gestation could be useful for early detection of Se deficiency and, given the number of abortion cases in which the etiology remains undetermined, may be included in the diagnostic panel when investigating abortions in cattle.

Author's contribution: NDG, NP, GT, CB and HK attended the clinical cases and designed this manuscript. PL conducted the pathological and histopathological examinations. EP, GF and SCC conducted the examinations for possible pathogens. ATK and KK conducted the selenium and vitamin determinations. All authors interpreted the data, revised the manuscript and approved the final version.

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