Relationship between Paratuberculosis and the microelements Copper, Zinc, Iron, Selenium and Molybdenum in Beef Cattle

F. Paolicchi¹,2, J. Perea¹, S. Cseh¹,2, C. Morsella²

¹Department of Animal Production, Faculty of Agricultural Sciences, National University of Mar del Plata, Buenos Aires Province, Argentina.

²Department of Animal Production, Estación Experimental Agropecuaria, Instituto Nacional de Tecnología Agropecuaria, Balcarce, Argentina.

Submitted: October 14, 2011; Approved: July 2, 2012.

Abstract

To study the deficiency of minerals and its relationship with Paratuberculosis, blood, serum, and fecal samples were obtained from 75 adult bovines without clinical symptoms of the disease and from two bovines with clinical symptoms of the disease, from two beef herds with a previous history of Paratuberculosis in the Province of Buenos Aires, Argentina. Serum samples were processed by ELISA and feces were cultured in Herrolds medium. Copper, zinc and iron in serum were quantified by spectrophotometry and selenium was measured by the activity of glutathione peroxidase. We also determined copper, zinc, iron and molybdenum concentrations in pastures and the concentration of sulfate in water. Mycobacterium avium subsp. paratuberculosis (Map) was isolated from 17.3% of fecal samples of asymptomatic animals and from the fecal samples from the two animals with clinical symptoms. All the Map-positive animals were also ELISA-positive or suspect, and among them, 84.6% presented low or marginal values of selenium and 69.2% presented low or marginal values of copper. The two animals with clinical symptoms, and isolation of Map from feces and organs were selenium-deficient and had the lowest activity of glutathione peroxidase of all the animals from both herds. All the animals negative to Map in feces and negative to ELISA had normal values of Se, while 13.8% of animals with positive ELISA or suspect and culture negative presented low levels of Se. Half of the animals that were negative both for ELISA and culture in feces were deficient in copper but none of them presented low values of selenium. The content of molybdenum and iron in pasture was high, 2.5 ppm and 1.13 ppm in one herd and 2.5 ppm and 2.02 ppm in the other, respectively, whereas the copper:molybdenum ratio was 1.5 and 5.2, respectively. These results do not confirm an interaction between imbalances of the micronutrients and clinical Paratuberculosis, but show evidence of the relationship between selenium deficiencies in animals with Map infection and ELISA positive results.

Key words: Paratuberculosis, micronutrients, selenium, molybdenum, copper.

Introduction

Paratuberculosis is a chronic infectious disease caused by Mycobacterium avium subsp. paratuberculosis (Map), which affects many domestic species such as cattle, sheep, and goats, and wild species such as foxes, deer and hares. Paratuberculosis is a common disease very frequent in dairy and beef cattle in Argentina (Paolicchi et al., 2003) and in all countries with a significant dairy industry, especially in areas with a moderate and humid climate (Barkema et al., 2010; Manning and Collins, 2001). Infected cows may have clinical signs such as persistent diarrhea which leads to the animal’s weight loss. The infected ani-
mals containing millions of Map in their stools contaminate the pasture, and asymptomatic animals may also shed Map in colostrum and milk (Streeter et al., 1995). In Argentina, estimates from some regions of the country show sero-prevalence percentages that vary between 7% and 19% in beef herds (Paolicchi et al., 2003). Particularly in dairy cattle, losses of milk production and poor body condition followed by death or culling are predominant (Hasanova and Pavlik, 2006). The economic losses due to Paratuberculosis in Argentina are estimated to be 22.0 million dollars for breeding cattle and 6.3 million dollars for dairy cattle in the breeding livestock area of Buenos Aires province (Paolicchi et al., 2003).

Map is characterized by slow growth in suitable culture media and dependence on mycobactin. The most important difference between Map and other mycobacteria is the repetitive and specific presence of the IS900 insertion sequence (Green et al., 1990). The conclusive diagnosis of Paratuberculosis requires the isolation of Map from feces, milk, semen or affected tissues from animals with clinical symptoms (Whitlock, 1998). Although ELISA tests detect circulating antibodies in serum from infected animals, the possibility to identify infected animals increases when they have clinical symptoms of the disease. In addition, the sensitivity of this test increases when the animals excrete high quantities of Map through feces. This coincides with periods that compromise the immune response of the animal like management in intensive exploitations, the stress of suckling, transport, nutritional alterations, and mineral deficiencies (Manning and Collins, 2001).

Causes of mineral deficiency are poor concentration of micronutrients in the diet (primary deficiency) or inability of the animal to absorb minerals from the diet, even when the concentration of micronutrients in the diet is normal (secondary deficiency) (Wikse et al., 1992). Deficiency of some minerals can affect the immunological system, diminishing the animal’s capacity to overcome infections. Complex inter-relationships exist between certain micronutrients, immune function and disease resistance in cattle. Several micronutrients have been shown to influence immune responses. Deficiencies of copper (Cu), selenium (Se), vitamin E and cobalt (Co) in cattle reduce the ability of isolated neutrophils to kill yeast and/or bacteria. Cu deficiency reduces antibody production, but cell-mediated immunity is generally not altered (Arthington, 2006). However, Cu deficiency appears to reduce production of interferon and tumor necrosis factor by mononuclear cells. Numerous studies have linked low vitamin E and/or Se status to increased susceptibility of dairy cows to intramammary infections (Arthington, 2006; Spears, 2000).

Cu is an important mineral in cattle nutrition and its deficiency causes a decrease in the resistance to diseases, since it diminishes the ability of the polymorphonuclear cells to phagocytize microorganisms (Ward and Spears, 1998). The use of Cu from the diet is greatly inhibited by the consumption of antagonists such as iron (Fe), sulfur (S), molybdenum (Mo), and zinc (Zn) (Ward et al., 1997).

Se is part of the glutathione peroxidase (GSH-Px), an enzyme that prevents damage to cellular membranes and participates in the immune response of animals. This element is an antioxidant and its deficiency is responsible for alterations in the function of the immune system, the metabolism of the thyroid gland of the reproductive system, and the modulation of inflammatory processes. Its deficiency diminishes the phagocytic capacity of polymorphonuclear cells (Lugton, 2004). Phillips and Humphries (1987) and Lepper et al. (1989) have shown that there is a relationship between Paratuberculosis, Fe excess, and Cu deficiency in the soil. In the USA, there are areas rich in Fe and deficient in Se, indicating multiple interactions between the minerals mentioned above and Paratuberculosis (Ward and Perez, 2004).

Lugton (2004) and Downs et al. (2008) have shown a possible link between mycobacterioses and micronutrient deficiency. These authors also analyzed the possible effects of different micronutrients from the bovine diet on cell-mediated immunity and on the pathogenesis of bovine Paratuberculosis and bovine Tuberculosis, respectively.

The objective of this work was to study the relationship between the presence of Paratuberculosis in two beef cattle herds and mineral imbalances in animals, soil, pasture or water.

Materials and Methods

Animals

Bovine older than 3 years old without clinical symptoms of Paratuberculosis were selected from two beef herds, herd 1 (H1) and herd 2 (H2), from two different regions of the Province of Buenos Aires, Argentina. The group from H1 included 45 Shorthorn adult cows whereas that from H2 included 30 Aberdeen Angus and Hereford-crossed adult cows, grazing on natural or implanted pastures with grass and legumes ad-libitum. Additionally, one cow from H1 and one cow from H2 with clinical symptoms of Paratuberculosis (diarrhea and emaciation) were euthanized and feces were taken for culture and the lymph node and ileocecal valve were examined by gross pathology and culture.

Sample collection

Blood and fecal samples were taken from all animals to identify and quantify the disease and the level of minerals, respectively. Pasture and drinking water samples were obtained from different areas for the quantitative analysis of minerals.

Feces

Feces were taken from the rectum of each animal in sterile containers, refrigerated at 4 °C and processed the following day for culture examination of Map.
Blood

Blood was obtained by venipuncture and divided into three aliquots: a) serum for ELISA test, b) serum for quantification of minerals, and c) blood collected in a tube containing heparin to quantify hemoglobin and determine GSH–Px enzyme activity.

Pasture

Pasture samples were taken from each location where animals had previously grazed and then kept at 4 °C until processing for the quantitative analysis of minerals.

Drinking water

Water samples from the watershed and drinking troughs were taken in 500 mL plastic bottles and stored at 4 °C until processing for the quantitative analysis of minerals.

Laboratory examination

Culture examination for the presence of Map

Fecal samples (10 g) were decontaminated in 100 mL of a 0.75% hexadecylpyridinium chloride solution (Sigma, USA) in sterile bi-distilled water, and then stirred for 30 min at room temperature and allowed to settle. An aliquot of 40 mL of the supernatant was kept overnight at room temperature and then centrifuged at 2000 rpm for 15 min. The pellet was re-suspended in 1 mL of phosphate buffer saline (PBS) and this suspension was used as an inoculum for the bacteriological culture. Four drops were placed in tubes containing Herrolds culture medium either alone or supplemented with 2 mg/L of mycobactin J (Allied Monitor, Missouri, USA), and pyruvate (4.1 g/L) and a mixture of antibiotics (100 µg/mL nystatin, 2.0 mg/L amphotericin B, 100 µg/mL vancomycin, and 3.0 mg/mL nalidixic acid). The tubes were incubated at 37 °C for 16 weeks to identify the development of colony forming units (cfu) (Paolicchi et al., 2003). The isolates of Map were processed by IS900 PCR to confirm the presence of Map (Paolicchi et al., 2003).

Serological testing

Sera were analyzed by indirect ELISA, with a slight modification of the technique described by Turnquist et al. (1991). The antigen used was a Paratuberculosis Protoplasmic Antigen (PPA-3 Allied Monitor, USA), a sterile-filtered, lyophilized protoplasmic cell extract of Mycobacterium sp, recommended for use in ELISA screening for the detection of antibodies produced against Map. We used 10 mg/mL of PPA-3 in carbonate buffer (pH 9.6) and this antigen was coated on plates (Immulon 1, USA) in a volume of 100 µL/well and incubated at 4 °C overnight. Sera were pre-treated with M. phlei to increase the specificity of the method. Each serum was diluted with PBS-TG (1:100) and 100 µL (in duplicate) was added to each well of the plate. Plates were incubated for 2 h at 15 °C and a 1:4000 dilution of the bovine antibody anti-IgG peroxidase (Sigma, USA) was added again and incubated for 1.5 h at 15 °C. Finally, 2.2-azino-di-ethyl-benz-thiazoline sulfate (ABTS, Sigma), diluted in citrate buffer (pH 4.0) was added and read in a spectrophotometer (Multiskan Plus, Helsinki, Finland) at a wavelength of 405 nm. Sensitivity and specificity (66% and 99%, respectively) defined by receiver operating characteristics (ROC) analysis using the MEDCALC program were determined previously (Paolicchi et al., 2003). An animal was considered positive seroreactor when the optical density (OD) reached a value of 2.1 units or greater, suspect when the value was between 1.5 to 2.0 units and negative when the value was less than 1.4 units of OD.

Biochemical analysis in blood

The concentration of Se in blood was estimated by the activity of GSH-Px, which was spectrophotometrically measured using cumene hydroperoxide as substrate. Results are expressed as units of enzymatic activity/g of Hb (Berret and Herbet, 1979), and this was quantified with the colorimetric method, which measures the formation of hemoglobin cyanide in blood (Laboratories Wiener, Argentina). Iron, Cu and Zn were measured by atomic absorption spectrophotometry (A.A.S) according to Perkin Elmer Manual Lab (Perkin Elmer, 1982). Briefly, for each of the minerals, an aliquot of serum diluted in distilled water was taken. The sample to analyze Fe was previously treated with acetic acid to precipitate proteins. Then, all the samples to measure Cu, Zn and Fe were read by AAS.

Pasture analysis

Samples were obtained from each herd and approximately 3 kg of pasture previously identified, was cut simulated grazing height. Samples were subsequently mixed, quartered and dried in a forced air oven at 60 °C and then ground in a laboratory mill equipped with a mesh 20 and stored until analysis. In order to quantify the contents of Cu, Zn and Fe by A.A.S., grass samples were previously mixed with a mixture of nitric, sulfuric, and perchloric acid (3/2/v:v:v), and heat treated to total destruction of organic material (Fick et al., 1979). Molybdenum was measured by colorimetric methods (Bingley, 1959).

Water analysis

The content of sulfates (SO₄) was quantified by a turbidimetric method (Cseh et al., 1993). The contents of total dissolved salts were measured by the gravimetric method. The pH of the samples was measured using a pH-meter (Perkin-Elmer, USA). Normal values of minerals in pasture, water and serum were obtained from historical analysis from Biochemical Laboratories from Instituto Nacional Tecnología Agropecuaria (INTA), Buenos Aires province, Argentina, for the last 10 years of mineral studies (S. Cseh, personal communication).
Statistical analysis

The optical density value in ELISA and the concentration of minerals in blood were analyzed by the “t” Student test, with 5% significance.

Results

Map isolation

A total of 15 Map strains were isolated from culture of feces and organs from all animals studied in H1 and H2, 13 Map strains from asymptomatic bovine (n = 75) and 2 Map strains from animals with clinical Paratuberculosis. The 13 Map isolates were recovered from asymptomatic animals of H1 (9 Map strains), and of H2 (4 Map strains) (Table 1). Based on the quantification of Map colonies per tube of the Herrold’s medium, cows were classified as moderate (50 colonies) or high shedders (100 colonies or more), but there was no relation with results in ELISA. Two Map strains were isolated after 7 weeks of incubation on Herrold’s medium from individual samples of feces and lymph nodes, ileocecal valve, and intestine from the two animals with clinical symptoms of Paratuberculosis, observing a high density of Map colonies (more than 100 colonies) on the culture tube. All Map isolates were confirmed positive by PCR in order to identify the IS900 insertion sequence.

ELISA results

From all the asymptomatic animals from H1 and H2 analyzed, 13 (17.3%) were ELISA positive and 29 (38.7%) had suspect results in ELISA test (Table 2). From the animals with positive or suspect ELISA results (n = 42), Map was isolated from 31% of fecal samples cultured (Table 1).

Analysis of micronutrients from animals

From all the animals with positive results in ELISA test, 4 (30.8%) were deficient in Cu, and 7 animals (53.8%) were deficient in Se (Table 2), while all these animals were positive to Map in culture on Herrold’s medium. From the animals with suspect results in ELISA, 14 (48.3%) were deficient in Cu, while 4 (13.8%) were deficient in Se (Table 2). Of the remaining 33 animals with negative results in ELISA, 16 (48.5%) were deficient in Cu and 4 (12.1%) were deficient in Se (Table 2). Additionally, the two animals with symptoms of Paratuberculosis and isolation of Map strains had normal levels of Cu but had the lowest enzymatic activity of GSH-Px of a total of animals studied (data not showed).

Analysis of micronutrients from pasture and water:
The content of Mo and Fe in the pasture was high in both herds: 2.5 ppm and 1.134 ppm in H1, and 2.5 ppm and 2.019 ppm in H2, respectively. The Zn values in the pasture consumed by animals from both herds were either normal or moderately high, whereas the Cu levels were very low in the animals from H1 and normal in the animals from H2, presenting a Cu:Mo relation of 1.5 in H1 and of 5.2 in H2. The amount of total dissolved salts and SO4 in the water samples was normal in both herds (Table 3).

Discussion

In Argentina there are no data about the relationship between the mineral status and the presence of Paratuberculosis in beef cattle. In this study, two cattle herds were studied for status of Paratuberculosis in serum and feces of animals and related to the concentration of oligoelements in blood, in drinking water and in pastures of each farm.

In the asymptomatic animals from H1, 9 Map strains were isolated from 45 individual samples (20.0%), while in the animals from H2 only 4 Map strains were isolated from 30 individual samples (13.3%). In both cases, the animals were asymptomatic to Paratuberculosis but showed positive results in serum by ELISA. The high values of OD observed in ELISA in the serum of animals from both herds were in relation with the positive isolation of Map in feces. Map recovery in feces from animals depends on the type of culture medium used and the decontamination procedure used. Due to the intermittent excretion of Map in feces, it is often necessary to take more than one sample, but in our work the Map isolation from one sampling from 20.0% of the animals without symptoms in H1 was considered a good result, since low bacterial recovery is frequent. The contamination was not present in the culture of feces from both herds, and culture samples from organs of the two euthanized animals with symptoms gave the best and most

Table 1 - Results from Map isolation, ELISA, Se and Cu quantification in blood from beef cattle of two herds with clinical history of Paratuberculosis.

|                        | GSH-Px Low / Marginal | (Se) Normal | Cu Low / Marginal | Normal |
|------------------------|-----------------------|-------------|-------------------|--------|
| Positive animals for Map culture | ELISA positive and suspect n = 13 (100%) | 11 (84.6%) | 2 (15.4%) | 9 (69.2%) | 4 (30.8%) |
| Negative animals for Map culture | ELISA positive /suspect n = 29 (47.5%) | 4 (13.8%) | 25 (86.2%) | 18 (62.0%) | 11 (38.0%) |
|                         | ELISA negative n = 33 (52.2%) | -            | 33 (100%) | 16 (48.5%) | 17 (51.5%) |
| Total of animals        |                       | 15 (20.0%) | 60 (80.0%) | 43 (57.3%) | 32 (42.7%) |
The greatest progression of Paratuberculosis may happen when animals have a very low activity of GSH-Px, as determined in the two animals with clinical symptoms, positive ELISA and Map isolation of feces. These lower levels of enzymatic activity in GSH-Px and Se deficiency in animals could make them less resistant to infectious diseases (Suttle and Jones, 1989) and cattle infected with Map isolates could thus manifest the clinical disease more easily. Selenium deficiency causes changes in the behavior of phagocytic cells of the immune system, diminishing the ability of neutrophils to phagocytize microorganisms (Boyne and Arthur, 1981). As for the activity of the GSH-Px enzyme, the rest of the animals in H1 showed no Se deficiency although animals that were ELISA positive represented the highest percentage of animals with a low activity of GSH-Px. This result could explain the tendency towards the relationship between the presence of disease and the low enzymatic activity (Grasso et al., 1990; Miller et al., 1993; Cao et al., 1993). On the other hand, we did not observe relationship between Map infection and enzymatic activity of GSH-Px in the animals of H2.

We also found low levels of Cu and high values of Mo in the pasture grazed by animals in H1, with a Cu:Mo relationship of 1.5. These results could be the cause of a primary and secondary Cu deficiency in the animals. In contrast, the pasture grazed by animals in H2 showed an adequate relationship (Cu:Mo 5.2). The contents of both Mo and Cu in H2 were increased, so the Cu:Mo ratio indicates that the pasture is not risky for the animal. In contrast, the values of Cu in H1 were very low, which makes Mo concentrations interfere with the metabolism of Cu. The results obtained in animals with deficiencies in Cu levels did not show a major relationships with the animals positives for cultures of feces but did demonstrate an inadequate Cu:Mo ratio in the soil and pastures grazed by animals with Map isolates. In this work, 80% of the ELISA positive animals in H2 had marginal or deficient Cu values, but 100% of the ELISA suspect animals had low values of Cu, possibly due to the excess of Mo with normal Cu levels in pasture grounds by these animals.

Suttle and Jones (1989) demonstrated that high levels of Mo could cause a secondary Cu deficiency and low resis-

---

### Table 2: Results of ELISA of Paratuberculosis, blood mineral levels and contents of hemoglobin in animals.

| ELISA | Cu (µg/mL) | Zn (µg/mL) | Fe (µg/mL) | GSH-Px (UI GSH-Px/g Hemoglobin) | Hemoglobin (mg/100 mL) |
|-------|------------|------------|------------|---------------------------------|-----------------------|
| Normal | 0.5-1.5    | 2-4        | 11-20      | > 30 UI GSH-Px/g Hemoglobin     | 9.5-14 mg/100 mL      |
| Low/Marginal | 0.2-0.5 | < 0.5      | 5-10       | 0.5-5 UI GSH-Px/g Hemoglobin   | 7-10 mg/100 mL       |
| NF     | < 0.2      | > 4.5      | < 5        | < 0.5 UI GSH-Px/g Hemoglobin   | < 7 mg/100 mL        |

| Herd 1 (n=45) | Cu | Zn | Fe | GSH-Px | Hemoglobin |
|---------------|----|----|----|--------|------------|
| (+) = 8       | 0.4±0.08 | 0.3±0.05 | 0.5±0.11 | 0.5±0.08 | 0.6±0.12 |
| (-) = 5       | 0.0±0.08 | 0.3±0.05 | 0.5±0.11 | 0.5±0.11 | 0.6±0.12 |

| Herd 2 (n=30) | Cu | Zn | Fe | GSH-Px | Hemoglobin |
|---------------|----|----|----|--------|------------|
| (+) = 5       | 0.4±0.08 | 0.3±0.05 | 0.5±0.11 | 0.5±0.11 | 0.6±0.12 |
| (-) = 5       | 0.0±0.08 | 0.3±0.05 | 0.5±0.11 | 0.5±0.11 | 0.6±0.12 |

The values show the mean ± standard deviation, range (*), and number of animals (n) in each herd. Reference values (Suttle, personal communication from Biochemical Lab, INTA): Cu: 0.5-1.5 µg/mL; Zn: 62-114 µg/mL; Fe: 69.8-591 µg/mL; Se: > 30 UI GSH-Px/g Hemoglobin; Hemoglobin: 11.6-13.8 mg/100 mL; NF: Not found.
tance of animals to infections. Copper deficiency in cattle can cause the lowest activity of neutrophils to phagocytize microorganisms (Boyne and Arthur, 1981; Ward et al., 1997; Ward and Spears, 1998). An excess of Zn in the diet could reduce Cu absorption and Cu concentration in plasma and the liver of animals, causing disease by diminishing immunity (Bremmer et al., 1976). The function of the immunological system is maintained when there are symptoms of hypocuprosis, but is affected after a long period of deficiency of this mineral (Ward and Spears, 1998). However, in other species used for experimental investigation such as the mouse, Cu deficiency considerably diminishes the immune response immediately (Ward et al., 1997). Copper deficiency due to the presence of antagonists such as Fe, Mo or SO4, and Se deficiency could negatively affect the immunological system of the animals, and the capacity of a response to infection with Map. There are evidences that implicate soil acidification, excess of Fe and Mo and marginal deficiencies in Cu and Se in the disease process in animals. A study of Paratuberculosis prevalence in cattle in Michigan (USA), found that an increase of 0.1 in soil pH was associated with a 5% decrease in the number of ELISA-positive animals with Paratuberculosis. Because cattle frequently ingest soil along with the pasture, it has been suggested that the dietary intake of Fe could allow the clinical expression of Paratuberculosis (Johnson-ifearulundu and Kannene, 1997). On the other hand, the Fe available in the soil has been proposed to enhance the survival or growth of Map in the environment and thereby improve the chances of transmission (Michel and Bastianello, 2000; Riviriego et al., 2000). Fe is involved in oxygen transport and storage and is a cofactor of enzymes like catalases, and a deficiency in Fe decreases phagocyte function, lymphocyte proliferation and natural killer cell activity (Hulsewe et al., 1999). In the rumen, Fe and S form a complex of iron sulfide which interacts with Cu in the abomasum, diminishing its availability and causing secondary Cu deficiency (Cabrera Torres et al., 2009). In this work, we found that the concentration of SO4 in the water in H2 was low, which would not cause interference with Cu metabolism. The results obtained in the present work cannot determine whether deficiencies in microelements play a definitive role or how they cause a predisposition in the relationships of Paratuberculosis in beef cattle. However, we consider that the lowest Se levels found in animals with Map isolation but without clinical symptoms of Paratuberculosis (n = 13 animals) are relevant, because 84.6% of these animals were Se deficient. Moreover, the two animals with evident clinical symptoms of Paratuberculosis, were also found to be severely deficient in Se. This result may indicate a possible relationship between infection with Map and Se-deficiency. Selenium has a role in important biological processes and there are at least 30 known selenium-containing proteins including deiodinases, selenoprotein P and GSH-Px, with a role in preventing cell and membrane damage induced by reactive oxygen intermediates and organic hydroperoxides (Lugton, 2004).
The results of Se deficiencies in animals with Paratuberculosis could indicate that these interactions are capable of showing clinical or subclinical Paratuberculosis in beef cattle. For optimal function of the immunological system, the composition of the diet and the micronutrient required by the animal appear to be important (Engle 2007, Downs et al., 2008) and it is possible that the clinical expression or immunological expression of Paratuberculosis or other mycobacterioses in the animal are more evident when micronutrient deficiencies are present.

Further epidemiological and experimental studies implicating excesses of Fe and deficiencies in Cu and Se and possibly other elements such as Zn, manganese and calcium in the clinical expression of Paratuberculosis and other mycobacterial diseases are necessary. Besides, more research directed at understanding the possible role of these micronutrients and their actual involvement in Paratuberculosis expression should be carried out.

References

Arthington J (2006) Trace mineral nutrition and immune competence in cattle. Proceeding of 17th Annual Florida Ruminant Nutrition Symposium. Gainesville, Florida, p 76.

Barkema H, Hesselink J, McKenna S, Benedictus G, Groenendaal H (2010) Global prevalence and economics of infection with Mycobacterium avium subsp paratuberculosis in ruminants. In: Paratuberculosis: Organism, Disease, Control. Ed: Marcel A. Behr and Desmond M. Collins. CABI, United Kingdom, pp 10-21.

Bartfay W (2003) Selenium status and the pathogenesis of iron overload cardiomyopathies: cause or consequence? Queen’s Health Science 6:40-46.

Berrett S, Herbet C (1979) A semi-quantitative spot test for gluta-thione peroxidase in blood of cattle and sheep for the assessment of biological selenium status. Vet Rec 105:145-146.

Bingley J (1959) Simplified determination of molybdenum in plant material by 4-methyl-1,2-dimercaptobenzene, Dithiol. Journal Agriculture Food Chemical 7:269-270.

Boyne R, Arthur J (1981) Effects of copper and selenium deficiency on neutrophil function in cattle. J Comp Pathol 91:271-276.

Bremmer I, Young B, Mills C (1976) Protective effect of zinc against copper toxicosis in sheep. Br J Nutr 36:551-561.

Cabrera Torres E, Sosa Rubio E, Castellanos Ruelas A, Gutierrez Baeza A, Ramirez Silva J (2009) Comparison of the mineral content in forage and soil of grazing areas in the state of Quintana Roo, Mexico. Veterinaria Mexico 40:167-179.

Cao Y, Maddox J, Mastro A, Scholz R, Hildebrandt G, Reddy C (1992) Selenium deficiency alters the lipoproteinase pathway and mitogenic response in bovine lymphocytes. J Nutr 122:2121-2127.

Cseh S, Ridao M, Yarrar M (1993) Determination of sulfates in water of consumption animal. Proceedings of the IX Annual Meeting AAFLD. Tandil, Argentina, p 47.

Downs S, Durr P, Edwards J, Clifton-Hadley R (2008) Trace micro-nutrients may affect susceptibility to bovine tuberculosis in cattle. Prev Vet Med 87:311-326.

Engle T (2007) The role of trace minerals in immunity and lipid metabolism in cattle. Department of Animal Sciences, Colorado State University, Fort Collins, USA. Available at http://en.engormix.com.

Fick K, Me Dowell L, Miles P, Wilkinson M, Kunk J, Conrad J (1979) Mineral analysis methods for tissue plants and animals. Universidad de Florida, Gainesville.

Grasso P, Scholz R, Erskine R, Eberhart R (1990) Phagocytosis, bactericidal activity and oxidative metabolism of milk neutrophils from dairy cow fed selenium-supplemented and selenium-deficient diets. Am J Vet Res 51:269-274.

Green E, Tizard M, Moss M, Thompson J, Winterbourne D, Mc. Fadden J, Hermon-Taylor J (1990) Sequence an characterization of IS900, an insertion element identified in a human Crohn’s disease isolate of Mycobacterium paratuberculosis. Nucleic Acid Res 17:9063-9073.

Hasanova L, Pavlik I (2006) Economic impact of paratuberculosis in dairy cattle herds: A review. Vet Med 51:193-211.

Hulsewe K, van Acker B, von Meyenfeldt M, Soeters P (1999) Nutritional depletion and dietary manipulation: effects on the immune response. World J Surg 23:536-544.

Johnson-Ifeearulundu Y, Kannene J (1997) Relationship between soil type and Mycobacterium paratuberculosis. J Am Vet Med Assoc 210:1735-1740.

Johnson-Ifeearulundu Y, Kannene J (1999) Distribution and environmental factors for paratuberculosis in dairy cattle herd in Michigan. Am J Vet Res 60:589-596.

Lugton I (2004) Review of possible links between the clinical expression of paratuberculosis and deficiency of macro and micronutrients. Aust Vet J 282:490-496.

Lepper A, Embury D, Anderson D, Lewis V (1989) Effect of altered diets iron intake in Mycobacterium paratuberculosis infected dairy cattle: sequential observations on growth, iron and copper metabolism and development of paratuberculosis. Res Vet Sci 46:289-296.

Manning E, Collins M (2001) Mycobacterium avium subsp paratuberculosis: pathogen, pathogenesis and diagnosis. Review of Scientific Technology 20:133-150.

Michel A, Bastianello S (2000) Paratuberculosis in sheep: an emerging disease in South Africa. Vet Microbiol 77:299-307.

Miller J, Brzezinka-Slebdzinska E, Madsen F (1993) Oxidative stress, antioxidants and animal function. J Dairy Sci 76:2812-2823.

Paolicchi F, Zumarraga M, Gioffre A, Zamorano P, Morsella C, Verna A, Cataldi A, Alito A, Romano M (2003) Application of different methods for the diagnosis of Mycobacterium avium subsp paratuberculosis in a dairy cattle herd in Argentina. Journal of Veterinary Medicine Series “B” 50:20-26.

Perkin Elmer Manual Lab (1982) Analytical methods for atomic absorption spectrophotometry. Norwalk (CO): The Perkins Elmer Corporation, 410 pp.

Phillips M, Humphries W (1987) The effect of dietary molybdenum and iron on copper status and growth in cattle. Journal of Agricultural Sciences 109:315-320.

Riviriego F, Moreno M, Dominguez L (2000) Soil type as a putative risk factor of ovine and caprine paratuberculosis seropositivity in Spain. Prev Vet Med 43:43-51.
Spears J (2000) Micronutrients and immune function in cattle. Proc Nutr Soc 59:587-594. Available in: http://journals.cambridge.org/action.

Stabel J (2010) Immunology of Paratuberculosis infection and disease. In: Paratuberculosis: Organism, Disease, Control. Marcel A. Behr, Desmond M. Collins (eds) CABI, United Kingdom pp 230-243.

Streeter R, Hoffis G, Bech-Nielsen S, Shulaw W, Rings D (1995) Isolation of Mycobacterium paratuberculosis from colostrum and milk of sub clinically infected cows. Am J Vet Res 56:1322-1324.

Suttle N, Jones D (1989) Recent developments in trace elements metabolism and function: trace elements, disease resistance and immune responsiveness in ruminants. J Nutr 119:1055-1061.

Turnquist S, Snider III T, Kreeger J, Miller J, Hagstad H, Olcott B (1991) Serologic evidence of paratuberculosis in Louisiana beef cattle herds as detected by ELISA. Prev Vet Med 11:125-130.

Ward J, Gengelbach G, Spears J (1997) The effects of copper deficiency with or without high dietary iron or molybdenum on immune function of cattle. J An Sci 75:1400-1408.

Ward M, Perez A (2004) Association between soil type and Paratuberculosis in cattle herds. Am J Vet Res 1:10-14.

Ward J, Spears J (1999) The effects of low-copper diets with or without supplemental molybdenum on specific immune responses of stressed cattle. J An Sci 77:230-237.

Whitlock R (1998) Johnes disease (Paratuberculosis): diagnostic tests for individual animal and the herd. In: Junta Anembe (ed), V Cong Int Med Bov, Sitges, Cataluña, pp 69-72.

Wikse S, Herd D, Field R, Holland P (1992) Diagnosis of copper deficiency in cattle. J Am Vet Med Assoc 200:1625-1629.

All the content of the journal, except where otherwise noted, is licensed under a Creative Commons License CC BY-NC.