TURKISH JOURNAL OF VETERINARY RESEARCH

Vol: 4 No:1
Year 2020
E-ISSN: 2602-3695

TJVR
http://dergipark.gov.tr/tjvr

TURKISH JOURNAL OF VETERINARY RESEARCH

E-ISSN:2602-3695

Year	Volume	Issue / Number
2020	4	1

Publisher

Prof. Dr. Ebubekir CEYLAN

All articles in this journal are available free of charge from
http://dergipark.gov.tr/tjvr
TJVR is published biannually

Abstracting & Indexing:

The **Turkish Journal of Veterinary Research (TJVR)** is indexed and abstracted by the following indexes and platforms (Alphabetical order); *Academic Keys, Asos Index, Bielefeld Academic Search Engine (BASE), CAB Abstracts, CiteFactor, COSMOS IF, DOJI indexing, DRJI, Entireweb, Eurasian Scientific Journal Index, General Impact Factor, Google Scholar, IdealOnline, Index Copernicus, International Innovative Journal Impact Factor, IP Indexing, i2or, J-Gate, JIFACTOR, Journal Factor, Journal TOCs, JournalSeek, Open Access Library (oalib), OpenAIRE, Pmi2Cite, ResearchBib, Rootindexing, Scholar Article Journal Index, Sindex, Systematic Impact, Worldcat, UDL-Edge (i-Journals and i-Focus), Ulrichsweb.*

Original Article

TJVR 2020; 4 (1): 1-8

Turkish Journal of Veterinary Research

http://www.dergipark.gov.tr/tjvr

e-ISSN: 2602-3695

Relationship between oxidative stress status and glycoprotein-associated pregnancy concentrations during the early pregnancy period in dairy cows

Abdelhanine Ayad[1] Priya Yuvaraju[2] Sumaya Beegam[3] Abderrahim Nemmar[4]

[1] *Department of Biological Sciences of the Environment, Faculty of Nature and Life Sciences, University of Bejaia, Bejaia, Algeria*
[2] *Departments of Physiology, College of Medicine and Health Sciences, United Arab Emirates University, Ai Ain, United Arab Emirates.*
[3] *Departments of Physiology, College of Medicine and Health Sciences, United Arab Emirates University, Ai Ain, United Arab Emirates.*
[4] *Departments of Physiology, College of Medicine and Health Sciences, United Arab Emirates University, Ai Ain, United Arab Emirates.*

Correspondence: *Abdelhanine Ayad (hanine06@gmail.com)*

Received: 13.10.2019 Accepted: 02.01.2020

ABSTRACT

Objective: This study was planned to assess the possible relationships with pregnancy-associated glycoproteins (PAG) concentrations by the determination of the biomarkers of oxidative stress in the plasma of dairy cattle during the early period of gestation.

Materials and Methods: Blood samples were collected from coccygeal vessels in pregnant (n = 54) and non-pregnant (n = 45) cows. Measurement of biomarkers of oxidative stress (LPO, GSH and SOD) was carried out in females using spectrophotometric method.

Results: Plasma PAG concentrations increased and continuously over the both periods investigated. There were significant differences between pregnant and non-pregnant groups ($P < 0.001$). The concentration of SOD were significantly lower ($P< 0.05$) in pregnant females from day 25 to 35 (7.08 ±0.31 U.ml^{-1}) and day 36 to 50 after AI (6.6 ± 0.29 U.ml^{-1}) compared with non-pregnant cows (7.59 ± 0.35 U.ml^{-1}). Concerning the concentrations of LPO and GSH, the values obtained were also significant lower ($P < 0.05$) in pregnant females in the period 25-35 days post AI (122.7 ±10.27 µM and 6.46 ±1.24 µmol/min.ml^{-1}, respectively) and 36-50 days post AI (108.05±6.17 µM and 6.2±0.77 µmol/min.ml^{-1}, respectively) than in the non-pregnant females (124.8 ± 12.16 µM and 6.96 ± 0.92 µmol/min.ml^{-1}, respectively).

Conclusion: It was observed that the markers of oxidative stress tended to be higher in non-pregnant females compared with pregnant females during the early period of gestation in dairy cattle. Our results suggest the existence of a relationship among the concentration of oxidative stress markers and PAG during early pregnancy.

Keywords: *Bovine; Pregnancy; Pregnancy-associated glycoproteins, Oxidative stress*

INTRODUCTION

During pregnancy, several hormones and proteins are synthesized and secreted in the maternal circulation by ovaries, the placenta and the foetus. Some of them are specific to gestation and being detected in maternal blood from the moment when the conceptus becomes more closely attached to the uterine wall and the formation of placentomes (Chavatte-Palmer and Tarrade, 2016).

Pregnancy-associated glycoproteins (PAG) constitute a large family of molecules specifically expressed in the outer epithelial cell layer of the placenta in eutherian species. Their functions are not fully understood and they tend to be enzymatically inactive because of a mature in their active site (Sousa *et al*, 2006). Some immune

suppressive properties of bovine PAGs and PAG-like molecules as pregnancy specific protein-B (PSPB) have been suggested in cattle (Hoeben *et al*, 2000). In particular, diminished immune functions of peripheral blood polymorphonuclear neutrophils (PMN) have been detected after PAGs peak at parturition (Dosogne *et al*, 1999). In a recent study, a clear relationship between high plasma PAG levels and elevated total leukocyte and neutrophil counts has been shown (Abdelfatah-Hassan *et al*, 2012). Neutrophils play an important role in host defense mechanisms and are known to cause tissue damage at the inflammatory site (Sordillo and Aitken, 2009). The activation of PMN is characterized by the production of reactive oxygen species (ROS) causing oxidative stress (Nathan and Cunningham-Bussel, 2013). The last may result from increased production of free radicals and reactive oxygen species, and/or a decrease in antioxidant defense which leads to damage of biological macromolecules and disruption of normal metabolism and physiology (Trevisan *et al*, 2001). It is well known that toxic ROS and oxidative stress are of primary importance in immune and inflammatory mechanisms (Codoñer-Franch *et al*, 2011) involved in the majority of diseases (Poston *et al*, 2011; Tuuli *et al*, 2011). Several studies confirmed the occurrence of oxidative stress during pregnancy, parturition (Fainaru *et al*, 2002; Lopez-Gatius *et al*, 2007) as well as during post-parturient period (Wall *et al*, 2002).

Pregnancy is a physiological event characterized by a drastic increase in energetic demands, to ensure an adequate fetal development and growth, thus, both mother and fetus are likely to experience oxidative stress (Garrel *et al*, 2010) with increased oxygen free radicals production (Mohanty *et al*, 2006). In normal pregnancy, there is a physiological oxidative balance where vitamin C, vitamin D and E gradually increase leading to a maintained oxidative balance throughout pregnancy (Bomba-Opon *et al*, 2014). There are several studies to support the concept that oxidative stress is a significant underlying factor to dysfunctional host immune and inflammatory responses that can increase the susceptibility dairy cattle to a variety of health disorders (Castillo *et al*, 2005; Wilde, 2006). Very early embryo mortality depends on blastocyst development capacities and uterine environment features (Gilbert, 2011) and it is well known in humans, ruminants and other animal species, that reactive oxygen species ROS are involved in embryo/fetal loss (Talukder *et al*, 2014).

Oxidative stress can be monitored with several biomarkers which can be assessed in plasma such as lipid peroxidation (LPO) and antioxidants including reduced glutathione (GSH) and superoxide dismutase (SOD). Whether oxidative status (LPO, GSH and SOD concentration) is related to trophoblast secretory properties (PAG secretion) during embryonic and early fetal development remains to be elucidated. To verify our hypothesis, we evaluated oxidative status and assessed the possible relationships with PAG concentrations by measuring LPO, GSH and SOD in Holstein Friesian dairy cattle during the early period of gestation.

MATERIALS and METHODS

Animals

The experiment was carried out from March to August 2011 in different dairy herds in Bass Kabylie area (36°34'N, 5°04'E), Algeria. This research was approved by the Scientific Council of the Faculty of Nature and Life Sciences (Report of Faculty Scientific Council Nbr. 07 dated December 14, 2010), University of Bejaia, Algeria). Concerning the ethical aspects, the experimental procedure was performed *in vitro* and the blood sampling of females was performed according to good veterinary practice under farm conditions. A total of 99 Holstein Friesian dairy cattle with mixed age (06 months and 12 years) and parity (0–9) were used in this study. The body condition score (BCS) was determined during the blood sampling period using a 5-points scale as described previously (Green *et al*, 2014). The BCS of experiment females was between 2.5 to 4.5. Fifty-four and forty-five Holstein Friesian females constituted the pregnant and non-pregnant groups, respectively. The pregnancies of females were diagnosis by ultrasonography (AgROSCAN A14, sondebi frequency 3.5 and 5.0 MHz) at 35-40 days post-artificial insemination (AI) (Wéré *et al*, 2012) or by rectal exploration approximately at 2 to 3 months after AI.

Blood sampling

Blood samples were collected in the in pregnant females between 25 to 50 days. For the control group, samples were randomly collected during a stabling period and in the absence of males. Samples collected from coccygeal vessels were transferred into a tube containing EDTA (Sarstedt®, Numbrecht, Germany). Plasma was obtained by centrifugation (1,500 × *g* for 15 min) immediately after collection and was stored at -20 °C until assay.

Measurement of pregnancy-associated glycoprotein (PAG) concentrations

The PAG concentrations in pregnant and non-pregnant groups were determined in plasma by Enzyme-linked immunosorbent assay (ELISA) performed in duplicates, as previously validated (Ayad *et al*, 2014). The detecting antibody used was a rabbit biotin-conjugated anti-PAG IgG. A spectrophotomer reader was used according to the kit instruction (Ref. Code E.G.7. CER. Marloie, Belgium). The enzyme substrate was avidin-horseradish peroxidase (HRP). The standard curve ranged from 0 to 2 ng/ml.

The basis of the test is a sandwich reaction involving two antibodies raised against PAG: the first one is coated on a 96 micro-plate whereas the second one is conjugated to biotin and detected using avidin-horseradish peroxidase (HRP). Briefly, dilution buffer is added just before adding PAG standards and serum samples. Afterwards, it is followed by an overnight incubation at room temperature. Micro titer wells are washed before addition of biotinylated anti-PAG. The washing step is followed by an incubation of 20 min at 37 °C with avidin-HRP. After the second washing, the substrate/chromogen solution is added to the wells and incubated 30 min at room temperature. The addition of the stopping reagent transforms the blue coloration into a yellow compound. Finally, the absorption at 450 nm was measured and the optical density was found to be proportional to the PAG concentration.

The threshold of 0.8 ng/ml was used to discriminate between pregnant and non-pregnant females (Ayad *et al*, 2009). The intra- and inter-assay coefficients of variation of PAG-ELISA were 2.78 and 6.08 %, respectively.

Measurement of the levels of superoxide dismutase (SOD), lipid peroxidation (LPO) and reduced glutathione (GSH) in plasma blood

Measurement of SOD was carried out in pregnant and non-pregnant females using spectrophotometric method with commercially available kits (Cayman Chemical). NADPH-dependent membrane lipid peroxidation was measured as thiobarbituric acid reactive substance using malonedialdehyde as standard (Sigma-Aldrich Fine Chemicals, St Louis, MO) (Nemmar *et al*, 2013). Measurement of GSH concentrations was carried out in pregnant and non-pregnant females according to the method described by commercially available kit (Sigma-Aldrich Fine Chemicals, Munich, Germany).

Statistical analysis

Data were analyzed using a mixed model for repeated measurements (Statview Software, Version 4.55) taking into account an autocorrelation between data obtained successively on the same animal. The data (± SE) were expressed as values of the PAG concentration and biomarkers of oxidative status. The Fisher's exact test was used to determine whether there was a significant difference between pregnant (P) cows (P-1: 25-35 days post AI; P-2: 36-50 days post AI) and non-pregnant (NP) cows. The values were statistically different when the *P*-value was ≤ 0.05.

RESULTS

Plasma PAG concentrations increased and continuously over the both periods investigated. Pregnant cows had the lowest levels of PAG (3.24±0.25 ng.ml^{-1}) in the period 25-35 days post-AI (Fig. 1). Thereafter, PAG concentrations increased significantly (P=0.05) in the period 36-50 days after fertilization (3.92±0.21 ng.ml^{-1}). The results of this experiment showed a very high PAG concentration (3.59±0.16 ng.ml^{-1}) in pregnant compared with non-pregnant cows (0.53±0.03 ng.ml^{-1}). There were significant differences between pregnant and non-pregnant groups (P < 0.001, Fig. 1).

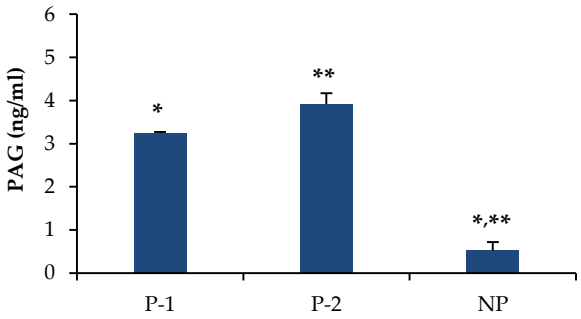

Figure 1. Plasma pregnancy associated glycoprotein concentration (mean ± SE) at early pregnancy period after IA in pregnant (P) cows (P-1: 25-35 days post AI; P-2: 36-50 days post AI) and non-pregnant (NP) cows. *,**: Significant difference in mean concentrations between P-1, P-2 and NP groups (P<0.05)

The levels of SOD (Fig. 2A) were significantly lower (P≤0.05) in pregnant cows at day 36 to 50 after AI (6.6±0.29 U.ml^{-1}) compared with non-pregnant cows (7.59±0.35 U.ml^{-1}). Concerning the concentrations of

LPO (Fig. 2B), the values obtained were significant lower ($P \leq 0.05$) in pregnant cows in the period 25-35 days post AI (122.7±10.27 µM) and 36-50 days post AI (108.05±6.17 µM) than in the non-pregnant cows (124.8±12.16 µM). On the other hand, GSH (Fig. 2C) concentrations were also significantly lower ($P \leq 0.05$) in pregnant cows during the period 36-50 days post AI (6.2±0.77 µmol/min.ml^{-1}) than in the non-pregnant cows (6.96±0.92 µmol/min.ml^{-1}).

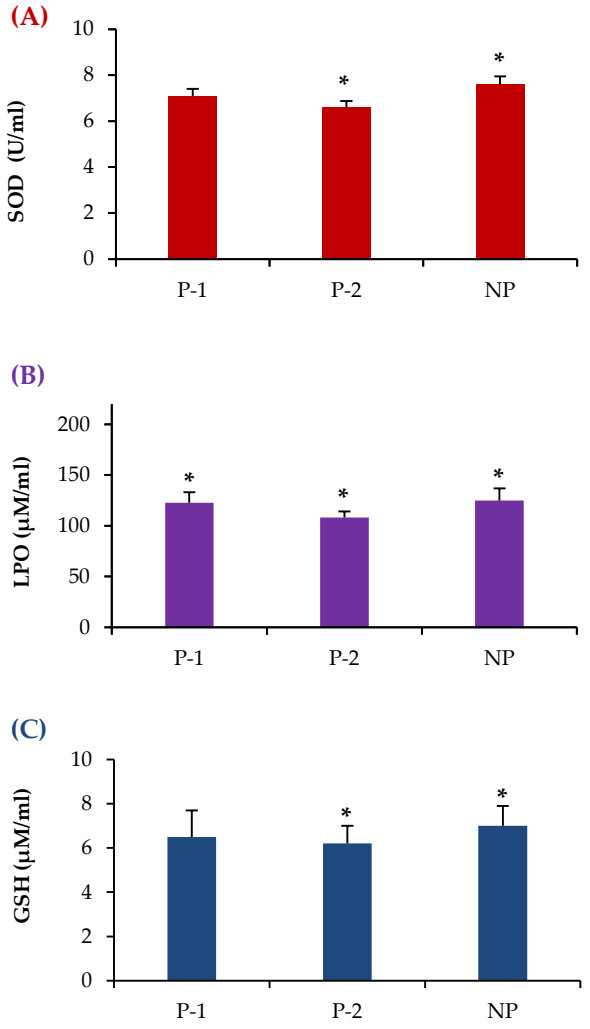

Figure 2. Plasma concentration (mean ± SE) of SOD (2A), LPO (2B) and GSH (2C) levels at early pregnancy period after IA in pregnant females (P-1: 25-35 days post AI; P-2: 36-50 days post AI) and non-pregnant females. * Significant difference in mean concentrations between P-1, P-2 and NP groups ($P<0.05$)

Positive correlation between LPO concentrations and SOD levels was r=0.6 ($P \leq 0.05$, Table 1). There was no significant correlation between PAG and other oxidative stress parameters.

Table 1. Correlation coefficients (r) between of pregnancy associated glycoprotein, SOD, LPO and GSH concentrations at early pregnancy period from day 25 to 50 after AI in pregnant females ($^{*}P < 0.05$)

	SOD	LPO	GSH
LPO	**0.6***		
GSH	-0.25	-0.14	
PAG	0.12	0.05	-0.11

DISCUSSION

This study investigated whether the concentrations of markers of oxidative stress during the early period of pregnancy in dairy cattle could be related to trophoblastic secretory function based on the measurement of PAG concentrations in pregnant cows.

Bovine pregnancy-associated glycoproteins are mainly secreted by trophoblastic binucleate cells (Wooding *et al*, 2005) and they have been detected in the blood pregnancy cows from day 25 after AI (Green *et al*, 2014). Therefore, these glycoproteins have been used as a biochemical marker of pregnancy (Gajewski *et al*, 2014), monitoring of placental secretory function in ongoing pregnancies (Breukelman *et al*, 2012), detection of placental abnormalities (Constant *et al*, 2011) and investigation of embryonic and fetal mortalities (Karen *et al*, 2014). Detection of PAG by radioimmunoassay method (García-Ispierto *et al*, 2015) or ELISA (Piechotta *et al*, 2011) is currently used as a specific serological method for pregnancy diagnosis in cattle from 28 day after breeding, with a threshold level for pregnancy of 0.8 ng/ml (Ayad *et al*, 2009).

The results of this experiment showed that the mean PAG concentrations measured at early gestation period by ELISA method in the pregnant cows agreed with those of Friedrich and Holtz (2010). There was only a significant difference at period 25-30 days after AI. The lack of significant difference in PAG values from 30 to 50 of gestation appears to be probably attributed to the high individual variation in PAG concentrations. A high variability of individual PAG levels has been documented in the literature. Lobago and co-workers (2009) reported that the PAG profiles were considerably influenced by factors such as breed, weight and parity status of the dam. Likewise, it was reported that PAG concentrations during the early fetal pregnancy period are correlated with some factors such as milk production in high

producing dairy cows (Lopez-Gatius *et al,* 2007). In other study, Ayad *et al.* (2009) observed that PAG concentrations tended to be higher in primiparous than nulliparous or multiparous females. However, according to Ledezma-Torres *et al* (2006) parity had no significant effect in plasma PAG levels. As shown in figure 1, the difference between pregnancy and non-pregnant cows were very significant. In the non-pregnant group, ELISA method detected PAG concentration in all females was lower than 0.8 ng/ml (Ayad *et al,* 2009).

Oxidative stress corresponds to an imbalance between the rate of oxidants production and that of their degradation (Sorg, 2004). All mechanisms linked to pregnancy are under control of steroid hormones, prostaglandins as well as other biologically active factors (Kindahl *et al,* 2004). These mechanisms are based on metabolic pathways which are usually aerobic and may be related to the production of certain amounts of ROS. The blastocyst losses the zona pellucid 9-10 days before implantation in the bovine specie (Ayad *et al,* 2006). This is accompanied by an increase in ROS generation, related to an augment in the cell to cell contact and to NADPH oxydase activation. Mitochondrial ROS generation increases during embryogenesis because an imbalance exists between ROS generation and its modulation, with ROS generation being overwhelming (Aurousseau *et al,* 2004).

To our knowledge, this is the first report on the relationship between markers of oxidative stress and PAG concentrations from bovine plasma at early stages of pregnant cows. The activities of the LPO is one the most important expression of oxidative stress induced by ROS, and as well as antioxidant enzymes, namely SOD and GSH. In the present study, LPO, SOD and GSH were determined in plasma sample of pregnant and non-pregnant cows.

Oxidative stress seems to have a role in the cause and progression of a number of reproductive events in both humans and animals, such as fertilization and early embryo development (Al-Gubory *et al,* 2010). Oxidative stress during early placental development is associated with pregnancy-related disorders in humans (Agarwal and Allamaneni, 2004; Gupta *et al,* 2007). The depletion of placental antioxidant systems has been suggested as a key factor in early human pregnancy failure (Liu *et al,* 2006). Some data published suggested that enhanced activities of key antioxidant enzymes with gestational ages may act as protective mechanism against oxidative stress during early human (Qanungo *et al,* 2000) and sheep (Garrel *et al,* 2010) placental development and growth.

Antioxidant enzymes such as superoxide dismutase and glutathione peroxidase could be beneficial in enhancing implantation and maintaining pregnancy by antagonizing the harmful oxygen free radical (Smith *et al,* 1998). Superoxide dismutase enzyme is believed to play a major role in the first line of antioxidant defense by catalyzing the dismutation of superoxide anion radicals to from hydrogen peroxide (H_2O_2) and molecular oxygen **(**Soehnlein *et al,* 2009). Glutathione peroxidase is a selenoprotein that reduce lipidic or non lipidichydroperoxides as well as H_2O_2 while oxidizing GSH (Sordillo *et al,* 2009). It is important to note that mitochondria are the major site of endogenous O_2^- production (Wallace, 2005). $O_2^-$is converted to H_2O_2 by mitochondrial matrix SOD.

The conceptus develops a good defense mechanism against free radicals, possessing high concentrations of antioxidants at 5 weeks of gestation such as vitamin A and E; and increased expression of catalase, Cu, Zn-SOD, Mn-SOD in villous placenta at twelve weeks (Jauniaux *et al,* 2003). The plasma markers of oxidative stress measured in the present study were statistically different between non-pregnant than in pregnant group. These differences could play a role of successful pregnancy during the early period in dairy cattle. It is difficult to compare these results with others reported in literature because of the lack of studies on oxidative stress during the early pregnancy period in dairy cattle. Preliminary results obtained in the present study suggest that PAG concentrations seem have an indirect effect on markers oxidative stress levels in pregnant females during the early period of gestation. Previous studies suggesting that advanced oxidative protein product (AOPPs), as markers of protein oxidation is not recent (Witko-Sarsat *et al,* 1996). The AOPPs are higher in dairy cows suffering from embryonic mortality than in pregnant females (Celi *et al,* 2012). These results are in disagreement with those reported in literature which showed that a SOD level was significantly higher in pregnancy positive than in pregnancy negative in women (Younis *et al,* 2012).

It is known that the polymorphonuclear (PMN) leukocytes play an important role in host defense mechanisms and are known to cause tissue damage at the inflammatory site. The activation of PMN is characterized by the production of reactive oxygen

species (ROS) such as superoxide anion radical (O_2^-), singlet oxygen ($^1O_2$), hydrogen peroxide (H_2O_2), and the highly reactive hydroxyl radical (OH) (Sordillo *et al,* 2009). Considering that the activity of monocytes and macrophages is increased during pregnancy, and that the concentration of several markers of oxidative stress is concomitantly increased, it could be argued that pregnancy is characterized by a pro-inflammatory state (Ness, 2004). It is interesting that decreased SOD, LPO and GSH activities in pregnant females. In this study, during the early period of pregnancy was associated with an increase of PAG concentrations compared with non-pregnant group. Several investigations carried out in order to detect relations between PAG or PSPB and a local immunological function. Dosogne and associated (2000) reported the succession of the very high concentrations of PAG and the decrease of the oxidation activity of the polymorphonuclear neutrophils. In other study, the results indicate that bPAG may directly or indirectly affect the respiratory burst activity of bovine neutrophils in the periparturient period (Hoeben *et al,* 2000).

CONCLUSION

In conclusion, this study presents a first report on the relationship between oxidative stress and PAG concentrations during the first trimester of pregnancy in dairy cattle. It was observed that the markers of oxidative stress tended to be higher in non-pregnant females (PAG concentration 0.53±0.03 ng.ml^{-1}) compared with pregnant females (PAG concentration, 3.59±0.16 ng.ml^{-1}) during the early period of gestation in dairy cattle. However, further studies including large-scale investigations are needed to confirm our results, as well as to investigate maternal concentrations of both oxidative stress parameters and PAG in pregnant females experiencing interrupted pregnancies.

ACKNOWLEDGMENTS

Conflict of Interests: The authors declared that there is no conflict of interests.

Financial Disclosure: The authors declared that this study has received no financial support.

REFERENCES

Abdelfatah-Hassan A, Almería S, Serrano B, de Sousa NM, Beckers JF, López-Gatius F. The inseminating bull and plasma pregnancy-associated glycoprotein (PAG) levels were related to peripheral leukocyte counts during the late pregnancy/early postpartum period in high-producing dairy cows. *Theriogenology* 2012; 77(7): 1390–1397.

Agarwal A, Allamaneni SSR. Role of free radicals in female reproductive diseases and assisted reproduction. *Reprod Biomed Online* 2004; 9: 338–347.

Al-Gubory KH, Fowler PA, Garrel C. The roles of cellular reactive oxygen species, oxidative stress and antioxidants in pregnancy outcomes. *Int J Biochem Cell Biol* 2010; 42(10): 1634-1650.

Anastasakis E, Papantoniou N, Daskalakis G, Mesogitis S, Antsaklis A. Screening for pre-eclampsia by oxidative stress markers and uteroplacental blood flow. *J Obstet Gynaecol* 2008; 28 (3), 285-289.

Aurousseau B, Durand D, Gruffat D. Contrôle des phénomènes oxydatifs pendant la gestation chez les monogastriques et les ruminants. *INRA Prod Anim* 2004; 17, 339–354.

Ayad A, Delahaut P, Yahi K, Iguer-Ouada M, Benbarek H. Validation of a new Elisa for measuring bovine pregnancy-associated glycoproteins in plasma and its application for early pregnancy diagnosis. *Reprod Dom Anim* 2014; 49, 55-56.

Ayad A, Sousa NM, Sulon J, Hornick JL, Iguer-Ouada M, Beckers JF. Correlation of five radioimmunoassay systems for measurement of bovine plasma pregnancy-associated glycoprotein concentrations at early pregnancy period. *Res Vet Sci* 2009; 86(3), 377-382.

Ayad A, Sousa NM, Sulon J, Hornick JL, Touati K, Iguer-Ouada M, Beckers JF. Endocrinologie de la gestation chez la vache: Signaux embryonnaires, hormones et protéines placentaires. *Ann Méd Vét* 2006; 150(4), 212-226.

Ayad A, Sousa NM, Sulon J, Iguer-Ouada M, Beckers JF. Comparison of five radioimmunoassay systems for PAG measurement: ability to detect early pregnancy in cows. *Reprod Dom Anim* 2007; 42, 433-440.

Bomba-Opon D, Samaha RBB, Kosinski P, Kozlowski S, Wegrzyn P, Borowski D, Bartoszewicz Z, Bednarczuk T, Wielgos M. First trimester maternal serum vitamin d and markers of preeclampsia. *J. Matern Fetal Neonatal Med* 2014; 27(10), 1078-1079.

Breukelman SP, Perényi Z, Taverne MAM, Jonker H, Van der Weijden GC, Vos PLAM, De Ruigh L, Dieleman SJ, Beckers JF, Szenci O. Characterization of pregnancy losses after embryo transfer by measuring plasma progesterone and bovine pregnancy-associated glycoprotein-1 concentrations. *Vet J* 2012; 194, 71–76.

Castillo C, Hernandez J, Bravo A, Lopez Alonso M, Pereira V, Benedito JL. Oxidative status during late pregnancy and early lactation in dairy cows. *Vet J* 2005; 169, 286–292.

Celi P, Merlo M, Barbato O, Gabai G. Relationship between oxidative stress and the success of artificial insemination in dairy cows in a pasture-based system. *Vet. J* 2012, 193(2), 498–502.

Chavatte-Palmer P, Tarrade A. Placentation in different mammalian species. *Ann Endocrinol* 2016; 77, 67–74.

Codoñer-Franch P, Valls-Bellés V, Arilla-Codoñer A, Alonso-Iglesias E. Oxidant mechanisms in childhood obesity: the link between inflammation and oxidative stress. *Transl Res* 2011; 158(6), 369-84.

Constant F, Camous S, Chavatte-Palmer P, Heyman Y, De Sousa N, Richard C, Beckers JF, Guillomot M. Altered secretion of pregnancy-associated glycoproteins during gestation in bovine somatic clones. *Theriogenology* 2011; 76, 1006–1021.

Dosogne H, Burvenich C, Freeman AE, Kehrli MEJr, Detilleux JC, Sulon J, Beckers JF, Hoeben D. Pregnancy-associated glycoprotein and decreased polymorphonuclear leukocyte function in early post-partum dairy cows. *Vet Immunol Immunopathol* 1999; 67(1), 47–54.

Dosogne H, Massart-Leen AM, Bervenich C. Immunological aspects of pregnancy associated glycoproteins. Adv. *Exp Cell Biol* 2000; 480, 295–305.

Fainaru O, Almog B, Pinchuk I, Kupferminc MJ, Lichtenberg D, Many A. Active labour is associated with increased oxidisibility of serum lipids *ex vivo*. *BJOG* 2002; 109 (9), 938-941.

Friedrich M, Holtz W. Establishment of an ELISA for measuring bovine pregnancy associated glycoprotein in serum or milk and its application for early pregnancy detection. *Reprod Dom Anim* 2010; 45, 142–146.

Gajewski Z, Petrajtis-Golobow M, De Sousa NM, Beckers JF, Pawlinski B, Wehrend A. Comparison of accuracy of pregnancy-associated glycoprotein (PAG) concentration in blood and milk for early pregnancy diagnosis in cows. *Schweiz. Arch Tierh* 2014; 156, 585-590.

García-Ispierto I, Rosselló-Visa MA, Serrano-Pérez B, Mur-Novales R, De Sousa NM, Beckers JF, López-Gatius F. Plasma concentrations of pregnancy-associated glycoproteins I and II and progesterone on day 28 post-AI as markers of twin pregnancy in dairy cattle. *Livest Sci* 2015; 192, 44–47.

Garrel C, Fowler PA, Al-Gubory KH. Developmental changes in antioxidant enzymatic defences against oxidative stress in sheep placentomes. *J Endocrinol* 2010; 205, 107–116.

Gilbert RO. The effects of endometritis on the establishment of pregnancy in cattle. *Reprod Fertil Dev* 2011; 24, 252–257.

Green JA, Parks TE, Avalle MP, Telugu BP, McLain AL, Peterson AJ, McMillan W, Mathialagan N, Hook RR, Xie S, Roberts RM. The establishment of an ELISA for the detection of pregnancy-associated glycoproteins (PAGs) in the serum of pregnant cows and heifers. *Theriogenology* 2005; 63, 1481–1503.

Green LE, Huxley JN, Banks C, Green MJ. Temporal associations between low body condition, lameness and milk yield in a UK dairy herd. *Prev Vet Med* 2014; 113(1), 63-71.

Gupta S, Agarwal A, Banerjee J, Alvarez JG. The role of oxidative stress in spontaneous abortion and recurrent pregnancy loss: A systematic review. *Obstet Gynecol Sur* 2007; 62, 335-347.

Hoeben D, Monfardini E, Opsomer G, Burvenich C, Dosogne H, De Kruif A, Beckers JF. Chemiluminescence of bovine polymorphonuclear leucocytes during the periparturient period and relation with metabolic markers and bovine pregnancy-associated glycoprotein. *J Dairy Res* 2000; 67, 249–259.

Jauniaux E, Gulbis B, Burton GJ. The human first trimester gestational sac limits rather than facilities oxygen transfer to the foetus: a review. *Placenta* 2003; 24, S86–S93.

Karen A, Bajcsy ÁC, Minoia R, Kovács R, De Sousa NM, Beckers JF, Tibold J, Mádl I, Szenci O. Relationship of progesterone, bovine pregnancy-associated glycoprotein-1 and nitric oxide with late embryonic and early fetal mortalities in dairy cows. *J Reprod Dev* 2014; 60(2), 162-167.

Kindahl H, Kornmatitsuk B, Gustafsson H. The cow in endocrine focus before and after calving. *Reprod Dom Anim* 2004; 39, 217–221.

Ledezma-Torres RA, Beckers JF, Holtz W. Assessment of plasma profile of pregnancy-associated glycoprotein (PAG) in sheep with a heterologous (anti-caPAG55+59) RIA and its potential for diagnosing pregnancy. *Theriogenology* 2006; 66(4), 906-912.

Liu AX, Jin F, Zhang WW, Zhou TH, Zhou CY, Yao WM, Qian YL, Huang HF. Proteomic analysis on the alteration of protein expression in the placental villous tissue of early pregnancy loss. *Biol Reprod* 2006; 75, 414–420.

Lobago F, Bekana M, Gustafsson H, Beckers JF, Yohannes G, Aster Y, Kindahl H. Serum profiles of pregnancy-associated glycoprotein, oestrone sulphate and progesterone during gestation and some factors influencing the profiles in Ethiopian Borana and crossbred cattle. *Reprod Dom Anim* 2009; 44(4), 685-692.

Lopez-Gatius F, Garbayo JM, Santolaria P, Yaniz J, Ayad A, De Sousa NM, Beckers JF. Milk production correlates negatively with plasma levels of pregnancy-associated glycoprotein (PAG) to plasma pregnancy-associated glycoprotein (PAG) during the early fetal period in high producing dairy cows with live fetuses. *Dom Anim Endocrinol* 2007; 32(1), 29-42.

Mocatta TJ, Winterbourn CC, Inder TE, Darlow BA. The effect of gestational age and labour on markers of lipid and protein oxidation in cord plasma. *Free Radical Res* 2004; 38, 185–191.

Mohanty S, Sahu PK, Mandal MK, Mohapatra PC, Panda A. Evaluation of oxidative stress in pregnancy induced hypertension. *Indian J Clin Biochem* 2006; 21(1), 101-105.

Nathan C, Cunningham-Bussel A. Beyond oxidative stress: an immunologist's guide to reactive oxygen species. *Nat Rev Immunol* 2013; 13(5), 349-361.

Nemmar A, Raza H, Yuvaraju P, Beegam S, John A, Yasin J, Hameed RS, Adeghate E, Ali BH. Nose-only water-pipe smoking effects on airway resistance, inflammation, and oxidative stress in mice. *J Appl Physiol* 2013; 115(9), 1316-1323.

Ness RB. The consequences for human reproduction of a robust inflammatory response. *Q Rev Biol* 2004; 79(4), 383–393.

Piechotta M, Bollwein J, Friedrich M, Heilkenbrinker T, Passavant C, Branen J, Bollwein H. Comparison of commercial ELISA blood tests for early pregnancy detection in dairy cows. *J Reprod Dev* 2011; 57(1), 72-75.

Poston L, Igosheva N, Mistry HD, Seed PT, Shennan AH, Rana S, Karumanchi SA, Chappell LC. Role of oxidative stress and antioxidant supplementation in pregnancy disorders. *Am J Clin Nutr* 2011; 94, 1980S-5S.

Qanungo S, Mukherjea M. Ontogenic profile of some antioxidants and lipid peroxidation in human placental and fetal tissues. *Mol Cell Biochem* 2000; 215, 11–19.

Smith SK, Charnock-Jones DS, Sharkey AM. The role of leukemia inhibitory factor and interleukin-6 in human reproduction. *Hum Reprod* 1998; 13(3), 237–43.

Soehnlein O, Weber C, Lindbom L. Neutrophil granule proteins tune monocytic cell function. *Trends Immunol* 2009; 30, 538–546.

Sordillo LM, Aitken SL. Impact of oxidative stress on thehealthandimmune function of dairy cattle. *Vet Immunol Immunopathol* 2009; 128(1-3), 104-109.

Sorg O. Oxidative stress: a theoretical model or a biological reality?. *C R Biol* 2004; 327, 649–662.

Sousa NM, Ayad A, Beckers JF, Gajewski Z. Pregnancy-associated glycoproteins (PAG) as pregnancy markers in the ruminants. *J Physiol Pharmacol* 2006; 57(8), 153-171.

Talukder S, Kerrisk KL, Ingenhoff L, Gabai G, Garcia SC, Celi P. Changes in plasma oxidative stress biomarkers in dairy cows after oestrussynchronisation with controlled internal drug release (CIDR) and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$). *Anim Prod Sci* 2014; 54(9), 1490–1496.

Trevisan M, Browne R, Ram M, Muti P, Freudenheim J, Carosella AN, Armstrong D. Correlates of markers of oxidative status in the general population. *Am J Epidemiol* 2001; 154, 348–356.

Tuuli MG, Longtine MS, Nelson DM. Review: oxygen and trophoblast biology—a source of controversy. *Placenta* 2011; 32 (2), S109–S118.

Wall PD, Pressman EK, Woods JRJr. Preterm premature rupture of the membranes and antioxidants: the free radical connection. *J Perinat Med* 2002; 30, 447–457.

Wallace DC. A mitochondrial paradigm of metabolic and degenerative diseases, aging, and cancer: a dawn for evolutionary medicine. *Ann Rev Gen* 2005; 39, 359–407.

Wéré P, Moussa Z, Hamidou B, Laya S, Pascal L, Beckers JF, Messanvi G, Etude de l'estrus et de la fertilité après un traitement de maîtrise des cycles chez les femelles zébus. *Int J Biol Chem Sci* 2012; 6, 257–263.

Wilde D. Influence of macro and micro minerals in the peri-parturient period on fertility in dairy cattle. *Anim Reprod Sci* 2006; 96, 240–249.

Witko-Sarsat V, Friedlander M, Capeillère-Blandin C, Nguyen-Khoa T, Nguyen AT, Zingraff J, Jungers P, Descamps-Latscha B. Advanced oxidation protein products as a novel marker of oxidative stress in uraemia. *Kidney Int* 1996; 49, 1304–1313.

Wooding FB, Roberts RM, Green JA. Light and electron microscope immunocytochemical studies of the distribution of pregnancy-associated glycoproteins (PAGs) throughout pregnancy in the cow: possible functional implications. *Placenta* 2005; 26, 807–827.

Younis A, Clower C, Nelsen D, Butler W, Carvalho A, Hok E, Garelnebi M. The relationship between pregnancy and oxidative stress markers on patients undergoing ovarian stimulations. *J Assist Reprod Gen* 2012; 29(10), 1083–1089.

Original Article

TJVR 2020; 4 (1): 9-16

Turkish Journal of Veterinary Research

http://www.dergipark.gov.tr/tjvr

e-ISSN: 2602-3695

Anatomic Characteristics and Locations of Nutrient Foramen in Humerus of Domestic Animals

Osman Yılmaz[1]

[1] *Department of Anatomy, Faculty of Veterinary Medicine, Van Yuzuncu Yil University, Van, Turkey*

Correspondence: *Osman Yılmaz (osman_40_5@hotmail.com)*

Received: 31.01.2020 Accepted: 11.02.2020

ABSTRACT

Objective: This study aimed to determine the numbers, directions, localizations, diameters, morphometric values of the nutrient foramina (NF) in humerus of domestic mammals and to reveal the differences between the right and left humerus in animal species.

Materials and methods: In the study, a total of 223 humerus, large ruminants (56), small ruminants (60), equidae (29), sus (24), carnivora-dog (42), and carnivora-cat (12), were examined in the Department of Anatomy, Faculty of Veterinary Medicine, Van Yuzuncu Yil University. The numbers, shapes, directions, localization sites and localized surfaces of the NF's were observed with the naked eye, and recorded. The locations of the NF's were confirmed by calculating the Foraminal Index (FI). The diameters of the NF's were measured using 1.2 mm (18 Gauge: G), 0.9 mm (20 G), 0.7 mm (22 G), 0.55 mm (24 G), and 0.1 mm (34 G) needles. In animal species, morphometric measurements were taken such as total length of the humerus (TLH), distance between the NF with the proximal end of the humerus (NFP), distance between the NF with the distal end of the humerus (NFD), FI and performed statistical analysis of the measured values.

Results: There was found a single NF in 99% of the examined humerus in the study. In general, it was seen that the NF's were directed downwards, and located in the middle 1/3 with lower 1/3 segments. NF's were determined to be localized to the facies caudalis in 100% of sus, in 93% of large ruminants and carnivora-dogs, and in 85% of small ruminants; however, in equidae and carnivora-cats were all localized to the margo medialis. According to the statistics, no statistically significant difference ($p>0.05$) was observed between the right and left humerus NF measurement values in terms of morphometric properties. But only, the diameter of the NF in the small ruminants was statistically significant ($p<0.05$).

Conclusion: It was found that the morphological and morphometric differences of NF's in right and left humerus of domestic mammals. Moreover, in these animals, it is thought that the study may help veterinary clinicians and surgeons in evaluating of the pathological conditions related to humeral NF and planning of the operative applications to be performed in this region.

Keywords: *Anatomy, Foraminal indeks, Humerus, Nutrient foramen*

INTRODUCTION

The humerus, one of the bones that make up the ossa membri thoracici, is a long and thick bone that connects to the scapula with the articulatio humeri in the proximally and also the extremity by means of the articulatio cubiti with the antebrachium (radius-ulna) in the distally (Dursun, 2002; Dyce et al., 2002; Bahadır and Yıldız, 2008).

Foramen nutricium, which is one of the important anatomical features of long bones such as humerus, is clinically important as it is the site of entry into the feeding vessels of the bone and guiding the

identification of weak areas sensitive to bone fractures (Uzuner et al., 2018; Zahra et al., 2018).

In general, there is an nutrient artery and vein that enters and leaves the diaphysis of the long bone from a nutrient foramen (NF). The nutrient artery (arteria nutricia; NA), which enter the bone through the NF and nutrient canal (NC), is divided into proximal and distal branches when they reach to the medullar cavity (cavum medullare), and these separated branches that provides feeding of the bone marrow and the adjacent cortical bone regions in both during the embryonic phase and the normal growth period (Rhinelander, 1968; Martini et al., 2015; Zahra et al., 2018). NA's are responsible for 70-80% of the blood supply of the bones. When this blood supply is restricted in various interventional procedures such as any surgical or operative procedures applied to the region, it may result in necrosis, especially bone ischemia, of the bone marrow and cortex (Shim et al., 1968).

Knowing the localization of NF in long bones is important in applications requiring evaluation of bone development and preservation of blood circulation (Campos et al., 1987). Foraminal Index (FI), used to describe the localization of NF's, is obtained by dividing the distance between the proximal end of the bone and the NF to the total bone length (Hughes, 1952; Gümüsburun et al., 1994; Kizilkanat et al., 2007; Kara et al., 2011). In addition, knowing the morphological features of NF is clinically critical importance for the evaluation of various pathological conditions such as developmental abnormalities and hematogenic osteomyelitis in these bones (Skawina and Wyczolkowski, 1987), and the planning of surgical and orthopedic applications such as bone fracture, bone graft and bone implant applications (Piermattei et al., 2006; Kizilkanat et al., 2007; Kara et al., 2011; Sim and Ahn, 2014; Zahra et al., 2018). Furthermore, in the development of new techniques in the field of bone transplantation and resection, it is of great benefit to know the position of the foramen nutricium and arteria nutriticia that supply blood to the bone (Kizilkanat et al., 2007; Xue et al., 2016).

When considering the importance of the clinical and morphological of NF, It is important medically to investigate the characteristics of NF in domestic mammals. This study aimed to determine the numbers, directions, localizations, diameters, morphometric values of the nutrient foramina (NF) in humerus of domestic mammals, and to reveal the differences between the right and left humerus in animal species.

MATERIALS and METHODS

In the study, a total of 223 humerus, large ruminants (56), small ruminants (60), equidae (29), sus (24), carnivora-dog (42), and carnivora-cat (12), were examined in the Department of Anatomy, Faculty of Veterinary Medicine, Van Yuzuncu Yil University. The bones were chosen so that they did not have any anatomical disorder. Age and sex characteristics of the examined bones were not known. The foramen nutricium on the bone was observed with the naked eye. Only well-defined the NF's were accepted in the diaphysis of the bone. The foramina nutricium at both ends of the bone were ignored.

Morphological and Morphometric Evaluation of the NF's in Humerus Diaphysis

Foramen nutricium in the diaphysis of the humerus in domestic mammals (Figure 1.) was evaluated using the following measurements:

Direction of the bone: Right and left directions of the humerus were determined first.

Number of NF's: The NF's in the diaphysis of the bones were counted.

Direction of NF's: It was separated downwards, upwards and horizontally.

Localizations of NF's: Localizations of NF's were calculated using FI. To calculate the localization of all the NF's; The formula FI = (NFP /TLH)) x 100 was used. TLH: total length of the humerus, NFP: distance between the NF and the proximal end of the humerus (Hughes, 1952; Gümüsburun at al., 1994; Kizilkanat et al., 2007; Kara et al., 2011).

According to the FI, the locations of the NF's were determined as follows: The proximal 1/3-Type1 (FI up to 33.33), the middle 1/3-Type2 (FI from 33.33 up to 66.66), and the distal 1/3-Type3 (FI above 66.66).

Localized surfaces of the NF's were divided into four types as follow: Facies cranialis, facies caudalis, margo lateralis, and margo medialis.

Diameters of NF's (FD): It was measured using 1.2 mm (18 Gauge: G), 0.9 mm (20 G), 0.7 mm (22 G), 0.55 mm (24 G), and 0.1 mm (34 G) needles.

Total length of the humerus (TLH): The distance between the proximal end and the distal end of the humerus.

NFP: Distance between the NF and the proximal end of the humerus.

NFD: Distance between the NF and the distal end of the humerus.

Nomina Anatomica Veterinaria (2017) was used as terminology in the study. Morphometric measurements were made by using digital caliper. Pictures of the study materials were taken with the Sony Digital DSC-W830.

Statistical Analysis

Descriptive statistics for studied variables were presented as median, mean, standard deviation, minimum and maximum values. In the study, categorical variables were expressed as numbers and percentages. Shapiro-Wilk (n<50) was used to determine whether the continuous variables in the study were normally distributed or not. In general, nonparametric tests were performed because these variables were not normally distributed. The Mann-Whitney U test was used to compare the measurements according to right-left groups. Statistical significance levels were considered as 1% and 5%. The SPSS (IBM SPSS Statistics for Windows, Ver. 23) was used for all statistical computations.

RESULTS

Direction of the humerus and Number of NF's

The number of the right and left humerus of domestic mammal in animal species and the number of NF's in the diaphysis of this bone are given in Table 1. According to this, it was observed that the NF's were the only one in 98% of the humerus of large and small ruminants; in the all of the equidae, sus, and carnivora. In general, there was found a single NF in 99% of the examined humerus in the study. However, NF was not found in only 1% of the examined bones.

Direction of NF's

The directions of the NF in the diaphysis of the examined humerus by animal species are given in Table 2. According to this, it was seen that the NF's were directed downwards in 93% of large ruminants; in the all of small ruminants, equidae, sus, and carnivora. Nevertheless, in 7% of large ruminants was detected that the horizontal direction. In domestic mammals, no humerus was found with the upward direction of the NF's.

According to the FI, the locations of the NF's

According to the FI, the locations of the NF's in the diaphysis of the examined humerus by animal species are given in Table 3. In general, it was seen that the NF's were located in the middle 1/3 with lower 1/3 segments. NF's were determined to be localized to the middle 1/3- type 2 in 91% of large ruminants, in 88% of small ruminants, in 34% of equidae, in 96% of sus, in 98% carnivora-dogs, and in 75% of carnivora-cats; the distal 1/3-type 3 in 9% of large ruminants, in 12% of small ruminants, in 66% of equidae, in 4% of sus, in 2% of carnivora-dogs, and in 25% of carnivora-cats.

Table 1. Numbers observed NF in the diaphysis of the humerus.

Animal	Direction	n	Numbers of the NF (%)	
Large ruminant	Left	29	1	28
	Right	27	0	27
	Total	56	1 (2)	55 (98)
Small ruminant	Left	31	1	30
	Right	29	0	29
	Total	60	1 (2)	59 (98)
Equidae	Left	14	0	14
	Right	15	0	15
	Total	29	0 (0)	29 (100)
Sus	Left	11	0	11
	Right	13	0	13
	Total	24	0 (0)	24 (100)
Carnivora-dog	Left	22	0	22
	Right	20	0	20
	Total	42	0 (0)	42 (10)
Carnivora-cat	Left	6	0	6
	Right	6	0	6
	Total	12	0	12 (100)
Grand total (all bones)		223	2 (1)	221 (99)

NF: Nutrient Foramen, n: Number

Localized surfaces of the NF's

Localized surfaces of the NF's in the diaphysis of the examined humerus by animal species are given in Table 4. According to this, NF's were determined to be localized to the facies caudalis in 100% of sus, in 93% of large ruminants and carnivora-dogs, and in 85% of small ruminants; however, in equidae and carnivora-cats were all localized to the margo medialis.

Diameters of NF's (FD)

The diameters of the NF's in the diaphysis of the examined humerus by animal species are given in Table 5. According to this, the diameters of the examined NF's were observed to be 1.2 mm in 76%, and 0.9 mm in 24% of large ruminants; 0.9 mm in 2%, 0.7 mm in 22%, and 0.55 mm in 76% of small ruminants; 1.2 mm in 79%, and 0.9 mm in 21% of equidae; 1.2 mm in 83%, and 0.9 mm in 17% of sus; 1.2 mm in 24%, 0.7 mm in 43%, and 0.55 mm in 33% of carnivora-dogs; 0.1 mm in 100% of carnivora-cats.

Table 2. Direction of NF's

Animal	Direction	n	Direction of NF's n (%) Downwards	Upward	Horizontal
Large ruminant	Left	28	24 (86)	0 (0)	4 (14)
	Right	27	27 (100)	0 (0)	0 (0)
	Total	55	51 (93)	0 (0)	4 (7)
Small ruminant	Left	30	31 (100)	0 (0)	0 (0)
	Right	29	29 (100)	0 (0)	0 (0)
	Total	59	60 (100)	0 (0)	0 (0)
Equidae	Left	14	14 (100)	0 (0)	0 (0)
	Right	15	15 (100)	0 (0)	0 (0)
	Total	29	29 (100)	0 (0)	0 (0)
Sus	Left	11	11 (100)	0 (0)	0 (0)
	Right	13	13 (100)	0 (0)	0 (0)
	Total	24	24 (100)	0 (0)	0 (0)
Carnivora-dog	Left	22	22 (100)	0 (0)	0 (0)
	Right	20	20 (100)	0 (0)	0 (0)
	Total	42	42 (100)	0 (0)	0 (0)
Carnivora-cat	Left	6	6 (100)	0 (0)	0 (0)
	Right	6	6 (100)	0 (0)	0 (0)
	Total	12	12 (100)	0 (0)	0 (0)

Table 3. According to the FI, the Locations of the NF's.

Animal	Direction	n	Locations of the NF's n (%) Proximal 1/3-Type 1	Middle 1/3-Type 2	Distal 1/3-Type 3
Large ruminant	Left	28	0	25	3
	Right	27	0	25	2
	Total	55	0 (0)	50 (91)	5 (9)
Small ruminant	Left	30	0	27	3
	Right	29	0	25	4
	Total	59	0 (0)	52 (88)	7 (12)
Equidae	Left	14	0	5	9
	Right	15	0	5	10
	Total	29	0 (0)	10 (34)	19 (66)
Sus	Left	11	0	10	1
	Right	13	0	13	0
	Total	24	0 (0)	23 (96)	1 (4)
Carnivora-dog	Left	22	0	21	1
	Right	20	0	20	0
	Total	42	0 (0)	41 (98)	1 (2)
Carnivora-cat	Left	6	0	5	1
	Right	6	0	4	2
	Total	12	0 (0)	9 (75)	3 (25)

FI: Foraminal Index;

Table 4. Localized Surfaces of the NF's

Animal	Direction	n	Localized Surfaces of the NF's n (%) Facies cranialis	Facies caudalis	Margo lateralis	Margo medialis
Large ruminant	Left	28	2	25	1	0
	Right	27	0	26	1	0
	Total	55	2 (3.5)	51 (93)	2 (3.5)	0 (0)
Small ruminant	Left	30	0	24	6	0
	Right	29	1	26	2	0
	Total	59	1 (2)	50 (85)	8 (13)	0 (0)
Equidae	Left	14	0	0	0	14
	Right	15	0	0	0	15
	Total	29	0 (0)	0 (0)	0 (0)	29 (100)
Sus	Left	11	0	11	0	0
	Right	13	0	13	0	0
	Total	24	0 (0)	24 (100)	0 (0)	0 (0)
Carnivora-dog	Left	22	1	20	0	1
	Right	20	0	19	0	1
	Total	42	1 (2)	39 (93)	0 (0)	2 (5)
Carnivora-cat	Left	6	0	0	0	6
	Right	6	0	0	0	6
	Total	12	0 (0)	0 (0)	0 (0)	12 (100)

Table 5. Diameters of NF's (mm)

Animal	Direction	n	Diameters of NF's n (%) 1.2 (18G)	0.9 (20G)	0.7 (22G)	0.55 (24G)	0.1 (34G)
Large ruminant	L	28	20	8	0	0	0
	R	27	22	5	0	0	0
	T	55	42 (76)	13 (24)	0 (0)	0 (0)	0 (0)
Small ruminant	L	30	0	0	3	27	0
	R	29	0	1	10	18	0
	T	59	0 (0)	1 (2)	13 (22)	45 (76)	0 (0)
Equidae	L	14	11	3	0	0	0
	R	15	12	3	0	0	0
	T	29	23 (79)	6 (21)	0 (0)	0 (0)	0 (0)
Sus	L	11	9	2	0	0	0
	R	13	11	2	0	0	0
	T	24	20 (83)	4 (17)	0 (0)	0 (0)	0 (0)
Carnivora-dog	L	22	0	7	8	7	0
	R	20	0	3	10	7	0
	T	42	0 (24)	10 (0)	18 (43)	14 (33)	0 (0)
Carnivora-cat	L	6	0	0	0	0	6
	R	6	0	0	0	0	6
	T	12	0 (0)	0 (0)	0 (0)	0 (0)	12 (100)

G: Gauge; D: Direction; n: Number; L: Left; R: Right; T: Total.

Table 6. Descriptive Statistics and Comparison of Characteristics of the NF's

Animal	Parameter	Direction	Mean	Median	Std. Dev.	Min.	Max.	*p.
Large ruminant	TLH	L	28.53	28.85	2.03	24.10	32.30	0.076
		R	29.80	29.80	2.42	25.10	35.00	
	NFP	L	17.20	16.95	2.37	13.60	22.10	0.209
		R	17.84	17.90	1.73	14.50	20.60	
	NFD	L	11.33	11.85	2.18	5.70	15.90	0.341
		R	11.96	12.10	1.55	9.40	14.90	
	FI	L	60.29	59.88	7.19	48.87	79.50	0.840
		R	59.89	59.67	3.79	52.92	67.02	
	FD	L	1.11	1.20	0.14	0.90	1.20	0.385
		R	1.14	1.20	0.12	0.90	1.20	
Small ruminant	TLH	L	16.39	16.40	1.74	13.50	21.10	0.903
		R	16.49	16.50	1.94	12.50	21.00	
	NFP	L	10.11	9.80	1.49	7.60	14.60	0.347
		R	9.81	9.60	1.82	6.40	15.10	
	NFD	L	6.29	6.30	0.78	4.80	8.10	0.108
		R	6.67	6.60	1.00	4.30	9.10	
	FI	L	61.51	61.29	4.23	52.41	69.23	0.071
		R	59.27	59.03	5.84	49.35	71.90	
	FD	L	0.56	0.55	0.05	0.45	0.70	0.033
		R	0.61	0.55	0.10	0.45	0.90	
Equidae	TLH	L	27.61	29.20	3.73	21.40	33.00	0.631
		R	27.51	28.70	3.51	21.50	33.00	
	NFP	L	18.54	19.15	2.34	14.30	23.00	0.965
		R	18.55	18.30	2.36	14.50	23.20	
	NFD	L	9.08	9.55	1.81	5.90	11.90	0.793
		R	8.96	9.50	1.81	5.60	11.60	
	FI	L	67.31	67.56	3.54	60.42	72.81	0.827
		R	67.59	67.44	4.19	59.58	74.07	
	FD	L	1.14	1.20	0.13	0.90	1.20	0.926
		R	1.14	1.20	0.12	0.90	1.20	
Sus	TLH	L	23.03	24.00	3.00	15.20	26.00	0.283
		R	21.95	22.30	3.01	15.40	26.30	
	NFP	L	13.67	13.90	2.33	9.00	18.10	0.643
		R	13.08	14.10	1.87	9.40	15.80	
	NFD	L	9.35	9.30	2.06	6.20	13.80	0.324
		R	8.86	8.80	2.18	6.00	15.10	
	FI	L	59.43	60.00	6.43	45.02	69.62	0.685
		R	59.88	60.73	5.64	42.59	66.36	
	FD	L	1.12	1.20	0.14	0.90	1.20	0.484
		R	1.15	1.20	0.11	0.90	1.20	
Carnivora-dog	TLH	L	17.62	18.00	3.20	12.10	23.80	0.830
		R	17.80	18.50	3.09	12.10	22.20	
	NFP	L	10.01	9.90	2.35	6.70	15.70	0.480
		R	10.17	10.30	1.88	6.00	13.90	
	NFD	L	7.61	8.00	1.34	4.90	9.50	0.890
		R	7.63	7.65	1.72	4.60	11.20	
	FI	L	56.53	57.05	4.85	48.05	71.36	0.378
		R	57.24	57.64	5.32	46.88	65.48	
	FD	L	0.72	0.70	0.14	0.55	0.90	0.418
		R	0.68	0.70	0.12	0.55	0.90	
Carnivora-cat	TLH	L	10.00	10.00	0.68	8.90	10.80	0.998
		R	10.00	10.05	0.72	8.80	10.80	
	NFP	L	6.27	6.30	0.66	5.50	7.10	0.809
		R	6.32	6.40	0.52	5.60	6.80	
	NFD	L	3.73	3.60	0.62	2.90	4.60	0.936
		R	3.68	3.60	0.62	3.00	4.70	
	FI	L	62.69	63.57	5.42	56.57	70.10	0.997
		R	63.27	63.30	4.61	55.66	69.07	
	FD	L	0.10	0.10	0.00	0.10	0.10	1.000
		R	0.10	0.10	0.00	0.10	0.10	

Mann-Whitney U Test Results, * $p<0.05$: Statistically significant; TLH: Total length of the humerus; NFP: Distance between the NF and the proximal end of the humerus; NFD: Distance between the NF and the distal end of the humerus; FI: Foraminal Indeks; FD: Diameters of NF's.

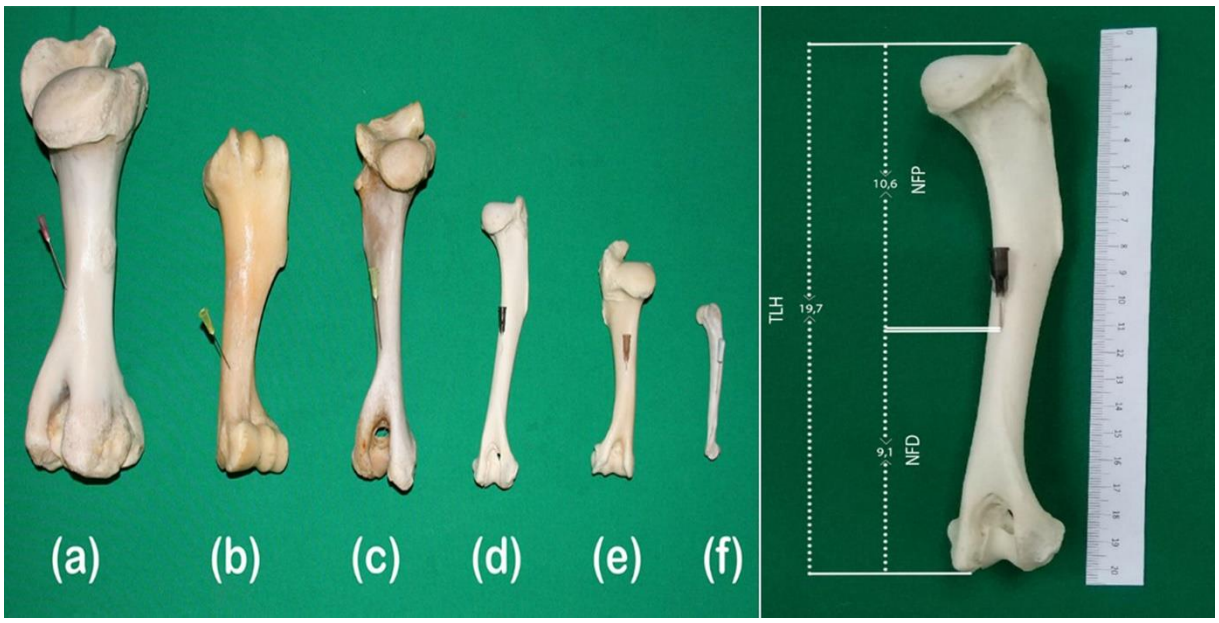

Figure 1. The diaphyseal foramen nutricium in the humerus of domestic mammals (a: Large ruminant, b: Equidae, c: Sus, d: Carnivora-dog, e: Small ruminant, f: Carnivora-cat, TLH: Total length of the humerus, NFP: Distance between the NF and the proximal end of the humerus, NFD: Distance between the NF and the distal end of the humerus)

Descriptive statistics and comparison of characteristics of the NF's

The descriptive statistics of the NF's in the diaphysis of the examined humerus by animal species are given in Table 6. According to the statistics, no statistically significant difference ($p>0.05$) was observed between the right and left humerus NF measurement values in terms of morphometric properties. But only, the diameter of the NF in the small ruminants was statistically significant ($p<0.05$).

DISCUSSION

It is important to know the distribution and location of foramen nutricium and canalis nutricium in long bones such as humerus, so as not to cause any damage to the nutritional vessels of the bone during surgical procedures (Kumar et al., 2013). In operative interventions to the long bones require vascularization or protection of these vessels to improve healing more quickly (Wavreille et al., 2006). Morever, knowing the number and position of NF's in these bones plays an important role in orthopedic surgery such as bone grafting, humeral diaphyseal transplantation, bone fracture repair, microvascular bone surgery, and joint replacement therapy (Kizilkanat et al., 2007).

While there are many studies on anatomical characterization and localization of NF's in human long bones (Shulman, 1959; Kawahara et al., 1967; Mysorekar, 1967; Longia et al., 1980; Ajmani, 1982; Campos et al., 1987; Skawina and Wyczolkawski, 1987; Gümüşburun et al., 1994; Kizilkanat et al., 2007; Kumar et al., 2013; Xue et al., 2016; Uzuner et al., 2018; Zahra et al., 2018; Sukumar, 2019); this issue has so far attracted little attention or has been studied without going into much detail in domestic mammals. In the veterinary field, Hughes (2016) on the directions of NF and NC in the long bones of various birds and mammals, Payton (1934) on the direction, development and position of NF's in pigs, Daniel et al. (2008) on the relationship between NF's and diseases in sesame bones of hounds, Ahn (2013), Evans and de Lahunta (2013), Sim and Ahn (2014) studied the localization, direction and localization of NF's and the vessels passing through them in the long bones of the forelimb and hindlimb of the dogs. In addition, Garita and Rapoff (2003), Bassage and Ross (1998) on equidea bones, Siddiqui et al. (2008) on goat bones, Rohlan et al. (2018) on bull bones was mentioned about the NF's in their studies. Morever, in the study performed by Johnson et al. (2017) the differences between the morphological and morphometric characteristics of NF's in the humerus and femur bones of human, sheep and pigs were revealed. This study is very important for forensic anthropology.

In general, there was found a single NF in 99% of the examined humerus in the our study. Compatibility with literature information shows that the NF's in the diaphyseal part of humerus are often singularity (Hughes, 1952; McLeod et al.,

1958; Getty, 1975; Kizilkanat et al., 2007; Sim and Ahn, 2014; Xue et al., 2016).

Generally, the NF's on the humerus were reported to be localized to the facies medialis, and the lower 1/3 segments of the bone in the horses (Getty, 1975); the facies caudalis, and the middle 1/3 segments of the bone in the sheeps (Getty, 1975) and the black bengal goats (Siddiqui et al., 2008); the margo lateralis, and the lower 1/3 segments of the bone in the cattle (McLeod et al., 1958) and blue bull (Rohlan et al., 2018); the facies caudalis, and the junction of the middle and lower 1/3 of the bone in the pigs (Payton, 1934); the facies caudalis, and the lower 1/3 segments of the bone in the elephant (Ahasan et al., 2016), Indian Blackbuck (Choudhary and Singh, 2016), Ox (Raghavan, 1964), and dogs (Miller et al., 1964; Sim and Ahn, 2014). In our study, NF's were determined to be localized to the facies caudalis in 100% of sus, in 93% of large ruminants and carnivora-dogs, and in 85% of small ruminants; however, in equidae and carnivora-cats were all localized to the margo medialis. Morever, the localizations of NF's were calculated using FI. In general, it was seen that the NF's were located in the middle 1/3 with lower 1/3 segments. NF's were determined to be localized to the middle 1/3- type 2 in 91% of large ruminants, in 88% of small ruminants, in 34% of equidae, in 96% of sus, in 98% carnivora-dogs, and in 75% of carnivora-cats; the distal 1/3-type 3 in 66% of equidae.

Literature shows that the growing end of the long bone grows twice as fast as the other end. Therefore, obliquity of NF's and NC's is directed away from the growing end. This can be explained by the 'Berard's rule' or growing end theory which suggests that the nutrient artery or NC is directed away from the growing end (Mysorekar, 1967; Longia et al., 1980). In general, this theory is mostly valid for the NF's in the diaphysis of long bones such as the humerus, and the direction of the NF or CN's is directed towards the elbow joint (downward-distal) (Hughes, 1952). In this study, in accordance with this information, it was seen that the NF's in the diaphysis of the examined humerus were directed downwards in 93% of large ruminants; in the all of small ruminants, equidae, sus, and carnivora.

Xue et al. (2016) reported that the diameter of the NF's on the humerus is approximately 1.11 ± 0.32 mm in human. Also, Sim and Ahn (2014) determined that this value was between 0.5-1.0 mm in their study on German Shepherd Dogs. However, in the literature review, no study was found to clearly identify the diameters of NF's in other animal species. In our study, the diameters of the examined NF's were observed to be 1.2 mm in 76%, and 0.9 mm in 24% of large ruminants; 0.9 mm in 2%, 0.7 mm in 22%, and 0.55 mm in 76% of small ruminants; 1.2 mm in 79%, and 0.9 mm in 21% of equidae; 1.2 mm in 83%, and 0.9 mm in 17% of sus; 1.2 mm in 24%, 0.7 mm in 43%, and 0.55 mm in 33% of carnivora-dogs; 0.1 mm in 100% of carnivora-cats. According to the statistics, no statistically significant difference ($p>0.05$) was observed between the right and left humerus NF measurement values in terms of morphometric properties. But only, the diameter of the NF in the small ruminants was statistically significant ($p<0.05$).

CONCLUSION

In conclusion, it was found that the morphological and morphometric differences of NF's in right and left humerus of domestic mammals. Moreover, in these animals, it is thought that the study may help veterinary clinicians and surgeons in evaluating of the pathological conditions related to humeral NF and planning of the operative applications to be performed in this region.

ACKNOWLEDGMENTS

This manuscript was presented as an oral presentation at the 2nd International & 11th National Veterinary Anatomy Congress, Oct 26-29, 2019, Crypus.

Conflict of Interests: The authors declared that there is no conflict of interests.

Financial Disclosure: The authors declared that this study has received no financial support.

REFERENCES

Ahasan AMSL, Quasem MA, Rahman ML, Hasan RB, Kıbrıa ASMG, Shıl SK. Macroanatomy of the bones of thoracic limb of an Asian Elephant (Elephas maximus). *Int J Morphol,* 2016; 34(3):909-917.

Ahn D. Anatomical study on the diaphyseal nutrient foramen of the femur and tibia of the German shepherd dog. *J Vet Med Sci*, 2013; 75:803-808.

Ajmani ML. A study of diaphysial nutrient foramen in human long bones. *Anat Anz,* 1982; 151:305-314.

Bahadır A, Yıldız H. Veteriner Anatomi: Hareket Sistemi & İç Organlar. Turkey: Bursa, Ezgi Kitabevi, 2008.

Bassage LH, Ross MW. Enostosis-like lesions in the long bones of 10 horses: scintigraphic and radiographic features. *Equine Vet J,* 1998; 30(1):35-42.

Campos FF, Pellico LG, Alias MG, Valencia RF. A study of the nutrient foramina in human long bones. *Surg Radiol Anat*, 1987; 9:251-255.

Choudhary OP, Singh I. Morphometrical studies on humerus of Indian black buck (Antilope cervicapra). *J Wildlife Res*, 2016; 4(1):06-09.

Daniel A, Read RA, Cake MA. Vascular foramina of the metacarpophalangeal sesamoid bones of Greyhounds and their relationship to sesamoid disease. *Am J Vet Res*, 2008; 69:716-721.

Dursun N. Veteriner Anatomi I. Turkey: Ankara, Medisan Yayınevi, 2002.

Dyce KM, Sack WO, Wensing CJG. Textbook of veterinary anatomy, 3rd ed. United States: Philadelphia, Saunders, 2002.

Evans HE, de Lahunta A. Miller's Anatomy of the Dog. 4th ed. St. Louis, Saunders, 2013; pp: 129-136.

Garita B, Rapoff AJ. Osteon trajectories near the equine metacarpus nutrient foramen. Proc ASME Summer Bioeng Conf., USA: Florida, 2003.

Getty R. Sisson and Grossman's. The Anatomy of the Domesticated Animals. Vol. I, 5th ed. Philadelphia: WB Saunders Co, 1975; pp: 273-296.

Gümüsburun E, Yücel F, Ozkan Y, Akgün Z. A study of the nutrient foramina of lower limb long bones. *Surg Radiol Anat*, 1994; 16:409-412.

Hughes H. The factors determining the direction of the canal for the nutrient artery in the long bones of mammals and birds. *Acta Anat,* 1952; 15:261-80.

Johnson V, Beckett S, Márquez-Grant N. Differentiating human versus non-human bone by exploring the nutrient foramen: implications for forensic anthropology. *Int J Legal Med*, 2017; 131:1757–1763, doi: 10.1007/s00414-017-1662-y.

Kara ME, Sevil-kilimci F, Onar V. Foraminal index on the dog femora. *Ankara Üniv Vet Fak Derg*, 2011; 58:229-232.

Kawahara G, Matsuda M, Kitai T, Sugiyama K, Aihara A. Anatomical observations on the foramen nutricium of the long bone (tubal bone) of the Japanese (further study) its location number and direction in the bone. *Kaibogaku Zasshi,* 1967; 42:132-145.

Kizilkanat E, Boyan N, Ozsahin ET, Soames R, Oguz O. Location, number and clinical significance of nutrient foramina in human long bones. *Ann Anat,* 2007; 189:87-95.

Kumar R, Mandloi RS, Singh AK, Kumar D, Mahato P. Analytical and morphometric study of nutrient foramina of femur in Rohilkhand Region. *IJMHS*, 2013; 3:52-54.

Longia GS, Ajmani ML, Saxena SK, Thomas RJ. Study of diaphyseal nutrient foramina in human long bones. *Acta Anat (Basel),* 1980; 107:399-406.

Martini FH, Timmons MJ, Tallitsch RB. Human Anatomy. 8th edition. Illinois: Pearson Benjamin Cummings, 2015.

McLeod WM, Trotter DM, Lumb JW. Bovine Anatomy. Burgess: Minneapolis, 1958; pp: 643-657.

Miller ME, Christensen GC, Evans HE. Anatomy of the Dog. USA: Philadelphia, WB Saunders Co, 1964; pp: 234-245.

Mysorekar VR. Diaphysial nutrient foramen in human long bones. *J Anat,* 1967; 101:813–822.

Nomina Anatomica Veterinaria. Prepared by the international committes on veterinary gross anatomical nomenclature and authorized by the general assambly of the world association of veterinary anatomists (6th Edition). The Editorial Committee Hanover (Germany), Ghent (Belgium), Columbia, MO (U.S.A.), Rio de Janeiro (Brazil), 2017.

Payton CG. The position of the nutrient foramen and direction of the nutrient canal in the long bones of the Madder-fed pig. *J Anat,* 1934; 68:500-510.

Piermattei DL, Flo GL, Decamp CE. Handbook of small animal orthopedics and fracture repair. USA: Missouri, St. Louis, 2006; pp: 818.

Raghavan D. Anatomy of Ox. New Delhi: Indian Council of Agricultural Research, 1964; pp: 97-117.

Rhinelander FW. The normal microcirculation of diaphyseal cortex and its response to fracture. *J Bone Joint Surg,* 1968; 50:784-800.

Rohlan K, Mathur R, Dangi A, Shringi N, Ganguly S, Kumar V. Morphometrical studies on humerus of Blue Bull (Boselaphus tragocamelus). *Int J Livest Res,* 2018; 8(6):177-184, doi: 10.5455/ijlr.20170829060005.

Shim SS, Copp DH, Patterson FP. Measurement of the rate and distribution of the nutrient and other arterial blood supply in long bones of the rabbit. A study of the relative contribution of the three arterial systems. *J Bone Joint Surg Br,* 1968; 50(1):178-183.

Shulman SS. Observations on the nutrient foramina of the human radius and ulna. *Anat Rec,* 1959; 134:685-697.

Siddiqui MSI, Khan MZI, Sarma M, Islam MN, Jahan MR. Macro-anatomy of the bones of the forelimb of Black Bengal Goat (Capra hircus). *BJVM,* 2008; 6(1):59-66.

Sim J, Ahn D. Anatomy of the diaphyseal nutrient foramen in the long bones of the pectoral limb of German Shepherds. *Korean J Vet Res,* 2014; 54(3):179-184.

Skawina A, Wyczolkowski M. Nutrient foramen of humerus, radius and ulna in Human Fetuses. *Folia Morphol*, 1987; 46:17–24.

Sukumar CD. A study on the anatomical variations in diaphyseal nutrient foramina of humerus and its clinical implications. *Int J Cur Res Rev,* 2019; 11(15):16-22, doi: http://dx.doi.org/10.31782/IJCRR.2019.11154.

Uzuner MB, Ocak M, Geneci F, Kocabıyık N, Sargon MF, Al-Shouk A. Quantitative and morphometric evaluation of the foramina nutricia in the long bones of the upper and lower extremities in anatolian population. *Kafkas J Med Sci,* 2018; 8(1):39–44, doi: 10.5505/kjms.2017.39259.

Wavreille G, Dos Remedios C, Chantelot C. Anatomic bases of vascularized elbow joint harvesting to achieve vascularised allograft. *Surg Radiol Anat,* 2006; 28:498–510.

Xue Z, Ding H, Hu C, Xu H, An Z. An anatomical study of the nutrient foramina of the human humeral diaphysis. *Med Sci Monit*, 2016; 22:1637–1645, doi: 10.12659/MSM.898361.

Zahra SU, Kervancioğlu P, Bahşi İ. Morphological and topographical anatomy of nutrient foramen in the lower limb long bones. *Eur J Ther,* 2018; 24:36-43, doi: 10.5152/EurJTher.2017.147.

Original Article

TJVR 2020; 4 (1): 17-22

Turkish Journal of Veterinary Research

http://www.dergipark.gov.tr/tjvr

e-ISSN: 2602-3695

The study of some hematologic and biochemical parameters in chickens vaccinated with inactivated dual Newcastle-Influenza vaccine

Mehdi Ezdiyadi[1] Ali Akbar Abolfathi[2] Aref Hoshyari[1]
Abolfazl Jafari-Sales[3] Farhad Farhadi[1]

[1] *Department of Research and Development, Razi Vaccine and Serum Research Institute, Agric. Res. Education and Extension Org, Marand, Iran*
[2] *Department of Biological Science, Ahar Branch, Islamic Azad University, Ahar, Iran.*
[3] *Department of Microbiology School of Basic Sciences, Kazerun Branch, Islamic Azad University, Kazerun, Iran.*

Correspondence: *Mehdi Ezdiyadi (Ezdiyadi.m@Chmail.com)*
Received: 26.09.2019 Accepted: 19.02.2020

ABSTRACT

Objective: Newcastle and Influenza diseases are important viral diseases and its occurrence and virulence in Iran has increased in recent years. The purpose of this study was to evaluate the humoral immune responses of chickens vaccinated with inactivated dual oil emulsion Newcastle disease and avian influenza vaccine in two methods of intramuscular and subcutaneous injection as well as to assess the possible changes in serum biochemical factors.

Materials and Methods: In this study, after subcutaneous and intramuscular inoculation of inactivated dual oil emulsion Newcastle-Influenza vaccine, the serum antibody level and hematobiochemical factors of these avian were analyzed on 7, 14, 21, and 28 days after vaccine injection.

Results: The results showed that the values of biochemical parameters such as albumin, glucose, total protein, triglyceride, cholesterol, ALT, AST, and ALP enzymes, sodium and potassium minerals, and hematocrit and hemoglobin levels in vaccinated and non-vaccinated chickens were not significantly different. Also, in chickens injected with antigen, Newcastle disease and influenza antibody titers were significantly different with those groups containing adjuvant.

Conclusion: The results of this study indicate that the intramuscular and subcutaneous injection of dual combination vaccine is similar in terms of changes in biochemical and hematological factors and Newcastle disease and influenza antibody titers. To ensure safety in inactivated vaccines, the presence of proper adjuvant is essential for immune response.

Keywords: *Avian influenza virus, Newcastle virus, Biochemical factors*

INTRODUCTION

Vaccination is the most effective method to prevent infectious diseases in both humans and animals. Generally, in inactivated vaccines, either whole organisms are killed or the subunit vaccines are effective for their adjuvant addiction (Aguilar and Rodriguez, 2007). Adjuvants are chemical or biological compounds that enhance the function of the immune system. Newcastle is also called "Multiple face disease", due to its several forms. Paramyxoviridae is a family of membranous viruses containing RNA, which have successive single-stranded genomes and negative sense. The disease is caused by a group of very close viruses that form avian paramyxovirus serotype 1 (PMV-1) (Abraham et al., 1986; Adair et al., 1989). The virus is a human pathogen and the most common sign in human infection is conjunctivitis that develops within 24 hours of Newcastle disease virus (NDV)

exposure to the eye (Swayne and King, 2003). The most important way of transmitting Newcastle virus to the flock is through airborne particles. The ELISA method is used to identify antibodies to poultry diseases due to the fact that most of the stages are automated and the results are achieved fast. One of the features of the RT-PCR technique is to be a very rapid detection of the presence of the virus. The oropharynx swab is used as a selective sample. NDV has both haemagglutination and neuraminidase activities. The action of haemagglutination is very important in Newcastle disease and the HI assay is the haemagglutination inhibition assay, which is routinely used to determine the NDV titration. Influenza viruses are classified into three types A, B and C on the basis of their antigen and the presence or absence of common group antigens. All avian influenza viruses are categorized as Type A (EFSA, 2005). This virus is a spherical particle with a diameter of 80-120 nm and a filament form that can be several microns in length. Serologic tests such as haemagglutination inhibition assay and neuraminidase inhibition assay are used to determine the type of influenza. Direct contact with infected birds or with a contaminated discharge and feces is necessary to transmit the infection. The super-acute H7 and H5 influenza viruses and the low-pathogenicity H9 viruses are more important because of their direct transmission to humans and the creation of a novel influenza pandemic. The ability of the virus to diffuse depends on the amount of viruses received by the respiratory or digestive tract of the animal. The National Veterinary Services Laboratory (NVSL) uses a brain-heart infusion broth without any antibiotic to collect the trachea and cloak swabs. Positive allantoic fluid is used for haemagglutination to detect the virus. NA subgroups are detected by micro-NI assay with antiserum prepared against 9 surface antigens (Palmer et al., 1975; Van Deusen et al., 1983; Alizadeh-Arsi et al., 2018). In serological monitoring programs, double immunodiffusion assay is often used to detect anti-NP antigens. There is currently no practical way and specific treatment for the infection caused by avian influenza virus in commercial poultry. It has been experimentally shown that amantadine is effective in reducing mortality (Lang et al., 1970; Beard and Easterday, 1973; Dolin et al., 1982; Webster et al., 1985; Easterday et al., 1997). Newcastle-Influenza Vaccine is an oil-killed vaccine containing inactivated avian influenza (H9N2) serotype with native origin and inactivated Newcastle (V4) serotype produced to prevent Newcastle disease and influenza (H9N2). The purpose of this study was to investigate some hematologic and biochemical parameters in chickens vaccinated with inactivated dual Newcastle-Influenza vaccine.

MATERIALS and METHODS

Chickens hatched from specific pathogen free eggs were used for this study. In this study, eight groups (Five chicks in each group) of 21-day chickens were randomly sampled from a flock of chickens. The sampling steps include: Blood collection from all chickens; blood re-sampling from the wing's vein during the first to fourth weeks, i.e., during 7, 14, 21 and 28 days; and then the HI test to evaluate the response of the chickens to the vaccine in terms of immunogenesis; the assessment of changes in liver enzymes such as Aspartate aminotransferase (AST), Alanine aminotransferase (ALT) and Alkaline phosphatase (ALP); and finally, the measurement of the biochemical parameters such as sodium, potassium, albumin, glucose, cholesterol, triglyceride and total protein. Hemoglobin and hematocrit tests were performed for hematological tests. Viral antigens were prepared according to conventional standard procedures. Auto Analyzer device (Orense, USA) and Pars kits were used to measure the biochemical parameters, and Cell Counter device (Excel, USA) was used to measure hematological parameters, hemoglobin and hematocrit percentage; and Electrolyte Analyzer (Convergent, Italy) device was applied to measure sodium and potassium contents. For the HI test, Newcastle and influenza antigens, Red blood cell 1% and 96 Well microplates were used. Data was put in SPSS software (version 20.0) and ANOVA and Tukey's test were used for analysis. P value of <0.05 was determined as significant.

RESULTS

The humoral immune responses of chickens vaccinated with inactivated dual combination oil Newcastle-influenza vaccine were evaluated in two methods of intramuscular and subcutaneous injection. This study was performed within four weeks after injection and afterwards the results were as follows:

The 7th day results after injection

There was no significant difference between the vaccinated and non-vaccinated (control) chickens in biochemical factors such as albumin, glucose, total

protein, triglyceride, cholesterol, ALP, AST and ALT enzymes, sodium and potassium salts and hematocrit and hemoglobin values after separating the serum of the chickens in each intramuscular and subcutaneous injection group and reading the serum results. Also, in the first week after intramuscular and subcutaneous injection, no significant difference was found in the HI antibody titer.

The 14th day results after injection

According to the isolating serum of chickens by researchers, there was no significant difference between the vaccinated and control chickens. As well as, no significant difference was observed between vaccinated and control chickens in the hematocrit and hemoglobin values in the fourteen days after injection in the blood, in which the anticoagulant substance was added. Although, in the vaccinated chickens, the antibody titer was significantly increased in both Newcastle disease and avian influenza, no antibody titer of Newcastle disease and avian influenza was detected in control chickens.

The 21th day results after injection

Biochemical factors had no significant difference in the results of serum isolated from vaccinated and control chickens within three weeks after subcutaneous and muscular injection. Although, in blood of chickens prepared with anticoagulant, there was no significant difference between vaccinated and non-vaccinated chickens, there was a significant difference between vaccinated and non-vaccinated chickens in terms of the Newcastle disease and avian influenza antibody titer.

The 28th day results after injection

The results of four weeks after subcutaneous and intramuscular injection in chickens showed that there was no significant difference in biochemical parameters between vaccinated and non-vaccinated chickens based on the serum isolated from chickens' blood. As well as, no significant difference was observed in the blood of chickens with anticoagulant substance of vaccinated and non-vaccinated chickens (Figure 1). However, there was a significant difference in Newcastle disease and avian influenza between vaccinated and non-vaccinated chickens (Figures 2 and 3). It should be indicated that subcutaneous and intramuscular injection of dual Newcastle-influenza vaccine produced by Razi Institute has the same results in terms of changes in biochemical factors, hematology and Newcastle disease and influenza titer. The presence of an appropriate adjuvant such as Montanide (ISA-70) oil is essential for immune response in order to create immunity in inactivated vaccines.

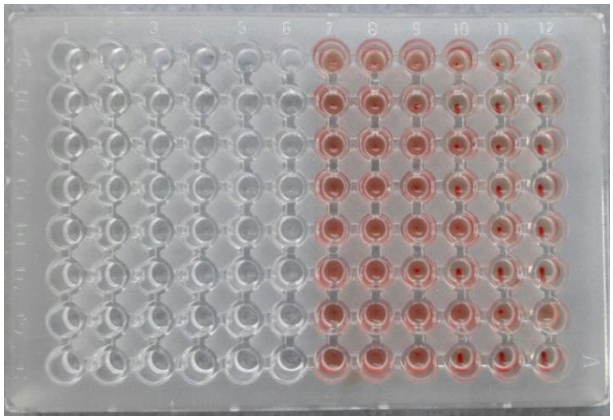

Figure 1. HI Test on the twenty-eighth day

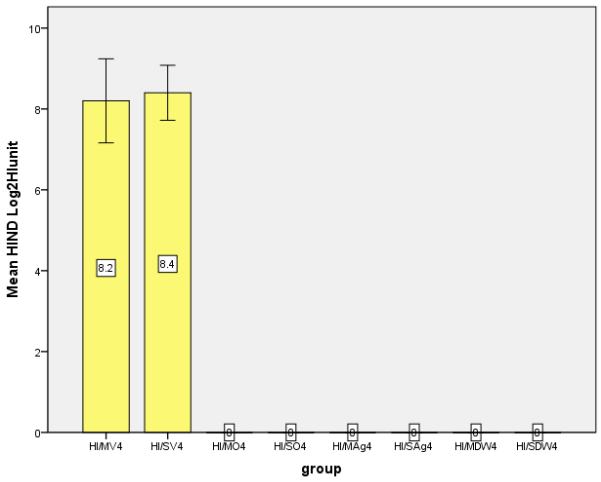

Figure 2. HI Test for Newcastle disease on the twenty-eighth day

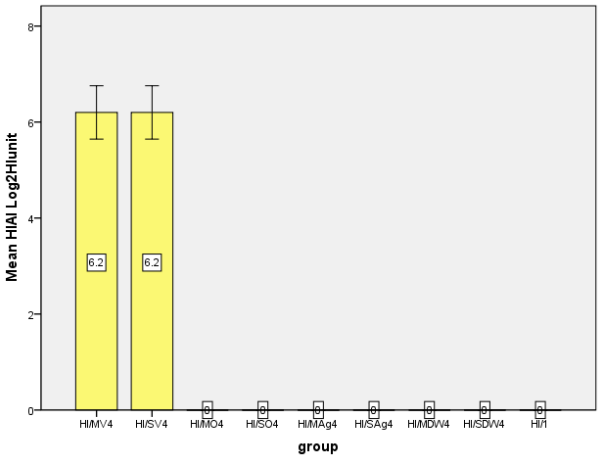

Figure 3. HI Test for avian influenza on the twenty-eighth day

Our results showed that there was no significant difference between vaccinated and non-vaccinated birds in terms of biochemical parameters, and this probably means that injection of inactivated dual combination Newcastle disease and avian influenza vaccines could not change these parameters compared to control birds.

DISCUSSION

We applied the HI assay for Newcastle disease antibody titer in our study and the results showed that Newcastle disease antibody titer can be detected from the second week by this method. Nidhin et al. (2009) in their study, by using HI method to determine the titer showed that the Newcastle disease antibody titer in the egg yolk is significantly more than its amount in serum. We also used the HI method in our study for the influenza antibody titer, and the results showed that the influenza antibody titer could be detected from the second week by this method. Trampel et al. (2006) in a study, used ELISA and AGID to determine the antibody titers. They concluded that antibody levels appear earlier in serum, reach sooner to the peak and remain for a long time; so they preferred antibody monitoring in serum more than that on yolk samples. Alexander et al. (2014) conducted a study, used the HI assay to search and determine the antibody in the egg yolk and showed that by detecting the antibody in the egg yolk, the Newcastle virus turning can be determined in the population of geese. We used the ISA70 oil adjuvant, which could stimulate the immune system and make it to produce antibody by providing the antigen. Iqbal et al. (2008) conducted they prepared the concentrations of 50% and 60% Montanide ISA70 and evaluated the immunogenicity with haemagglutination inhibition (HI) test after injection to layer birds. And they eventually reported that the immune response of the vaccine with 60% oil was better than the vaccine containing 50%. The vaccine was used in our study had 70% adjuvant. Silva et al. (2009) conducted a study, used oil Montanide ISA-70 as an adjuvant in the ratio of 70% to make the vaccine. They concluded that this vaccine protects the vaccinated chickens against the H5N1 virus and provides long-term immunogenicity in these birds. Liu et al. (2011) used three types of adjuvants, including ISA-70, ISA-206 and mineral oil; and they used vaccine injection into SPF chickens to test the constructed vaccines; and after challenging the highly acute influenza virus, they showed that the vaccine produced with the adjuvant ISA-70 and mineral oil could protect 100% of the chickens and the vaccine produced with ISA-206 protected only 40% of the chickens. Finally, they concluded that ISA-70 is the best adjuvant for the construction of an oil-based influenza vaccine that this type of vaccine was also used in our study.

Our results did not show any significant difference in the biochemical parameters between vaccinated and non-vaccinated groups, which was not consistent with other researchers. For example, Talebi (2006) conducted a study, used live Bronchitis, Gumboro and Newcastle vaccines at different ages in the treatment group. Then, blood samples were collected from different groups at different times and the parameters of albumin, calcium, chloride, cholesterol, glucose, magnesium, phosphorus, triglyceride and total protein were examined in the study. He concluded that the physiological values of the biochemical parameters in both groups of one-day chickens are different from its values during breeding period; and cholesterol levels on day zero are close to their values during the breeding period, while the values of other parameters are much lower than their values during the breeding period. Comparison of triglyceride, total protein and albumin values in the vaccinated group showed a significant difference with the recorded values for the control group, but other parameters were not significantly different between the two groups.

The dual Newcastle-Influenza vaccine, with the NewFluRazi brand, was used in the present study that ISA-70 has been used as an adjuvant in its construction. The aim of this study was to evaluate the changes of some hematological and biochemical parameters in the serum of injected chickens in both intramuscular and subcutaneous methods. Silva et al. (2009) assessed the immunogenicity of the inactivated oil emulsion influenza vaccine and stated that the immunity created by the oil-based vaccine in two-month chickens will remain to twelve months; and they announced the maximum visible immunity of the vaccine on the 28th day after injection, and influenza titer began to decline from 150th day. In the present study, our immunogenicity results are consistent with the above results. An effective vaccine is required to have a good antigen as well as a preferred adjuvant. Adjuvant is required to create humoral and cellular immunity, but adjuvants may also have side effects such as inflammation of the tissue, damage and pain (Silva et al., 2009). Kudair and Al-Hussary (2010)

investigated the effects of vaccination on some biochemical parameters in broiler chickens. In their study, they concluded that vaccination against Newcastle disease, infectious bronchitis and Gumboro disease had significant differences in some biochemical parameters between vaccinated and non-vaccinated groups as well as in different ages; and the effect of age was clearer in the vaccinated groups. Their examination also showed that vaccination had no significant effect on the values of glucose, total protein, lipid, triglyceride, lipoprotein, high-density cholesterol, low-density lipoprotein and alanine aminotransferase activity, but the vaccinated chickens showed a significant reduction in albumin values, albumin/globulin ratio and alkaline phosphatase activity compared to control groups. However, the globulin values and the activity of the alkaline aspartate aminotransferase enzyme and lactate dehydrogenase were higher than non-vaccinated groups; no significant difference was observed among different groups in our study.

Although one of the hypotheses was the probability of the impact of Montanide oil on biochemical factors as well as the creating the effective stress on the bird, the results showed that the same results were obtained in the parameters in both subcutaneous and intramuscular injections. As well as, the results of antibody titer showed that Montanide oil was effective in its immunity and durability; and the desired antigens were not able to show the measurable titer by HI assay without that; and the immune system is probably not able to provide a detectable immune response in this way without adjuvant. The similarity of the results of distilled water injections in both subcutaneous and intramuscular injections show that contrary to the assumption, the volume of injections presents the same results, the vaccine, therefore, has the same efficacy in terms of immunogenicity and biochemical and hematological factors in both injection methods. The results of this study did not present any reason for the recommendation of subcutaneous injection. Therefore, despite the use of intramuscular injection in poultry units and vaccine producers, the recommendation for subcutaneous injection seems unnecessary; and according to these results, the vaccine can also be injected into muscle. Of course, it should be noted that this study does not comment about the adjuvant residue in muscle injection, and it certainly requires further investigating and providing documentations that the subcutaneous injection is much safer compared to the intramuscular injection.

CONCLUSION

The results showed that there was no significant difference between vaccinated and non-vaccinated birds in terms of biochemical and hematological parameters. So the vaccine injection failed to change the parameters in comparison with the control birds. Also, the results showed that from the second week, Hi test could detect Newcastle and Influenza Antibody Titer.

ACKNOWLEDGMENTS

Conflict of Interests: The authors declared that there is no conflict of interests.

Financial Disclosure: The authors declared that this study has received no financial support.

REFERENCES

Abraham A, Sivanandan V, Halvorson D.A. and Newman J.A. Standardization of enzyme linked immunosorbent assay for avian influenza virus antibodies in turkeys. *Am J Vet Res*, 1986; 47: 561–566.

Adair BM, McNulty MS, Todd D, Connor TJ, Burns K. Quantitive estimation of Newcastle disease virus antibody levels in chickens and turkeys by ELISA. *Avian Pathol*, 1989; 18:175-192.

Aguilar JC, Rodriguez EG. Vaccine adjuvants revisited. *Vaccine*, 2007; 25(19): 3752-62.

Alexander Y, Alekseee V, Kirill A, Sharsho V, Vasiliy Y, Marchenko ZL. Antibodies to Newcastle disease virus in egg yolks of Great Cormorant (Phalacrocorax carbo) at Qinghai Lake. *Adv Infect Dis*, 2014; 4:194-197.

Alizadeh-Arsi L, Hoshyari A, Ameghi-Roodsary A, Ghaniei A, Jafari B, Jafari SA. Evaluation of antibody titer changes against Newcastle disease and influenza (H9N2) in the egg yolk during incubation period and its impact on the replication of the viruses. *New Findings in Veterinary Microbiology*, 2018; 1(1): 15-27.

Beard CW, Easterday BC. A Turkey Oregon 71, an avirulent influenza isolate with the hemagglutinin of fowl plague virus. *Avian Dis*, 1973; 17: 173–181.

Dolin R, Reichman RC, Madore HP, Maynard R, Linton PN, Webber Jones J. A controlled trial of amantadine and rimantadine in the prophylaxis of influenza A infection. *N Engl J Med*, 1982; 307:580–584.

Easterday BC, Hinshaw VS, Halvorson DA. Influenza. In: Calnek BW, Barnes HJ, Beard CW, McDougald LR, Saif YM, (eds). *Diseases of Poultry*. 10th edition. Iowa State University Press Ltd, 1997;583–605.

Iqbal M, Nisar MA, Noor S, Gill ZJ. Evaluation of oil based avian influenza vaccine (H5NI) prepared with different concentrations of adjuvant. *Pakistan Veterinary Journal*. 2008;28(4).

Kudair IM, Al-Hussary NAJ. Effect of vaccination on some biochemical parameters in broiler chickens. *Iraqi J Vet Sci,* 2010; 24(2):59-64.

Lang G, Narayan O, Rouse BT. Prevention of malignant avian influenza by 1-adamantanamine hydrochloride. *Archiv fur die Gesamte Virusforschung*, 1970; 32:171–184.

Liu CG, Liu M, Liu F, Liu DF, Zhang Y, Pan WQ, et al. Evaluation of several adjuvants in avian influenza vaccine to chickens and ducks. *Virology journal.* 2011;8(1):321.

Nidhin R, Praseena P, Surya PS, Chintu R, Mathew S. Comparison of antibody titres of newcastle disease virus in randomly collected sera and egg yolk of layers. *J Vet Anim Sci*, 2009; 40:20-21.

Palmer DF, Coleman MT, Dowdle WD, Schild GO. Advanced laboratory techniques for influenza diagnosis. Immunology series no. 6. U.S. Department of Health, Education and Welfare, Public Health Service, Centers of Disease Control: Atlanta, GA. 1975.

Silva BC, Martins ML, Jatobá A, et al. Hematological and immunological responses of Nile tilapia after polyvalent vaccine administration by different routes. *Pesquisa Veterinária Brasileira*, 2009; 29(11), 874-880.

Swayne DE, King DJ. Avian influenza and Newcastle disease. *J* Am *Vet Med Assoc,* 2003; 222(11):1534–1540.

Talebi A. Biochemical parameters in broiler chickens vaccinated against ND, IB and IBD. *Int J Poultry Sci*, 2006; 5(12):1151-1155.

Trampel DW, Zhou EM, Yoon KJ, Koehler KJ. Detection of antibodies in serum and egg yolk following infection of chickens with an H6N2 avian influenza virus. Journal of veterinary diagnostic investigation. 2006;18(5):437-42.

Van Deusen RA, Hinshaw VS, Senne DA, Pellacani D. Micro neuraminidase inhibition assay for classification of influenza A virus neuraminidases. *Avian Dis*, 1983; 27:745-750.

Webster RG, Kawaoka Y, Bean WJ, Beard CW, Brugh M. Chemotherapy and vaccination: A possible strategy for the control of highly virulent influenza virus. *J Virol*, 1985; 55:173-176.

Original Article

TJVR 2020; 4 (1): 23-27

Turkish Journal of Veterinary Research

http://www.dergipark.gov.tr/tjvr

e-ISSN: 2602-3695

Investigation of some biochemical and haematological parameters in sheep infected with *Dicrocoelium dentriticum*

Vural Denizhan[1] Ayşe Karakuş[2]

[1] *Department of Medical Laboratory Technician, Özalp Vocational High School, University of Van Yuzuncu Yıl, Van, Turkey*
[2] *Department of Parasitology, Faculty of Veterinary Medicine, Van Yuzuncu Yıl University 65080 Van, Turkey.*

Correspondence: *Ayşe Karakuş (vetci.sona@hotmail.com)*

Received: 07.02.2020 Accepted: 20.02.2020

ABSTRACT

Objective: The aim of this study was to investigate the changes in some biochemical and haematological parameters due to liver damage caused by parasites in sheep infected with *Dicrocoelium dendriticum*.

Material and Methods: The study was conducted on 10 healthy sheep and 60 sheep infected with *D. dendriticum*. After the blood was taken from the sheep brought to the slaughterhouse in Van Municipality, the liver and gall bladder were examined after the slaughter, and the blood of sheep infected with *D. dendriticum* was brought to the laboratory and examined for some haematological and biochemical parameters.

Results: As a result of the examination, some changes were observed in the biochemical and haematological parameters. In statistical analysis: Haematologically compared with the healthy group of sheep infected with *D. dentriticum*; WBC, Neu, Eo and Mon values were higher than the control group's values, while RBC, Hct, Hb and MCV values were lower and no significant change was found in the Lym level.

Results of biochemical parameters of sheep infected with *D. dentriticum* according to statistical analysis; ALT, AST and GGT values were higher than of the health group sheep, while serum TP, Alb and glucose values were found than lower.

Conclusion: As a result; WBC, Neu, Eo, Mon, ALT, AST and GGT levels in infected sheep due to dicrocoeliasis which cause important pathological disorders in liver that have functions such as synthesis, metabolism and detoxification in the organism were found to be significantly higher compared to the same values of the healthy group. It is predicted that these parameters may be useful in determining the degree and prognosis of liver damage and will shed light on the studies to be performed in this field.

Keywords: *Dicrocoeliosis, Sheep, Biochemical parameters, Haematologic parameters*

INTRODUCTION

Hepatic trematode infections in sheep cause significant economic losses due to slow growth, decreased meat, milk and wool yields, suppression of the immune system and death of animals in severe infections. *Dicrocoelium dentriticum* Rudolphi, 1819, one of the liver trematodes, causes dicrocoeliasis. Dicrocoeliasis is an important disease that causes liver damage in ruminants and causes significant economic losses in countries where sheep, goat, buffalo (Alvarez et al., 2009) and cattle breeding worldwide (Yang et al., 1998).

In dicrocoeliasis infections, it can cause severe infection in cases where the number of parasites is too high in the definitive host (Boch and Supperer, 2000; Akyol, 2001).

There is a direct relationship between the number of parasites and the symptoms that occur in animals

with dicrocoeliasis (Jithendran and Bhat, 1996). Experimentally, hepatic enzyme activities such as aspartate amino transferase (AST) and alanine amino transferase (ALT) were observed in lambs infected with 1000 and 3000 metacercariae of *D. dendriticum*. A significant correlation was found between increased hepatic enzyme activities and parasite density, and the highest activity was reported in lambs with the highest parasitic number (Manga-González and Ferreras, 2014).

Pathological injuries to the liver and gallbladder of recent hosts with dicrocoeliasis are probably caused by the toxic metabolites of the parasite and mechanical stimulation of the bile duct walls (Manga-González and Ferreras, 2014). *Dicrocoelium dendriticum,* AST, ALT, gamma glutamyl transferase (GGT), alkaline phosphatase (ALP), albumin and total bilirubin levels increase. Infection has been reported to cause an increase in leukocyte and neutrophil counts and a decrease in lymphocyte count (Sanchez-Campos et al., 1999; Gonzalez-Lanza et al., 2000; Otranto and Traversa, 2002). In addition, it has been reported that malondialdehyde (MDA), an indicator of lipid peroxidation, increases and this causes oxidative stress (Şimşek et al., 2006).

Haematological and biochemical parameters in animal health are an important diagnostic tool for the assessment of disease by veterinarians and are also important for assessing the effectiveness of treatment in infected animals. Changes in enzyme activity in the blood are used in the clinical diagnosis of many diseases. Tissue damage in invasions caused by parasites such as *D. dendriticum* result in changes in serum enzyme activities. Especially in the toxic destruction of the liver, changing enzyme activities in the serum before shaping the clinical picture is of great importance in terms of early detection (Dağoğlu et al., 1995).

Dicrocoeliasis can also be diagnosed with ELISA, immunoelectrophoresis techniques, and changes in the liver's pathology in recent years, as well as the detection of typical trematode eggs in the stool (Ducháček and Lamka, 2003).

The aim of this study is to reveal the changes occurring in some haematological and biochemical parameters in naturally infected sheep with *D. dentriticum*, which causes significant pathological disorders in the liver.

MATERIALS and METHODS

After the anamnesis and general examinations of sheep brought to the slaughterhouse of Van Municipality, 5 ml blood samples were taken from Vena jugularis for haematological and biochemical analyses and transferred to anticoagulant and non-anticoagulant tubes. 30-50 grams of feces were taken from the rectum for stool examination. As a result of liver, gallbladder and fecal examinations, 60 sheep blood detected as infected by dicrocoeliasis were identified as infected group, while 10 sheep blood that were detected negative for dicrocoeliasis as a result of liver, gallbladder and stool examinations were determined as healthy group.

Fecal examination

After the liver and gall bladder were examined, the stool samples of the sheep detected to be infected were brought to Van Yuzuncu Yil University, Faculty of Veterinary Medicine, Department of Parasitology, and kept at –20 °C until necessary analyses were made. Benedek sedimentation method was used in the examination of stool samples (Toparlak and Tüzer, 1994).

Haematological analysis

Blood samples taken into tubes with anticoagulants were brought to Van Yuzuncu Yil University, Faculty of Veterinary Medicine, Department of Internal Medicine on the same day and red blood cell (RBC), white blood cell (WBC), lymphocyte (Lym), monocytes (Mon), neutrophil (Neu), eosinophil (Eo), MCV (mean cell volume), hematocrit (Hct) and hemoglobin (Hb) haematological parameters were analysed by blood counting device (Veterinary MS4-S-Melet).

Biochemical analysis

Blood samples taken into anti-coagulated tubes were centrifuged at 3000 g for 5 minutes. The sera obtained were stored at -20 °C until biochemical parameters were analysed. ALT, AST, GGT, GLU (Glucose), albumin (Alb) and total protein (TP) levels were measured by commercial kits using auto analyser (BS-120 Vet-Mindray).

Statistical Analysis

Descriptive statistics for the characteristics of haematological and biochemical parameters of infected and healthy sheep with *Dicrocoelium dentriticum*; Mean, standard deviation were given as minimum and maximum values. Student's t test was used to compare the groups. Pearson correlation analysis was performed to determine

linear relationships between variables. Statistical significance level was accepted as 5% and SPSS (ver: 13) statistical program was used for all statistical calculations.

RESULTS

The results of haematological parameters of sheep infected with healthy sheep group and *D. dentriticum* are given in Table 1. In statistical analysis; WBC, Neu, Eo and Mon values of sheep infected with *D. dentriticum* were higher than those of the healthy sheep group, while RBC, Hct, Hb and MCV values were lower than the healthy sheep group.

Table 1. Haematological parameters of healthy and *Dicrocoelium dentriticum* infected sheep

Parameters	Healthy Group	Infected Sheep	p<
WBC (m/mm³)	8.60 [a]	14.12[b]	0.001
Lym (m/mm³)	3.64 [a]	4.31[a]	0.057
Mon (m/mm³)	0.57 [a]	0.69 [b]	0.001
Neu (m/mm³)	5.16 [a]	9.19 [b]	0.001
Eo (m/mm³)	0.39 [a]	0.74 [b]	0.001
RBC (m/mm³)	10.41 [a]	9.13 [b]	0.001
MCV (m/mm³)	27.91 [a]	23.93 [b]	0.001
Hct (%) (m/mm³)	26.33 [a]	22.38 [b]	0.001
Hb (g/dl) (m/mm³)	12.94 [a]	8.77 [b]	0.001

a, b: p<0.05 and a, a: p>0.05 were defined as statistical significance between the parameters on the same line and named with different letters

Table 2. Biochemical parameters of healthy and *Dicrocoelium dentriticum* infected sheep

Parameters	Healthy Group	Infected Sheep	p<
ALT (U/L)	17.17 [a]	32.08[b]	0.001
AST (U/L)	92.77 [a]	154.68 [b]	0.001
GGT (U/L)	52.24 [a]	81.21 [b]	0.001
TP (g/dl)	6.57 [a]	4.79 [b]	0.001
Alb (g/dl)	2.64 [a]	1.71 [b]	0.001
GLU (mg/dl)	71.75 [a]	59.18 [b]	0.004

a, b: p<0.05 was defined as the statistical significance between the parameters on the same line and named with different letters

Results of biochemical parameters of sheep infected with healthy sheep group and *D. dentriticum* are given in Table 2. According to statistical analysis, ALT, AST and GGT values of sheep infected with *D. dentriticum* were found to be high according to the same parameters of healthy sheep, while serum TP, Alb and glucose (GLU) values were found low.

DISCUSSION

Studies on haematological and biochemical parameters in sheep that are naturally infected with *D. dendriticum* are limited and little information is available. Changes in biochemical parameters of animals have been reported in parasitic infections (Değer et al., 1997; Şahin and Akgül, 2006). Experimentally, an increase in serum ALT and AST levels has been reported in mice infected with *D. dentriticum* (Sánchez-Campos et al., 1999). Yuksek et al. (2007), reported that there was an increase in serum ALT and AST levels in sheep infected with endoparasites. In *D. dentriticum* infected sheep, RBC, Lym, Hb and Hct values were significantly lower compared to the same parameters of healthy animals, WBC, neutrophil, eosinophil and MCV values were significantly higher than infection. Also, no significant changes were reported in MCH, monocyte and basophil counts. (Kaneko et al., 1997; Kramer, 2000; Matanović et al., 2007). In this study, in addition to the changes in serum Tp, Alb, GLU, AST, ALT and GGT levels in sheep infected with *D. dentriticum*; changes in WBC, Lym, Mon, Neu, Eo, RBC, MCV, Hc and Hb concentrations were examined. In the study, WBC, Neu, Eo and Mon levels of sheep infected with *D. dentriticum* were significantly higher than healthy sheep group. In addition, RBC, Hct, Hb and MCV of infected sheep were significantly lower than those of the healthy sheep group, but there was no significant change in Lym. In our study, haematologic data obtained from sheep infected with *D. dentriticum* were similar to those of the researchers (Kaneko et al., 1997; Kramer, 2000; Matanović et al., 2007).

D. dentriticum, which is the liver trematode of sheep, causes damage to the liver parenchymal tissue and fibrosis in the bile duct (Calleja et al., 2000). Serum AST and LDH activities reflect damage during the passage of young parasites throughout the liver parenchyma, while GGT increases provide information on the penetration of trematodes in the bile ducts (Gonzalo-Orden et al., 2003). Serum ALT is an enzyme normally found only in hepatocytes, while the AST enzyme is restricted to hepatocytes, red blood cells, cardiac and skeletal muscles; GGT is limited to hepatocytes; ALP bile ducts, bone and an intra hepatic enzyme found in the lining cells of the placenta. While serum ALT is normally found only in hepatocytes, AST enzyme is limited to hepatocytes in hepatocytes, red blood cells, cardiac and skeletal muscles, while GGT is limited to hepatocytes, while ALP and an intra-hepatic enzyme found in the lining cells of the placenta.

These enzymes are used as biological markers of hepatic disorders (Manga-González et al., 2004; Al-Hadithy et al., 2013). Serum glutamate dehydrogenase (GLDH) and gamma glutamyl transpeptidase (GGT) have been reported as the most sensitive markers of liver cell damage (Anderson et al., 1977). Some investigators have reported that plasma GLDH and GGT activities are more sensitive indicators of liver cell damage than AST activity in chronic and subclinical dicrocoeliasis (Sykes et al., 1980). However, GGT stability is reported to be a more important indicator in determining the level of damage in liver tissue due to the higher trematode infections (Blood et al., 1989; Gonzalo-Orden et al., 2003; Phiri et al., 2007; Raadsma et al., 2008). This decrease in plasma enzyme activities can be used to monitor the effectiveness of treatment in trematode infections (Gaasenbeek et al., 2001). In this study, ALT, AST and GGT values of sheep infected with *D. dentriticum* were found to be higher than those of healthy sheep. Increases in ALT, AST, and GGT concentrations of sheep infected with *D. dentriticum* have been investigated by researchers (Gaasenbeek et al., 2001; Gonzalo-Orden et al., 2003; Phiri et al., 2007; Severin et al., 2012).

Liver trematodes reduce glycogen reservoir in organ due to tissue damage both in hepatocytes and during migration of parenchymal tissue (Phiri et al., 2007). In studies on liver diseases, serum glucose levels are reported to be significantly lower than in healthy animals (Yadav and Sharma, 1986; Baghshani et al., 2012). In this study, the serum GLU levels of sheep infected with *D. dentriticum* were found to be lower than the healthy sheep group. It is thought that low GLU concentration in sheep with dicrocoeliasis may result from the use of GLU by *D. dentriticum* and the lack of sufficient glycogen reservoir due to hepatic dysfunction caused by *D. dentriticum* infection. The data obtained on the serum GLU levels of sheep infected with *D. dentriticum* support the data of the researchers (Yadav and Sharma, 1986; Phiri et al., 2007; Baghshani et al., 2012).

Hypoalbumin has been detected in hepatic infections and injuries and liver trematode infections (Thomas, 2000; Bosy-Westphal et al., 2001; Matanović et al., 2007). In studies, biochemical parameters in non-infected healthy sheep group were reported to be within the reference range (Kaneko et al., 1997; Kramer, 2000). In the study of sheep with dicrocoeliasis, serum total protein and albumin concentrations were reported to be lower than in healthy animals (Thomas, 2000; Matanović et al., 2007). In this study, total serum and albumin levels of sheep with dicrocoeliasis were found to be low, and it is in line with the results of the investigators (Thomas, 2000; Matanović et al., 2007).

CONCLUSION

D. dendriticum, which can be overlooked in organ aspects due to its morphological small size, causes both loss of yield in live animals and destruction of the liver due to the damage it causes in the liver, and in the case of infection with this parasite in animals, disturbances in the body's natural chemistry can occur. In this study, changes in some biochemical parameters in sheep with dicrocoeliasis were examined and evaluated statistically. In particular, increase in WBC, Neu, Eo and Mon values and decrease in RBC, Hct, Hb and MCV values were found to be statistically significant.

In the study, it has been determined that some haematological and biochemical parameters cause significant changes in sheep infected with *D. dentriticum* and we believe that it will shed light on the future studies.

ACKNOWLEDGMENTS

Conflict of Interests: The authors declared that there is no conflict of interests.

Financial Disclosure: The authors declared that this study has received no financial support.

REFERENCES

Akyol VÇ. Bursa ortak girişim tesislerinde (Etba) kesilen koyunlarda Distamatosis'in yayılışı. *J Fac Vet Med* 2001; 20:23-27.

Al-Hadithy HAH, Badawi NM, Mahmood MM. Estimation of serum liver enzymes activities in Awassis heep. *Iraqi J Vet Med* 2013; 37:115-120.

Alvarez L, Moreno G, Moreno L, Ceballos L, Shaw L, Fairweather I, *et al.* Comparative assessment of albendazole and triclabendazole ovicidal activity on *D. dentriticum* eggs. *Vet parasitol* 2009; 164(2):211-216.

Anderson PH, Berrett S, Brush PJ, Hebert CN, Parfitt JW, Patterson DSP. Biochemical indicators of liver injury in calves with experimental fasciolasis. *Vet Rec* 1977; 15:43-45.

Baghshani H, Razmi GR, YaghfouriS, Dezaki AA. Investigation of selected biochemical parameters in sheep naturally infected with theileriosis. *Comp Clin Path* 2012; 21(6):1417-1420.

Blood DC, Radostits OM, Arundel JH, Gay CC. Veterinary medicine. In: *Diseases Caused by Helminth Parasites*. 7th ed. London: Bailliere Tindall; 1989.

Boch BJ, Supperer R. Veterinär medizinische Parasitologie. 5. Auflage. Berlin, Parey Buchverlag 2000; 353-354.

Bosy-Westphal A, Petersen S, Hinrichsen H, Czech N, Müller MJ. Increased plasma homocysteine in liver cirrhosis. *Hepatol Res* 2001; 20:28-38.

Calleja C, Bigot K, Eeckhoutte C, Sibille P, Boulard C, Galtier P. Comparison of hepatic and renal drug metabolising enzyme activities in sheep given single or two fold challenge infections with *Fasciola hepatica*. *Int J Parasitol* 2000; 30:953-958.

Dağoğlu G, Değer S, Akgül Y, Aksoy A, Şekeroğlu R, Tarakçıoğlu R. Koyunlarda Doramectinin antiparaziter etkinliği ve serum enzimleri üzerine etkisi. *Yüzüncü Yıl Üniv Sağ Bil Derg* 1995; 1:1-5.

Değer Y, Gül A, Bildik A, Dede S, Yur F, Değer S. Parazitli kopeklerin bazı kan parametreleri ile plazma vitamin C düzeylerinde görülen değişiklikler. *Türkiye Parazitol Derg* 1997; 21:195-198.

Ducháček L, Lamka J. Dicrocoeliosis the presentstate of knowledge with respect to wild life species. *Acta Vet Brno* 2003; 72(4):613-626.

Gaasenbeek CP, Moll L, Cornelissen JB, Vellema P, Borgsteede FH. An experimental study on triclabendazole resistance of *Fasciola hepatica* in sheep. *Vet Parasitol* 2001; 95:37-43.

Gonzalez-Lanza C, Manga-Gonzalez MY, Compo R, Del-Pozo P, Sandoval H, Oleaga A, *et al*. IgG antibody responseto ES or somatic antigens of *Dicrocoelium dendriticum* (Trematoda) in experimentally infected sheep. *Parasitol Res* 2000; 86:472-479.

Gonzalo-Orden M, Millan L, Alvarez M, Sanchez-Campos S, Jimenez R, Gonzalez-Gallego J, et al. Diagnostic imaging in sheep hepatic fascioliasis: ultrasound, computer tomography and magnetic resonance findings. *Parasitol Res* 2003; 90:359-364.

Jithendran KP, Bhat TK. Prevalence of dicrocoeliosis in sheep and goats in HimachalPradesh. India. *Vet Parasitol* 1996; 61(3-4):265-271.

Kaneko JJ, Harvey JW, Bruss ML. Clinical Biochemistry of Domestic Animals. 5th ed. San Diego: Academic; 1997.

Kramer JW. Normal hematology of cattle, sheep and goats. In: Feldman BF, Zinkl JG, Jain NC (eds). *Schalm's Veterinary Hematology*. 5th ed. Philadelphia: Lippincott Williams &Wilkins, 2000; 1075-1084.

Manga-González MY, Ferreras MC. Dicrocoeliidae family: majör species causing veterinary diseases. *Adv Exp Med Biol* 2014; 766:393-428.

Manga-González MY, Ferreras MC, Campo R, González-Lanza C, PérezV, García-Marín JF. Hepatic marker enzymes, biochemical parameters and pathological effects in lamb sex perimentally infected with *Dicrocoelium dendriticum*. *Parasitol Res* 2004; 93(5):344-355.

Matanović K, Severin K, Martinkovic F, Simpraga M, Janicki Z, Barisic J. Hematological and biochemical changes in organically farmed sheep naturally infected with *Fasciola hepatica*. *Parasitol Res* 2007; 101:1657-1661.

Otranto D, Traversa D. A review of dicrocoeliosis of ruminants including recent advances in the diagnosis and treatment. *Vet Parasitol* 2002; 107(4):317-335.

Phiri IK, Phiri AM, Harrison LJS. The serum glucose and β-hydroxy butyrate levels in sheep with experimental *Fasciola hepatica* and *Fasciola gigantica* infection. *Vet Parasitol* 2007; 143(3):287-293.

Raadsma HW, Kingsford NM, Suharyanta Spithill TW, Piedrafita D. Host responses during experimental infection with *Fasciola gigantica* and *Fasciola hepatica* in Merinos sheep. II. Development of a predictive index for *Fasciola gigantica* worm burden. *Vet Parasitol* 2008; 154:250-261.

Sánchez-Campos S, Tunon MJ, Gonzales P, Gonzales-Gallego J. Oxidative stress and changes in liver antioxidant enzymes induced by experimental dicrocoeliosis in hamsters. *Parasitol Res* 1999; 85:468-474.

Severin K, Mašek T, Janicki Z, Konjević D, Slavica A, Marinculić A, *et al*. Liver enzyme sand blood metabolites in a population of free ranging red deer (Cervus elaphus) naturally infected with *Fascioloides magna*. *J Helminthol* 2012; 86(2):190-196.

Sykes AR, Coop RL, Robinson MG. Chronic subclinic alovine fascioliasis: Plasma glutamate dehydrogenase, gamma-glutamyl liver lipids in fatty liver of dairy cows. *ASJ* 1980; 77:347-351.

Şahin T, Akgül Y. Investigation of some trace element level sand biochemical parameters in sheep with endoparasite. *YYU Sağ Bil Derg* 2006; 9:100-106.

Şimşek S, Yuce A, Utuk AE. Determination of serum malondialdehyde levels in sheep naturally infected with *Dicrocoelium dendriticum*. *FÜ Sağlık Bil Dergisi* 2006; 20:217-220.

Thomas JS. Over view of plasma proteins. In: Feldman BF, Zinkl JG, Jain NC, (eds). *Schalms Veterinary Hematology*. Philadelphia: Lippincott Williams &Wilkins, 2000; 891-898.

Toparlak M, Tüzer E. Paraziter Hastalıkların Tanısında Laboratuvar Teknikleri. İstanbul: İstanbul Üniversitesi Veteriner Fakültesi Parazitoloji Anabilim Dalı; 1994.

Yadav CK, Sharma NN. Changes in blood chemical components during experimentally induced *Theileria annulata* infections in cattle. *Vet Parasitol* 1986; 21:91-98.

Yang Q, Mao WH, Ferre I, Bayón JE, Mao XZ, González-Gallego J. Plasma aspartate amino transferase (AST), glutamate dehydrogenase (GLDH) and gamma-glutamyl transpeptidase (GGT) activities in water buffaloes with experimental subclinical fasciolosis. *Vet Parasitol* 1998; 78(2):129-136.

Yuksek N, Altuğ N, Gul A. Therapeutic effect of the combination of trichlobendazole and levamisole in sheep with endoparasite infection. *YYU Vet Fak Derg* 2007; 18:19-24.

Original Article

TJVR 2020; 4 (1): 29-32

Turkish Journal of Veterinary Research

http://www.dergipark.gov.tr/tjvr

e-ISSN: 2602-3695

Characterization in Two Indonesian *Bos indicus* Cattle Breeds Based on Morphometrical Measurements

Widya Pintaka Bayu Putra[1] Fiqy Hilmawan[2] Johar Arifin[3]

[1] *Indonesian Institute of Science, Bogor, West Java, Indonesia*
[2] *South Kalimantan Assessment Institute for Agricultural Technology, Banjarbaru, South Kalimantan, Indonesia*
[3] *Faculty of Animal Science, Padjajaran University, Jatinangor, West Java, Indonesia.*

Correspondence: *Widya Pintaka Bayu Putra (widya.putra.lipi@gmail.com)*

Received: 05.02.2020 Accepted: 03.03.2020

ABSTRACT

Objective: The aim of this study was carried out to obtain the discriminant variables between Pasundan and Ongole bulls through morphometrical measurements.

Materials and Methods: Six morphometric traits of chest girth (CG), withers height (WH), body length (BL), chest depth (CD), hip height (HH) and rump width (RW) were performed in this study. Total of 110 bulls (72 Pasundan and 38 Ongole) from West Java Province of Indonesia were used in this study.

Results: The stepwise discriminant analysis showed that three morphometrical measurements of CG, BL and CD had strong discriminating power to characterize of both breeds. The discriminant function obtained correctly classified 87.5% for Pasundan and 100% for Ongole (with canonical correlation of 0.81).

Discussion: Pasundan and Ongole bulls can be characterized using discriminant analysis with at least three morphometrical measurements of CG, BL and CD. Therefore, about 12.5% of morphological characteristics in Pasundan bulls was closed to Ongole bulls.

Keywords: *Bos indicus, Discriminant analysis, Morphometrical measurements, Indonesia*

INTRODUCTION

Pasundan and Ongole cattle were included of *Bos indicus* breed that adapted well in Indonesia. Both cattle were kept by the farmers as beef cattle. Selection program in both cattle can be started with breed characterization. Breed characterization is important to evaluate livestock performance for developing breed standard in the future. Breed characterization can be performed based on molecular genetic and morphometric. Hartatik et al. (2019) reported that Pasundan and Bali cattle (*Bos javanicus*) had similar of Cytochrome-b gene (mtDNA) sequence. According to Cytochrome Oxidase Sub-unit 1 (COI) gene (mtDNA) Pasundan cattle closed to Madura (*Bos indicus*) cattle (Wulandari et al., 2019). Thus, Agung et al. (2019) reported the genetic distance of Pasundan cattle was closed to the Madura (0.89) and followed by Ongole (1.96) based on microsatellite markers. However, the characterization of livestock must be supported by morphometric analysis. Breed characterization using morphometrical measurements is easily and cheaply to apply in the smallholds.

Breed characterization by morphometrical measurements can be performed with many statistical analyses such as multivariate analysis (cluster analysis), principal component anaysis (PCA) and canonical discriminant analysis (Carneiro et al., 2010). The morphometrical measurements has been widely used for breed characterization of livestock (Cazar, 2003). Hence,

the discriminant analysis is one of statistical method has been proved to be assessing the variation within population or breed and can characterize of different population or breed based on morphomerical measurements (Boujenane et al., 2016).

Previous studies worked with morphometrical measurements to characterize breeds of ruminant livestock i.e. cattle (Pundir et al., 2015; Yakubu et al., 2014), goat (Bolaceli et al., 2017; Hamdan et al., 2018), sheep (Carneiro et al., 2010; Asamoah-Boaheng and Sam, 2016; Dauda et al., 2018) and buffalo (Rezende et al., 2017). Recently, study of breed characterization among Indonesian native cattle based on morphometrical measurements was not reported. Therefore, the aim of this study was to obtain morphological characterization in two *Bos indicus* cattle breeds of Indonesia (Pasundan and Ongole) using discriminant analysis. The results of this study can be used as supported information for phylogenetic studies in Indonesian native cattle in the future.

MATERIALS and METHODS

Data collection

Data used in this study were obtained from 110 *Bos indicus* bulls consisted of 72 Pasundan and 38 Ongole bulls (Figure 1). The morphometric data of Ongole bulls and 15 Pasundan bulls were collected from breeding station (BPPIB-SP Ciamis, West Java). This station located at longitude 108°20′ to 108°40′ E and latitude 7°40′20″ to 7°41′20″ S with 20-500 above the sea level. The air temperature about 25-35°C with air humidity 45-80% and rainfall intensity about 1,680 mm/year. Meanwhile, morphometric data of the other Pasundan bulls were collected from smallholders. In addition, all animals had 2 or 3 pairs permanent incisors.

Studied traits

Six morphometrical measures were taken from each animals i.e. chest girth (CG) withers height (WH), body length (BL), chest depth (CD), hip height (HH) and rump width (RW). CG was measured with a tape measure as circumference of the chest just behind the foreleg (fourth *os costa*). WH was measured from the behind of *os scapulla* at dorsal point to the ground. BL was measured from from *tuber humerus* to *tuber ischium.* CD was measured from from the behind of *os scapulla* at dorsal to ventral points. HH was measured from from *os coxae* or *tuber coxae* to the ground. RW was measured from left and right of the *tuber coxae.* All measurements were taken by measuring stick and taken from the right side of the animal.

Statistical analysis

Canonical discriminant analysis were used to obtain some variable as the discriminator variable based on the canonical structure or canonical correlation values. This analysis has been used for the genetic distance estimation between livestock populations or breeds through Mahalanobis distance analysis (Hamdan et al., 2018). The genetic distance of Mahalanobis as the minimum quadratic square distance (D^2) and can be presented in the following model:

$$D^2 = \left(\overline{X}_i - \overline{X}_j\right) C^{-1} \left(\overline{X}_i - \overline{X}_j\right)$$

In this model, $\overline{X}_i$ is the vector of average observation for i^{th} group in each variable; $\overline{X}_j$ is the vector of average observation for j^{th} group in each variable and C^{-1} is the inverse matrix of variance-covariance between variables (Nei, 1987). Each discriminant function consisted of a linear combination (Z) of the independent variable (Y_i) in order to maximize the correlation between Z and Y_i. Linear combination of a discriminant function can be represented in the following model:

$$Z = \mu_0 + \mu_1 Y_1 + \mu_2 Y_2 + \mu_3 Y_3 + \ldots\ldots + \mu_i Y_i$$

In this model, μ_i is the estimated canonical coefficient for the i^{th} data and Y_i is the independent variables of i^{th} data (Arandas et al., 2017). Discriminant analysis has been used to characterize species into statistically different groups based on morphometric traits. The canonical discriminant analysis was performed in this study using SPSS 16.0 statistical package.

RESULTS AND DISCUSSION

The average of CG, CD, HH and RW of Ongole bulls were higher than Pasundan bulls but not significantly different as presented in Table 1. The stepwise discriminant analysis showed that CD, BL and CG were the most discriminating variables between Pasundan and Ongole bulls (Table 2). When the three most important morphometrical measurements for discriminating the two cattle breeds were selected, Wilk's Lambda (λ) and Mahalanobis distance (D^2) dropped to 0.37 and 7.30 respectively with a significant difference between the two cattle breeds (F = 9.49). The unstandardized stepwise discriminant function was used to classify individual cattle. The three discriminating variables variables earlier extracted were the variables included in the discriminant (D) equation: D = -

5.06+0.05(CG)-0.05(BL)+0.10(CD) as presented in Table 3. Hence, the canonical correlation in this study was 0.81 (very high category). While 100% of Ongole bulls were classified into their source population.

Table 1. Descriptive statistic of the morphometric traits of Pasundan and Ongole bulls

Morphometric (cm)	Pasundan (Mean±SD)	Ongole (Mean±SD)
Chest girth	135.20±9.71	154.13±11.97
Withers height	125.31±6.56	124.74±6.70
Body length	128.46±11.02	123.97±11.58
Chest depth	39.69±8.28	58.37±5.16
Hip height	128.49±6.54	130.82±6.39
Rump width	31.07±3.44	33.42±3.57

Table 2. Morphometrical characters selected by stepwise discriminant analysis to characterize Pasundan and Ongole bulls

Step	Variables entered	Tolerance	F-remove	Min. D Squared	Wilk's Lambda (λ)
1	Chest depth	0.67	30.42	5.52	0.44
2	Body length	0.74	16.40	6.64	0.40
3	Chest girth	0.52	9.49	7.30	0.37

Table 3. Canonical discriminant function coefficients

Parameter	Function 1
Constant	-5.06
Chest girth	0.05
Body length	-0.05
Chest depth	0.10

Table 4. Percentage (%) of individual classification per breed based on discriminant analysis

Breed	Predicted group membership (N)		Total (N)
	Pasundan	Ongole	
Pasundan	87.5 (63)	12.5 (9)	100.0 (72)
Ongole	0.0 (0)	100.0 (38)	100.0 (38)

N: number of animal

In Pasundan bulls, 87.5% of the individuals (63 animals) were classified into their origin populational, and 12.5% were classed as individuals of Ongole bulls (9 animals) as presented in Table 4. According to discriminant score (D), the breed characterization in this study was obtained two groups with positive D score for Ongole breed and negative D scores for Pasundan breed (Figure 2).

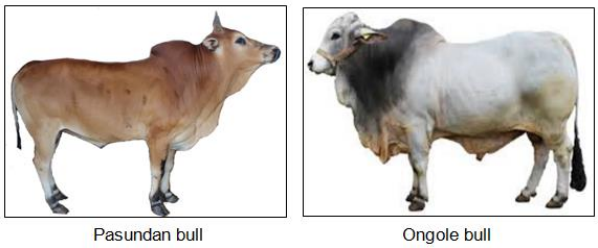

Figure 1. Two *Bos indicus* cattle breeds of Indonesia

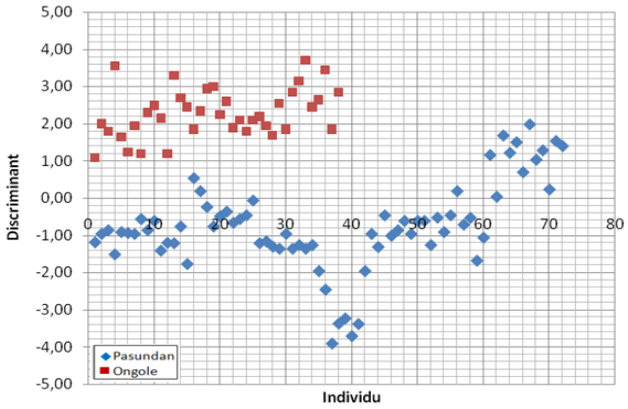

Figure 2. Canonical discriminant plot in the morphological characterization between Pasundan and Ongole bulls

Three morphological measurements of CD, BL and CG were important variable to characterize Pasundan and Ongole bulls. Yakubu et al. (2010) obtained three morphometrical measurements of RW, WH and face length (FL) as the strong variables to characterize Bunaji and Sokoto Gudali cattle. Pundir et al. (2015) obtained 6 morphometrical measurements of WH, BL, FL, ear length (EL), tail length (TL) and paunch girth (PG) as the strong variables to characterize Tripura, Mizoram and Manipur cattle. Dauda et al. (2018) obtained canonical correlation of 0.71 (high category) with 14 morphometrical measurments to characterize Balomi, Karoji, Uda and Yankasa sheeps. Nafti et al. (2014) obtained canonical correlation of 0.51 (moderate) with six morphometrical measurements to characterize Arbi and Serti goats. Asamoah-Boaheng and Sam (2016) obtained canonical correlation of 0.89 (very high) with 16 morphometrical measurements to characterize Djallonke, Sahel and crosses sheeps. In addition, Yakubu et al. (2010) obtained the D^2 value of 7.19 between Bunaji and Sokoto Gudali cattle and close to this study. Pundir et al. (2015) obtained the D^2

value among three Indian cattle breeds about 9.73 (Tripura-Mizoram), 5.72 (Tripura-Manipur) and 4.65 (Mizoram-Manipur) using eight variables. In addition, Rezende et al. (2017) obtained the D^2 value among three Bralizian buffalo breeds that lower than in this study i.e. 4.48 (Jafarabadi-Murrah), 3.31 (Jafarabadi-Mediterranean) and 0.37 (Murrah-Mediterranean) using 11 variables. The tolerance values obtained in the present study were greater than 0.1 and indicated no collinearity problem among discriminator variables (Yakubu et al., 2010) among discriminator variables. The three discriminator variables extracted were sufficiently to characterize two cattle breeds. The morphometrical measurements may considerably increase the reliability of the classification of different cattle breeds. In addition, Yakubu et al. (2010) obtained 85.48% (Bunaji) and 96.55% (Sokoto Gudali) of individual cattle into their different groups. Pundir et al. (2015) used discriminant analysis to classify Tripura (84.13%), Mizoram (82.09%) and Manipur (79.87%) cattle in their source population. Nine Pasundan bulls in this study showed misclassification and can be caused by genetic flow from Ongole cattle as reported by Agung et al. (2019).

CONCLUSION

Pasundan and Ongole bulls can be characterized using discriminant analysis with at least three morphometrical measurements of CG, BL and CD. Therefore, about 12.5% of morphological characteristics in Pasundan bulls was closed to Ongole bulls. The morphometrical measurements in this study can be used for field assessment, management and conservation in both cattle breeds to obtain phenotypically pure local genetic resources and breeding improvement strategies in the future.

ACKNOWLEDGMENTS

Conflict of Interests: The authors declared that there is no conflict of interests.

Financial Disclosure: The authors declared that this study has received no financial support.

REFERENCES

Agung PP, Saputra F, Zein MSA, Wulandari AS, Putra WPB, Said S, *et al.* Genetic diversity of Indonesian cattle breeds based on microsatellite markers. *Asian-Australas J Anim Sci* 2019; 32:467-476.

Arandas JKG, da Silva NMV, Nascimento RB, Filho ECP, Brasil LHA, Ribeiro MN. Multivariate analysis as a tool for phenotypic characterization of an endangered breed. J Appl Anim Res 2017; 45:152-158.

Asamoah-Boaheng M, Sam EK. Morphological characterization of breeds of sheep: a discriminant analysis approach. *SpringerPlus* 2016; 5:69.

Bolacali M, Ozturk, Y, Yilmaz O, Kucuk M, Karsli MA. Investigation of some morphological traits of Boer x Hair F1 crossbred and pure hair goat kids raised in semi-intensive conditions. Int J Morphol 2017; 35:1502-1511.

Boujenane I, Derqaoui L, Nouamane G. Morphological differentiation between two Marocco goat breeds. *J Livest Sci Tech* 2016; 4:31-38.

Carneiro H, Louvandini H, Paiva SR, Macedo F, Mernies B, McManus C. Morphological characterization of sheep breeds in Brazil, Uruguay and Colombia. *Small Rum Res* 2010; 94:58-65.

Cazar RA. An exercise on chemometrics for a quantitative analysis course. *J Chem Edu* 2003; 80:1026-1029.

Dauda A, Abbaya HY, Ebegbulem, V.N. Application of multifactorial discriminant analysis of morphostructural differentiation of sheep. *J Genet Eng* 2018; 2:11-16.

Hamdan, Saputra H, Mirwandhono E, Hasnudi, Sembiring I, Umar S, *et al.* Genetic distance estimate and variable factors distinguishing between goat Kacang, Muara and Samosir. *Earth Env Sci* 2018; 122:012130.

Hartatik T, Hariyono DNH, Adinata Y. Genetic diversity and phylogenetic analysis of two Indonesian local cattle breeds based on cytochrome-b gene sequences. *Biodiversitas* 2019; 20:17-22.

Nafti M, Khaldi Z, Haddad B. Multivariate characterization of morphological traits in local Tunisian oases goats. *Anim Genet Res* 2014; 55:29-38.

Nei M. Molecular Evolutionary Genetic. New York, NY: Columbia University Press; 1987.

Pundir RK, Singh PK, Sadana DK. Multivariate analysis of morphometric traits of three different indigenous cattle populations from north east states of India. *JITV* 2015; 20:79-86.

Rezende MPG, Ferraz PC, Carneiro PLS, Malhado CHM. Phenotypic diversity in buffalo cows of the Jafarabadi, Murrah and Mediterranean breeds. *Pesq Agr Bras* 2017; 52:663-669.

Wulandari A, Nurgiartiningsih, VMA, Kuswati, Susilorini TE, Agung PP. Kinship of several Indonesian local cattle by using DNA mitochondrial COI (Cytochrome Oxidase Sub-unit I). *Int Res J Adv Eng Sci* 2019; 4:165-167.

Yakubu A, Idahor KO, Haruna HS, Wheto M, Amusan S. Multivariate anaysis of phenotypic differentiation in Bunaji and Sokoto Gudali cattle. *Acta Agric Slov* 2014: 96:75-80.

Yakubu A, Salako AE, Imumorin IG, Ige AO, Akinyemi MO. Discriminant analysis of morphometric differentiation in the West African Dwarf and Red Sokoto goats. *South African J Anim Sci* 2010: 40:381-387.

Case Report

TJVR 2020; 4 (1): 33-37

Turkish Journal of Veterinary Research

http://www.dergipark.gov.tr/tjvr

e-ISSN: 2602-3695

Effect of Pendulous Crop on Certain Clinicopathological and Biochemical Parameters in Japanese Quails

Özgür Yaşar Çelik[1] Kıvanç İrak[2]

[1] *Department of Internal Medicine, Faculty of Veterinary Medicine, Siirt University, Siirt, Turkey*
[2] *Department of Biochemistry, Faculty of Veterinary Medicine, Siirt University, Siirt, Turkey.*

Correspondence: *Özgür Yaşar Çelik (oyc@siirt.edu.tr)*

Received: 24.02.2020 Accepted: 10.03.2020

ABSTRACT

In this case report, clinicopathological findings and certain biochemical parameters were presented in quails with the pendulous crop. The animal material of the study consists of a total of 3 Japanese quails (*Coturnix coturnix japonica*). The quails were examined clinically first, followed by the post-mortem examinations. The investigations of Na, K, Mg, total TP and Alb were conducted using autoanalyzer. The plasma levels of sodium, potassium, magnesium, total protein and albumin in the blood samples collected from the animals were found to be higher than the averages for quails. Postmortem examination of quails has revealed that the veins of their crops were prominently pronounced and that the crops were filled with a yellowish, foul-odor liquid containing whole grain particles. Furthermore, the crop mucosa of an animal contained ulcerative lesions. As a result, it was found that certain mineral levels and protein profiles of animals were affected by the pendulous crop phenomenon, and that laboratory findings should be considered alongside physical findings when dealing with it.

Keywords: *Quail, Pendulous crop, Clinicopathologic, Biochemical parameters*

INTRODUCTION

The pendulous crop is a physiological disorder characterized by abnormal dilatation of the crops of poultry animals, and its etiology is still unknown (Ebling et al., 2015; Qasim et al., 2015). Once the crop dilates and prolapses it can't return to its normal posture which is the characteristic symptom of the disease. It is reported that this phenomenon is most commonly encountered turkeys (Arda et al., 1990; Venkatasivakumar et al., 2016; Gonder, 2018), but its incidence in broilers have also reportedly increased in recent years (Ebling et al., 2015), and it can even rarely be encountered in quails (Al-Soudi et al., 1974; Hurst, 1978).

While the cause of the disease is not exactly known, it is reported that hereditary causes are the most significant factor (Hinshaw and Asmundson, 1936; Asmundson and Hinshaw, 1937; Willems et al., 2014; Gonder, 2018). Besides genetic susceptibility, extended periods of hot weathers and excessive water consumption due to this heat are reported as preparative factors (Asmundson and Hinshaw, 1937; Willems et al., 2014). Furthermore, in some cases of the disease encountered in broilers, it was found to be related to the presence of Marek's disease or parasites (Ebling et al., 2015).

The feed consumption of diseased animals does not change significantly, but the digestion slows down nevertheless, and the contents can't be sent to the gizzard at the normal rate, causing malnourishment and dehydration (Arda et al., 1990; Ebling et al., 2015). The weakening of the crop and its supportive tissues in animals in this condition causes the crop to sag, resulting in the pendulous crop (Willems et al., 2014). The enlargement rate of the crop depends

on the course and extent of the disease. In most cases, a semi-liquid content is present inside the crop (Arda et al., 1990; Qasim et al., 2015; Venkatasivakumar et al., 2016). In some cases, the crop content is watery and sour-smelling, while in others it may have the consistency of mud (Venkatasivakumar et al., 2016; Gonder, 2018). In advanced forms of the disease, necrotic ulcers can be noticed. Scraping of the necrotic tissue over the crop reveals the bleeding parts below (Arda et al., 1990). In most cases, treatment of the diseased poultry is in vain (Bacon, 1968; Qasim et al., 2015). Hereditary methods and management are reported as the two main approaches to preventing the disease (Bacon, 1968; Willems et al., 2014). The animals with pendulous crop should be removed from the facilities, and they shouldn't be used for breeding, whether they are male or female. Improvement of animal raising conditions is most important, and attention to shadow areas, water presence and, proper diet is also significant in hot regions (Bacon, 1968).

In this case, it was aimed to investigate the clinicopathological findings and certain biochemical parameters of quails diagnosed with the pendulous crop.

CASE HISTORY

The animal material of the study consists of a total of 3 Japanese quails (*Coturnix coturnix japonica*) of 4 to 6-week ages, of which 2 were females and 1 was a male. The quails were examined clinically first, followed by the post-mortem examinations. The blood samples for the biochemical analyzes were collected during the slaughtering of the animals. The samples were taken into non-anticoagulant tubes, which were centrifuged in 3000 rpm for 10 minutes and were transferred into 1.5 ml microtubes. The samples were kept in -20 ◦C till their analysis. The investigations of sodium (Na), potassium (K), magnesium (Mg), total protein (TP) and albumin (Alb) were conducted using autoanalyzer (ADVIA 1800 Chemistry System, Germany).

Inspections have revealed that the ventral part of the animals' necks was abnormally dilated, and their crops were full and bulging (Fig. 1A). Dehydration was also detected in the quails. Postmortem examination of quails has revealed that the veins of their crops were prominently apparent and the crops were filled with a yellowish, foul-odor liquid containing whole grain particles (Fig. 1B). Ulcerative areas were detected in the crop mucosa of a quail (Fig. 1C). Duodenum, jejunum, and ileum of the animals were found to be empty (Fig. 1D). The results of biochemical examinations are shown in Table 1. As can be seen in Table 1, the Na, K, Mg, TP, and Alb levels of both male and female quails were found to be higher than their counterparts in the literature.

Table 1. Serum biochemical parameters in japanese quails.

Biochemical Parameters	Cases			References
	I (Male)	II (Female)	III (Female)	
Na (mmol/l)	144	145	147	142.60 ± 8.55[a]
K (mmol/l)	4.50	3.60	3.50	2.40 ± 0.76[a]
Mg (mg/dL)	5.07	4.26	5.41	3.77 ± 0.16[b]
TP (g/dL)	2.60	2.90	3.50	2.40 ± 0.10[c]
Alb (g/dL)	1.20	1.30	1.50	0.90 ± 0.00[c]

[a:] (Suchý et al., 2010), [b:] (Karabulut and Eren, 2006), [c:] (Tufan et al., 2015)

DISCUSSION AND CONCLUSION

While the pendulous crop disease is reported to be most commonly encountered in turkeys (Venkatasivakumar et al., 2016; Gonder, 2018), its incidence in broilers have also reportedly increased in recent years (Ebling et al., 2015). There are a limited number of studies reporting the disease for the quails (Al-Soudi et al., 1974; Hurst, 1978).

Some researchers have reported that extended periods of hot weathers played an important role for the occurrence of the disease, besides the genetic susceptibility of the animals (Hinshaw and Asmundson, 1936; Asmundson and Hinshaw, 1937; Willems et al., 2014). Pendulous crop cases in this study were detected in Siirt province. The weather of the province has the semi-arid climate classification. The lowest and highest average temperatures in summers are 36.9 ◦C and 18.9 ◦C, respectively. Water shortages are common during summer months. It is possible that the very hot summer months of the Siirt province climate could have played a role in the occurrence of the pendulous crop disease in quails.

In their studies of the disease in turkeys, the researchers (Bacon, 1968; Qasim et al., 2015) have reported that the crops were initially slightly swollen, and the swelling increased as the disease progressed. Qasim et al. (2015) have reported that one of the turkeys with pendulous crop was languid, anorexic, and recumbent, and a foul-odor

liquid was being discharged from its mouth. Similar to the findings of these researchers, abnormally swollen ventral neck sections were present in our study as well, and palpations revealed content-filled and swollen crops.

In their postmortem inspections, Qasim et al. (2015) have found that one of the turkeys with pendulous crop condition had an enlarged crop filled with soft content, and the veins of the crop were prominently apparent. When they incised the crop, the content was noticeably full of a liquid containing whole grain particles, while the duodenum, jejunum, and ileum of the animal were found to be empty. Bacon (1968) reports that postmortem crop content had a sour odor and the walls of the crop were thinner than normal. Rigdon et al. (1960) have reported that some degenerative objects had caused ulcers in the inner part of the crop, and the muscles around the crop were weaker than usual. The findings of the swollen crop, prominent crop veins, crop content containing whole grain particles, and empty duodenum, jejunum, and ileum in our study, along with the ulcerative areas in the crop mucose of one of the quails, is consistent with the findings of the other researchers.

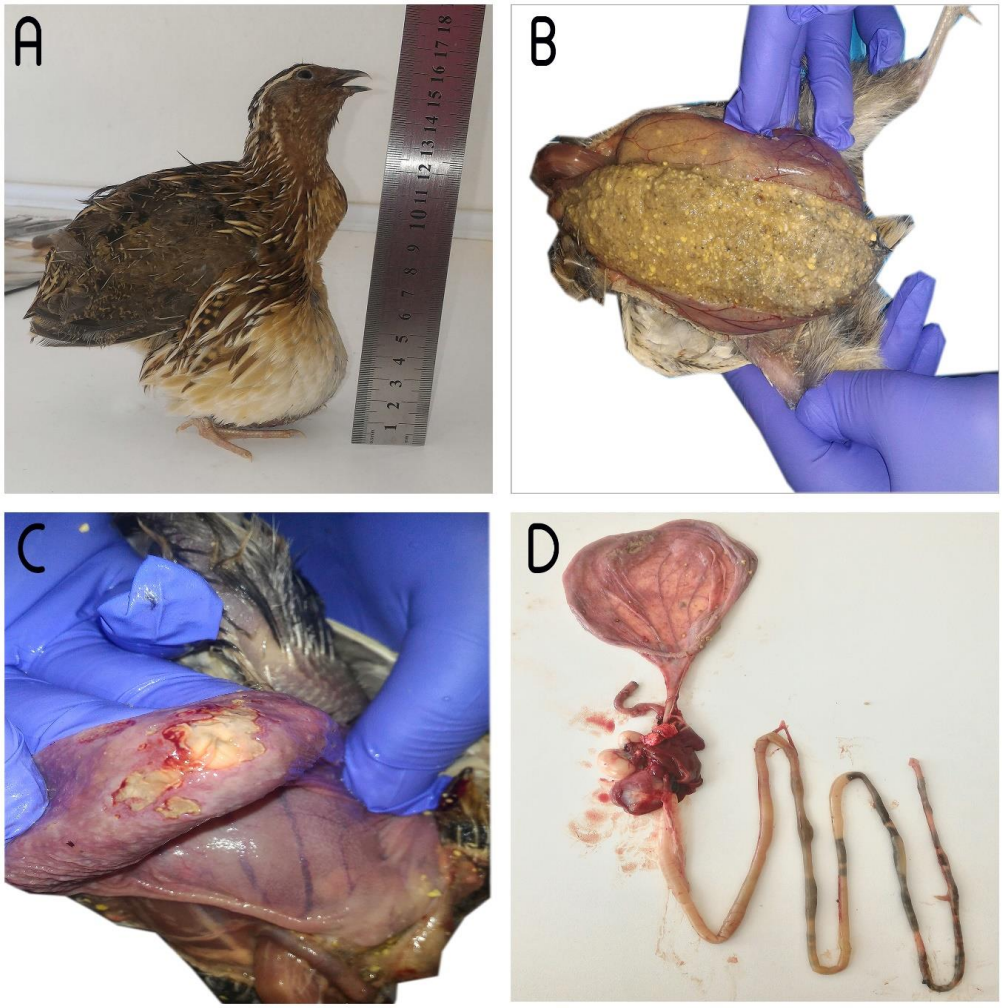

Figure 1. (A) Pendulous crop in male quail, (B) semi-liquid crop content with grain particles, (C) ulcerative areas in the crop mucosa, (D) Empty bowels

Al-Soudi et al. (1974) report that the quail chicks that were separated into a group for a low protein (17.9%) glucose monohydrate-soybean meal diet displayed 44.0% pendulous crop occurrence, compared to 11.8% of the control group fed with normal ration. Wheeler et al. (1957) were able to experimentally create pendulous crop in animals by using either starch or diet purified by glucose monohydrates. Since the content of the feed used for feeding the quails in this study was in accordance with the requirements of the National Research Council (NRC, 1994), it can be surmised that the pendulous crops that occurred in our study couldn't have originated from the diet.

The monitoring of biochemical indicators in blood samples from poultry is now a routine part of experimental studies in veterinary medicine and animal husbandry. Blood parameters are influenced by many factors such as genotype, age, physiological status, gender, diet, micro and macro climate conditions, growth method, seasons and pathological factors (Suchý et al., 2010).

In a study that investigated the clinicopathologic characteristics of pendulous crops observed in turkeys, it was found that the protein and glucose levels of diseased turkeys had slightly increased compared to the reference values (Qasim et al., 2015). In the present study, total protein and albumin levels were found to be higher than the values reported by Tufan et al. (2015).

In broiler chicks, the muscles around the crop are damaged due to the increased tension caused by the sagging, and as the weakened muscles are unable to transfer the nutrients to the proventriculus, dehydration and malnourishment begin to occur (Ebling et al., 2015). Dehydration is basically defined as the reduction in total intra and/or extracellular liquids due to reduced liquid intake or increased liquid discharge and is a common symptom that develops during the course of numerous diseases. In addition to increased hematocrit, hemoglobin, serum TP and albumin concentrations in dehydrated animals, significant deviations can occur in the serum electrolyte concentration and acid-base balance of the blood. In cases of dehydration, it is possible that serum Na and K concentrations will stay at normal levels, but they may get increased or reduced as well (Aktaş, 2007). In this study, the increase in serum TP, albumin, Na, K, and Mg levels in male and female quails diagnosed with pendulous crop could be due to dehydration caused by the incapability of transferring the nutrients to the proventriculus.

Pendulous crop cases were detected in 1 male and 2 female quails in this study. This distribution might be caused by the fact that females usually consume more feed than males. This difference in feed consumption arises due to the hormonal changes occurring in the male quails in 30 to 40 days of age which initiate the sexual activities. This period also coincides with the formation of social hierarchy in the covey, causing increased stress in male quails. Due to all these developments, males of this age consume less feed compared to females (Seker et al., 2007; Bolacali and Irak, 2017; Tufan and Bolacali, 2017). The relatively higher feed consumption amount may explain why pendulous crop incidence is higher in the females.

As a result, in this case report, it was found that certain mineral levels and protein profiles of animals were affected by the pendulous crop phenomenon, and that laboratory findings should be considered alongside physical findings when dealing with it. It was also surmised that it could be possible to reduce the incidence of the disease by improving animal care and nutrition conditions.

ACKNOWLEDGMENTS

The study has been orally presented at I. Anatolian International Multidisciplinary Studies Congress on 28-29 December 2018, Diyarbakır/TURKEY.

Conflict of Interests: The authors declared that there is no conflict of interests.

Financial Disclosure: The authors declared that this study has received no financial support.

REFERENCES

Aktaş S. The effect of bicarbonated sodium chloride solution on haematological and biochemical parameters in dehydrated dogs (Master thesis). Aydın: Adnan Menderes University; 2007.

Al-Soudi K, Bernier P, Arscott G. Pendulous crop in Japanese quail (Coturnix coturnix japonica). *Poult Sci* 1974; 53:252-257.

Arda M, Minbay A, Aydın N, Akay Ö, İzgür M. Kursak Sarkması. *Kanatlı Hayvan Hastalıkları*. 1st ed. Ankara: Medisan Yayın Evi, 1990; 213-214.

Asmundson V, Hinshaw W. On the inheritance of pendulous crop in turkeys (Meleagris gallopavo). *Poult Sci* 1937; 17:276-285.

Bacon M. Pendulous crop of turkeys. *Journal of the Department of Agriculture, Western Australia, Series 4* 1968; 9:463-464.

Bolacali M, Irak K. Effect of dietary yeast autolysate on performance, slaughter, and carcass characteristics, as well as blood parameters, in quail of both genders. *S Afr J Anim Sci* 2017; 47:460-470.

Ebling P, Pontalti G, Villanueva A, Ribeiro A. Pendulous crop in broilers. *Braz J Poultry Sci* 2015; 17:395-398.

Gonder E. Pendulous Crop in Poultry. Avaiable at https://goo.gl/AfQHmG. Accessed July 15, 2018.

Hinshaw W, Asmundson V. Observations on pendulous crop in turkeys. *J Amer Vet Med Assoc* 1936; 41:154-165.

Hurst G. Crop Impactions in Bobwhite Quail in Louisiana. *J Wildl Dis* 1978; 14:355-357.

Karabulut N, Eren M. Besi bıldırcını yemlerine bor ilavesinin serum kalsiyum, inorganik fosfor ve magnezyum düzeyleri ile alkali fosfataz aktivitesine etkisi. *Erciyes Üniv Sağlık Bilim Derg* 2006; 15:8-12.

Meteorology. Turkish State Meteorological Service. Avaiable at https://goo.gl/ud7dBK. Accessed August 15, 2018.

NRC. Nutrient requirements of poultry (Vol. 9). Washington, D.C: National Academy Press, 1994; 155.

Qasim A, Musa U, Barde I, *et al.* Unobstructed pendulous crop in an adult turkey hen and the associated clinicopathological features: a case report. *Vom Journal of Veterinary Science* 2015; 10:155-158.

Rigdon R, Ferguson T, Couch J. Pendulous crops in turkeys anatomic and pathologic study. *AmJ Vet Res* 1960; 21:979-986.

Seker I, Bayraktar M, Kul S, Ozmen O. Effect of slaughter age on fattening performance and carcass characteristics of Japanese quails (Coturnix coturnix japonica). *J Appl Anim Res* 2007; 31:193-195.

Suchý P, Straková E, Kroupa L, Steinhauser L, Herzig I. Values of selected biochemical and mineral metabolism indicators in feathered game. *Acta Vet Brno* 2010; 79:9-12.

Tufan T, Arslan C, Sarı M, Kaplan O. Effect of black cumin (Nigella sativa L.) seeds or black cumin oil addition to Japanese quail diets on growth performance, carcass traits and some blood parameters. *Kafkas Univ Vet Fak Derg* 2015; 21:593-599.

Tufan T, Bolacali M. Effects of dietary addition of synbiotic on the performance, carcass traits, and serum parameters of Japanese quails. *Rev Bras Zootec* 2017; 46:805-813.

Venkatasivakumar R, Reddy ET, Reddy BS, Sekhar BS. Clinical Management of Pendulous Crop in a Turkey. *IJLR* 2016; 6:68-72.

Wheeler H, Crawford W, Couch J. Pendulous crop in turkeys. *Poultry Sci* 1957; 36.

Willems O, Buddiger N, Wood B, Miller SP. The genetic and phenotypic relationship between feed efficiency and pendulous crop in the Turkey (Meleagris gallopavo). Paper presented at the 10th World Congress of Genetics Applied to Livestock Production, Vancouver, Canada, 2014.

Case Report

TJVR 2020; 4 (1): 39-43

Turkish Journal of Veterinary Research

http://www.dergipark.gov.tr/tjvr

e-ISSN: 2602-3695

A Case of Pyometra In A 5-Month-Old Cat

Arzu Esen[1] Merve Menekşe Yıldırım[1] Berfin Kahraman[1]
Özgenur Kafkas[1] Ezgi Dikmeoğlu[1] İpek Mutluer[1]

[1] *Department of Obstetrics and Gynaecology, Faculty of Veterinary Medicine, Ankara University, Ankara, Turkey*

Correspondence: *Arzu Esen (aesen@ankara.edu.tr)*

Received: 04.03.2020 Accepted: 16.03.2020

ABSTRACT

Pyometra is an inflammation of the uterus which is characterized by purulent to sanguinopurulent intraluminal contents. Pyometra has been mostly observed in queens older than 6 years but it has no age predilection. The average age of cats with pyometra is between 5 - 7 years. Traditionally queens reach puberty at a bodyweight of 2.3-2.5 kg. Here in this case we have a 5-month-old cat which has not experienced oestrus but yet developed a pyometra. In this case ovariohysterectomy was performed on this queen. The treatment procedure continued with fluid therapy and antibiotics.

Keywords: *Pyometra, Queen, E. coli, Kitten*

INTRODUCTION

Pyometra is a common disease of adult intact female dogs and cats and it is characterized by an acute or chronic suppurative bacterial infection of the uterus (Dow, 1959; Hagman and Greko, 2005). Ovarian hormones are considered the main factors in pyometra development and progesterone is assumed to be the primary factor of its pathogenesis (Maria dos Anjos Pires et al., 2016). But, the etiopathogenesis of pyometra is still not fully understood (Hagman, 2018). Progesterone plays an important role in the pathogenesis of infection for this reason the disease generally develops in the luteal phase or during pseudopregnancy which is a phase of progesterone dominance that lasts approximately 40 days (Hagman, 2018; Hollinshead and Krekeler, 2016). Opportunistic bacterias are responsible for the disease and E. coli is the most frequently isolated one from these pathogens (Hagman and Greko, 2005). Other bacterias are also included in the pathogenesis of queen pyometra, including Streptococcus, Staphylococcus, Klebsiella, Pasteurella, Pseudomonas, and Proteus species (Rebordão et al., 2017).

The incidence of pyometra is thought to be lower in cats than dogs, because queens are induced ovulators. Pyometra can occur in queens between the ages of 1-20, most commonly around 5-7 years old (Hollinshead and Krekeler, 2016).

Purulent, sanguinopurulent vaginal discharge was observed in 50-86% of open feline pyometra. Vaginal discharge is not observed in closed pyometra and lethargy, anorexia, polydipsia, polyuria and vomiting are among the reported symptoms. Abdominal ultrasound is considered the best tool for the diagnosis of pyometra (Hollinshead and Krekeler, 2016; Davidson and Black, 2015.)

Medical or surgical treatment can be applied depending on the condition of the patient in pyometra cases. Broad-spectrum antibiotic therapy should start immediately after diagnosis in all pyometra cases. Surgery can be performed once the patient has been stabilized and surgical risk is

minimized. The most common treatment of pyometra is the surgical method.

The presented report describes the clinical presentation of pyometra in an intact 5-month-old queen and results of bacterial culture and sensitivity testing.

CASE

A 5-month-old kitten, weighing 2.3 kg, was admitted to Small Animal Hospital, Faculty of Veterinary Medicine in Ankara with a history of purulent vaginal discharge for a few days. And also the only knowledge about the patient's history is the cat fell down from the height a few months ago and the veterinarian used oral antibiotics and the owner has been using multi-vitamin paste for the cat about a month for treatment.

A general examination was performed and usual/specific symptoms of pyometra have not been noticed. During the ultrasonographic examination the uterus was distended with fluid. According to the owner of the patient, the cat hasn't experienced oestrus or received estrogen or progesterone compounds. The diagnosis of the case was open-cervix pyometra.

Ovariohysterectomy is one of the treatment procedures of pyometra. At the owner's request, ovariohysterectomy was our choice instead of medical treatment. Before the surgery, we collected blood samples for biochemical tests and Complete Blood Count (CBC) and the animal was found to be stable for surgery (Table 1, Table 2). General anesthesia was induced with medetomidine (Medetomidin, Domitor®, Pfizer®, Finland) and maintained with ketamine (ketamine hydrochloride, Ketasol® 10%, Richter Pharma Ag, Austria). We positioned the patient in dorsal recumbency for a ventral midline celiotomy, clipped the entire ventral abdomen and prepared for aseptic surgery. Ovariohysterectomy operation was performed and both ovaries and both horns of the uterus were removed. During surgery intravenous antibiotic (Ampicillin + Sulbactam, Sulbaksit 500 mg Im/IV, Tüm Ekip İlaç A.Ş., Türkiye) treatment was applied. 45 minutes after the medetomidine injection, atipamezole hydrochloride (Atipamezole, Antisedan®, Pfizer®, Finland) was injected. After the surgery horns were dissected and purulent discharge was collected (Figure 1). Escherichia coli and Klebsiella spp. have been isolated with microbiological examination.

Table 1. Serum Biochemical Analysis

Parameters	Measured Value	Laboratory Reference Range
Urea (mg/dl)	47.5	42.80 – 64.20
Creatinin (mg/dl)	0.72	0.80 -1.80
Albumin (g/dL)	2.36	2.40 – 3.80
ALT (IU/L)	27.0	0.00 – 50.00
AST (IU/L)	16.0	0.00 – 40.00
CK (IU/L)	80.0	0.00 – 130.00

ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, ALP: Alkaline phosphatase

Table 2. Hematological Analysis

Parameters	Measured Value	Laboratory Reference Range
WBC	12.50	5.50 – 19.50
LYM	3.60	1.10 – 7.00
MONO	1.00	0.20 – 1.50
NEUT	7.60	2.80 – 13.00
EOS	0.30	0.10 – 99.90
LYM%	28.90	15.00 – 60.00
MONO%	7.70	0.50 – 11.00
NEUT%	61.10	25.00 – 85.00
EOS%	2.30	0.10 – 99.90
RBC	7.77	5.00 – 11.00
HGB	11.50	8.00 – 15.00
HCT	25.60	25.00 – 45.00
MCV	32.90	39.00 – 50.00
MCH	14.80	12.50 – 17.50
MCHC	45.10	31.00 – 38.50
RDWa	17.40	20.00 – 35.00
RDW%	16.70	14.00 – 18.50
PLT	156.00	200.00 – 500.00
MPV	11.10	8.00 – 12.00

WBC: White blood cells, LYM: Lymphocytes, MONO: Monocytes, NEUT: Neutrophil, EOS: Eosinophils, LYM%: Lymphocytes percent, MONO%: Monocytes percent, NEUT%: Neutrophil percent, EOS%: Eosinophils percent, RBC: red blood cells, HGB: Haemoglobin, HCT: Haematocrit, MCV: Mean corpuscular volume, MCHC: Mean corpuscular hemoglobin concentration, RDWa: Red blood cell distribution, RDWa%: Red blood cell distribution percent, PLT: Platelet, MPV: Mean platelet volume

According to antibiogram test results bacterial isolates were found to be susceptible to sulfamethoxazole, ampicillin, enrofloxacin; semi-susceptible gentamicin and resistant to neomycin sulfate. The medical treatment continued with fluid therapy. Amoxicillin-clavulanic acid (Amoklavin; Suspension) 25 mg/kg BID was administered after surgery for five consecutive days until the cat's

health condition returned to normal. Post-operation controls were made on day 3 and 7 after the operation and at the end of 7th day, stitches were removed.

Figure 1. Uterine content after operation

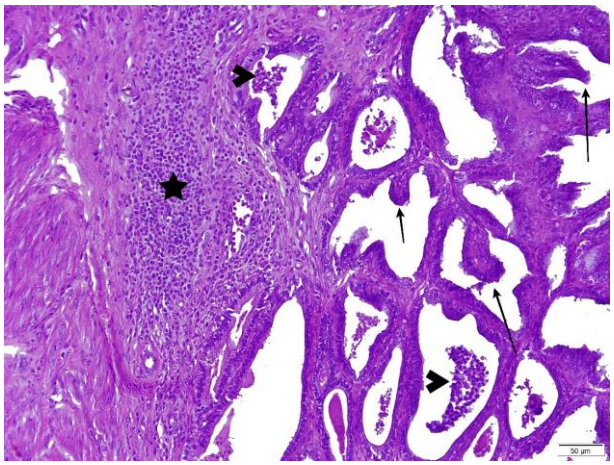

Figure 2. Living and dead neutrophil leukocytes (Arrowhead), mononuclear cell infiltrations (Stars) and Papillar extensions (Arrows) in gland lumens. HE staining.

After the surgery uterus and ovaries were put in 10% formaldehyde for histopathologic examination. After detection of the uterus tissue in 10% formaldehyde brought to the Ankara University Department of Pathology, it was trimmed and placed in the cassettes and subsequently washed for 12 hours in running water. Then, tissues were taken to the routine tissue tracer (Leica TP1020) and blocked in paraffin (Thermo Electron Corp. Shandon Histocentre 3). Sections prepared from each block with a microtome (Leica RM2255) of 5 µm thickness; after deparaffinization and dehydration steps in an automatic dyeing machine (Leica Autostainer XL), by Harris's Hematoxylin-Eosin (HE) method; It was closed with lamella in the automatic capping machine (Leica CV5030). All findings were evaluated under a light microscope (Olympus BX51) and their diagnoses were made and the necessary areas were photographed (Olympus DP71).

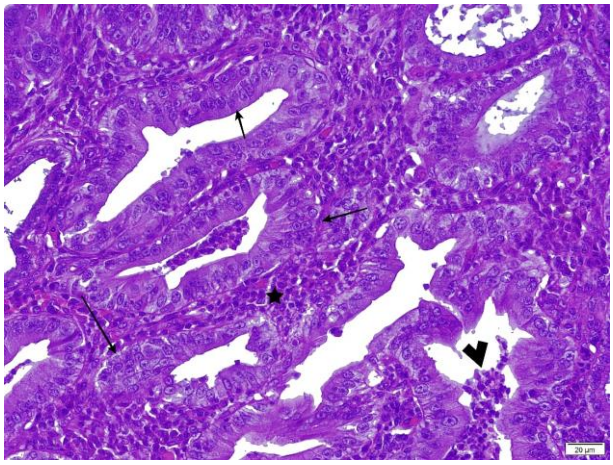

Figure 3. Epithelial hyperplasia (Arrows) in the uterine glands, mononuclear cell infiltration (Star) between the glands, and dead and living neutrophil leukocytes (Arrowhead) in the gland lumen. HE staining.

In histopathological examination, intensive dead and living neutrophil leukocyte accumulations were observed in the uterine lumen (Figure 2). The neutrophil leukocytes in question were also detected in the gland lumens from place to place with a slight severity (Figures 2 and 3). The glands in the endometrium were enlarged, their number had increased. Mononuclear cell infiltrations, both in the endometrium between the glands and in the myometrium, as multifocal areas, attracted attention (Figures 2 and 3). In the histopathological examination of ovaries, follicles were detected at various stages. According to this report, the diagnosis is metritis subacute.

DISCUSSION

Generally, pyometra occurs in queens between the age of 5-7 (average 7.6 years, range 1-20) but it can be observed any time after puberty (Ettinger & Feldman, 2010). Clinical signs usually occur within 4 weeks after the onset of oestrus in queens that are either mated, spontaneously ovulate or are induced to ovulate (mechanical stimulation or hormone induction) (Hagman, 2018). In this case the queen has never experienced oestrus or medically

stimulated with any other drugs or injection and the age of 5-month-old.

Pyometra is most common in dogs but more fatal in queens. Progesterone-mediated pathological proliferation and the formation of cystic endometrial hyperplasia are believed to predispose to pyometra, but two disorders can develop independently (Hagman, 2018). In this case no cystic endometrial hyperplasia was observed. One of the reasons for low mechanical stimulation (e.g excessive petting or vaginal palpation), thus no recurrent estrogen and progesterone effects on the uterus. Another reason is that the uterus is exposed to progesterone in queens (40-50 days) than in bitches (over 60 days) (Agudelo, 2005; Smith, 2014).

According to our knowledge pyometra occurs when the uterine environment during the luteal phase is suitable for pregnancy but also for microbial growth. Progesterone stimulates growth and proliferation of endometrial glands, increased secretion, cervical closure, and suppression of myometrial contractions. In this case the oestrus cycle hasn't occurred so that suppression of functions of uterus by progesterone cannot be the reason for bacterial infection. It may be caused by spontaneous ovulation which is a rare occasion in queens (Lawler et al., 1993). Despite many hypotheses, the reason behind the spontaneous ovulation might be because of the breed of the cat. Recent studies showed Oriental purebred cats having a higher incidence of pyometra than domestic and mixed-breed cats. It has also been observed by many authors that Oriental breed cats have a short interoestrus intervals compared to domestic cat breeds (Hollinshead and Krekeler, 2016).

In fact, progesterone measurement is useful, but this was not necessary because the patient did not show oestrus therefore we didn't include progesterone measurement for this study.

In many studies with microbiological examinations Escherichia coli, Klebsiella spp., Streptococcus spp. are commonly isolated. The main reason for this is that these are the opportunistic bacterial population on the urinary and intestinal tracts of cats (Hagman, 2018). In this case Escherichia coli, Klebsiella spp., were also isolated. Klebsiella spp. is a common bacterium in dogs and cats, one report documented the isolation of this pathogen from 2 dogs with diarrhea. Some strains of K. pneumoniae have been shown to produce an enterotoxin, similar to enterotoxic strains of Escherichia coli, which stimulate secretion of fluid by mediating activation of the guanylcyclase–cyclic guanosine monophosphate mechanism (Olsen et al, 1985). According to this we have continued to administer Amoxicillin-clavulanic acid (Amoklavin; Suspension) 25 mg/kg BID for 5 days.

The postoperative complication includes peritonitis, dysfunction of the kidneys and liver, deterioration in health condition (Hagman, 2018). In our case we did not have any complications relating to peritonitis or any other health problems but we have continued with fluid therapy to prevent any kind of complications after the operation.

In conclusion pyometra has a complex pathology so it should not be disregarded when dealing with feline species or in animals of an age that is not typical for pyometra.

ACKNOWLEDGMENTS

We are grateful to Dr. İnci Başak Müştak for her contribution to microbiological analysis in our research and also we are grateful to Prof. Dr. Rıfkı Hazıroğlu and Res. Assist. Kürşat Filikci for their contribution to histopathologic examination in our research.

Conflict of Interests: The authors declared that there is no conflict of interests.

Financial Disclosure: The authors declared that this study has received no financial support.

REFERENCES

Dos Anjos Pires M, Vilhena H, Miranda S, Pereira MT, Seixas F, Saraiva AL. Proliferative endometrial lesions hidden behind the feline pyometra. Insights from Animal Reproduction, 2006; 227.

Dow C. The cystic hyperplasia-pyometra complex in the bitch. J Comp Pathol, 1959; 69:237-57

Ettinger SJ, Feldman EC. Textbook of Veterinary Internal Medicine (Vol. 2). St. Louis, Missouri: Saunders. 2010; 4542-4552

Hagman R, Greko C. Antimicrobial resistance in Escherichia coli isolated from bitches with pyometra and from urine samples from other dogs. Vet Rec, 2005; 157:193-197.

Hagman R. Pyometra in small animals. Vet Clin Small Anim, 2018; 48:639–661.

Lawler DF, Johnston SD, Hegstad RL, et al. Ovulation without cervical stimulation in domestic cats**.** J Reprod Fertil Suppl 1993; 47: 57–61.

Agudelo CF. Cystic endometrial hyperplasia-pyometra complex in cats. A review, Veterinary Quarterly, 2005; 27:4, 173- 182.

Smith FO, Bonagura JD, Twedt DC. Kirk's Current Veterinary Therapy XV. St. Louis, Missouri: Saunders, 2014; pp. 967- 969

Hollinshead F, Krekeler N. Pyometra in the queen to spay or not to spay? J Feline Med Surg, 2016; 18:21–33.

Davidson J, Black D. Small Animal Pyometra. In: Aronson LR (ed) Small animal surgical emergencies, 1rd edn. Wiley-Blackwell, New Jersey, USA, 2015; pp 397-401.

Rebordão MR, Alexandre-Pires G, Carreira M, et al. Bacteria causing pyometra in bitch and queen induce neutrophil extracellular traps. Vet Immunol Immunopathol, 2017; 192:8-12.

Olsen P, Hedhammar A, Faris A, et al. Enterotoxigenic Escherichia coli (ETEC) and Klebsiella pneumoniae isolated from dogs with diarrhoea. Vet Microbiol, 1985; 10:577–589.

TURKISH JOURNAL OF VETERINARY RESEARCH
E-ISSN:2602-3695

Turkish Journal of Veterinary Research (TJVR)

Aim and scoop:

The aim of the Turkish Journal of Veterinary Research (TJVR) is to publish, in a timely manner, peer-reviewed reports of the highest quality research that has the clear potential to enhance the health, welfare, and performance of animals and humans. The journal will maintain the highest ethical standards of scientific journalism and promote such standards among its contributors. In addition, the journal will foster global interdisciplinary cooperation in veterinary medical research.

The *Turkish Journal of Veterinary Research* supports the collaborative exchange of information between researchers and clinicians by publishing novel research findings that bridge the gulf between basic research and clinical practice or that help to translate laboratory research and preclinical studies to the development of clinical trials and clinical practice. The journal welcomes submission of high-quality original studies, review articles and other academic papers in a wide range of scientific fields, including anatomy, anesthesiology, animal welfare, behavior, epidemiology, genetics, heredity, infectious disease, molecular biology, oncology, pharmacology, pathogenic mechanisms, physiology, surgery, theriogenology, toxicology, and vaccinology. Species of interest include production animals, companion animals, equids, exotic animals, birds, reptiles, and wild and marine animals. Reports of laboratory animal studies and studies involving the use of animals as experimental models of human diseases are considered only when the study results are of demonstrable benefit to the species used in the research or to another species of veterinary interest. Other fields of interest or animal species are not necessarily excluded from consideration, but such reports must focus on novel research findings. Submitted papers must make an original and substantial contribution to the veterinary medicine knowledge base.

EDITORIAL POLICIES

Authorship

Individuals should be listed as authors only if they

(1) made a substantial contribution to the conception and design of the study, the acquisition of the data used in the study, or the analysis and interpretation of that data; (2) were involved in drafting or revising the manuscript critically for important intellectual content; and

(3) approved the submitted version of the manuscript and will have an opportunity to approve subsequent revisions of the manuscript, including the version to be published.

All 3 conditions must be met. Each individual listed as an author must have participated sufficiently to take public responsibility for the work. Acquisition of funding, collection of data, or general supervision of the research team does not, alone, justify authorship.

Prior Publication

A manuscript is received with the understanding that the information has not been published or submitted for publication in any compiled printed (eg, journals, symposia, proceedings, newsletters, or books) or electronic (eg, websites, CD-ROMs, DVDs, or blogs) format in English or any other language and will not be published or submitted for publication elsewhere while the manuscript is under consideration by TJVR. Any exceptions must be clearly described at the time of manuscript submission.

A manuscript containing previously published information may be rejected on the grounds of prior publication. Publication of abstracts containing less than 250 words will not be considered to constitute prior publication, but publication of longer abstracts may be. Authors are encouraged to consult the guidelines for preparation of scientific abstracts when preparing scientific abstracts for publication. In general, figures, tables, footnotes, and references should not be included in abstracts.

At the time of manuscript submission, the corresponding author must include copies of any abstracts of the manuscript that have been published or submitted for publication or that are expected to be submitted for publication, along with copies of any closely related manuscripts or manuscripts with substantially similar content.

The Editor-in-Chief has the discretion to reject the manuscript straight off before or after sending it to the reviewers for reviewing.

Copyright

TJVR is covered by copyright. All authors will be required to transfer copyright to the editorial board prior to publication of any manuscript or letter. Requests to copy, reprint, or use portions of published material (including information in figures and tables) should be addressed to the editor-in-chief.

Authors must obtain and submit a statement of permission from the copyright holder (most often, the author or publisher) if they wish to include items such as figures, tables, or appendices that appeared or will have appeared in other published reports prior to publication of the manuscript, regardless of the originating source.

Original artwork (eg, drawings or photographs) that was created specifically for use in the manuscript must be accompanied by a letter explaining the conditions under which the work was created. The letter must be signed by the artist and specify the rights given to the authors for use of the artwork and the rights retained by the artist (if any). If rights are retained by the artist, the letter must include a statement that allows the journal to use the material for publication in print and online.

Funding and Support

All funding, other financial support (eg, grant support), and material support (eg, provision of equipment or supplies) received directly or indirectly (via an author's institution) from any third party (eg, any government agency, foundation, or commercial enterprise) in connection with the study or writing of the manuscript must be clearly and completely described in the*Acknowledgments* section of the manuscript. If no third-party funding or support was received, the following statement or an equivalent may be included:

No third-party funding or support was received in connection with this study or the writing or publication of the manuscript.

The authors must also include a relevant statement in the *Acknowledgments* section if any funding organization or sponsor had any role in the design or conduct of the study; collection, analysis, or interpretation of the data; writing or approval of the manuscript; or decision to submit the manuscript for publication. Alternatively, the following statement or an equivalent may be included:

Funding sources did not have any involvement in the study design, data analysis and interpretation, or writing and publication of the manuscript.

Failure to fully disclose sources of financial and other support may be grounds for rejection or retraction of the manuscript.

Conflicts of Interest and Financial Disclosures

A conflict of interest exists whenever an individual has financial interests or personal relationships that might consciously or unconsciously influence his or her decisions. Conflicts of interest are ubiquitous and cannot be completely eliminated; they do not, by themselves, indicate improper behavior, wrongdoing, or scientific misconduct.

Financial relationships are the most easily identifiable conflicts of interest and include, among other things, ownership, employment, consultancies, honoraria, paid expert testimony, grants, patents, stock ownership or options, and service as an officer or board member. Other types of conflicts of interest include personal relationships, academic competition, and intellectual beliefs.

All authors must disclose in the Acknowledgments section of the manuscript any financial or personal relationships that could be perceived to influence or could give the appearance of influencing information in the submitted manuscript. This includes detailed information about all relevant financial interests, activities, relationships, and affiliations (other than affiliations listed on the title page of the manuscript) occurring at the present time or within the 3 years prior to manuscript submission. In this context, relevant financial interests, activities, relationships, and affiliations should be interpreted broadly. For example, authors should disclose relationships they have not only with companies that manufacture products that are the subject of research described in the manuscript but also with companies that manufacture competing products. If no such conflicts of interest existed, the following statement or an equivalent may be included:

The authors declare that there were no conflicts of interest.

The editors reserve the right to reject any manuscript because of conflicts of interest. Failure to fully disclose conflicts of interest may be grounds for rejection or retraction of the manuscript.

Humane Animal Care and Use

To be considered for publication in TJVR, all research studies involving animals must have been performed in compliance with guidelines outlined in the Animal Welfare Act (http://awic.nal.usda.gov/government-and-professional-resources/federal-laws/animal- welfare-act), US Public Health Service Policy on the Humane Care and Use of Laboratory Animals (http://grants.nih.gov/grants/olaw/references/phspol.htm), National Research Council's Guide for the Care and Use of Laboratory Animals (http://www.nap.edu/read/5140/chapter/1), or Guide for the Care and Use of Agricultural Animals in Research and Teaching (http://adsa.org/Publications/FASS2010AgGuide.aspx) or in compliance with equivalent guidelines. If animals were euthanized, the method of euthanasia must be indicated in the manuscript. If a method not recommended, a justification for use of this method must be provided. A manuscript containing information that suggests animals were subjected to adverse, stressful, or harsh conditions or treatments will not be considered for publication unless the authors demonstrate convincingly that the knowledge gained was of sufficient value to justify these conditions or treatments.

Publication Fees

Publication of the accepted manuscripts is **free of charge**.

MANUSCRIPT CATEGORIES

Original Research: An Original Research article is a research paper that provides new information derived from original data and usually includes extensive statistical analysis.

Review Article: Review Article is an in-depth analysis of the current status of a particular topic. It is not an original article with new data but represents a well-balanced summary of a timely subject with reference to the literature.

Technical Innovation: Technical Innovation is a paper that describes a new method, procedure, technology, technique, or treatment with a practical approach.

Case Series: Case Series is a paper that describes a group of cases or particular observations with unique imaging features or involving novel procedures. Case Series are usually retrospective and not associated with extensive statistical analysis.

Commentary: Commentary is a short paper that describes the personal views of the author on a particular subject. It includes some review of the literature in making a point, although it is clearly an opinion piece rather than a review of the subject.

Letter to the Editor: Letter to the Editor offers brief comments on published material and should be objective, constructive, and educational.

Clinical Letter: Clinical Letter is a brief description of a single unique or unusual case. The manuscript should include a title, authors' full names, degrees, and affiliations, a brief text description/discussion without headings, up to 10 references if applicable, and figure legends; note that each figure part (ie, Figure 1A or 1B) is counted as a separate image. No more than 4 images total may be included. It should not contain tables or data analysis. Clinical Letters are generally not peer reviewed and are published at the discretion of the editor based on space available; publication is not guaranteed.

Short Communication: Short Communication article is a rather short paper reporting preliminary observations, new interpretations of old data, simple new techniques or devices, or points of historical interest.

ORGANIZATION OF THE MANUSCRIPT

Manuscripts should be organized in the following order, as applicable: *title page, structured abstract or summary (when applicable; letters to the editor and review articles do not have a structured abstract), full text, acknowledgments, footnotes, references, tables, figure legends, and figures*. All text elements and tables should be combined in a single document, double- spaced, with line numbering.

Title Page

This first page should contain the complete title of the article, manuscript category, names and credentials of all authors, institutional affiliations of all authors, and a short running title (≤60 characters). The corresponding author should be indicated, including a complete mailing address, telephone number, and e-mail address.

Structured Abstract or Summary

An abstract is required for Original Research articles. The abstract should be divided into *Objectives, Animals (or Sample), Procedures, Results, and Conclusions and Clinical Relevance* sections. It should not exceed 250 words. Three to 6 key words should be provided at the end. The abstract should not contain figures, tables, or reference citations, trade names, or manufacturer names. Statements of statistical significance should include P values or 95% confidence intervals. The abstract should not include data that are not given in the full text.

In place of an abstract, a summary is required for Review Articles, Pictorial Essays, Technical Innovations, and Case Series. Content should adhere to the guidelines for abstracts but should not be divided into sections. It should not exceed 100 words and should give a brief overview of the article. Three to six key words should be provided at the end.

Full Text

For Original Research articles, the full text of the manuscript should be divided into the following sections: *Introduction, Materials and Methods, Results, and Discussion*. For other articles, the text may be divided into sections if desired.

Acknowledgments

On a separate page preceding the references, contributions made by colleagues who are not authors of the paper should be acknowledged. This page should also include acknowledgments of published material reproduced with permission, previous presentations of the work at meetings, related grant support received, and disclosures of relevant commercial interests.

Footnotes

Footnotes are to be used when referencing each of the following types of information:

• Abstracts

• Conference presentations

• Online databases

• Personal communications

• Products, drugs, equipment, and other materials • Statistical and computer software

• Theses and dissertations

Specific products, equipment, or drugs should be included in the footnotes only if they were essential to the outcome of the report or study. Products, equipment, and drugs that are commonly used materials in veterinary medicine need not be footnoted.

Footnotes should be cited in the text as superscript letters and listed alphabetically after the Acknowledgments section and before the references. If more than 26 footnotes are required, continue the sequence with double letters (eg, aa, bb, and cc). For products and equipment, provide complete information in the footnote, including manufacturer's name and location (ie, city, state, and country).

References

Each reference must be cited as (surname, year of publication) and listed in the alphabetical order. Duplicate references should not be listed; rather, subsequent citations of a reference should be listed only once. When more than 6 authors are included in a reference, ***et al*** replaces the names of all authors after the first 3. Inclusive page ranges should be given where applicable. Accepted work in press includes the journal title or the publisher if it is a book. Material not accepted for publication cannot be included as a reference and should be cited parenthetically in the text as unpublished data, including all participants' initials and surnames and the year. Abbreviations of journal titles should conform to those used in the *National Library of Medicine* (http://www.ncbi.nlm.nih.gov/nlmcatalog/journals). The style and punctuation of references should follow the formats illustrated by the following examples:

Journal Article With Up to 6 Authors

1. Luo W, Zhou X, Gong X, Zheng M, Zhang J, Guo X. Study of sequential histopathologic changes, apoptosis, and cell proliferation in rabbit livers after high- intensity focused ultrasound ablation. *J Ultrasound Med* 2007; 26:477–485.

Journal Article with More Than 6 Authors

2. Lamont LA, Bulmer BJ, Sisson DD, et al. Doppler echocardiographic effects of medetomidine on dynamic left ventricular outflow tract obstruction in cats. *J Am Vet Med Assoc* 2002; 221:1276–1281.

Journal Article Published Online Ahead of Print

3. Cummisford K, Sundar S, Hagberg R, Mahmood F. Realtime three-dimensional transesophageal echocardiography and a congenital bilobar left atrial appendage [published online ahead of print June 24, 2009]. *J Cardiothorac Vasc Anesth.* doi:10.1053/j.jvca. 2009.04.005.

Abstract of a Paper Presented at a Meeting

4. Shipp TD, Bromley B, Benacerraf BR. Comparison of the fundal endometrial diameters using 3-dimensional rendering for those with embedded and nonembedded intrauterine devices [abstract]. *J Ultrasound Med* 2009; 28 (suppl): S47.

Whole Book

5. Cardenosa G. Breast Imaging. 1st ed. Philadelphia, PA: Lippincott Williams & Wilkins; 2004.

Edited Book

6. McGahan JP (ed). Interventional Ultrasound. Baltimore, MD: Williams & Wilkins; 1990.

Chapter in a Book

7. Muir P, Johnson KA, Manley PA. Fractures of the pelvis. In: Birchard SJ, Sherding RG, (eds). *Saunders Manual of Small Animal Practice*. 2nd ed. Philadelphia: WB Saunders Co, 2000;1126–1132.

Website

8. Animal and Plant Health Inspection Service. Bovine spongiform encephalopathy (BSE). Available at: www.aphis.usda.gov/lpa/issues/bse/bse.html. Accessed Feb 18, 2003.

Tables

Tables must be numbered consecutively with Arabic numerals in the order in which they appear in the manuscript. Each table should appear on a separate page and should contain a title that includes the table number. No vertical lines or borders may appear in a table. Tables must contain all necessary information so that they may stand alone, independent of the text. Data given in the text should not be duplicated in tables. All tables must be cited in numerical order in the text. Tables should be included in the text document and should not be submitted as graphics.

Figure Legends

For initial submissions, figure legends should be provided with their corresponding images at the end of the text document. Figures must be numbered consecutively with Arabic numerals in the order in which the figures appear in the article. All figures must be cited in numerical order in the text.

Abbreviations

Abbreviations of common clinical and technical terms listed in the *American Medical Association Manual of Style, 10th Edition*, may be used if they appear 3 or more times in the full text and abstract/summary, respectively. Each abbreviation should be defined at first use in the full text and abstract/summary, respectively. Use of other abbreviations is discouraged unless they are essential to the clarity of the manuscript.

Supplemental Materials

Additional materials that are not in themselves essential to the understanding of the article but provide an important expansion of the article contents may be submitted for publication as supplemental materials. Examples include extended descriptions of experimental methods, extended bibliographies, additional supporting data or results (eg, tables and figures), reporting checklists, copies of survey instruments or questionnaires, handouts, forms, and multimedia representations (eg, video clips) of relevant content.

Supplemental materials must be useful to readers and relevant to the article; redundant and extraneous content will not be accepted. Whether supplemental materials will be accepted for publication is solely at the discretion of the editors. Supplemental materials accepted for publication will not appear in the printed version of the journal but will be posted on the journal's website. Ideally, supplemental materials will be sent with the manuscript to external reviewers for peer review. Whether supplemental materials have or have not undergone peer review will be indicated on the landing page where the supplemental materials are posted. Supplemental materials should be prepared in compliance with the general guidelines for manuscript style. Although supplemental materials may undergo minor copy editing or formatting, they generally will not undergo the same substantive editing provided for manuscripts. Therefore, the authors are responsible for ensuring clarity and accuracy of the content.

MANUSCRIPT SUBMISSION PROCESS

New Manuscript Submissions

Submit manuscripts through the journal's online system. To begin, go to *Manuscript Submission Page* at **http//dergipark.gov.tr/tjvr**

Prepare version 1 manuscripts as follows:

Cover Letter: Please supply along with your manuscript files and forms. Be sure to include the e-mail address of each listed author.

Text: Microsoft Word is preferred. All text should be double spaced, and all text elements, including tables, should be combined in a single document. Manuscripts should include continuous line numbering.

Images and Graphics: For initial submissions, please embed images into the text file at the end of the document. Please clearly label images and place the corresponding legend with each image.

Guidelines for Images

All figures should be embedded at the end of the manuscript with the name of the figure used as the file name (eg, Figure 1). Gray scale or black and white should be used; color should be used only when important information would otherwise be lost (eg, when certain tissue-staining patterns are poorly visible in gray scale or when a color-flow Doppler ultrasonogram is provided). For figures that include multiple panels, each panel should be sequentially labeled with a capital letter in the same corner of each panel. If a figure contains 2 or more rows of panels, the letter labels should be applied sequentially from left to right in the first row, then from left to right in the second row, and so on.

Permission to Reproduce Published Material

Written permission from the author and publisher of any previously published figure, table, or lengthy passage of text included in the manuscript must be submitted with the paper. If any form of preliminary publication, other than an abstract of no more than 400 words, has occurred or is contemplated, a reprint or manuscript of the actual or proposed preliminary publication should accompany the manuscript.

AUTHORITY/RESPONSIBILITY

The intellectual content of the paper is the responsibility of the author; the editor and the TJVR accept no responsibility for the opinions and statements of authors. While every effort is made by the editor and editorial staff to avoid any inaccurate or misleading information, they accept no liability for the consequences of wrong information. The authors agree to keep the editor and publisher fully indemnified against any liability or claim that may arise from the publication of incorrect or misleading data.

INSTITUTIONAL REVIEW BOARD APPROVAL (ETHICAL REPORT) AND INFORMED CONSENT

With the exception of reports of retrospective studies based solely on reviews of medical records, manuscripts describing studies that involved the use of animals, including studies that involved the use of privately owned animals (eg, animals owned by clients, staff members, students, or private entities), must include a statement in the *Methods* section that the study protocol was reviewed and approved by an appropriate oversight entity (eg, an animal care and use committee or institutional review board) or was performed in compliance with institutional or other (eg, governmental or international) guidelines for research on animals.

Manuscripts describing prospective studies that involved privately owned animals must also include a statement indicating that informed owner consent was obtained. Manuscripts describing research involving human subjects must include a statement that the research was performed under appropriate institutional review board oversight.

For those investigators who do not have formal ethics review committees, the principles outlined in the Declaration of Helsinki should be followed. Manuscripts that do not comply with these rules will not be accepted for publication.

EDITING

Accepted manuscripts will be copyedited to eliminate grammatical and typographical errors and to ensure conciseness and clarity. The corresponding author will receive proofs of the copyedited article before publication.

PERMISSION REQUESTS

CONTACTING THE JOURNAL

The Turkish Journal of Veterinary Research Editorial Office may be contacted through the following:

Prof. Dr. Ebubekir CEYLAN

Turkish Journal of Veterinary Research

Ankara Üniversitesi Veteriner Fakültesi

İç Hastalıkları ABD Dışkapı, 06110 Ankara, TURKEY

E-mail: *ebubekirceylan@gmail.com*, *infotjvr@gmail.com*

Manuscripts are considered for publication with the understanding that they are submitted solely to the Turkish Journal of Veterinary Research and are not under consideration elsewhere.

TURKISH JOURNAL OF VETERINARY RESEARCH
E-ISSN:2602-3695

Article Copyright Transfer Agreement

We, the undersigned researchers, certify that; the article we have sent; is original, wasn't sent to or disapproved of potential publication by any other journal, has not initially published, and we bear the responsibility concerning the Scientific content and Ethical values related to the article, and transfer any kind and form of copyright related to the Article to Turkish Journal Veterinary Research (TJVR) since it is published in the journal, and accept that we will not make any changes wholly or partly in the article and chose ……..……..…… named author as the authorized researcher.

Title of the article: ……..……..……

……..……..……..……

……..……..……..……

……..……..……..……

	Authors Name	Date	Signature
1.			
2.			
3.			
4.			
5.			
6.			

Authorized Researcher

Title, Name-Surname :

Full Address :

e- mail :

Tel, Fax :

Date and Signature :