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JIVS JOURNAL OF ISTANBUL VETERINARY SCIENCES

# Emphysematous cystitis in a non-diabetic cat with *staphylococcus* spp.

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**ABSTRACT**

Emphysematous cystitis (EC) is uncommon infection of urinary tract which is characterized by air within the bladder wall and lumen. A 3 years old male cat presented with polyuria/polydipsia, lethargy, and inappropriate urination. Temperature, pulse and respiratory rates were within normal limits on physical examination. Blood work revealed leukocytosis and increased levels of blood urea nitrogen and creatinine. Urinalysis revealed persistent proteinuria in urine dipstick and bacteria under microscopical examination of urine. The presence of air in the bladder were seen in latero-lateral abdominal view. Based on lab work and imaging abnormalities, a diagnosis of emphysematous cystitis was made. The use of diagnostic imaging and lab work are necessary in the diagnosis and treatment of this rare disease, and will be described in this report.

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## Introduction

Emphysematous cystitis (EC) is uncommon bacterial infection of urinary tract which is characterized by air within the bladder wall and lumen. EC cases have been occurred in humans, dogs, cats and a cow (Moon et al., 2014). EC has reported in dogs more than cats. Although no sex predilection has been reported in animals, female patients more likely to be affected by EC in dogs (Moon et al., 2014; Petite et al., 2006).

Etiology is multifactorial and pathogenesis is poorly understood. These infections are often reported with Diabetes mellitus in humans, and animals. Slightly more than half of the cases (15 of 26) had underlying diabetes mellitus in dogs and cats (Moon et al., 2014). Also EC has been diagnosed with primary renal glycosuria (Fanconi's syndrome), urinary tract obstruction, chronic urinary tract infections, neurogenic bladder dysfunction, morphologic abnormalities, iatrogenic (e.g., cystocentesis, catheterization, cystoscopy) and immunosuppression (Moon et al., 2014). Early recognition of EC is important in order to prevent progression of the infection to emphysematous pyelonephritis and urosepsis (Fabbi et al., 2016).

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## Case

A 3 years old male cat presented with polyuria, polydipsia, lethargy, and inappropriate urination. On the initial physical examination, the patient was dehydrated. Temperature, pulse and respiratory rates were within normal limits.

**Table 1**: Routine blood tests on the day of presentation and after 40 days of treatment

| | Normal Values | Before Treatment | After Treatment |
|---|---|---|---|
| RBC (1x $10^6$µL) | 6-10 | 8.3 | 6.8 |
| HGB (g/dl) | 9.5-15 | 11.4 | 9.2 |
| HCT (%) | 29-45 | 34 | 30 |
| WBC (x$10^3$µL) | 6-19.5 | 21 | 10.1 |
| PLT (1x$10^3$µL) | 150-600 | 55 | 159 |
| MCV | 41-54 | 41 | 44 |
| MCH (pg) | 13-17.5 | 14 | 14 |
| MCHC (%) | 31-36 | 34 | 31 |
| ALT (IU/L) | 28-76 | 54 | 48 |
| AST (IU/L) | 5-55 | 80 | 21 |
| BUN (mg/dL) | 15-34 | 324 | 24 |
| CRE (mg/dL) | 0.8-2.3 | 10.4 | 1 |
| GLU (mg/dL) | 70-150 | 103 | 119 |

CRE = Creatinin, GLU = Glucose

Initial diagnostics included a complete blood count, serum biochemical profile, urinalysis, and abdominal radiographs. Blood work revealed leukocytosis and increased levels of blood urea nitrogen and creatinine. Glucose was within normal levels (Table 1). Urinalysis, obtained by cystocentesis, and revealed proteinuria (3+) and hemoglobinuria (3+) in urine dipstick. A large amount of erythrocytes and bacteria, 8-10 leukocytes/high-power field (hpf), 3-5 epithelial cells/hpf and 1-2 struvite crystals/hpf were detected in the microscopic examination. A urine sample was submitted for culture and antibiotic sensitivity.

In abdominal radiograph a huge amount of gas detected in the middle of the urinary bladder lumen (Figure 1a). An iodine-based, water soluble contrast media was given to the cat by urinary catheter which can be seen at the caudo-dorsal of the bladder. Multiple round gas opacities within contrast media in the bladder and urethra was detected (Figure 1b).

Based on lab work and imaging abnormalities, a diagnosis of emphysematous cystitis (EC) was made. Initial treatment started with Enrofloxacin (5-10 mg/kg, IM BID) and bladder drainage.

Urine culture was positive for *Staphylococcus spp*, and found resistant to Ampicillin, Amoxicillin/clavunolic acid, Efoperazon, Ciprofloxacin, Cefovecin, Gentamicin, Marbofloxacin. Sensitive to Oxytetracycline, Enrofloxacin, and Streptomycin. According to these results initial treatment continued. After 10 days of the treatment, the patients' complications resolved and after 20 days there was a little gas in the bladder on x-rays (Figure 2a). 40 days after the diagnosis, a routine blood analysis, urinary test and radiography were repeated (Table 1). Leukocyte count, blood urea and creatinine levels were decreased and there was a resolution of the gas in the x-rays (Figure 2b).

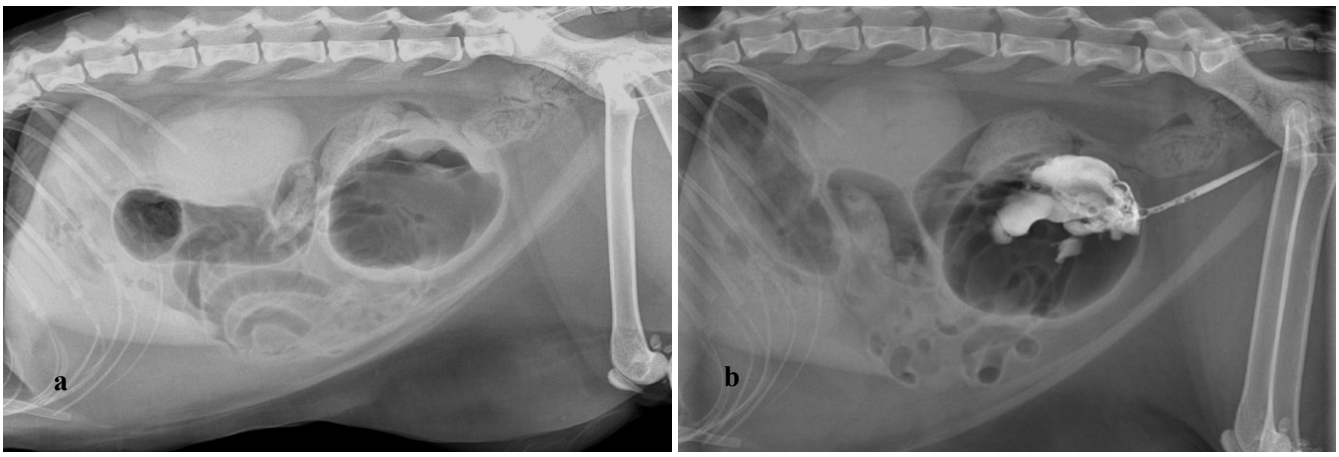


**Figure 1: a**- abdominal laterolateral view of the cat, **b**- positive contrast abdominal laterolateral view with an iodine-based, water soluble contrast media.

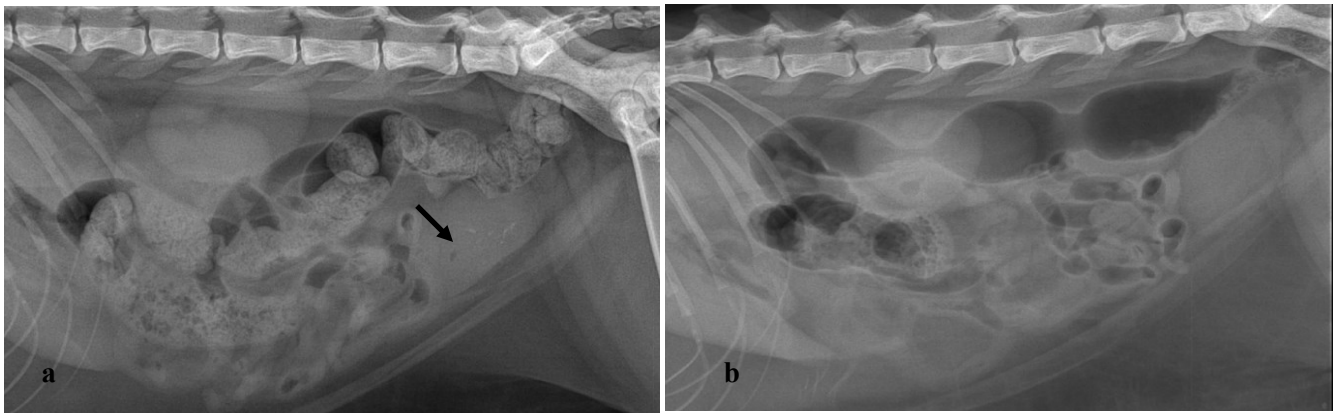


**Figure 2: a**- abdominal view after 20 days of the treatment, arrow: small amount of gas in the bladder. **b**-after 40 days of the treatment.

## Discussion

The pathogenesis is not completely understood in EC cases, it has been suggested that the high tissue glucose levels and immunodeficiency can led to infection (Moon et al., 2014). It is caused by fermentation of an underlying bacterial infection mostly by *Escherichia coli* (60%) and *Klebsiella pneumoniae* (10-20%). Other microorganisms isolated in human EC cases include *Aerobacter, Enterobacter, Proteus, Citrobacter, Staphylococcus, Streptococcus, Nocardia, Clostridium* and *Candida* but these cases are rare (Amano and Shimizu, 2014; Quint et al., 1992). *Clostridium* spp., *Escherichia coli*, *Klebsiella* spp., *Proteus* spp., *Aerobacter aerogenes* have been isolated in dogs and cats (Moon et al., 2014; Lobetti and Goldin, 1998). Petite et al. (2006), reviewed the medical records of dogs with ultrasonographic diagnosis of EC and isolated *Proteus mirabilis* in all dogs. This case exhibits *Staphylococcus* spp. which is rare isolated bacteria in EC cases.

It has been suggested that high glucose consumption by the microorganisms produce carbon dioxide and hydrogen thorough the natural fermentation process. In non-diabetic patients increased levels of urinary albumin, lactose, or tissue proteins can result in gas formation. Also bacterial endotoxin release contribute to the process (Bos et al., 2014; Fabbi et al., 2016).

The clinical signs of EC disease are often characterized by non-specific and very variable clinical symptoms, with little or no diagnostic clues. The most common ones are abdominal pain and hematuria which was not detected in our case (Moon et al., 2014; Amano and Shimizu, 2014). However other symptoms like dysuria and urinary frequency occur in 50% of human patients that compatible with the cat symptoms (Amano and Shimizu, 2014).

The most common imaging method for obtaining a definitive diagnosis is abdominal radiography (Amano and Shimizu, 2014). However the sensitivity is low because it is sometimes difficult to distinguish from intestinal gases (Fabbi et al., 2016). Although radiographic classification of EC has not been established for animals, we used the human classification system in our case. According to this; grade 1 is characterized by gas in the urinary bladder wall. It is classified as grade 2, when there is a thickening and irregularity in the wall. Gas both in the bladder lumen and wall is classified as grade 3 (Moon et al., 2014; Petite et al., 2006). The cat presented in this case classified as grade 3.

Ultrasonography can be useful for detection of early cases. Ultrasonographic findings include a hyperechoic stripe with reverberation artifact (Moon et al., 2014; Petite et al., 2006). However a large amount of gas has been identified in the bladder on radiographs taken at initial presentation in our case so it not useful to make an ultrasonography. Also positive contrast cystogram was performed to the cat and multiple gas opacities was detected in the urethra via this method. Maybe it'll be useful in EC cases.

Management of this disease depends on it severity and mostly consists of antibiotics, bladder drainage and glycemic control (Petite et al., 2006). The prognosis of EC is good when appropriate antibiotic therapy is given (Fabbi et al., 2016). Duration of treatment is not well established (Petite et al., 2006). Grupper et al. (2007) found that a median length of 10 days for treatment is effective. Although after 10 days there was a resolution of the complications, the gas in the urinary bladder resolved after 40 days in our case.

No significant clinical signs strongly suggestive of EC have been reported to the authors knowledge; hence EC can easily be missed. A delayed diagnosis can result an over helming infection of the upper urinary tract and develop emphysematous pyelonephritis that difficult to treat and able to cause death (Amano and Shimizu, 2014). More awareness of the condition is important for the future diagnosis and treatment.

## References

Amano, M., & Shimizu, T. (2014). Emphysematous cystitis: a review of the literature. *Internal medicine, 53*(2), 79-82.

Bos, D., Patal, P., & Ditullio, S. (2014). Emphysematous cystitis: An atypical multi-organism presentation. *Canadian urological association journal, 8*(3-4), 210-212.

Fabbi, M., Manfredi, S., Bianchi, E., Gnudi, G., Miduri, F., & Volta A. (2016). Emphysematous pyelitis and cystitis associated with vesicoureteral reflux in a diabetic dog. *Canadian veterinary journal. 57*(4): 382–386.

Grupper, M., Kravtsov, A., & Potasman, I. (2007). Emphysematous cystitis: illustrative case report and review of the literature. *Medicine, 86*(1), 47-53.

Lobetti, R. G., & Goldin, J. P. (1998). Emphysematous cystitis and bladder trigone diverticulum in a dog. *Journal of small animal practice, 39*(3), 144-147.

Moon, R., Biller, D. S., & Smee, N. M. (2014). Emphysematous cystitis and pyelonephritis in a nondiabetic dog and a diabetic cat. *Journal of the American animal hospital association 50*(2), 124-129.

Petite, A., Busoni, V., Heinen, M. P., Billen, F., & Snaps, F. (2006). Radiographic and ultrasonographic findings of emphysematous cystitis in four nondiabetic female dogs. *Veterinary Radiology & Ultrasound*, *47*(1), 90-93.

Quint, H. J., Drach, G. W., Rappaport, W. D., & Hoffmann, C. J. (1992). Emphysematous cystitis - a Review of the spectrum of disease. *Journal of urology, 147*(1), 134-137.

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# Effect of milk replacer added *Macleaya cordota* extract calf body weight and health*

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**ABSTRACT**

This experiment was conducted on 40 newborn Holstein female calves comprising two experimental groups, with 20 calves in each group to investigate the effects of *Macleaya cordata* extract (MCE), on calf body weight and health which added to milk replacer. In both group calves fed on the same terms. Differently, 10 g /head/ day dosages MCE added to the milk replacer of the trial group from day 3 to day 25. Afterward, calves continued to feed until 3 months old and weighed on the birth, 15th, 40th, 60th and 90th days. At the end of the experiment, there was no significant difference at the weights on the birth and 15th days. However, on the 40th, 60th and 90th days weighing in favor of the control group, a significant difference was found ($P<0.05$). At the end of the experiment in favor of the trial group, there was a significant difference with clinical pneumonia and aspiration pneumonia ($P<0.05$). The results obtained in the research; MCE which added the milk replacer as a feed additive indicated no positive effect on calf body weight. From a health point of view, MCE has reduced the incidence of respiratory diseases in particular.

**Keywords:** calf, phytobiotic, performance, respiratory disease

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## Introduction

During the past decades, there were renewed interest in developing natural compounds and understanding their target specificity for drug development instead of antibiotics. The recent demand for reduction of antibiotic use in animal production and the ban on their use as feed additives in the European Union (Regulation 1831/2003/ EC), has led to the development and evaluation of alternatives for improving animal performance and health status. Plant-derived natural bioactive compounds have a large variety of active ingredients and thus represent one of the most promising alternatives to antibiotics. However, the results from these studies have largely been inconsistent and the mechanisms are still inconclusive with limited resources (Liu et al. 2014). For example; gradual or suddenly weaned calves were fed essential oils blend (EOB) as feed additives, results showed that EOB had the potential to improve growth

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performance and to decrease the effect of stressful events in suckling calves (Jeshari et al., 2016). In contrast to this, oregano oil extract did not affect performance and blood parameters in lambs (Ünal and Kocabağlı, 2014). Macleaya *cordata extract* (MCE) is of great scientific and practical interest to researchers, due to its antimicrobial and anti-inflammatory responses within experimental animals (Newton et al., 2002). This extract has a natural appetite enhancer effect on swine, cattle, poultry and even aquaculture (Vieira et al., 2008). MCE is a natural plant-derived (Sangrovit®) supplement, standardized the weight to 1.5% as used in animal nutrition, containing manybenzophenanthidrine alkaloid compounds, the most abundant of which is sanguinarine (Dvořak et al., 2006). Antimicrobial (Namkung et al., 2004; Walker et al., 1990), antifungal, immunomodulator (Chaturvedi et al., 1997) and anti-inflammatory (Namkung et al., 2004; Yui et al., 1993) effects were determined in different studies.

Many studies in poultry (Vieira et al., 2008; Matulka et al., 2018), beef cattle (Aguilar Hernández et al., 2016) and pigs (Kantas et al., 2015; Liu et al., 2016; Zhao, et al., 2017) has been reported positive effect of sanguinarine-like alkaloids on performance. A Study conducted especially in piglet extract of MCE has been reported as an alternative to antibiotics with diarrhea prevention and a positive effect on immunity (Liu et al., 2016). There are no reported studies on the use of the MCE as feed additives in Turkey until today. Thus, the aim of this study was to examine the effect of *Macleaya cordata* extract on calf body weight and health which added to milk replacer.

## Materials and methods

This research project was conducted at Uluova Dairy Farm (in Ezine, Çanakkale/Turkey) from September 2017 to January 2018; all procedures were approved by Çanakkale 18 Mart University Animal Experiments Local Ethics Committee (decision number: 2017 / 08-01). In the study, 40 female Holstein calves were used which has born in the date between September-October 2017 and selected calves considering the weights and divided into two groups for the experiment.

The first three days, calves were fed with colostrum , then they transferred to the individual boxes and fed with milk and milk replacer mixture (mixture content 50 g of commercial milk replacer with 2 liters of milk) and fed with 3 times a day. Calves had free access to calf starter feed + roughage + corn flakes mixture and fresh water all times during the trial. A total of 2.5 kg of this mixture formed by 200 g chopped alfalfa hay, 500 g corn flakes and, 1800 g calf starter feed. This mixture was prepared and given every day to the animals in front of their individual boxes. We added 10 g/ head/ day dosage MCE (Sangrovit® CS, Phytobiotics Futterzusatztoffe GmbH, Rosengasse9, 65343 Eltville, Germany) in milk and milk replacer mixture to the trial group. This process adds it into the morning feeding. In conclusion; Macleaya cordata extract added for 3 weeks to the trial group.

The label information of the milk replacer which is used in research (Halavit 440, Maabarot Products Ltd., Post Maabarot 4023000, Israel) contains Crude protein 23%, Casein 16%, Crude Fat 15%, Crude Ash 7%, Crude Cellulose 0.05%, Sodium 0.5%. This milk replacer mixed in 2 liters milk with 50 g dosage milk replacer.

During the research, we recorded the health parameters and weight of animals in the control and trial group. Body weights were taken on the day birth, 15th, 40th, 60th and 90th. Farm veterinarians made the diagnosis of the diseases in the control and trial group.

**Table 1.** Average nutrient values of milk which used for calf feeding during the trial

| **Nutrients** | **%** |
|---|---|
| Dry Matter | 12.06 |
| Fat | 3.40 |
| Protein | 3.01 |
| Fat/Protein | 1.13 |
| MUN | 14.33 |
| Urea | 30.69 |
| Fat-Free Dry Matter | 8.47 |
| Casein | 2.24 |
| Lactose | 4.90 |
| Acetone | 0.11 |

**Statistical analysis:** Independent samples T-test were used for the difference between control and trial group importance in terms of weights on birth, 15th day, 40th day, 60th day and 90th day. We used the Chi -square test to compare the health data of the control and trial group. It was used SPSS 13.0 statistical calculation program. Statistical significance was defined according to $P<0.05$.

## Results

It has provided the standard error measurements, significance levels and detected average calf weights of trial and control group on 15th, 40th, 60th and 90th days on Table 2. There was no significant difference in the detected weight gains on birth and 15th days (P>0.05). But on the 40th, 60th and 90th days weighing, the difference was significant (P<0.05), (Table 2).

**Table 2.** Calf weights of trial and control group during the experiment (n=20)

| Day | *Macleaya cordata* | Control | *P* Values |
|---|---|---|---|
| 0 | 36.10 ± 1.24 | 38.90 ± 1.24 | 0.119 |
| 15 | 40.55 ± 1.16 | 43.41 ± 1.28 | 0.107 |
| 40 | 53.63 $^{b}$ ± 1.01 | 58.18$^{a}$ ± 1.12 | 0.004 |
| 60 | 68.98 $^{b}$ ± 1.23 | 73.84 $^{a}$ ± 1.04 | 0.004 |
| 90 | 83.30$^{b}$ ± 1.34 | 98.82$^{a}$ ± 0.54 | 0.000 |

a-b : The average values with different letters on the same line have significantly difference.

With the purpose to detect the MCE effect on calf health, we recorded the animals which have aspiration pneumonia, clinical pneumonia, chronic pneumonia, feed induced diarrhea and calf diarrhea in the trial and control group. A number of the sick calves in the trial and control group have shown on Table 3 according to disease type.

**Table 3.** Number of sick calves in the experimental and control groups according to disease type

| | *Macleaya cordata* (n=20) | | Control (n=20) | | Chi-Square *P* Value |
|---|---|---|---|---|---|
| **Disease** | n | % | n | % | |
| Aspiration Pneumonia | 0$^{b}$ | 0.0 | 4$^{a}$ | 2.0 | 4.444 (0.035) |
| Clinical Pneumonia | 9$^{b}$ | 45.0 | 17$^{a}$ | 85.0 | 7.033 (0.008) |
| Chronic Pneumonia | 3 | 15.0 | 3 | 15.0 | 0.000 (1.000) |
| Feed Induced Diarrhea | 7 | 35.0 | 11 | 55.0 | 1.616 (0.204) |
| Calf Diarrhea | 1 | 5.0 | 4 | 20.0 | 2.057 (0.151) |

a-b: The average values with different letters on the same line have significantly difference.

We recorded diseases, treatments, date of onset of disease and the date of treatment in trial and control group calves. SPSS cross- table rates of detected calf diseases in trial and control groups have shown in Table 4.

**Table 4.** SPSS cross-table rates of detected calves diseases in trial and control groups

| **Disease** | **Group** | **Status** | |
|---|---|---|---|
| | | Patient (%) | Healthy (%) |
| Clinical Pneumonia | *Macleaya cordata* | 22.5 | 27.5 |
| | Control | 42.5 | 7.5 |
| Feed Induced Diarrhea | *Macleaya cordata* | 17.5 | 32.5 |
| | Control | 27.5 | 22.5 |
| Chronic Pneumonia | *Macleaya cordata* | 7.5 | 42.5 |
| | Control | 7.5 | 42.5 |

In order to determine the risk of developing diseases whatever connect to MCE application, as a result of the chi-square test, the difference between the groups was founded significant in clinical pneumonia and aspiration pneumonia (P<0.05). However, there was no significant difference between the trial and control group which calves have feed induced diarrhea, chronic pneumonia and calf diarrhea (P>0.05).

## Discussion

It has known the fact that using phytobiotics in animal nutrition, has positive effects on various performance parameters. In this study, there are no positive effect on 40th, 60th and 90th day weighings of calves weight despite applied MCE recommended dosages for 21 days (Table 1). Calsamiglia et al. (2007) shown that adding essential oil to milk replacer, decreases feed intake due to the flavor problems in the feed. In this study, due to the taste of MCE, it decreases of milk replacer intake observed. Therefore, the results obtained in this study in line with Calsamiglia et al. (2007)'s study results.

In many studies that applied different essential oils to ruminants, not determines significant statistical differences and was not observed positive results in terms of performance. In the study conducted by Santos et al. (2015) with applied essential oils in the milk replacer, not reported any change in feed consumption and calf weight. Vakili et al. (2013) applied 5 g/ head/ day phytobiotic containing cinnamon and thyme to Holstein calves who have an average weight of 217 kg and reported no significant effect on daily weight gain, feed consumption and feed conversation ratio. The results obtained from these studies support our findings.

There are also studies reporting that phytogenic ingredients have a positive effect on ruminants (Ruben et al., 2015). A study carried out by Estrada Angulo et al. (2016) in sheep during the finishing period on the use of MCE; not detected significant statistical differences in daily weight gains, despite reported that trial group which applied MCE provide 11% more weight gain than the control group.

Results of the studies on animal health of feed additives with phytogenic ingredients show differences. Bampidis et al. (2006) applied feed additives to black pied breed calves which have diarrhea. They applied oregano essential oil with dosage a 10 mg/ day/ kg BW to one group and applied neomycin sulfate as antibiotics with dosage 10 mg/ day/ kg BW. There was no difference in diarrheal days, diarrhea severity and mortality rate between thyme essential oil and antibiotic applied group. Unlu et al. (2011) detected that addition of 250 mg oregano and garlic essential oils in to the full fat milk per day, did not cause any positive results on fecal coliform, Escherichia coli and Lactobacillus spp. numbers, fecal score and diarrhea treatment days. Liu et al. (2016) reported that no significant difference in performance when compared control group to the group which applied MCE for 90 days to weaned piglets (P>0.05). But in the same study, the group with *Macleaya cordata* significantly increased volumes of ZO-1 and claudin-1, particularly in comparison with the pigs in the control group. Therefore researchers reported that MCE increases intestinal barrier function and can be used as an alternative to antibiotics for piglets (Liu et al., 2016). When we looked to the health results in our trial, in terms of aspiration and clinical pneumonia observed significant results in favor of MCE treated group (P<0.05). However, there was no significant difference in feed induced diarrhea, chronic pneumonia and calf diarrhea (P>0.05). These positive results weldable antimicrobial effect of the extract with based on Liu et al. (2016) reviews.

The results obtained in the study; MCE which use as a feed additive and added to milk replacer, has shown no positive effect on calf weight gain. When an evaluation is made in terms of health, MCE especially decreased the incidence of respiratory diseases. For this reason, MCE recommended for use as a preservative especially for the respiratory disease for the first stage of life.

In conclusion, MCE has the potential to decrease the incidence of respiratory diseases in calves. Future research is needed to determine the benefit of feeding MCE if it can be used as an alternative to antibiotics for suckling calves.

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## References

Aguilar Hernández, J. A., Urías Estrada, J. D,. López Soto, M. A., Barreras, A., Plascencia, A., & Montaño, M. (2016). Evaluation of isoquinoline alkaloid supplementation levels on ruminal fermentation, characteristics of digestion, and microbial protein synthesis in steers fed a high-energy diet. *Journal of Animal Sciences, 94*(1), 267-274.

Bampidis, V. A., Christodoulou, V., Florou – Paneri, P., & Christaki, E. (2006). Effect of dried oregano leaves versus neomycin in treating new born calves with colibacillosis. *Journal of Veterinary Medicine, 53*(3), 154-156.

Calsamiglia, S., Busquest, M., Cadozo, P., Castikkejos, L., & Ferret, A. (2007). Essential oils as modifiers of rumen microbial fermentation: a review. *Journal of Dairy Science*, *90*(6), 2580-2595.

Chaturvedi, M. M., Kumar, A., Darnay, B., Chainy, G. B. N., Agarwal, S., & Aggarwal, B. B. (1997). Sanguinarine (Pseudochelerythrine) is a potent inhibitor of NF-κB activation, IκBα phosphorylation, and degradation. *Journal of Biological Chemistry, 272* (48), 30129-30134.

Dvŏřak, Z., Sovadinov´a, I., Bl´aha, L., Giesy, J. P., & Ulrichov´a, J. (2006). Quaternary benzo[c] phenathridine alkaloids sanguinarine and chelerythrine do not affect transcriptional activity of aryl hydrocarbon receptor: analyses in rat hepatoma cell line H4IIE.luc. *Food and Chemical Toxicology, 44* (9), 1466-1473.

Estrada Angulo, A., Aguilar Hernández, A., Osuna Pérez, M., Núñez Benítez, V. H., Castro Pérez, B. I., & Silva Hidalgo, G. (2016). Influence of quaternary benzophenantridine and protopine alkaloids on growth performance, dietary energy, carcass traits,

visceral mass, and rumen health in finishing ewes under conditions of severe temperature – humidity index. *Asian-Australasian Journal of Animal Sciences, 29*(5), 652-658.

Jeshari, M., Riasi, A., Mahdavi, A. H., Khorvash, M., & Ahmadi, F. (2016). Effect of essential oils and distillation residues blends on growth performance and blood metabolites of Holstein calves weaned gradually or abruptly. *Livestock Science,* 185, 117-122.

Kantas, D., Papatsiros, V. G., Tassis, P. D., Athanasiou, L. V., & Tzika, E. D. (2015). The effect of a natural feed additive (*Macleaya cordata*), containing sanguinarine, on the performance and health status of weaning pigs. *Animal Science Journal, 86(*1), 92-98.

Khadem, A., Soler, L., Everaert, N., & Niewold, T. A. (2014). Growth promotion in broilers by both oxytetracycline and Macleaya cordata extract is based on their anti-inflammatory properties. *British Journal of Nutrition, 112*(7), 1-9.

Liu, G., Guan, G., Jun, F., Martínez, Y., Chen, S., & Bin, P. (2016). Macleaya cordata extract decreased diarrhea score and enhanced intestinal barrier function in growing piglets. BioMedical Research International, 7 pages.

Liu, Y., Song, M., Che, T. M., Bravo, D., Maddox, C. W., & Pettigrew, J. E. (2014). Effects of capsicum oleoresin, garlic botanical, and turmeric oleoresin on gene expression profile of ileal mucosa in weaned pigs. *Journal of Animal Science, 92*(8), 3426-3440.

Matulka, R. A., von Alvensleben, S., Morlacchini, M. & Fusconi, G. (2018). Tolerance study for standardized *Macleaya cordata* extract added to chicken layer diet. *Open Journal of Animal Sciences*, *8*(1), 104-117.

Namkung, H., Li, M., Gong, J., Yu, H. & Cottrill, M. (2004). Impact of feeding blends of organic acids and herbal extracts on growth performance, gut microbiota and digestive function in newly weaned pigs. *Canadian Journal of Animal Science, 84*(4), 697 -704.

Newton, S.M., Lau, C., Gurcha, S.S., Besra, G.S. & Wright, C.W. (2002). The evaluation of forty-three plant species for in vitro antimycobacterial activities: Isolation of active constituents from Psoralea corylifolia and Sanguinaria canadensis. *Journal of Ethnopharmacology, 79*(1), 57-67.

Ruben, B., Billy, J., Cervantes, I.R., Aljandro Plascencia, J., Alejandro, C. & Marco, A. (2015). Supplementary levels of Macleaya cordata plant extract Sangrovit RS on feedlot performance and carcass traits of finishing bullocks. American Dairy Science Association Ruminant Nutrition Beef I, Poster M 323.

Santos, F. H. R., De Paula, M. R., Lezier, D., Silva, J. T., Santos, G., & Bittar, C. M. M. (2015). Essential oils for dairy calves: effects on performance, scours, rumen fermentation and intestinal fauna. *Animal Consortium, 9*(6), 958-965.

Ünal, A., & Kocabağlı, N. (2014). Effect of different dosages of oregano oil on performance and some blood parameters in lambs. *Veterinary journal of Ankara university*, *61*(3), 199-204.

Ünlü, H. B., & Erkek, R. (2011). Effects of oregano and garlic essential oil on performance of calves, and some parameters of faeces and blood. *PhD thesis,* Ege University, Izmir, Turkey.

Vakili, A. R., Khorrami, B., Danesh Mesgaran, M., & Parand, E. (2013). The effects of thyme and cinnamon essential oils on performance, rumen fermentation and blood metabolites in Holstein calves consuming high concentrate diet. *Asian Australasian Journal of Animal Science, 26*(7), 935 – 944.

Vieira, S. L., Oyarzabal, O. A., Freitas, D. M., Berres, J., Peña, J. E. M., Torres, C. A., & Coneglian, J. L. B. (2008). Performance of broilers fed diets supplemented with sanguinarine-like alkaloids and organic acids. *Journal of Applied Poultry Research, 17*(1), 128–133.

Zhao, L., Von Alvensleben, S., Fusconi, G., & Morlachini, M. (2017). Safety evaluation of a standardized Macleaya cordota extract in a ninety day feeding study in weaned piglets. Journal *of Animal Sciences, 7*, 213-231.

JIVS JOURNAL OF ISTANBUL VETERINARY SCIENCES

# Intestinal parasitic infection in wild animals of a zoological garden in Alborz, Iran

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**ABSTRACT**

Here we studied the intestinal parasites in animals at a zoological garden in Alborz, Iran. A total of 83 fecal samples from various captive wild animals, consisting of 21 different species were collected randomly and analyzed for the presence of the different stages of parasites by direct smear preparation and zinc sulfate flotation followed by Ziel-Neelsen staining method. The examined animals in this study consist of 7 species of carnivores (26 samples), 10 species of herbivores (46 samples), and 4 species of different groups of birds (11 samples). Examination of fecal samples revealed that 22 (26.50%) of animals, that belonging to 7 animal species, were infected with different intestinal parasites. Among gastrointestinal parasites positive captive wild animals 18 samples (21.68 %) belong to herbivores and 4 samples (4.81 %) to Aves. Among captive wild animals the prevalence of parasites was higher in herbivores (21.68%) followed by Aves (4.81). Results indicated that out of 22 animal samples that parasites were encountered, 14 (16.86%) were infected with helminths (Trichuris spp., *Nematodirus* spp., *Ascaridia galli* and some unknown Nematodes eggs) and 8 (9.63%) were infected with protozoa (Oocysts of *Eimeria* sp.). In the conclusion, it could be resulted that there is a need of control measures against the spread of infectious parasitic diseases among animals within the zoo.

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## Introduction

Zoos are places where a great number of valuable animal species are put together taken out of their natural habitats (Panayotova-Pencheva, 2013) and these Zoological collections are represented with exotic animal species which would never or rarely meet certain parasites amongst natural circumstances. Keepers may play the role of mechanical vector of parasites and improper feeding systems can encourage the parasite infection. Parasite control, due to the specific nature of zoological collection, is one of the pillars of preventive health care of zoo animals (Kvapil et al., 2017).

Browsing animals forced to graze or pick up food from the ground are at a greater risk of infection with geohelminths. Serious cases of parasite infection may then arise if a parasite is introduced in a new environment where fully susceptible suitable hosts are available (Borgsteede, 1996).The same situation

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applies to wild animals in captivity, which are normally kept in the same enclosure for prolonged periods of time, with space limitations and under constant stress, leading to immunosuppression and consequent higher susceptibility to parasitic infection (Mir et al., 2016).

In addition, as zoos are institutions which are opened to the public, close contact with humans ,which would not happen in the natural environment of the captive animals, rises the risk of development of anthropozoonosis (Panayotova-Pencheva, 2013). This significantly augments the risk of spreading the parasitic zoonoses posing a threat to the health of the animals themselves, the personal of the zoos and of course to the visitors (Panayotova-Pencheva, 2013). Some studies have revealed that gastrointestinal parasites of wild animals in captivity include zoonotic species to humans and raise public health concerns (Adejinmi & Ayinmode, 2008; Ajibade et al., 2010; Akinboye et al., 2010; Levecke et al., 2007; Opara et al., 2010; Otegbade & Morenikeji, 2014).

Regular coprological examinations seem to be an efficient tool to control the parasite burden in most of the animals, especially in wild animals that were kept in captivity conditions. By using a system of preventive and therapeutic means, parasitic infections in zoos are reduced to a minimum ,but the absence of the natural biological balance due to the artificial amassment of various animals in one and the same location can also result in development of parasites in such animals which normally are not specific host to them (Panayotova-Pencheva, 2013).

Previously, we carried out a survey to establish the gastrointestinal parasites profile in animals at the Eram zoological garden in Tehran, Iran, that according to our study, examination of fecal samples revealed that 24 (16.7 %) of animals were infected with intestinal parasites. Out of 24 parasites encountered, 10 (41.6 %) and 14 (58.4 %) were helminths and protozoa respectively. Cryptosporidium spp. infection was detected in 6 (4.1 %) of samples (Nasiri et al., 2017).

In a recently published study (Kiani et al., 2018), one hundred fresh fecal samples were collected from 35 species of animal lived in Eram park zoo, Tehran, Central Iran during Oct 2015 to Jun 2015. 65.7% (23/35) of zoo animal species were infected with intestinal parasites. The superfamily *Trichostrongyloidea* (6/16) and *Strongylus* sp. (16/4) were the most prevalent helminthic infections, while *Blastocystis* sp. (6/14), *Entamoeba cyst* (3/14) and Eimeria sp. (3/14) were the common protozoan parasites. For the first time, *Bivitellobilharzia nairi* egg was identified an elephant at Iran. They indicated that intestinal parasitic infections were apparently circulating among animals of the Eram park zoo (Kiani et al., 2018).

To have a better understanding about the prevalence of the parasites those affecting zoo animals, the present study was carried out to establish the gastrointestinal parasite profile of the captive wild animals of a central zoological garden in Alborz, Iran.

## Materials and Methods

**Animals Sample Collection and Study site:** The zoological garden of this study is one of the zoological gardens in Iran with different numbers of wild animal species. Between May and August 2018, freshly faecal samples were collected from 83 zoo animals representing 21 different species. Animals were classified into herbivorous, carnivores and aves. Information about the examined animals was obtained from zoo labels on the cages of each species. When it was possible and where animals kept separately in cages, the samples were collected individually, but where animals kept in the groups in a cage, samples were collected randomly from each cage. All samples were labeled with related animal species and were collected in 50 ml clean vials and then transported to the Parasitology Laboratory of Razi Vaccine and Serum Research Institute and were stored at +4◦C immediately upon arrival.

**The laboratory procedures and techniques:** Samples were examined macroscopically, to verify the presence of nematodes, cestodes, and/or fragments of parasites, and then were processed by qualitative methods of faecal sample examination. All samples were examined by direct wet mount preparation, formalin ethyl acetate concentration, zinc sulfate flotation and Ziehl Neelsen stain technique within 24 hours of collection. Slides were microscopically screened at 100x ,400x and 1000x magnification and detected parasites were identified by their morphometric characteristics as mentioned in references (Bowman, 2014; Soulsby, 1982; Yamaguti, 1961; Zajac & Conboy, 2012). Collected parasites were deposited in the Museum of Parasitology Department, Razi Vaccine and Serum Research Institute, Karaj, Alborz, Iran.

**Ethics Statement:** This research was carried out accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the Razi Vaccine and Serum Research Institute and all animals experiments were approved by Institutional Animal Care and Research Advisory Committee of the Razi. Vaccine and Serum Research Institute based on the Specific National Ethical Guidelines for Biomedical Research issued by the Research and Technology Deputy of Ministry of Health and Medicinal Education of Iran.

## Results

A total of 83 fecal samples from various captive wild animals, consisting of 21 different species were collected randomly and analyzed for the presence of the different kinds and stages of parasites. Scientific and common names of zoo animals that were sampled are listed in Tables 1, 2 and 3. Data showed that the examined animals were consist of 7 species of carnivores (26 samples), 10 species of herbivores (46 samples), and 4 species of different groups of birds (11 samples) that were listed in Table 4. Examination of fecal samples revealed that 22 (26.50 %) of animals, that belonging to 7 animal species, were infected with different intestinal parasites. Table 5 presents the list of detected gastrointestinal parasites according to the captive wild animals' species in this research. Among gastrointestinal parasites positive captive wild animals, 18 samples (21.68 %) belong to herbivores (Figure 1-4) and 4 samples (4.81 %) belong to Aves (Figure 5-6). Types, numbers and percentages of different species of parasites indicated in table 6. Among captive wild animals the prevalence of

**Table1.** The taxonomic characterization of 7 species of examined carnivores.

| Scientific Name | Common name | Number of examined carnivores |
|---|---|---|
| *Panthera leo* | African lion | 5 |
| *Hyaena hyaena* | Striped hyena | 2 |
| *Procyon lotor* | Raccoon | 3 |
| *Ursus arctos* | Brown bear | 5 |
| *Felis chaus* | Jungle cat | 4 |
| *Lynx lynx* | Black eared | 4 |
| *Canis lupus familiaris* | Siberian husky | 3 |
| Total | 7 Species | 26 |

**Table 2.** The taxonomic characterization of 10 species of examined herbivores

| Scientific Name | Common name | Number of examined animals |
|---|---|---|
| *Lama glama* | Llama | 8 |
| *Equus hemionus onager* | Asiatic wild ass (onager) | 5 |
| *Camelus ferus* | Wild Bactrian camel | 4 |
| *Cervus elaphus maral* | Maral or red deer | 5 |
| *Dama dama* | Fallow deer | 4 |
| *Equus ferus caballus* | Horse | 5 |
| *Equus ferus caballus* | Falabella miniature horse | 2 |
| *Ovis orientalis* | Wild sheep | 4 |
| *Capra aegagrus* | Wild goat | 5 |
| *Gazella subgutturosa* | Goitered gazelle or Persian gazelle | 4 |
| Total | 10 Species | 46 |

**Table 3.** The taxonomic characterization of 4 species of different groups of examined birds.

| ***Scientific name*** | ***Common name*** | ***Number of examined bird*** |
|---|---|---|
| *Struthio camelus* | *Ostrich* | 2 |
| *Pavo cristatus* | *Tavous* | 5 |
| *Alectoris chukar (Perdicinae)* | *Kabk* | 3 |
| *Aquila chrysaetos* | *Golden Eagle* | 1 |
| Total | 4 Species | 11 |

**Table 4.** The type, species and number of examined and positive animals.

| Animal types | Number of species | Number of animals | Number of positive animals | Percentage ofpositive animals |
|---|---|---|---|---|
| Carnivores | 7 | 26 | 0 | 0 % |
| Herbivores | 10 | 46 | 18 | 21.68 % |
| Aves | 4 | 11 | 4 | 4.81 % |
| Total | 21 | 83 | 22 | 26.50 % |

**Table 5.** Positive number and percentage of different species of examined animals

| Scientific name of animals | Number of examined animals | Number of positive animals | Detected parasite with number of infected animals | Percentage of positive animals (in species/in all) |
|---|---|---|---|---|
| *Ovis orientalis* | 4 | 4 | Oocysts of *Eimeria* sp. | 100 (4.81) |
| *Capra aegagrus* | 5 | 4 | Oocysts of *Eimeria* sp. | 80 (4.81) |
| *Lama glama* | 8 | 2 | *Trichuris* spp. egg (1)<br>*Nematodirus* spp. egg (1) | 25 (2.40) |
| *Camelus ferus* | 4 | 3 | *Nematode eggs* | 75 (3.61) |
| *Equus ferus caballus* | 5 | 5 | *Nematode eggs and Larvae* | 100 (6.02) |
| *Pavo cristatus* | 5 | 2 | *Nematode eggs* | 40 (2.40) |
| *Alectoris chukar* | 3 | 2 | *Ascaridia galli* | 66.66 (2.40) |
| Total | 34 | 22 | | 64.70 (26.50) |

parasites was higher in herbivores (21.68 %) followed by Aves (4.81). Results indicated that out of 22 animal samples that parasites were encountered, 14 (16.86%) were infected with helminths and 8 (9.63 %) were infected with protozoa (Table 6).

**Table 6.** Types and numbers of different species of parasites.

| Kinds of parasites | Type of Detected parasites | Number(percentage) of positive animals |
|---|---|---|
| Protozoa | *Eimeria* spp. | 8 (9.63) |
| Helminthes | *Nematodes* spp. eggs | 14 (16.87) |
| All parasites | *Total* | 22 (26.50) |

## Discussion

Although wild animals are usually infected with several species of parasites, but, natural resistance against parasitic diseases and a state of equilibrium between host and parasite generally prevent the development of clinical disease, unless in stress conditions (Mir et al., 2016).

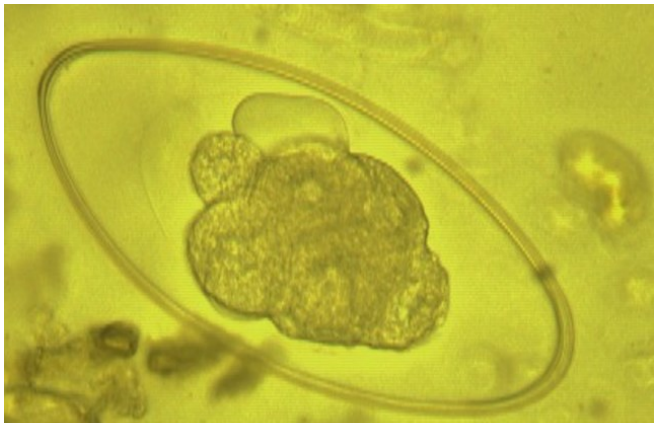

**Figure 1.** The detected *Nematodirus* spp. eggs from *Lama glama* (Lama) (×400 magnification).

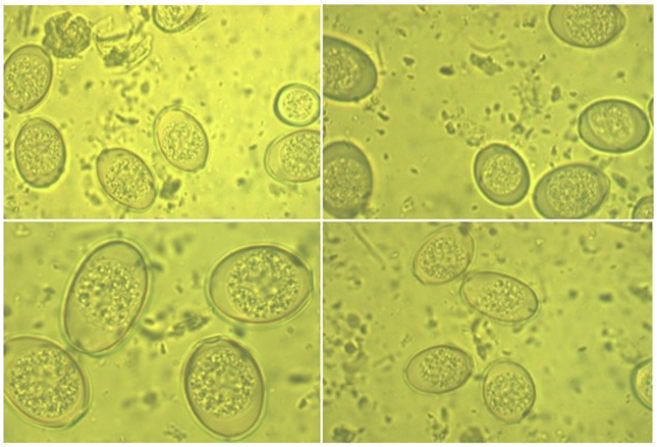

**Figure 2**. The detected Eimeria sp. from *Capra aegagrus* (Wild goat) (×1000 magnification)

In the present research, wild animal species in a national park of Alborz province were investigated for

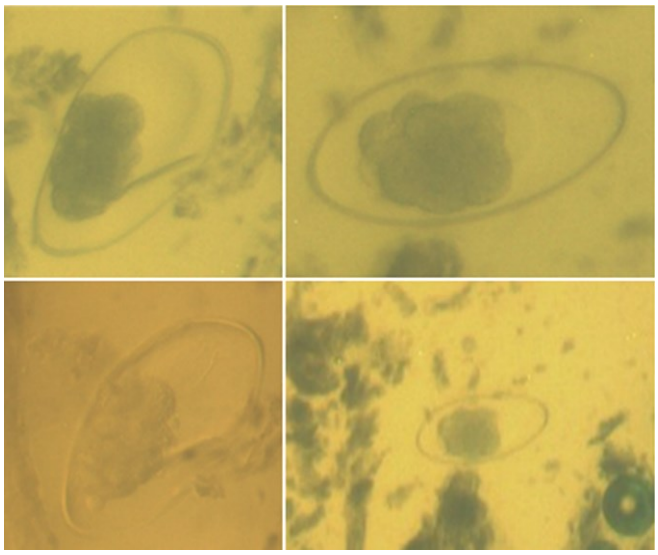

**Figure 3.** The detected *Nematodirus* spp. eggs from *Camelus ferus* (Wild Bactrian camel) (×1000 magnification).

gastrointestinal parasites by examination of faecal samples. The overall prevalence of these parasites in the animals at zoological garden, showed an infection rate of 26.50 %. The prevalence of gastrointestinal helminths (16.86 %) were almost higher than protozoans (9.63 %) and the gastrointestinal helminths comprised mainly of nematodes that this finding agrees with the reports of other researchers that nematodes were responsible for most of the helminthic diseases of veterinary importance, because they don't need intermediate hosts (Otegbade & Morenikeji, 2014). All the parasites genus identified in this research have previously been identified and described in captive wild animals by other authors (Lim et al., 2008).

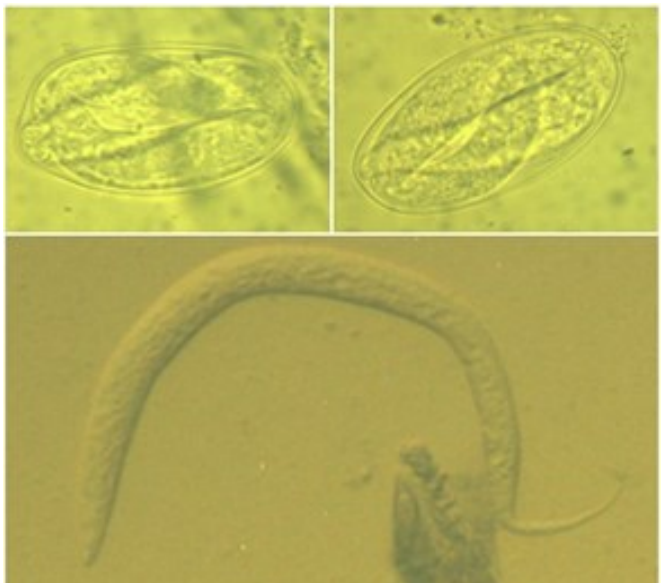

**Figure 4.** The detected Nematode larvae and eggs from Equus ferus caballus (horse)(×1000 magnification).

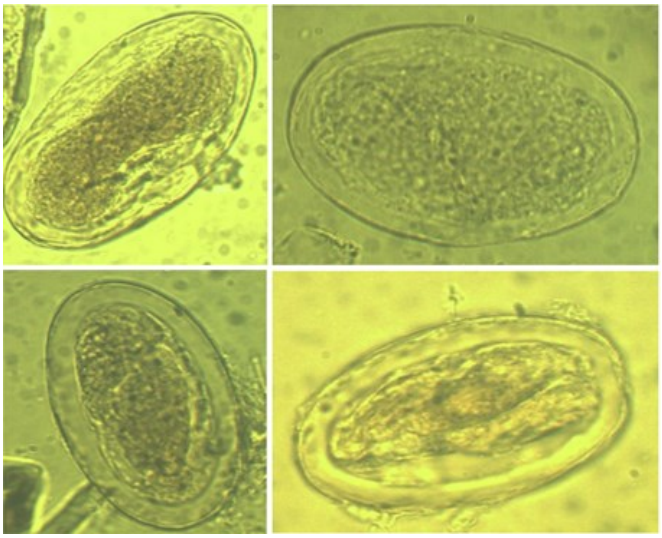

**Figure 5.** The detected nematode eggs from *Pavo cristatus* (Tavous) (×1000 magnification).

According to previous researches, as animal were apparently healthy during the period of examination and there was no reported mortality and clinical signs, the observed prevalence indicates probable subclinical infection, which may flare up under stress conditions and can cause pathogenicity (Mir et al., 2016). Based on the prevalence of gastrointestinal parasites and by administration of desired anti helminthic drugs to the captive wild animals periodically that coupled with better sanitary measures, we would be able to reduce the parasitic infection in the zoos (Thawait et al., 2014). The Parasitic prevalence survey is a way of monitoring the impact on the health and maintenance of wild animals' population (Allwin, 2015), and the prevalence of gastrointestinal parasites recorded in the wild animals in this study shows the need to design and implement a control program for parasite elimination.

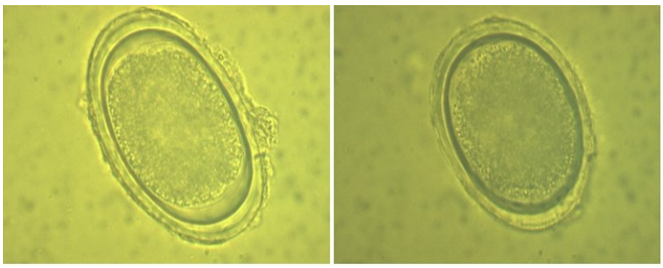

**Figure 6**. The detected *Ascaridia galli* eggs from *Alectoris chukar* (Perdicinae) (×1000 magnification).

### Conclusions

In conclusion, the findings of this study reported that both protozoan and helminth gastrointestinal parasites are prevalent in the wild animals of this zoo that they can serve as potential reservoirs of some zoonotic parasite for transmission to humans. It should pay attention that among husbandry procedures and diseases preventive measures, the routine monitoring of parasitic diseases and the use of selective treatments can represent crucial measures for the control of gastrointestinal parasitic infections in zoological gardens. The high prevalence of gastrointestinal parasites found in zoo animals examined in this study emphasizes the importance of controlling these parasitic diseases in order to keep animals, especially in the case of endangered species, in healthy conditions and prevent probable infection of humans working with these animals to zoonotic parasites.

### Conflict of interest statement

We declare that we have no conflict of interest.

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## References

Adejinmi, O. J., & Ayinmode, A. B. (2008). Preliminary investigation of zooanthroponosis in a Nigerian Zoological Garden. *Veterinary Research, 2*(3), 38–41.

Ajibade, W. A., Adeyemo, O. K., & Agbede, S. A. (2010). Coprological survey and inventory of animals at Obafemi Awolowo University and University of Ibadan Zoological Gardens. *World Journal of Zoology, 5*(4), 266-271.

Akinboye, D. O., Ogunfetimi, A. A., Fawole, O., Agbolade, O., Ayinde, O. O., Atulomah, N. O. S., Livingstone, R. (2010). Control of parasitic infections among workers and inmates in a Nigerian zoo. *Nigerian Journal of Parasitology, 31*(1), 93-97

Allwin, B. (2015). Prevalence of gastrointestinal parasites in gaur (*Bos gaurus*) and domestic cattle at interface zones of the Nilgiri Hills, Tamil Nadu, India. *Journal of Veterinary Science & Technology, 7*(1), 1-6

Borgsteede, F. H. (1996). The effect of parasites on wildlife. *The Veterinary Quarterly*, 18 Suppl 3(sup3), S138-40.

Bowman, D. D. (2014). *Georgis' Parasitology for Veterinarians-E-Book. 10th ed*. St Louis: US. Elsevier Health Sciences.

Kiani, H., Mirzapour, A., Mobedi, I., Spotin, A., Tabaei, S. J. S., & Rahimi, M. (2018). Frequency of intestinal parasites among zoo animal by morphometric criteria and first report of the *Bivitellobilharzia nairi* from elephant (*Elephasmaximus maximus*) in Iran. *Iranian Journal of Parasitology, 13*(4), 611–617.

Kvapil, P., Kastelic, M., Dovc, A., Bártová, E., Cížek, P., Lima, N., & Štrus, Š. (2017). An eight-year survey of the intestinal parasites of carnivores, hoofed mammals, primates, ratites and reptiles in the Ljubljana zoo in Slovenia. *Folia Parasitologica, 64*, 1-6.

Levecke, B., Dorny, P., Geurden, T., Vercammen, F., & Vercruysse, J. (2007). Gastrointestinal protozoa in non-human primates of four zoological gardens in Belgium. *Veterinary Parasitology, 148*(3–4), 236–246.

Lim, Y. A. L., Ngui, R., Shukri, J., Rohela, M., & Naim, H. R. M. (2008). Intestinal parasites in various animals at a zoo in Malaysia. *Veterinary Parasitology, 157*(1-2), 154–159.

Mir, A. Q., Dua, K., Singla, L. D., Sharma, S., & Singh, M. P. (2016). Prevalence of parasitic infection in captive wild animals in Bir Moti Bagh mini zoo (Deer Park), *Patiala, Punjab. Veterinary World, 9*(6), 540–543.

Nasiri, V , Jameie, F , Soltani, S, Pashaei, M, F. M. (2017). *Gastrointestinal parasitic infection in captive wild animals at the Eram Zoological Garden*, Tehran, Iran. Paper presented at In 3rd International and 10th national congress of parasitology and parasitic diseases of Iran (NICOPA10) Şiraz, Iran.

Opara, M., Osuji, C., & Opara, J. (2010). Gastrointestinal parasitism in captive animals at the Zoological Garden, Nekede Owerri, Southeast Nigeria. *Ostrich, 2*(5), 21–28.

Otegbade, A. C., & Morenikeji, O. A. (2014). Gastrointestinal parasites of birds in zoological gardens in south-west Nigeria. Tropical *Biomedicine, 31*(1), 54–62.

Panayotova-Pencheva, M. S. (2013). Parasites in captive animals: a review of studies in some European zoos. *Der Zoologische Garten, 82*(1-2), 60-71.

Soulsby, E. J. L. (1982). *Helminths, arthropods and protozoa of domesticated animals*. *7th ed.* London: UK. Bailliere Tindall.

Thawait, V. K., Maiti, S. K., & Dixit, A. A. (2014). Prevalence of gastro-intestinal parasites in captive wild animals of Nandan Van Zoo, Raipur, Chhattisgarh. *Veterinary World, 7*(7), 448–451.

Yamaguti, S. (1961). *Systema Helminthu: Vol. 3. The Nematodes of Vertebrates*. London: UK. Intersicence Publishers.

Zajac, A. M., & Conboy, G. A. (2012). *Veterinary clinical parasitology*. *8th ed*. New Jersey : US. John Wiley & Sons.

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# The effect of serum β-hydroxybutyric acid and calcium levels on left displaced abomasum in Holstein cows on transition period

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**ABSTRACT**

In this study, the effect of serum β-hydroxybutyric acid and calcium concentrations, which are the parameters used in the diagnosis of ketosis and hypocalcemia in lactation period, on left displaced abomasum (LDA) has been investigated. The lactation period covering the 3 weeks before and after parturition, known as the transition period, is highly important for high yield dairy cows (Holstein Friesian cattle). Hormonal and metabolic changes occur in this period. The energy requirement, which increases in direct proportion with the milk yield at the beginning of lactation, cannot be met with insufficient dry matter consumption, however, it is compensated with the mobilization of body fat. Ketosis and fatty liver are nutritional diseases that are observed in animals with high milk yield resulting from the disturbances in energy metabolism. Hypocalcemia and ketosis are the most important risk factors in the development of left displaced abomasum (LDA) in high-yield milk cows. Hypocalcemia and ketosis are also the most important nutritional diseases in the transition period. In this study, 17 Holstein Friesian cattle were used in group 1 that were diagnosed with left displaced abomasum after hearing the ''ping'' sound, and 17 healthy, randomly selected Holstein Friesian cattle were used in group 2. Blood analyses (BHBA and Ca) were performed in group 1 and 2 after parturition

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## Introduction

Left displaced abomasum (LDA) is an economically significant nutritional disease that is common in dairy cows in gestation period. The lactation period covering the 3 weeks before and after parturition, known as the transition period, is highly important for high yield dairy cows (Holstein Friesian cattle). Hormonal and metabolic changes occur in this period. The energy requirement, which increases in direct proportion with the milk yield at the beginning of lactation, cannot be met with insufficient dry matter consumption, however, it is compensated with the mobilization of body fat. Nutritional diseases observed in animals with high milk yield such as displaced abomasum, hypocalcemia, ketosis, fatty liver, mastitis, metritis, retained placenta, acidosis and laminitis are all associated with one another, and presentation of one of these causes the appearance of others.

Nutritional diseases observed in high yield dairy

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cows in transition period due to inaccurate care and nutrition are: Energy and mineral metabolism disorders and immune system disorders experienced due to the negative energy balance that occurs upon insufficient food and dry matter intake. The appearance of one of these nutritional diseases in high milk yield cows triggers another, and causes the presentation of diseases that are associated with each other. The incidence of left displaced abomasum is high in cows with hypocalcemia and ketosis. The most important factor in displaced abomasum is abomasal atony. Meanwhile, atony is caused by traumatic reticuloperitonitis, ulcer, metritis, mastitis, retained placenta, acidosis, increased volatile fatty acids and low blood calcium levels. In left displaced abomasum, uterus expands towards the end of pregnancy, gets under the rumen and lifts the rumen up. Abomasum enters the space previously occupied by rumen. Shrinking in size after parturition, the uterus retreats back to its own space, and rumen descends toward the space left by the uterus again, leaving the abomasum stuck in the place it entered before. As being stuck between the rumen and left abdominal wall, and the abomasum gets "displaced" to the left. The abomasum is displaced to the left in 75% of cases (Geishauser, 1995; Van Winden and Kuiper, 2003; Alaçam, 2011)

There is a positive correlation between non-esterified fatty acids, which took form after fatty acid mobilization, and negative energy level and nutritional diseases. Increases in non-esterified fatty acids in the blood, hormonal changes, increased requirement of nutrients (a pregnant cow needs 75% more nutrients than non-pregnant cow at the same weight), and increases in feed consumption are observed upon parturition (while a non-pregnant cow consumes dry matter at 2% of its live weight, a pregnant cow consumes 1.4% of its live weight in dry matter). Negative energy balance is observed in 80% of high yield milk cows. At negative energy balance, anestrus period is extended, corpus luteum functions are weakened, and cystic ovary and metritis cases are high (Alaçam, 2011; Overton and Waldron, 2004; Alaçam et al., 2008; Van Saun, 2004). Immune system is suppressed as a result of the excretion of minerals and fat-soluble vitamins with milk, and it results in immune dysfunction (Curtis et al., 1983).

Ketosis is a nutritional disease characterized with degeneration of the liver, decreased blood glucose concentration, increased level of ketone bodies in the blood and other body tissues (acetoacetic acid, β-hydroxybutyric acid and acetone) observed in postpartum period. As a result of body fat mobilization in negative energy table, plasma concentration of free fatty acids is increased in various tissues. Free fatty acids in the liver are limited with the oxidation capacity of the liver. Upon exceeding this capacity, free fatty acids are converted into triglycerides, and accumulated in the liver. The prevalence of ketosis increases when plasma concentration of free fatty acids exceeds 1000 mEq/ L (Arslan and Tufan, 2010).

Depending on the energy deficit of the animal in negative energy level, increased blood levels of non-esterified fatty acids resulting from the mobilization of body fat storage are transported to the liver and undergo incomplete oxidation, resulting in the formation of ketone bodies that participate in milk formation in mammary glands, and thus the milk fat ratio is increased. Due to low levels of fermentable non-structural carbohydrates in the ration feed, inability of rumen bacteria to multiply, insufficient energy in rumen, insufficient microbial protein synthesis in rumen and decreased level of metabolized proteins in the small intestine causes insufficient amino acid level in mammary glands. This causes decreases in milk protein ratio (Hayırlı and Çolak, 2011).

In a non-pregnant cow, abomasum resides on the ventral wall in abdominal cavity, slightly to the right of the median line. With pregnancy, the space occupied by the uterus increases in the abdominal cavity. The uterus pushes rumen forward and the abomasum shifts to the left, and after parturition, abomasum goes back to its original place. The gases formed as a result of the fermentation of feed in the abomasum are sent back to the rumen. As a result of feeding the cow with a ration that includes higher level of concentrate feed compared to roughage, the level of gas formed in abomasum increases and the abomasum expands, decreasing its capacity to move. Expanded abomasum starts to spread out to the left, and gets between rumen and abdominal wall. Limited feed consumption causes decreased fullness in the rumen, and this poses a risk for displaced abomasum (Biricik, 2012).

In this study, the effect of ketosis and hypocalcemia on left displaced abomasum (LDA) was investigated.

## Materials and Methods

**Sample collection:** In this study, there were 17 Holstein Friesian cattle in group 1 that were diagnosed with left displaced abomasum (LDA) in the auscultation performed between 11th-13th intercostal space in the first postpartum 30 days, by

hearing the ''Ping'' sound with tympanic resonance, and there were 17 Holstein Friesian cattle in group 2 that were healthy, randomly selected and gave birth within the last 30 days.

**Biochemical analysis:** Serum NEFA level is not used in our study since nonesterified fatty acids (NEFA) are esterified after parturition and converted into triglycerides (reesterified triglycerides), or ketone bodies as β-hydroxybutyric acid (BHBA). Serum BHBA level was designated as BHBA≥ 1.200 mmol/L in the diagnosis of ketosis, and total Ca level was designated as 1.875- 2.075 mmol/L in the diagnosis of hypocalcemia. Blood samples were taken from V. Coccygea of the cows in group 1 that were diagnosed with displaced abomasum and the healthy cows in group 2 that gave birth in the last month, samples were taken to the laboratory by cold chain, separated from the serum was obtained by centrifugation at 3500 rpm for 5 min, and analyzed Serum Beta hydroxy butyric acid (BHBA) and total calcium (Ca) levels have been analyzed in Biotecnica Instruments BT 3500 device by using commercial kits.

**Ration analysis:** In this study, cows were fed with Total Mixed Ration (TMR). The contents of TMR were 25 kg of corn silage, 3 kg of wheat straw, 2 kg of hay and 9 kg of dairy cattle feed. The cows have consumed 39 kg of natural feed daily, 19.6 kg on dry matter basis. The ratio of roughage / concentrated feed in the ration was: 61.2/ 38.8. Nutrient composition of TMR is presented in Table 1 on dry matter basis.

**Statistical analysis:** Obtained results have been evaluated with SPSS V.21 package program. The distribution of results was controlled with Levene's test, calcium level with t test, and BHBA levels were evaluated with Mann Whitney U test. Mean results of groups and standard errors are presented in Table-2. Box plots were established for visualizing the distribution of data. Statistical significance level was determined as p<0.001. Serum BHBA and total Ca levels of the cows in group 1 that were diagnosed with displaced abomasum and the healthy cows in group 2 have been compared, and the differences between the groups were determined to be significant at P < 0.001 level for both features. The approval of local ethics committee dated 12.03.2018 nr. 2018/ 5 has been obtained from Etlik Veterinary Control Central Research Institute Ankara /Turkey.

## Results

Definition of left displaced abomasum is ''Ping'' sound on abdominal percussion. The symptoms of ketosis are decrease in milk yield, reduced feed intake and decreased appetite/refusing concentrate intake, excessive loss of body condition, constipation, ketone odor in breath /milk and nervous signs (weakness, mania, apparent blindness and pica). The cows in this study were fed with Total Mixed Ration (TMR), and nutrient composition of TMR is presented in Table 1 on dry matter basis. Statistical results of the cows in group 1 that were diagnosed with displaced abomasum and the healthy cows in group 2 that gave birth in the last month for serum BHBA and total Ca levels are also presented. As it is known, hypocalcemia was diagnosed within the first postpartum 3 days by checking the symptoms and serum total Ca level, and ketosis was diagnosed within the first postpartum week by checking the symptoms and serum BHBA level.

**Table 1.** Nutrient composition of TMR (DM %)

| | |
|---|---|
| DM (dry matter) % | 50.7 |
| CP % (Crude protein) | 12.8 |
| CF % (Crude fat) | 2.6 |
| CC % (Crude cellulose) | 20.6 |
| NDF % (Neutral detergent fiber) | 44.2 |
| CA % (Crude ash) | 6.6 |
| NEL Mcal/kg (Net energy lactation) | 1.39 |

Figure 1 shows the BHBA level in cows that were diagnosed with left displaced abomasum. BHBA level was determined to be higher in cows diagnosed with displaced abomasum compared to healthy cows. The difference between cows diagnosed with displaced abomasum and healthy cows was determined to be statistically significant with regard to BHBA level (p<0.001). Calcium results are presented in Figure 2. Upon comparing mean results between the two groups, Ca level was determined to be higher in healthy cows compared to cows diagnosed with displaced abomasum. The difference between the Ca levels of cows diagnosed with left displaced abomasum and healthy cows was determined to be statistically significant (p<0.001).

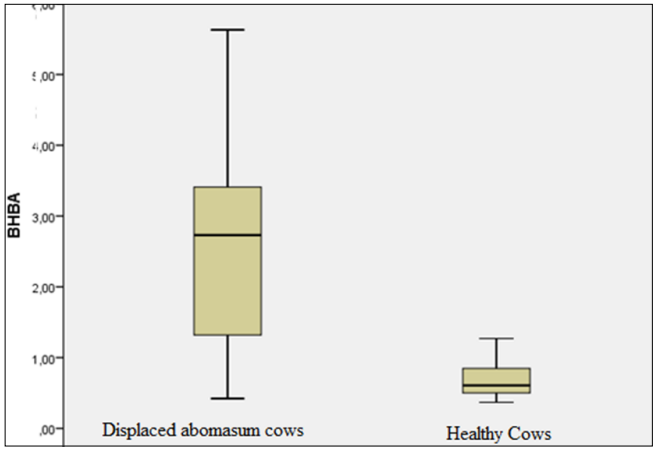


**Figure 1.** BHBA levels in cows diagnosed with left displaced abomasum and healthy cows

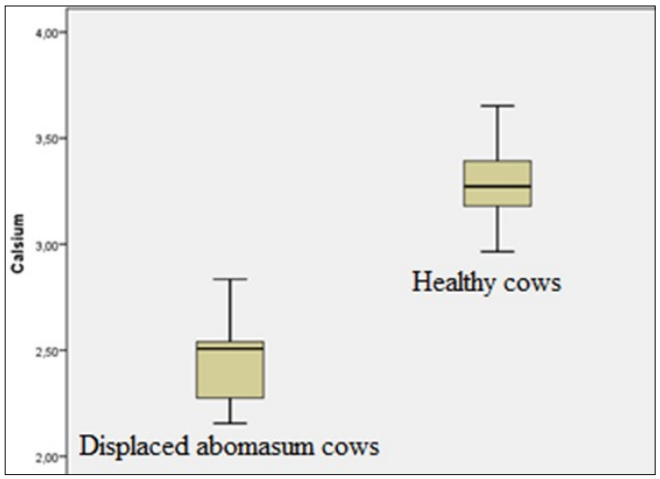


**Figure 2.** Calcium levels in cows diagnosed with left displaced abomasum and healthy cows.

## Discussion

With the start of lactation, serum glucose and insulin levels are decreased in dairy cows while beta hydroxy butyric acid (BHBA) and non-esterified fatty acid (NEFA) levels increase (Van Winden et al., 2003).

It has been stated that the risk of displaced abomasum increases with the decreased consumption of roughage and dry matter in the last two weeks of dry period, slow increase of dry matter and concentrated feed consumption with parturition, and decreased fullness capacity of rumen as a result of decreased roughage/concentrated feed ratio. As a result of the decrease in roughage, and the increase in concentrated feed and volatile fatty acids upon parturition, decreased rumen motility reduces the absorption of volatile fatty acids. Gases accumulated in the rumen proceed into the abomasum and allows the abomasum to move. Also, there is a risk of LDA in cows with hypocalcemia upon parturition (Shaver, 1997).

The risk of displaced abomasum in cows with hypocalcemia is 4.8 times higher compared to those that do not have hypocalcemia. It has been indicated that LDA risk increases if total serum Ca level falls below 2.04 upon parturition (Massey et al., 1993).

Rumen activity is lost upon 2.2 mg/dl blood calcium level. Decreased rumen activity causes gas accumulation in rumen and abomasum, and decreases in abomasum activity (Jorgensen et al., 1998).
Subclinical BHBA level varies between 1.200- 1.400 mmol/L (Suthar et al., 2013). Clinical BHBA level is above 1.400 mmol/L. A high rate of blood BHBA concentrations has been determined in cows with displaced abomasum. BHBA levels have been found as 1.56 and 0.90 mmol/L, respectively, in cows with displaced abomasum compared to control group (Stengarde et al., 2010). In our study, mean serum BHBA level was determined as 2.51 mmol / L in cows diagnosed with displaced abomasum.

BHBA concentration was increased on postpartum day 3 in cows diagnosed with displaced abomasum, however, Ca concentration was determined to be decreased (Hädrich, 2006). These results are determined to be in line with the results of our study.

In a study, BHBA level was determined as 1.29±0.13mmol/L for subclinical ketosis, and BHBA level was determined as 2.49±0.17 mmol/L for clinical ketosis (Şentürk et al., 2016). It has been stated that hypokalemic abomasal displacement cows have important deviations in their energy and fat metabolism for acid-base and electrolyte status compared to normokalemic cows, and negative events are experienced in the course of disease for this reason. BHBA has been stated to cause a negative effect on abomasal displacement DA cows (Alexandra, 2014).

It was indicated that increased BHBA concentration and decreased Ca concentration in the herd after parturition was associated with increased DA, and DA risk was elevated as a result. It was indicated that 1.400 mmol/L higher BHBA level and 2.1 mmol/L lower Ca level in the herd after parturition increased the possibility of displaced abomasum in the herd, and that 800 mmol/L lower BHBA level and 2.1 mmol/L lower Ca level was associated with decreased milk yield (Chapinal et al., 2012).

In a study comparing left displaced abomasum (LDA) cows and healthy cows, a statistical difference was observed between serum BHBA levels after parturition (p=0.001) (Cardoso et al., 2008). These results are determined to be in line with the results of our study.

In the study performed by Sarashina et al. (1989), the gas in the abomasum has originated from the rumen. While ruminal CO2/CH4 gas ratio is 2.01 in healthy cows, CO2/CH4 gas ratio is 0.44 in abomasum. Ruminal CO2/CH4 gas ratio dropped to 1.62 in displaced abomasum, and abomasal CO2/CH4 ratio has dropped to 0.35. CO2 has been absorbed from the abomasal wall, and therefore CO2/CH4 gas ratio in the abomasum has changed. Unlike the gas in the rumen, abomasal CH4 has been found to be higher than CO2 gas both in normal and DA cows (Sarashina, 1990).

In general, 1.70 mmol/L serum Ca concentration has been reported to cause decreased feed consumption due to the decrease in ruminal activity

(Batmaz, 2015), and Ca concentration below 1.2 mmol/L has been reported to cause decreased abomasal motility (Madison and Troutt, 1998). This value was suggested to be associated with displaced abomasum as a result of decreased abomasal motility.

Eight times higher displaced abomasum prevalence has been reported in cows that had serum BHBA levels of > 1.200 mmol/L after parturition. Elevation of serum BHBA level after parturition has been significantly associated with the subsequent increase in LDA risk. Displaced abomasum is a more important risk factor than ketosis and hypocalcemia. Prepartum nonesterified fatty acid (NEFA) value both shows and reflects the severity of negative energy balance, and plays a key role in LDA etiology (Leblanc et al., 2005).

In general, feed consumption and milk yield drops in cows with DA, and serum Ca level is decreased while BHBA level in increased (Van Winden et al., 2003).

It has been stated that DA risk is 13.6 times higher in cows with ≥1000 mmol/L serum BHBA level, compared to cows with lower serum BHBA levels. It has also been indicated that ketosis risk is 4.7 times higher in cows with ≥1200 mmol/L serum BHBA level. It has been determined that 2.2 mmol/L lower serum total Ca concentration after parturition increases the risk of displaced abomasum (Seifi et al., 2011). These results are determined to be in line with the results of our study.

Postpartum BHBA concentration at critical threshold value of 1.000 mmol/ L should be assessed as a risk factor for displaced abomasum (Ospina et al., 2010).

## Conclusion

Consequently, as a reflection of the negative energy balance after parturition, high BHBA level causes the presentation of ketosis. Reducing nutritional diseases bears vital importance for the health and yield of the herd. It has been concluded that subclinical and clinical hypocalcemia reference levels should be re-interpreted due to the high yield of dairy cows and increased dry matter consumption. There is a strong relation between the manifestation of ketosis after parturition and the presentation of displaced abomasum in the first postpartum month. Ketosis is observed as a result of the negative energy balance presented due to dry matter consumption and decreased appetite. It is considered that left displaced abomasum is inevitable after the diagnosis of ketosis with the increased ruminal fullness and decreased abomasum motility. Furthermore, feeding cows with negative ration cation-anion balance in dry period will decrease the risk of hypocalcemia.

## References


Alaçam, E., Tuncer, Ş. D., Salmanoğlu, M. R., Küçükersan, S., Küçükersan, M. K., & Özlüer, A. (2008). The effects of nutritionally unbalanced diet on some blood and postpartum fertility parameters in dairy cows. *Turkish Journal of Veterinary and Animal Sciences, 32*(2), 99-106.

Alaçam, E. (2011). Transition period and it's importand problems in dairy cows. *Turkiye Klinikleri Journal of Veterinary Sciences, 2*(2), 85-95.

Alexandra, M. M. (2014, June 5) Serum potassium concentration in dairy cows with abomasal displacement in relation to the course of disease Large Animal Clinic for Internal Medicine, Faculty of Veterinary Medicine, University of Leipzig Submitted in November. Retrieved from http:// ul.qucosa.de/api/qucosa%3A12487/attachment/ ATT-0/

Arslan, C., & Tufan, T. (2010). Geçiş dönemindeki süt ineklerinin beslenmesi II. Bu dönemde görülen metabolik hastalıklar ve besleme ile önlenmesi. *Journal of the Faculty of Veterinary Medicine, Kafkas University, 16*(1), 159-166.

Batmaz, H. (2015). *Sığırlarda Sürü Sağlığı ve Yönetimi*. Bursa ,Turkey: Alfa Aktüel Yayınları.

Biricik, H. (2012). Abomasal Displacement in Dairy Cattle. *Turkiye Klinikleri Journal of Veterinary Sciences, 3*(1), 32-37.

Cardoso, F. C., EstevesI, V. S., OliveiraI, S. T., LastaI, C. S., ValleII, S. F., CamposIII, R., & González, F. H. D. (2008). Hematological, biochemical and ruminant parameters for diagnosis of left displacement of the abomasum in dairy cows from Southern Brazil. *Pesquisa Agropecuária Brasileira, 43*(1),141-147.

Chapinal, N., LeBlanc, S. J., Carson, M. E., Leslie. K. E., Godden, S., Capel. M., Santos, J. E. P., Overton, M.W., & Duffield, T. F. (2012). Herd-level association of serum metabolites in the transition period with disease, milk production, and early lactation reproductive performance. *Journal of Dairy Science, 95*(10), 5676–5682.

Curtis, C. R., Erb, H. N., Sniffen, C.. J., Smith, R. D., Powers, P. A., Smith, M. C., White, M.E., Hillman, R.B., & Pearson, E. J. (1983). Association of parturient hypocalcemia with eight periparturient

disorders in Holstein cows. *Journal of American Veterinary Medical Association, 183*(5), 559-561.

Geishauser, T. (1995). Abomasal displacement in the bovine - A review on character, occurence, aetiology and pathogenesis. *Zentralblatt für Veterinärmedizin A, 42*(4), 229-251.

Hädrich, G. (2006, 1 July). İnvestigations of the body condition development, the periparturient metabolism and the morbidity in high yielding dairy cows. Large Animal Clinic for Internal medicine, Faculty of Veterinary Medicine, University of Leipzig, Retrieved from http://ul.qucosa.de/api/qucosa%3A10675/attachment/ATT-0/

Hayırlı, A., & Çolak, A. (2011). İneklerin kuru ve geçiş dönemlerinde sevk-idare ve beslenme stratejileri: Postpartum süreçte metabolik profil, sağlık durumu ve fertiliteye etkisi. *Turkiye Klinikleri Journal of Veterinary Sciences, 2*(1),1-35.

Jørgensen, R. J., Nyengaard, N. R., Hara, S., Enemark, J. M., & Andersen, P. H. (1998). Rumen motility during induced hyper- and hypocalcaemia. *Acta Veterinaria Scandinavica, 39*(3), 331-338.

Leblanc, S. J., Leslie, K. E., & Duffield, T. F. (2005). Metabolic predictors of displaced abomasum in dairy cattle. *Journal of Dairy Science*, *88*(1), 159-170.

Madison, J. B., & Troutt. H. F. (1988). Effects of hypocalcaemia on abomasal motility. *Research in Veterinary Science*, *44*(2), 264-266.

Massey, C. D., Wang, C., Donovan, G. A., & Beede, D. K. (1993). Hypocalcemia at parturition as a risk factor for left displacement of the abomasum in dairy cows, *Journal of American Veterinary Medical Association, 203*(6), 852-853.

Ospina, P. A., Nydam, D. V., Stokol, T., & Overton, T. R. (2010). Evaluation of nonesterified fatty acids and β-hydroxybutyrate in transition dairy cattle in the northeastern united States: Critical thresholds for prediction of clinical diseases. *Journal of Dairy Science,* 93, 546-554.

Overton, T. R., & Waldron, M. R. (2004). Nutritional management of transition dairy cows: Strategies to optimize metabolic health. *Journal of Dairy Science 87,* Sup. E105- E119.

Sarashina, T., Ichijo, S., Takahashi, J., & Osame, S. (1990). Origin of abomasum gas in the cows with displaced abomasum. *Japanese Journal of Veterinary Science*, 52, 371-378.

Seifi, H. A., LeBlanc, S. J., Leslie, K. E., & Duffield, T. F. (2011). Metabolic predictors of post-partum disease and culling risk in dairy cattle. *Veterinary Journal, 188*(2), 216-20.

Shaver, R. D. (1997). Nutritional risk factors in the etiology of left displaced abomasum in dairy cows: a review. *Journal of Dairy Science, 80*(10), 2449-2453.

Stengärde, L., Holtenius, K., Tråvén, M., Hultgren, J., Niskanen, R., & Emanuelson, U. (2010). Blood profiles in dairy cows with displaced abomasum. *Journal of Dairy Science, 93*(10), 4691-4699.

Suthar, V. S., Canelas-Raposo, J., Deniz, A., & Heuwieser, W. (2013). Prevalence of subclinical ketosis and relationships with postpartum diseases in European dairy cows. *Journal of Dairy Science, 96* (5), 2925-2938.

Şentürk, S., Cihan, H., Mecitoğlu, Z., Çatık, S., Akgül, G.D., Kasap, S., & Topal, O. (2016). Prevalence of ketosis in dairy herds in Marmara, Aegean and Mediterranean regions of Turkey. *Ankara Üniversitesi Veteriner Fakültesi Dergisi,* 63, 283-288.

Van Saun, R. J. (2004). Transition cow nutrition and management: The key to herd reproductive performance. Proceedings Czech Buiatrics Specialist Congress. Hradec Kralove: *Vetenerinary and Biomedical Science*, 13-21.

Van Winden, S. C, & Kuiper, R. (2003). Left displacement of the abomasum in dairy cattle: recent developments in epidemiological and etiological aspects. *Veterinary Research, 34*(1), 47-56.

Van Winden, S. C. L., Jorritsma, R., Muller, K. E., & Noordhuizen, J. P. T. M. (2003). Feed intake, milk yield, and metabolic parameters prior to left displaced abomasum in dairy cows. *Journal of Dairy Science, 86*(4), 1465-1471.

JIVS JOURNAL OF ISTANBUL VETERINARY SCIENCES

# Clinical and paraclinical changes in experimental colisepticemia in neonatal calves

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**Research Article**

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**ABSTRACT**

The present study aim was to assess changes in clinical signs and plasma cytokines in calves experimentally infected with *Escherchia coli* and use them as a tool to diagnose colisepticemia. Ten healthy Holstein-Frisian bull calves were used for this experimental study. Experimental colisepticemia was induced in calves with intravenous injection of 1.5 × 109 CFU of O111:H8 strain of E.coli. Clinical scores were recorded before induction of septicemia, every 30 minutes for 8 hours, every hour from 8 till 12 h, every 3 hours from 12 till 24 hours after bacterial challenge. Blood samples were collected to determine plasma concentration of tumor necrosis factor-α (TNF-α) and gamma interferon gamma (IFN-γ) before and after E.coli IV injection. Blood culture was performed before and after bacterial challenge to confirm colisepticemia. The present study showed that total clinical score of the calves increased with a simultaneous significant rise in plasma concentration of TNF-α and INF-γ during septicemia period (P< 0.05). Changes in the heart and respiratory rate during septicemia and using clinical scoring are not enough to assess the magnitude of infection and disease progress; therefore, it is recommended some laboratory tests be used for better evaluation of clinical status of the septic calves

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## Introduction

Septicemic colibacillosis is a highly fatal disease of calves less than 2 weeks of age and occurs mainly in newborn animals that are agammaglobulinemic due to not receiving enough colostrum on the first day of their life (Constable et al., 2017; Rezazadeh et al., 2004; Thomas et al., 2004) and inflicts significant loss on dairy farms. Calves are mostly infected from their early environment (Gay et al., 1994), during nursing attempts in heavily contaminated udder (Aldridge et al., 1993), and or through contact with infected feces (Rabbani et al., 2007). The disease is characterized by depression, weakness, listlessness, recumbency, poor response to external stimuli, cold extremities, and coma. Affected animals have a poor suckling reflex, an abnormal body temperature (low or high), cardiac dysfunction (tachycardia or bradycardia) and

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leukocyte abnormality. The sick animals will die due to hypovolemic shock (dehydration) and multiple organ insufficiencies soon after onset of clinical signs. Although bacteremia is the main cause of death in calves with colisepticemia, the early diagnosis is important for veterinarian and farm owners (Aldridge et al., 1993; Fecteau et al., 1997; Gay et al., 1994). Blood and postmortem tissue culture are sure methods to diagnose bacteremia but unfortunately this tool is time consuming, costly and needs technical skills all of which persuaded researchers to search for an easier method. Studies based on prospective and retrospective records developed a sepsis score to predict sepsis in human and equine neonate (Ballou et al., 2011; Brewer and Koterba, 1998; Fecteau et al., 1997). A clinical scoring based on the status of hydration, fecal consistency, scleral vessels appearance, general attitude and umbilical abnormality in calf to predict bacteremia with 75% sensitivity and 71% specificity was developed (Blackwell and Christman 1996). It has been shown that as well as changes of general status of calf during colisepticemia many inflammatory mediators such as tumor necrosis factor (TNF- α), interleukin-1 (IL-1), IL-6, alpha interferon (IFN- α), and eicosanoids are released in the blood (Annane and Bellissant, 2005). Adams et al. (1990) observed a rapid rise of TNF in the blood after IV administration of Escherichia coli lipopolysaccharide (LPS) (Adams et al., 1990). The blood elevation of TNF- α and IFN-ϒ after IV injection of LPS was also observed (Bieniek et al., 1998). Many deleterious and pathologic effects of LPS could be consequences of TNF- α release in the blood (Beutler et al., 1985), since TNF- α suppressors can inhibit some of the LPS effects such as fever (LeMay et al., 1990). The objective of this study was to use a clinical sepsis score in association with the serum TNF- α and IFN-ϒ to predict the status and early diagnosis of bacteremia in newborn calves.

## Material and methods

**Study design:** Ten Holstein-Frisian bull calves between 8 and 10 days of age with body weight ranging from 53 to 60 kg were studied. The calves were fed colostrum (10% BW) within six hours of birth and housed in individual metal pens (1 m × 1.5 m × 1m) with a chaff coated floor and were fed twice daily with whole milk at the rate of 10% of their body weight per day divided into 2 feedings at 7:0 and 16:0. Water and starter (composed of: barley 40%, corn 20%, bran 19%, soybean meal 18%, mineral 1.5% and vitamin supplement 1.5%) were provided ad libitum. The calves' vital signs (temperature, heart and respiratory rate) and clinical scores were checked at the arrival day, 5 days after that and the day before start of experiment. The E.coli strain of O111:H8 was chosen in the present study because of its availability and being rapidly phagocytized, producing a robust oxidative burst (Hulbert et al., 2011). This strain was cultured and after incubation at 37°C for 24h, DNA was extracted in boiling method and the bacteria were checked for expressing Intimin (eae) and Shiga-like toxin (STX1 and STX2). Antibiogram test was performed for choosing an appropriate antibiotic to treat the septicemia. All experimental procedures followed the guidelines on ethical standards for experimental processes in animals according to a protocol approved by the Animal Ethics Committee, University of Tehran, Iran. To induce septicemia experimentally, a catheter was then inserted in the jugular vein, an extension set was attached to the catheter, and the catheter and extension set were secured to the calf's neck. A suspension of E-coli (1.5 × 109 CFU) in 5 ml isotonic saline was administered as a bolus. The challenge dose was prepared about 1 hour before the start of experiment and checked with spectrophotometer for its concentration. For ethical reasons, an untreated control group in this study was thought to be unnecessary. Therefore, all calves were treated with a suitable antibiotic selected by antibiogram. Treatment began 24h after bacteria administration with ceftazidime (ZACZIDIM 1g vial by DAANA Pharma Co) at dose of 10 mg/kg IV every 8h for 3 days.

**Clinical assessment:** The clinical score procedure was adapted from a system previously described for scoring sepsis in neonatal calves (Fecteau et al., 1997). Seven clinically assessed criteria including appetite, dehydration, fecal nature, behavior, shock, standing ability, and suckling reflex were considered (Table1). Rectal temperature, the heart and respiratory rate were also measured. All the findings in physical examination were individually recorded by the same observer in each calf. After injection of bacteria, clinical scores were assessed at 30 min intervals for 8 h, hourly till 12 h, then every 3 hours till 24h. Each of the seven scores was classified as 0, 1, 2, and 3 so that 0 is normal state and 3 is the worst condition (Table 1). Respiratory and heart rate was examined with auscultation of the trachea and heart region in one minute, respectively, by 2 persons. Rectal temperature was taken with a digital thermometer.

**Laboratory examination:** Blood samples were collected into 6-mL tubes containing EDTA for determination of plasma TNF-α and IFN-ϒ before (day -10, day -5, and day -1) and after (0, 2, 8, and 24 hour) challenge and centrifuged at 2000 g for 15 minutes. Four mL of peripheral blood was collected into a sterile syringe and injected to a two-phasic media and

**Table1.** Clinical scoring system for calf neonatal sepsis used in the study (29)

| Parameter | Score | Criteria |
|---|---|---|
| Appetite | 0 | Normal |
| | 1 | Slightly decreased (<50%) |
| | 2 | Decreased (>50%) |
| | 3 | Anorexic |
| Dehydration | 0 | Normal hydration: a skin fold tented and twisted 90 ° for 1 sec. returns immediately to original position when released |
| | 1 | Slight (<5%): skin fold remains tented for up to 4 sec. |
| | 2 | Mild (5 to 10 %): skin fold remains tented for 4 to 8 sec. |
| | 3 | Severe(>10%): skin fold tented for more than 8 sec. |
| Nature of feces | 0 | Normal |
| | 1 | Semi-solid |
| | 2 | Liquid with solid particles |
| | 3 | Liquid |
| General behavior | 0 | Normal: calf is vigorous, alert, and responsive |
| | 1 | Dull: calf quiet, slow to respond, and/or move |
| | 2 | Depressed: calf is dull and markedly slow to respond |
| | 3 | Prostrated or coma |
| Shock | 0 | Absent |
| | 1 | Slight or early: dull, heart rate decreased |
| | 2 | Mild or advanced: weakness, pale/dry mucous membranes, oliguria, cold extremities |
| | 3 | Severe: weak rapid pulse, diminished heart sounds, coma |
| Ability to stand | 0 | Normal |
| | 1 | Able to stand but with difficulty |
| | 2 | Unable to stand without assistance |
| | 3 | Recumbent and unable to stand |
| Suckling reflex | 0 | Present |
| | 3 | Absent |

Minimum possible score = 0, Maximum possible score = 21

incubated at 37°C for 24 h for septicemia confirmation. Then pure culture was provided to detect the isolated bacteria and serotyping was performed to confirm the E-coli strain O111:H8. The plasma concentration of IFN-Ɣ and TNF-α was measured with commercial kits (AbD-serotec kit, Bio-Rad Company, UK and Vet Set kit, Kingfisher Biotech INC, USA), respectively. Statistical analysis of data was performed using SPSS version 13.0. Ordinal data were expressed as median values and changes occurring in different times were analyzed by non-parametric Friedman test. Descriptive data were expressed as mean and standard deviation (SD). Illness procedure and changes in cytokines levels were done with repeated measures ANOVA and significance level was considered as P less than 0.05.

## Results

Clinical examination performed on calves was normal before and at challenge time. Appetite, dehydration, behavior, and standing ability changed after septicemia induction and reached a peak at 3.5, 3, 3, 3 to 3.5h after challenge, respectively. Appetite in all calves during the adaptation period and at challenge time was normal (Score 0). As disease progressed, the calves became reluctant to eat and median score was 2 between 3 and 7.5 h post challenge. In 22% of calves (2 of 9), anorexia was observed (score 3) at 0.5h after challenge and in 33% (3 of 9) it appeared between 1 to 3.5h after challenge. Change proceeding in these times was significant ($P < 0.001$). Suckling reflex had median score 3 between 2.5 and 12h after challenge. Seventy-eight percent of calves (7 of 9) showed lack of suckling reflex at 3.5h after challenge. The changes were not significant ($P = 0.166$).

After colisepticemia induction and signs progression, feces became semisolid, and liquid with solid particles (score 2 and 3, respectively). The minimum fecal nature was score 2 (liquid with solid particles) and appeared in one calf between 2 and 6.5h and between 2.5 and 18h after challenge. Thirty-three percent of the calves had decrease in fecal nature from 12h after challenge to end of study, changing the score from 1 to 2. However, the changes were not significant ($P = 0.067$). Maximum dehydration score was 2 in 55% of the calves to 5h

after challenge. Twenty-two percent of calves from 0.5h after challenge had score 1, and 22% had no signs of dehydration. Also, 67% of the calves showed score 1 at hour 24. In the present study maximum score for dehydration was 2 and 78% of the calves had various degree of dehydration during study. Changes were significant in Freidman test ($P < 0.001$).

Median score for standing ability from first hour to end of the study was 1 and calves were able to stand, but with difficulty. Twenty-two percent of the calves showed score from 0.5 to 3.5h after challenge. Maximum weakness in standing appeared in 78% of the calves at 3 to 3.5h after challenge and changes proceedings were significant in Freidman test ($P < 0.001$). Thirty minutes after challenge, 44% of the calves showed behavioral changes. Maximum median score was 2 from 2.5h to 5h after challenge. Maximum score (score 3) for behavior was only observed in one calf (11%) at 3 and 3.5h. Change proceeding was significant ($P < 0.001$).

One out of 9 calves showed signs of a mild shock at 0.5h; on the other hand, 33% of the calves showed a score of 2 for shock at first hour after microbial challenge. Sixty-seven percent of calves had no signs of shock. Changes pertaining to shock were not significant ($P > 0.05$). Total score ranged from 0 to 21 (the dead calf) and its peak was observed at 3-3.5h after challenge. Median total score at 3 and 3.5h after challenge was 11. Maximum total score was 17 in one calf at 1 and 1.5h after challenge. Median total score was 5 at 24h. Changing of total score was significant ($P < 0.001$) and details are shown in Figure 1.

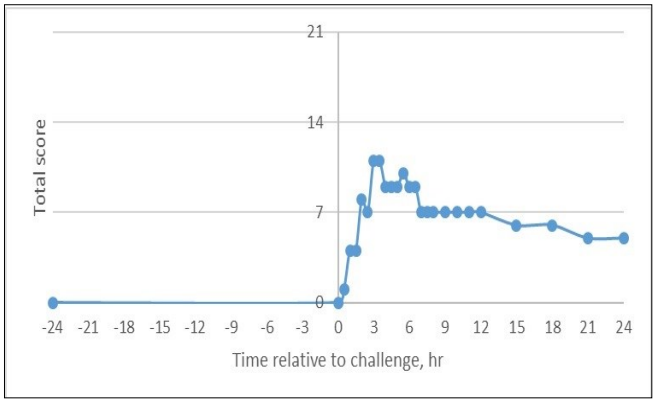


**Figure 1.** Effects of intravenous E.coli challenge dose on total score (Median).

Mean temperature during adaptation period and at the time of challenge was 38.6 and 38.7°C, respectively. Thirty minutes after colisepticemia, the temperature reached 39.2°C. In the current study, increase in body temperature after the challenge was significant at -6 h and maximum temperature was 39.7°C at hour six. In that time, 78% of calves had temperature more than 39.5°C. At the end of study, mean temperature was 39.5°C and 33% of the calves had rectal temperature more than 39.5°C. Maximum temperature recorded in this study was 40.9°C in one calf at hour 12 post challenge. In general, fever occurred in all the calves mostly between 3 and 12h after the challenge and showed reduction to normal after that (Figure 2). Repeated measures of ANOVA showed significant temperature changes ($P < 0.001$).

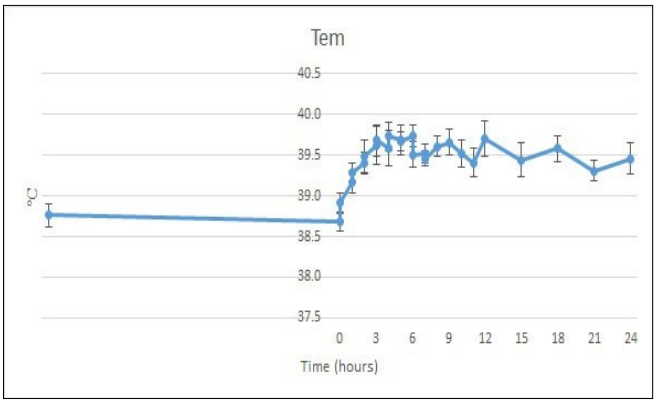


**Figure 2.** Effects of intravenous E.coli challenge dose on rectal temperature (Mean ± SE).

The highest and lowest mean heart rate was at 4h (116 bpm) and 1h (83 bpm) after challenge, respectively. Analysis of these changes showed significances ($P = 0.040$). Increasing respiratory rate was observed from challenge time to 2.5h later on and also at 6.5 and 12h after the challenge (Figure 3). Respiratory rate reached 48 rpm at 2.5h after challenge (respiratory rate before challenge was 27.4 rpm). In general, respiratory rate variations were significant ($P = 0.009$).

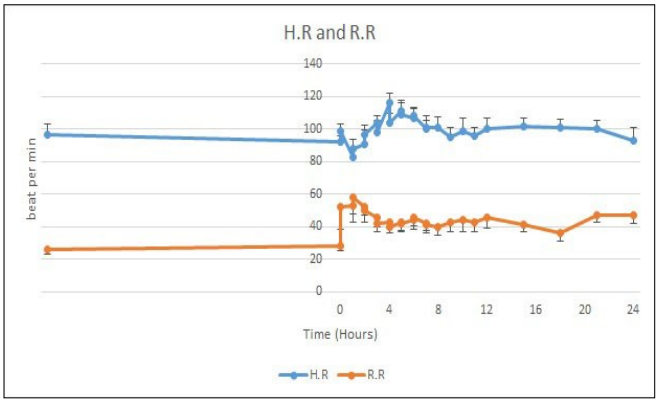


**Figure 3**. Effects of intravenous E.coli challenge dose on heart rate (H.R) and respiratory rate (R.R). (Mean ± SE).

Mean concentration of TNF-α increased 2h after the challenge from 484.9 to 682.6 pg/ml but no significant changes were observed after that. Mean plasma concentration of IFN-ϒ in adaptation period and 8 hours after the challenge time was 427.4 and 451.1 pg/ml respectively and reached pretreatment value after 24 hours. However, these changes were not significant (Table 2).

**Table 2.** Changes of plasma cytokines during experimental septicemia with E-coli O111:H8.

| | TNFα (pg/ml) | IFN- ϒ (pg/ml) |
|---|---|---|
| Hour -24 | 487 ± 18 | 426 ± 18 |
| Hour 0 | 449 ± 29 | 433 ± 16 |
| Hour 2 | 598 ± 32 | 448 ± 32 |
| Hour 8 | 487 ± 39 | 451 ± 33 |
| Hour 24 | 521 ± 13 | 428 ± 18 |

The blood cultures for all calves at the time of challenge (0h) were negative, and at 8, 15, and 24h later were positive for E.coli. One of 10 calves showed severe signs of shock and died 25 minutes after the challenge. Bacterial culture of tissue samples of the dead calf included lung, liver, heart, spleen, intestine, cerebrospinal fluid, and brain was positive for E.coli. No bacteria were isolated from bone marrow culture. Microscopic and macroscopic pathological examination revealed septic shock as the cause of death. Serotyping of isolated bacteria from blood and necropsy confirmed their strain O111:H8. The mortality rate in present study was 10%.

## Discussion

Two models for prediction and diagnosis of septicemia and bacteremia have been described by Fecteau et al. (1997) and Lofstedt (1999). These models are used on farm to assist in early and better diagnosis of sepsis. Clinical evaluation and experience are the major tools available for veterinarians to start treatment in a prompt but rational manner. An association between an objective clinical evaluation and bacteremia has been reported for bovine neonates, as it has for human infants and foals (Brewer and Koterba, 1988; Tollner 1982). Fecteau et al. (1997) suggested a model to predict bacteremia in ill calves based on clinical sepsis score (Fecteau et al., 1997). A clinical illness score system is commonly used in the clinical assessment of animals to measure the probability of a specific outcome (Amrine et al., 2013). In the present study, as disease progressed, the calves became reluctant to eat and median score for appetite was 2 at 3 to 7.5 h after the challenge. These findings are similar to Thomas et al. (2004) study which showed loss of appetite was a common sign in all septicemic calves and anorexia was reported in 38% of them (32 of 84). In another study on experimental endotoxemia in calves, severe inappetence was observed at 3h after endotoxin injection which is in accordance with our study (Gerros et al., 1993). Thomas et al. (2004) stated that lack of suckling reflex was observed in 54% of septicemic calves which is similar to our observations at 2.5, 4 to 5.5 and 8 to 12h after the challenge (Thomas et al., 2004). Gerros et al. (1993) reported maximum lack of the suckling reflex at 3h after E.coli LPS challenge. In another study, Lofstedt et al. (1999) reported that lack of suckling reflex or weak reflex in septic calves was 91.8% compared with non-septic calves (Lofstedt et al., 1999). In septicemic colibacillosis, diarrhea occurs in the final stages of disease in which the volume of feces increases, but it is not liquid excess (Fecteau et al., 2009). Thomas reported that 79% of septicemic calves had liquid feces which could be due to disease progression before the beginning of treatment (Thomas et al., 2004). The calves in the present study did not show significant fecal changes, which could be due to intravenous rout of infection. The best assessment of dehydration in calves is eyeball recession into the orbit and skin tent duration in the neck (normally < 2 sec). In the present study, score 2 was recorded as maximum score for dehydration and it was observed that, with this score 78% of the calves had various degree of dehydration. Using the same score, Thomas et al. (2004) reported 89% of calves were dehydrated and Lofstedt et al. (1999) stated 7.7% dehydration in 92% of septicemic calves.

Thirty minutes after the challenge, 44% of the calves showed behavioral changes and median score was 2 from 2.5h to 5h after challenge. Ballou et al. (2011) studied pathophysiological response in calves infused E.coli intravenously and stated that recumbency and lack of response to stimuli occurred in 67% of the calves at the time of the peak behavioral changes (Ballou et al., 2011). Thomas et al. (2004). also reported 83% incidence of depression in septic calves. In the present study, all calves had behavioral changes and 78% showed depression at 3 and 3.5h after challenge. In calves with experimental endotoxemia, maximum scores were observed in the first and second hours after endotoxin injection. Lofstedt et al. (1999) proposed a model to predict septicemia in calves with diarrhea and reported 68.8% depression, 29.9% coma, and 84.6% recumbency in septicemic calves (Lofstedt et al., 1999). Basoglu et al. (2004) studied the serum concentration of TNF-α in septic calves and reported depression in 70% of TNF-positive calves and 74% of TNF-negative calves similar to the present results. Basoglu et al. (2004), reported 30% of TNF-positive and 13% of TNF-negative calves were comatose (Basoglu et al., 2004). Smith and Halls (1968) reported rapid changes in behavior which lasted for 1-5h after disease induction in calves which received E.coli O78:K80

intravenously (Smith and Halls, 1968).

In our study, 67% of the calves had no signs of shock. However, Thomas et al (2004) reported shock in all calves using similar scoring system, but only 5% had marked signs. In the present study an increase in body temperature 3-6 h after the bacteria injection with maximum rise (39.7°C) at 6 hours after challenge occurred. In another study, Thomas showed 32% of septic calves were febrile (Thomas et al., 2004). Ballou et al. (2011) reported rise in body temperature in calves with experimental septicemia was 40.3 °C and 39.7°C 12 and 24 hours respectively after the challenge (Ballou et al., 2011). The calves in the present study had milder fever as compared with calves in the Ballou study. Mean rectal temperature of 39°C in 28 septic calves was also reported (Fecteau et al., 1997). Lofstedt et al (1999) stated mean rectal temperatures in septic calves and non-septic calves were 37.6 and 38.2°C, respectively. Hypothermia in calves in the Lofstedt study was not unexpected because only 29.5% of septic calves survived in their research. Mean temperatures of 37°C and 39.8°C, respectively were observed in TNF-positive and TNF-negative septic calves (Basoglu et al., 2004). Peripheral inflammation during septicemia and endotoxemia can directly stimulate central nervous system and affect the thermoregulatory center in the brain (Givalois et al., 1994). According to the literature, normal heart rate in calf is between 72 and 120 bpm (Constable et al., 2017). No significant changes in the heart rate of calves were observed after bacterial challenge in the present study. Fecteau (1997) reported mean heart rate in bacteremic and non-bacteremic calves in their study was 104.8 and 111.8 bpm, respectively (Fecteau et al., 1997). Kinsbergen and Bruckmaier (1994) observed increased heart rate in calves (145bpm) till 3 to 4h after endotoxin administration. Respiratory rate reached 48 rpm at 2.5h after the challenge. Mean respiratory rate of 58.2 and 57 rpm, respectively in bacteremic and non-bacteremic calves was reported. Basoglu et al (2004) stated that mean respiratory rate in TNF-positive and TNF-negative calves was 48 and 35 rpm, respectively. Kinsbergen and Bruckmaier( 1994) observed an increased respiratory rate lasting for 3 to 4 hours in calves after IV endotoxin injection (Kinsbergen and Bruckmaier, 1994). Lofstedt (1999) reported 44rpm as a predictor of septicemia in diarrheic calves (Lofsted et al., 1999). In another study no relationship of the vital signs (heart rate, respiratory rate, and rectal temperature) as marker to differentiate bacteremic from non bacteremic calves was observed (Fecteau et al., 1997). The autonomic nervous system controls heart rate, body temperature, respiratory rate, and other physiological adjustments to maintain a stable internal environment. This system, as well as immune system, is influenced by inflammatory responses. Their main interaction is through hypothalamus-pituitary-adrenergic axis (Chen et al., 2011).

TNF-α is a primary mediator of inflammation, and has been implicated in a large number of infectious and non-infectious inflammatory diseases. TNF-α plays various roles in acute phase of inflammation induced by the negative gram bacteria. A significant increase in the TNF-α of calves blood was observed 2 hours after bacterial challenge in the current research. In a study using chronically awake instrumented sheep, infusion of TNF-α resulted in pulmonary hypertension, decreased lung compliance, hypoxemia, and increases in pulmonary micro vascular permeability (Blackwell and Christman, 1996). Carroll et al in 2009 studied the profile of the bovine acute-phase response following an intravenous bolus-dose lipopolysaccharide challenge and found that the mean serum concentration of IFN-ϒ and TNF-α before challenge was 27±13 pg/ml and 85±49 pg/ml, respectively. Basoglu (2004) stated that TNF-α in healthy calves was 234±115 pg/ml. Ballou et al. (2011) used various doses of E.coli in calves to induce septicemia and only calves with injection of 1.5 × 109 CFU and more showed acute increase in TNF-α concentration 2-3h after challenge. However, this increase was not statistically significant. The lower doses of bacteria used in that study could be the reason for no enhancement in cytokines in the plasma. In a study a close relationionship between severity of disease and serum concentration of TNF-α has been shown (Basoglu et al., 2004). In the present study, the highest level of TNF-α was measured in 2-6h post challenge concurrently associated with marked increase in body temperature (3-6h post challenge). It has also been shown that some other clinical criteria including weakness or lack of suckling reflex, recumbency, no response to stimuli and comatose attitude, and mortality rate could be observed in calves with higher TNF-α level (Carrol et al., 2009; Kinsbergen and Bruckmaier, 1994). E.coli endotoxin infused to healthy volunteers significantly increased TNF-α with a peak 1h after infusion which is in agreement with the results of present study in the calves (Michie et al., 1988).

It has been shown that IFN-ϒ increases in the blood during septic shock. In the current study IFN-ϒ reached its peak at 8h after bacterial challenge and returned to baseline within 24h and changes of plasma IFN-ϒ were not significant. Other researchers showed a rise in peripheral IFN-ϒ with a peak at 4h post bacterial challenge (Ballou et al., 2011; Carrol et al., 2009). Carroll et al. (2009) reported that intravenous LPS

administration in beef calves caused a peak of IFN-ϒ after 3-4h, returning to base level within 12h.

In the present investigation 100 percent of the calves showed bacteremia and one of them died 25 minutes after the challenge. The mortality rate in the present study was 10%. Fecteau et al in two different experiments in 2011 and 1997 observed that 24 and 31 percent of severely ill calves were bacteremic and the mortality of blood culture positive calves was greater than 57.4% versus 15.1 % for blood culture negative calves. In another study, the same researchers reported that of the 51 bacterial isolates, 51% were Escherichia coli, 25.5% from other gram negative enteric, 5.9% gram negative anaerobes, 11.8% gram positive cocci and 5.9% gram positive rods (Fecteau et al., 2001). Although presumptive diagnosis can be made in a significant proportion of cases on the basis of clinical signs, particularly if quantified by a standardized clinical scoring system, blood culture remains the definitive diagnostic test for septicemia.

## Conclusion

It should be considered that unnecessary treatment or treatment of every ill calf with systemic antimicrobials may result in unnecessary expenses, treatment side effects, drug residues, and increased resistance to antimicrobials. Prediction of sepsis allows for a more accurate prognosis and estimates the treatment costs required for a successful treatment.

In the present study, the results of clinical scoring, laboratory tests, and statistical analysis showed that the total score in the colisepticemic calves increased. However, scores of some criterion such as shock may have no marked increase. Also, it seems that the heart and respiratory rates may show insignificant changes and are not appropriate to assess the infection magnitude and disease progression. Using observation and various examinations as an appropriate clinical tool leads to early diagnosis, but has moderate sensitivity and ability to predict the disease procedures and prognosis. It seems that additional laboratory tests combined with clinical scores will increase sensitivity and specificity of scoring systems for application on farms.

**Compliance with ethical standards:** All experimental procedures performed in this study involving animals were followed the guidelines on ethical standards for experimental processes in animals according to a protocol approved by the Animal Ethics Committee, University of Tehran, Iran.

**Author contribution:** Conceptualization: Samad Lotfollahzadeh, Mohammad Reza Mokhber Dezfoulie, Data curation: Gholamreza Nikbakht Boroujeni, Masoomeh Heidari, Supervision: Samad Lotfollahzadeh, Gholamreza Nikbakht Boroujeni, Writing - original draft: Masoomeh Heidari, Writing - review & editing: Samad Lotfollahzadeh.

**Conflict of interests:** Author, Samad Lotfollahzadeh declares that he has no conflict of interest. Author, Author Mohammad Reza Mokhber Dezfouli declares that he has no conflict of interest. Author Masoomeh Heidari declares that she has no conflict of interest, Author Gholamreza Nikbakht Boroujeni declares that he has no conflict of interest.

## References


Adams, J. L., Semrad, S. D.,& Czuprynski, C. J. (1990). Administration of bacterial lipopolysaccharide elicits circulating tumor necrosis factor-alpha in neonatal calves. *Journal of Clinical Microbiology, 28*(5), 998-1001.

Aldridge, B. M., Garry, F. B., & Adams, R. (1993). Neonatal septicemia in calves: 25 cases (1985-1990). *Journal of American Veterinary Medicine Association*, *203*(9), 1324-1329.

Amrine, D, E., White, B. J., Larson, R., Anderson D. E., Mosier D. A., & Cernicchiaro N. (2013). Precision and accuracy of clinical illness scores, compared with pulmonary consolidation scores, in Holstein calves with experimentally induced *Mycoplasma bovis* pneumonia. *Americam Journal of Veterinary Research, 74*(2), 310-315.

Annane, D., Bellissant, E., & Cavaillon, J. M. (2005). Septic shock. *Lancet, 365*(9453), 63-78.

Ballou M. A, Cobba C. J, Hulberta, L. E, & Carroll J. A. (2011). Effects of intravenous Escherichia coli dose on the pathophysiological response of colostrum-fed Jersey calves. *Veterinary Immunology and Immunopathology*, *141*(1-2 ), 76-83.

Basoglu, A., Sen, I., Sevinc, M., & Simsek, A. (2004). Serum Concentrations of Tumor Necrosis Factor-α in Neonatal Calves with Presumed Septicemia. *Journal of Veterinary Internal Medicine, 18*(2), 238-241.

Beutler, B., Greenwald, D., Hulmes, J. D., Chang, M., Pan, Y-C. E., Mathison, J., Ulevitch, R., & Cerami, A. (1985). Identity of tumour necrosis factor and the macrophage-secreted factor cachectin. *Nature, 316* (6028), 552-554.

Bieniek, K., Szuster-Ciesielska, A., Kamin´ska, T., Kondracki, M., Witek, M., & Kandefer-Szerszeń, M. (1998). Tumor necrosis factor and interferon activity in the circulation of calves after repeated injection of low doses of lipopolysaccharide. *Veterinary Immunology and Immunopathology, 62*(4), 297-307.

Blackwell, T. S., &Christman, J. W. (1996). Sepsis and cytokines: current status. *British Journal of Anaesthiology, 77*(1), 110-117.

Brewer, B. D., & Koterba, A. M., (1988). Development of a scoring system for early diagnosis of equine neonatal sepsis. *Equine Veterinary Journal, 20*(1), 18-22.

Carroll, J. A., Reuter, R. R., Chase, C. C. Jr., Coleman, S. W., Riley, D. G., Spiers, D. E., Arthington, J. D., & Galyean, M. L. (2009). Profile of the bovine acute-phase response following an intravenous bolus-dose lipopolysaccharide challenge. *Innate Immunology, 15*(2), 81-89.

Chen, X., Yin, Y., & Zhang, J. (2011). Sepsis and immune response. *World Journal of Emergency Medicine, 2*(2), 88-92.

Constable, P. D., Hinchcliff, K. W., Done, S., & Gruenberg, W. (2017). *Veterinary Medicine, A textbook of the diseases of cattle, horses, sheep, pigs and goats. 11th ed*. Philadelphia, USA: W. B. Saunders Ltd.

Fecteau, G., Fairbrother, J. M., Higgins, R., Van Metre, D. C., Paré, J., Smith, B. P., Holmberg, C.A., & Jang, S. (2001). Virulence factors in *E-coli* isolated from blood of bacteremic neonatal calves. *Veterinary Microbiology, 78*(3), 241-249.

Fecteau, G., Pare, J., Van Metre, D. C., Smith, B. P., Holmberg, C. A., Guterbock, W., & Jang, S. (1997). Use of a clinical sepsis score for predicting bacteremia in neonatal dairy calves on a calf rearing farm. *Canadian Veterinary Journal, 38*(2), 101-104.

Fecteau, G., Smith, B. P., & George, L. W. (2009). Septicemia and Meningitis in the Newborn Calf. *Veterinary Clinics of North America: Food Animal Practice, 25*(1), 195-208.

Gay, C. C., & Besser, T. E. (1994). Escherichia coli septicaemia in calves. In C. L. Gyles, (Ed.). *Escherichia coli in domestic animals and humans*. Wallingford, USA: CAB International.

Gerros, T. C., Semrad, S. D., Proctor R. A., & LaBorde A. (1993). Effect of dose and method of administration of endotoxin on cell mediator release in neonatal calves. *American Journal of Veterinary Research, 54* (12), 2121-2127.

Givalois, L., Dornand, J., Mekaouche, M., Solier, M. D., Bristow, A.F., Ixart, G., Siaud, P., Assenmacher, I., & Barbanel, G. (1994). Temporal cascade of plasma level surges in ACTH, corticosterone, and cytokines in endotoxin-challenged rats. American Journal of Physiology, 267(1), R164-R170.

Hulbert, L. E., Cobb, C. J., Carroll, J. A., & Ballou, M. A. (2011). Effects of changing feeding milk replacer from twice to once daily on Holstein calf innate immune responses before and after weaning. *Journal of Dairy Science, 94*(5), 2557-2565.

Kinsbergen, M., & Bruckmaier, R. M. (1994). Metabolic, endocrine and haematological responses to intravenous E.coli endotoxin administration in 1-week- old calves. *Zentralblatt für Veterinärmedizin . A, 41*(7), 530-547.

LeMay, L. G., Vander, A. J., & Kluger, M. J. (1990). The effects of pentoxifylline on lipopolysaccharide (LPS) fever, plasma interleukin 6 (IL 6), and tumor necrosis factor (TNF) in the rat. *Cytokine, 2*(4), 300-306.

Lofstedt, J., Dohoo, I. R., & Duizer, G. (1999). Model to predict septicemia in diarrheic calves. *Journal of Veterinary Internal Medicine, 13*(2), 81-88.

Michie, H. R., Manogue, K. R., Spriggs, D. R., Revhaug, A., O'Dwyer, S., Dinarello, C. A., Cerami, A., Wolff, S.M., & Wilmore, D. W. (1988). Detection of circulating tumor necrosis factor after endotoxin administration. *New England Journal of Medicine, 318*(23), 1481-1486.

Rabbani, M., Zahraie-Salehi, T., Dezfouli, M. R., Rezazadeh, F., Yoosefi Ramandi, F., & Bahonar, A. R. (2007). Detection of anti-E.coli, rotavirus and corona virus antibodies in sera samples of diarrheic and normal calves under 1 month of age. J*ournal of Veterinary Research, 62*(3), 145-149.

Rezazadeh, F., Zahraei-Salehi, T., Mokhber Dezfouli, M.R., Rabani, M., Morshedi, A., Khaki, Z., Nabian, S., Rahbari, S., & Bahonar, A. (2004). Clinincal, biochemical and microbiological findings of calves diarrhea in a dairy herd in Suburb of Tehran. *Journal of Veterinary Research, 59*(4), 301-308.

Smith, H. W., & Halls, S. (1968). The experimental infection of calves with bacteriaemia-producing strains of Escherichia coli: the influence of colostrum. *Journal of Medical Microbiology, 1*(1), 61-78.

Thomas, E., Roy, O., Skowronski, V., Zschiesche, E., Martin, G., & Böttner, A. (2004). Comparative field efficacy study between cefquinome and gentamicin in neonatal calves with clinical signs of septicaemia. *Revue Médicine Véterinair, 155*(10), 489-493.

Tollner, U. (1982). Early diagnosis of septicemia in the newborn. *European Journal of Pediatrics, 138*(4), 331-337.