

Case Report of Recovery from Theileriosis in Five Dairy Cows Naturally Infected with *Theileria orientalis*

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KEY WORDS: *Theileria orientalis*, Hemolytic Anemia, Recovery, Dairy Cows

ABSTRACT

Theileria orientalis is an opportunistic pathogen affecting cattle in most continents, with manifestations ranging from latent infection to severe hemolytic anemia. This report describes the hematological changes during recovery from hemolytic anemia in dairy cows. Oxytetracycline and buparva-

quone were administered. Red blood cell count began to increase a few days after the administration of antibiotics. This may indicate the damaged erythrocytes undergoing phagocytosis more than the newly produced erythrocytes for a short period after the first treatment. Although the hematocrit values normalized by about a month, other hematological values took longer to become normal.

Table 1. Information of 5 dairy cows showing hemolytic anemia by *T. orientalis*

Case	Age(Parity)	Clinical Signs except for Anemia	Calving date from Clinical signs of Theileriosis
1	3Y(Double)	Fever, Inappetence, Lethargy Reduced milk production	-8
2	2Y(Single)	Inappetence, Lethargy	-5
3	3Y(Double)	Inappetence, Lethargy	-11
4	2Y(Single)	Fever, Inappetence, Lethargy	-3
5	4Y(Double)	Inappetence, Lethargy	-7

Table 2. The hematological analysis in the dairy cows on calving date and after recovery

	RBC (M/ μ L)	HCT (%)	MCV (fL)	MCHC (g/dL)
reference range	4.47-9.35	22.5-39.9	40.4-56.4	30.2-33.5
Calving Date	3.72 \pm 1.35	23.76 \pm 2.55	70.18 \pm 22.01	31.22 \pm 3.08
Case 1	4.38	25	57.1	32.8
Case 2	2.44	21.5	88.1	28.4
Case 3	4.72	24.6	52.1	33.7
Case 4	4.97	26.9	54.1	33.8
Case 5	2.09	20.8	99.5	27.4
After Recovery	6.28 \pm 0.76	29.20 \pm 2.40	46.70 \pm 2.65	33.65 \pm 0.70
Case 1	6.34	31.1	49.1	33.1
Case 2	5.56	26.7	48	33
Case 4	7.31	31.4	43	34.4
Case 5	5.91	27.6	46.7	34.1

INTRODUCTION

Theileria orientalis (also termed as *T. buffeli* and *T. sergenti*) belongs to the intracellular hemoprotozoan parasite, which affects erythrocyte destruction.¹ Although infection with *T. orientalis* is sometimes asymptomatic, hemolytic anemia could manifest clinically with pale mucous membranes, jaundice, fever, tachycardia, tachypnea, lethargy, inappetence, reduced milk production, late-term abortion, dystocia, and mortality.^{2,3,4} *T. orientalis* infections cause economic losses in most continents, especially Far East Asian and Oceanian countries.^{4,5,6}

Even though *T. orientalis* has been investigated in many areas, we are not aware of any previous study addressing the hematological changes from the clinical manifestation to recovery. This paper is to report the

Figure 1. Pale vaginal mucous membrane of a dairy cow (case 1) with *T. orientalis*.

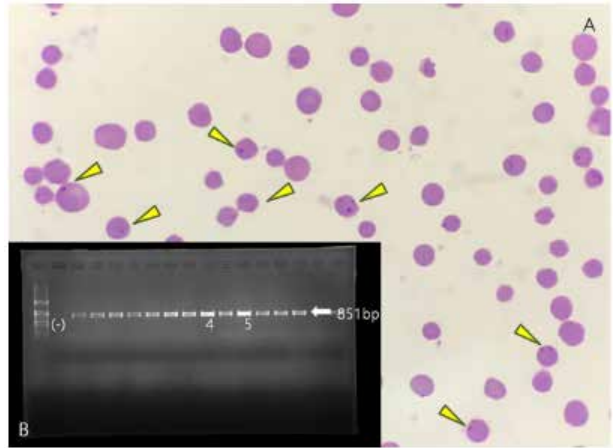


alterations in the hematological values during recovery from hemolytic anemia by *T. orientalis*.

CASE PRESENTATION

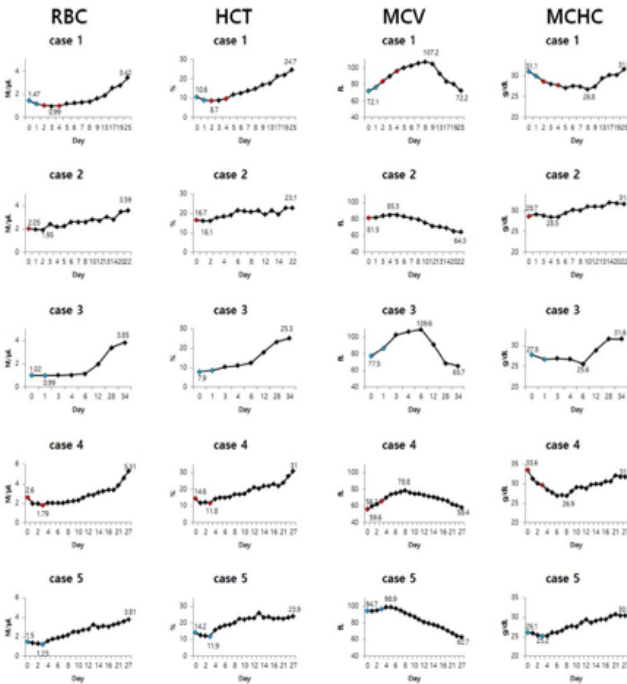
Five Holstein Friesian cows, among dairy cows raised in Cheonan city, South Chungcheong Province, Republic of Korea from 2017 to 2018, showed no appetite and low activity within 11 days postpartum (Table 1). The dairy cows had over 20% HCT right after the partum (Table 2), but HCT decreased rapidly during the transition period. The first and fourth cows had fever. Moreover, the first cow showed reduced milk production. Pale and jaundiced vaginal mucosa was noticed in the cows (Fig. 1). The cows were diagnosed to have theileriosis by *T. orientalis* Ikeda type through direct microscopy, polymerase

Figure 2. *Theileria orientalis* identified through direct microscopy and polymerase chain reaction. Arrowheads: *T. orientalis* in the erythrocytes (case 2), Modified Giemsa stain, 1000x (A); PCR was performed on blood samples (case 4, 5) on the basis of the major piroplasm surface protein (MPSP) gene (B).



chain reaction, and phylogenetic analysis (Fig. 2).

Figure 3. The changes of the hematological results. Blue points and red points separately indicate the usage of oxytetracycline and buparvaquone.



Intramuscular injections of oxytetracycline (20 mg/kg) or buparvaquone (2.5 mg/kg) were administered, following recommended dosage guidelines. The antibiotics were used from clinical onset to a maximum of 5 days, because the sick cows gradually recovered, and these drugs had withholding periods. Hematological values had been investigated since the first treatment began. A hematological analysis was performed with an automated hematology analyzer (Procyte Dx® Hematology Analyzer, Idexx Laboratories Inc., ME, USA) and a commercial chemistry analyzer (Catalyst One™, Idexx Laboratories).

RBC (Red Blood cell Count) and HCT (Hematocrit) levels (mean ± standard deviation) were 1.73 ± 0.61

M/ μ L, and $12.80 \pm 4.51\%$ respectively when the treatment began. RBC and HCT did not normalize rapidly. In fact, the level of RBC kept dropping, and only started rising a few days after the antibiotics were started. At their most severe, RBC and HCT levels were 1.39 ± 0.45 M/ μ L and $11.28 \pm 3.24\%$ respectively. The most serious values involved an RBC count of 0.99 M/ μ L and HCT of 7.9%. MCV (Mean Corpuscular Volume) increased for several days after antibiotic injection, and then decreased. MCHC (Mean corpuscular hemoglobin concentration) likely changed opposite MCV. Total bilirubin was 4.93 ± 2.55 mg/dL at the worst stage. Approximately one month later, the HCT and MCHC values returned into the reference range, but other hematological indices including MCV and mean corpuscular hemoglobin were still abnormal (Fig. 3).

Excluding the 3rd case cow that had been culled, the 4 cows were tested again for the presence of *T. orientalis* with PCR and complete blood count, on 4 December 2017 and 15 October 2018, which corresponded to about 5 months after the onset of treatment. Although all the cows were positive to *T. orientalis*, they had close to reference range complete blood count (CBC) values, and exhibited no clinical signs like fever, lethargy, inappetence, or reduced milk production. The average CBC levels were 6.28 ± 0.76 M/ μ L RBC, $29.20 \pm 2.40\%$ HCT, 46.70 ± 2.65 fL MCV and 33.65 ± 0.70 g/dL MCHC (Table 2).

DISCUSSION

Oxytetracycline differs from buparvaquone in the mode of action. Oxytetracycline inhibits mitochondrial/cytoplasmic protein synthesis in susceptible organisms whereas buparvaquone causes progressive cytoplasmic vacuolation by inhibiting mitochondrial electron transport in *Theileria* spp.^{7,8} The RBC levels increased a few days after the first dose of medication, in spite of the fact that both antibiotics have different pharmacodynamic properties.

The pathogenesis of hemolytic anemia resulting from infection with *T. orientalis*

is not clearly established.⁹ However, it was demonstrated that increased methemoglobin is linked to anemia caused by *T. orientalis*.¹⁰ Increased methemoglobin stimulates the release of superoxide radicals from hemoglobin, which causes oxidative injury to the erythrocyte. *T. orientalis* infection may damage antioxidant defence mechanism of the erythrocyte, similar to malaria-infected erythrocytes with decreased antioxidant molecules, which renders the erythrocyte susceptible to oxidative stress.¹¹ Oxidative-damaged erythrocytes are destroyed by the macrophages of the reticuloendothelial system at an increased rate.^{1,12,13} Considering Case 2, where Single dose was used, this may be the result of the damaged erythrocytes undergoing phagocytosis more than the newly produced erythrocytes for a short period after the first treatment.

This is the first report that examined hematological changes during recovery from hemolytic anemia by *T. orientalis* in dairy cows. The present report focused early recovery patterns. This information may be useful in predicting how dairy cows recover from hemolytic anemia by the intracellular hemoprotozoan parasite.

ACKNOWLEDGEMENTS

This work was conducted with the support of “Research on dairy cow disease and prevention (Project No. PJ01191503)” project at National Institute of Animal Science, Rural Development Administration, Republic of Korea. Furthermore, we would like to express our deepest gratitude to Alannah Boland for her helpful comments.

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