JIVS
The Chamber of Veterinary Surgeons, Istanbul
e-ISSN: 2602-3490
Abbr. Title: J Ist Vet Sci

Journal of Istanbul Veterinary Sciences (JIVS)

http://www.jivs.net Since 2017

JIVS JOURNAL OF ISTANBUL VETERINARY SCIENCES

Volume 2; Issue 3, December 2018 E-ISSN:

Indexes and Platforms

Turkey citation index, Google scholar, ResearchBib, Bielefeld Academic Search Engine, Cosmos IF, İnternational Institute of organized research(I2OR), Scientific İndexing service, CrossRef, Eurasian Scientific Journal Index, Open AIRE, General Impact factor, Directory of Research Journals Indexing (DRJI), ASOS index.

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JIVS JOURNAL OF ISTANBUL VETERINARY SCIENCES

Contents

JIVS JOURNAL OF ISTANBUL VETERINARY SCIENCES

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The effects of acute blood loss on electrocardiogram in male and female Swiss albino mice

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Research Article

Volume: 2, Issue: 3
December 2018
Pages: 66-71

ABSTRACT

The aim of the present study is to investigate the possible effects of acute blood loss (1% of body weight) on electrocardiogram (ECG) in male and female Swiss albino mice. Anesthesia was maintained with the ketamine-xylazine combination. The tail was cut using a scalpel blade and blood at a ratio of 1% body weight was collected in capillary tubes. ECG recording was done by using the standard lead II. P and T waves, intervals of PQ, QT, and RR, and QRS complex were measured after all ECG was recorded. The amplitude of P and QRS waves were significantly higher in males than that of females (P<0.005, P<0.05 respectively). PR intervals were also longer in male mice than those of females (P<0,001). Neither blood removal nor anesthesia affected the amplitude of the QRS complex in any gender. The duration of the QRS complex was longer in males than females in both groups (P<0,01). Blood removal led to a reduction in the duration of the QRS complex in male mice (P<0.01). Anesthesia caused the prolongation of the QT and QTc in all groups (P<0,001, P<0,05 respectively). Females had longer QT interval compared to male groups (P<0,05) and blood removal caused prolongation of the QT period in females (P<0,05). An acute blood loss of 1% of body weight in male mice led to a reduction in heart rate, whereas prolongation of the QT interval in female mice. It was concluded that gender is an important factor in terms of blood-loss associated ECG alterations.

Article History

Received: 13.08.2018
Accepted: 28.09.2018
Available online: 29.09.2018

Keywords: ECG, ketamine, xylazine, blood loss, mouse, gender

DOI: 10.30704/http-www-jivs-net.453151

To cite this article: Unsal, C., Canset Ozden, F., Unsal, H., Balkaya, M.**(2018). The The effects of acute blood loss on electrocardiogram in male and female Swiss albino mice.** ***Journal of Istanbul Veterinary Sciences*** **2(3), 66-71.**
Abbreviated Title: ***J Ist Vet Sci***

Introduction

Blood collection is required for performing hematological, biochemical, pharmacologic, and toxicological analyses (Raabe et al., 2011). The blood collection method depends on type of the analyses and duration and frequency of sampling (Gargiulo et al., 2012). Blood sampling procedures affect the data collected from a study conducted in smaller laboratory animals. The amount and frequency of blood collected have adverse effect on animals such as immunosuppression, reduction in blood pressure, anemia, hemorrhagic shock, or mortality (Xu et al., 2007). A typical rule is to not exceed removal of 10% of total blood volume (roughly 1% of body weight or 8 mL/kg) for every 2-week period (Gargiulo et al., 2012). The minor blood losses may be asymptomatic in animals. Loss of about 10% or less of the total blood volume causes baroreceptor-initiated reflexes which leads further release of adrenergics from the adrenal medulla and sympathetic nerve endings, which causes an increase in heart rate and construction in veins, venous reservoirs, and arteriolar beds in muscle and skin (McGuill & Rowan, 1989). The cardiac functions determined by electrocardiogram under anesthesia are found to be usually depressed compared with the cardiac functions found in the conscious state. The ideal drug must be safe and simple to use and induce

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Journal homepage: www.jivs.net
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reproducible sedation and immobility without causing any changes in cardiac function and heart rate (Lairez et al., 2013). The combination of ketamine and xylazine is popular for inducing anesthesia in many species including mice. This combination has some advantages such as to provide single injection, easier animal handling, well-controlled anesthetic depth and duration, and fewer side-effects after being awoken (Xu et al., 2007). However, previous studies have shown that lowered heart rate (HR), depressed contractile function, loss of animals with overt heart disease and functional instability may occur (Roth et al., 2002; Tan et al., 2003).

This study aimed to determine the effects of maximum amount of blood that can be taken at once without causing any adverse effects on the ECG in male and female mice.

Materials and methods

Animals

The study was approved by Adnan Menderes University Local Ethics Committee for Animal Experiments (Protocol number: 2010-60). A total of 28 Swiss Albino mice of both sexes, six months of age, were randomly divided into four groups of equal size: males and females from which blood were taken and control group of males and females. Mice were housed in the same room with a 12:12 h. light/dark cycle at a 22 ± 1 °C temperature and relative humidity of 40–50%. The mice were then allowed *ad libitum* access to water and standard laboratory rodent chow. Anesthesia was maintained by intraperitoneally injected ketamine-xylazine combination (ketamine (80 mg/kg, Alfamine, xylazine; 10 mg/kg, Alfazyne, EGE-VET®, Turkey). The induction of anesthesia was controlled by the loss of righting reflex. Depth of anesthesia was determined by checking the pedal withdrawal reflex. Mice were then laid down on their back immediately after having achieved anesthesia and placed on heating pads to maintain their body temperature at 37°C. The tail (1 cm from distal) was quickly cut by using a scalpel. Blood was collected at a ratio of 1% of body weight in capillary tubes.

ECG Recording

After the blood collection, bleeding was stopped by applying pressure to the bleeding area. ECG recordings were done using MP30 data acquisition system (BIOPAC®, USA) at a rate of 2 kHz and the data were collected in a personal computer until being analyzed. The standard lead II was used to record ECG. Bipolar surface electrodes (EL254S, EL254 BIOPAC®, USA) were placed on the right forelimb and on each hindlimb. The electrodes were then gently connected to the MP30 data acquisition system by using the ECG gel. ECG recording continued throughout the anesthesia. Recordings were analyzed by using BIOPAC BSL 3.7.2 program, a software for MP30 data acquisition system. All ECG frontal axis (P and QRS) and time intervals (PR, QRS, QT and RR) were calculated from each mouse in lead II. The QT intervals were also rate-corrected with adapted Bazett's formula (Mitchell et al., 1998).

Statistical Analyses

Data were presented as mean and standard error of the mean. Shapiro-Wilk and Levene tests were used for normal distribution of the data and homogeneity of variance. Data were analyzed by using SPSS 19.0 (IBM®, USA) software package. Repeated measures performed by three-way ANOVA were conducted to evaluate the effects of anesthesia, gender and blood loss. Post hoc multiple comparisons were done when any intervention are significant. The significance level was set at $p<0.05$.

Results

Results are shown in Table 1. It was found that the anesthesia led to a reduction in heart rate ($P<0.01$), which was more distinct in males compared to females ($P<0.05$). Furthermore, blood removal affected males more than females ($P<0.001$) in the first 10 minutes after onset of the blood collection. P wave amplitudes were lower in female mice than those of males for both study and control groups ($P<0.01$). The durations of P wave were not different in all groups. PR interval did not change by time and blood loss did not have any gender effect on PR interval. However, females had shorter PR interval than males in both the group from whose members blood were collected and in the control group ($P<0.01$). The amplitude of the QRS complex was higher in males than females ($P<0.05$). Neither blood removal nor anesthesia affected the amplitude of the QRS complex in any gender. The duration of the QRS complex was longer in males than females in both groups ($P<0.001$). Anesthesia affected the duration of the QRS complex in male and female mice ($P<0.001$). Blood removal led to a reduction in the duration of the QRS complex in male mice ($P<0.001$). Anesthesia caused the prolongation of the QT and QTc in all groups ($p<0.001$, $P<0.05$, respectively). Females had longer QT interval compared to male groups ($P<0.05$) and blood removal caused prolongation of the QT period in females ($P<0.05$). Any clear and separate T wave could be reliably distinguished from almost any ECG recordings.

Table 1. ECG parameters in mice

Variables	Time (min)	Groups			
		Experiment		Control	
		Female	Male	Female	Male
P Wave Amplitude (µV)	0	4.05 ± 0,61	6.76 ± 0.57	4.59 ± 1.06^{b}	10.78 ± 1.99^{b}
	10	2.67 ± 0.19	7.22 ± 0.70	4.33 ± 0.67^{b}	9.67 ± 1.59^{b}
	20	4.59 ± 1.36	5.98 ± 0.81	4.16 ± 0.50^{b}	9.62 ± 1.48^{b}
	30	2.34 ± 0.32^{a}	8.15 ± 0.76^{a}	4.61 ± 0.67^{b}	8.81 ± 1.07^{b}
	40	2.25 ± 0.39^{a}	7.91 ± 0.90^{a}	4.18 ± 0.68	8.14 ± 1.12
P Wave Duration (msec)	0	34.53 ± 1.10	39.64 ± 1.45	35.19 ± 0.96	39.83 ± 2.35
	10	36.29 ± 0.60	39.56 ± 1.43	42.97 ± 1.12	41.58 ± 1.21
	20	42.62 ± 0.99	39.07 ± 1.50	35.14 ± 2.01	42.17 ± 2.09
	30	38.50 ± 3.58	37.36 ± 1.82	37.10 ± 3.08	43.36 ± 3.12
	40	39.96 ± 2.59	40.58 ± 1.82	40.14 ± 2.05	38.07 ± 2.25
QRS Amplitude (µV)	0	47.04 ± 3.23^{a}	67.91 ± 6.20^{a}	40.02 ± 3.29^{b}	79.55 ± 7.78^{b}
	10	45.81 ± 3.40^{a}	68.64 ± 4.96^{a}	39.47 ± 2.55^{b}	82.68 ± 8.27^{b}
	20	47.18 ± 2.79^{a}	68.90 ± 5.20^{a}	40.79 ± 3.20^{b}	84.60 ± 8.31^{b}
	30	44.00 ± 3.25^{a}	79.10 ± 2.60^{a}	39.17 ± 3.91^{b}	86.83 ± 8.86^{b}
	40	43.53 ± 3.32^{a}	67.64 ± 5.60^{a}	40.51 ± 3.86^{b}	89.86 ± 8.25^{b}
QRS Duration (msec)	0	43.43 ± 1.19	47.21 ± 2.26^{c}	43.83 ± 0.78^{b}	61.02 ± 3.39^{bc}
	10	41.57 ± 1.86	51.96 ± 4.87^{c}	45.67 ± 2.63^{b}	55.04 ± 1.44^{bc}
	20	41.79 ± 1.29^{a}	58.80 ± 4.18^{ac}	43.83 ± 3.53^{b}	68.89 ± 3.65^{bc}
	30	44.71 ± 2.10^{a}	63.86 ± 2.91^{ac}	46.15 ± 2.90^{b}	72.60 ± 2.42^{bc}
	40	44.29 ± 1.15	54.12 ± 1.71^{c}	45.10 ± 4.40^{b}	79.78 ± 3.88^{bc}
Heart Rate (BPM)	0	266.1 ± 16.3	189.1 ± 21.4^{b}	288.2 ± 25.7	292.6 ± 22.3^{b}
	10	236.6 ± 12.4^{a}	126.5 ± 8.3^{ab}	211.3 ± 6.7	198.1 ± 12.5^{b}
	20	221.9 ± 10.8^{a}	108.3 ± 7.5^{ab}	229.0 ± 8.1^{c}	154.6 ± 10.4^{bc}
	30	210.9 ± 11.0^{a}	133.3 ± 21.1^{a}	214.17 ± 6.4^{c}	125.7 ± 8.9^{c}
	40	215.3 ± 10.6^{a}	128.8 ± 22.8^{a}	239.67 ± 12.0^{c}	111.7 ± 7.2^{c}
PR Interval (msec)	0	35.03± 0.99^{a}	46.83 ± 1.37^{a}	37.14 ± 0.91^{b}	45.50 ± 1.50^{b}
	10	34.90 ± 0.93^{a}	51.50 ± 2.36^{a}	43.37 ± 1.67	44.59 ± 1.81
	20	46.05 ± 2.30	45.42 ± 1.26	37.39 ± 2.13^{b}	50.17 ± 2.71^{b}
	30	38.58 ± 3.62^{a}	45.42 ± 2.47^{a}	39.32 ± 1.43^{b}	53.33 ± 2.82^{b}
	40	35.42 ± 1.89^{a}	48.50 ± 2.53^{a}	40.82 ± 2.01^{b}	52.42 ± 2.71^{b}
QT Interval (msec)	0	146.71 ± 5.66^{ab}	85.33 ± 4.53^{b}	102.75 ± 4.81^{ac}	78.97 ± 4.82^{c}
	10	160.60 ± 2.86^{ab}	82.00 ± 3.07^{b}	102.02 ± 2.52^{ac}	77.33 ± 1.60^{c}
	20	157.92 ± 2.85^{ab}	94.57 ± 5.27^{b}	97.32 ± 3.20^{a}	93.66 ± 3.80
	30	165.66 ± 4.73^{ab}	106.17 ± 6.71^{b}	100.25 ± 4.33^{a}	99.04 ± 3.42
	40	165.43 ± 4.73^{ab}	104.93 ± 1.77^{b}	115.42 ± 2.84^{ac}	98.94 ± 2.88^{c}
QTc Interval (msec)	0	97.01 ± 2.81	48.04 ± 5.15	71.23 ± 6.27	53.38 ± 3.54
	10	99.15 ± 1.93	37.31 ± 0.82	61.08 ± 1.21	44.35 ± 1.77
	20	95.83 ± 2.82	39.95 ± 2.46	60.24 ± 3.17	47.03 ± 0.83
	30	97.73 ± 2.46	49.27 ± 4.94	59.80 ± 2.42	45.02 ± 1.67
	40	98.52 ± 1.06	50.59 ± 4.05	72.88 ± 2.83	41.39 ± 0.97

Within rows, means followed by the same letter are significantly different (P< 0.05)

Discussion

Clinical and experimental studies have shown that gender is an important factor in cardiac electrophysiology in human and various animal species (Cheng, 2006). The differences in the ECG between men and women are that the P-wave and P-R intervals are slightly longer in men than in women. Women have higher resting heart rate than men do, but a longer rate-corrected QT (QTc) interval, indicating that there is intrinsic sex-related differences in the electrophysiological properties of the myocardium (James et al., 2007).

At the beginning of the adolescence, the resting heart rate is faster in males than females (Moss, 2010). However, some previous animal studies speculated that cardiac electrophysiological properties between male and female mice were generally similar (Appleton et al., 2004; Berul et al., 1998). We observed that the combination of ketamine -xylazine decreased the heart rate throughout the anesthesia in all groups. Different types of sedation and different doses of ketamine-xylazine combination cause variation on heart rates (Appleton et al., 2004; Erhardt et al., 1984; Mitchell et al., 1998; Roth et al., 2002; Stypmann, 2007). Xylazine (5-10 mg/kg ip) induces sedation, but it seems to have little analgesic effect when used alone in mice. The combination of ketamine (80-100 mg/kg ip) and xylazine (10 mg/kg ip) gives about 20-30 minutes of anesthesia (Flecknell, 2009). However, respiratory depression, reduction in heart rate and blood pressure might also be seen (Erhardt et al., 1984). Ketamine exerts negative inotropic effects on the heart (Rusy et al., 1990) and inhibits both Na+ and Ca++ channels (Hara et al., 1998). In our study, the heart rate was found lower compared to the studies mentioned above. This difference in the heart rate may depend on age, size and strain of the mice studied, and inter-strain differences in response to anesthesia and the anesthetic regimens used (Gargiulo et al., 2012). The male mice from which blood was withdrawn had a low heart rate in the first 20 minutes of the experiment. There is an initial arterial baroreceptor-mediated phase in acute blood loss (phase I, until 25–35% of blood volume is lost) characterized by a sympathoexcitatory response, resulting in maintenance of arterial blood pressure at baseline levels (Ryan et al., 2012; Schadt & Ludbrook, 1991). The hypotensive hemorrhage induces a short-lasting sympathetic excitation followed by a powerful sympathetic inhibition and bradycardia within 5-10 minutes in rats (Skoog et al., 1985). Slowing down of the heart rate when action potential is prolonged may lead to an increase in early afterdepolarization, which may trigger polymorphic ventricular tachyarrhythmias such as torsade de pointes (Jeron et al., 2000).

The amplitude and interval of P wave did not change during anesthesia and after blood withdrawal in the present study. We found that P wave duration of our mice was not similar to other studies conducted with the other mouse strains (Appleton et al., 2004). In human, duration of the P wave has been reported to be slightly longer in men than that of women and this gender difference may be related to smaller atrial size in women compared to men (Dhala et al., 2002).

In human, the QT and the QTc interval are longer and the amplitude and duration of QRS are larger in men than that of women at the beginning of the adolescence (Moss, 2010). The amplitude and duration of QRS are higher and longer, respectively, in male mice than those of females in present study. In human, the androgens and androgen-mediated increase in cardiac mass and left ventricular wall thickness cause larger QRS amplitude in males than females (Sachin Khane & Surdi, 2012). Blood withdrawal resulted in a reduction in the duration of the QRS complex in male mice. The cardiac K+ currents/channels which can be regulated by male sex hormones and androgens are the two major regulatory factors in cardiac repolarization in mice (Brouillette et al., 2003; Brouillette et al., 2005). In our analysis, females' QT intervals were longer than those of males. Mitchell et al. (1998) reported that ketamine anesthesia prolonged the QT interval in mice and this effect was for both gender as the QT and QTc interval were not different between the male and female mice. Speerschneider & Thomsen (2013) suggest the QTc interval is not useful in mice because the cardiac repolarization in the anaesthetized mouse is independent of paced heart rate and thus, this might cause overcorrection of the QT interval.

A well-defined T wave separated from the QRS is absent in murine (Danik et al., 2002). Although some important differences have been described, morphologically, M cells look similar to epicardial and endocardial cells, but functionally, they are more similar to Purkinje cells (Yan et al., 1998). M cells seem like absent in mouse, which makes the magnitude of the transmural repolarization gradient significantly smaller, eventually leading to the absence of a T wave in the murine ECG (Liu et al., 2004).

In conclusion, as reported in previous studies, there is a gender difference for electrocardiographic parameters in anesthetized mice. In addition, anesthesia is an important intervention factor on electrocardiogram. Therefore, electrocardiography should be performed both during and after onset of anesthesia. The effects of an acute blood loss of 1% of body weight on electrocardiogram vary depending on gender. It should be considered that blood sampling within the acceptable upper limits may have an effect on electrocardiographic variables and gender differences might also play another distinct role on the electrocardiographic variables.

Acknowledgment

We thank to Amanda Chilaka, technical assistant in Whitehead Institute, for language editing.

References

Appleton, G. O., Li, Y., Taffet, G. E., Hartley, C. J., Michael, L. H., Entman, M. L., Roberts, R. & Khoury, D. S. (2004). Determinants of cardiac electrophysiological properties in mice. *Journal of Interventional Cardiac Electrophysiology, 11*(1), 5-14.

Berul, C. I., Christe, M. E., Aronovitz, M. J., Maguire, C. T., Seidman, C. E., Seidman, J. G. & Mendelsohn, M. E. (1998). Familial hypertrophic cardiomyopathy mice display gender differences in electrophysiological abnormalities. *Journal of Interventional Cardiac Electrophysiology, 2*(1), 7-14.

Brouillette, J., Rivard, K., Lizotte, E. & Fiset, C. (2005). Sex and strain differences in adult mouse cardiac repolarization: importance of androgens. *Cardiovascular Rearch, 65*(1), 148-157.

Brouillette, J., Trepanier-Boulay, V. & Fiset, C. (2003). Effect of androgen deficiency on mouse ventricular repolarization. *The Journal of Physiology, 546*(Pt 2), 403-413.

Cheng, J. (2006). Evidences of the gender-related differences in cardiac repolarization and the underlying mechanisms in different animal species and human. *Fundamentals & Clinical Pharmacology, 20*(1), 1-8.

Danik, S., Cabo, C., Chiello, C., Kang, S., Wit, A. L. & Coromilas, J. (2002). Correlation of repolarization of ventricular monophasic action potential with ECG in the murine heart. *American Journal of Physiolology: Heart and Circulatory Physiology, 283*(1), H372-381.

Dhala, A., Underwood, D., Leman, R., Madu, E., Baugh, D., Ozawa, Y., Kasamaki, Y., Xue, Q., Reddy, S. & Multicenter, P.-R. S. (2002). Signal-averaged P-wave analysis of normal controls and patients with paroxysmal atrial fibrillation: a study in gender differences, age dependence, and reproducibility. *Clinical Cardiology, 25*(11), 525-531.

Erhardt, W., Hebestedt, A., Aschenbrenner, G., Pichotka, B. & Blumel, G. (1984). A Comparative-study with various anesthetics in mice (pentobarbital, ketamine-xylazine, carfentanyl-etomidate). *Research in Experimental Medicine, 184*(3), 159-169.

Flecknell, P. (2009). *Laboratory Animal Anaesthesia*. San Francisco, USA, Elsevier Science.

Gargiulo, S., Greco, A., Gramanzini, M., Esposito, S., Affuso, A., Brunetti, A. & Vesce, G. (2012). Mice anesthesia, analgesia, and care, Part I: anesthetic considerations in preclinical research. *ILAR Journal, 53*(1), E55-69.

Gargiulo, S., Greco, A., Gramanzini, M., Esposito, S., Affuso, A., Brunetti, A. & Vesce, G. (2012). Mice anesthesia, analgesia, and care, Part II: anesthetic considerations in preclinical imaging studies. *ILAR Journal, 53*(1), E70-81.

Hara, Y., Chugun, A., Nakaya, H. & Kondo, H. (1998). Tonic block of the sodium and calcium currents by ketamine in isolated guinea pig ventricular myocytes. *The Journal of Veterinary Medical Science, 60*(4), 479-483.

James, A. F., Choisy, S. C. & Hancox, J. C. (2007). Recent advances in understanding sex differences in cardiac repolarization. *Progress in Biophysics and Biophysical Chemistry, 94*(3), 265-319.

Jeron, A., Mitchell, G. F., Zhou, J., Murata, M., London, B., Buckett, P., Wiviott, S. D. & Koren, G. (2000). Inducible polymorphic ventricular tachyarrhythmias in a transgenic mouse model with a long Q-T phenotype. *American Journal of Physiolology: Heart and Circulatory Physiology, 278*(6), H1891-1898.

Lairez, O., Lonjaret, L., Ruiz, S., Marchal, P., Franchitto, N., Calise, D., Fourcade, O., Mialet-Perez, J., Parini, A. & Minville, V. (2013). Anesthetic regimen for cardiac function evaluation by echocardiography in mice: comparison between ketamine, etomidate and isoflurane versus conscious state. *Laboratory Animal, 47*(4), 284-290.

Liu, G., Iden, J. B., Kovithavongs, K., Gulamhusein, R., Duff, H. J. & Kavanagh, K. M. (2004). In vivo temporal and spatial distribution of depolarization and repolarization and the illusive murine T wave. *The Journal of Physiology, 555*(Pt 1), 267-279.

McGuill, M. V. & Rowan, A. N. (1989). Biological effects of blood loss: Implications for sampling volumes and techniques. *ILAR Journal, 31*(4), 5-20.

Mitchell, G. F., Jeron, A. & Koren, G. (1998). Measurement of heart rate and Q-T interval in the conscious mouse. *The American Journal of Physiology, 274*(3 Pt 2), H747-751.

Moss, A. J. (2010). Gender differences in ECG parameters and their clinical implications. *Annals of Noninvasive Electrocardiology, 15*(1), 1-2.

Raabe, B. M., Artwohl, J. E., Purcell, J. E., Lovaglio, J. & Fortman, J. D. (2011). Effects of weekly blood collection in C57BL/6 mice. *Journal of the American Association for Laboratory Animal Science, 50*(5), 680-685.

Roth, D. M., Swaney, J. S., Dalton, N. D., Gilpin, E. A. & Ross, J., Jr. (2002). Impact of anesthesia on cardiac function during echocardiography in mice. *American Journal of Physiolology: Heart and Circulatory Physiology, 282*(6), H2134-2140.

Rusy, B. F., Amuzu, J. K., Bosscher, H. A., Redon, D. & Komai, H. (1990). Negative inotropic effect of ketamine in rabbit ventricular muscle. *Anesthesia and Analgesia, 71*(3), 275-278.

Ryan, K. L., Rickards, C. A., Hinojosa-Laborde, C., Cooke, W. H. & Convertino, V. A. (2012). Sympathetic responses to central hypovolemia: new insights from microneurographic recordings. *Frontiers in Physiology,* 3, 110.

Sachin Khane, R. & Surdi, A. D. (2012). Gender differences in the prevalence of electrocardiogram abnormalities in the elderly: a population survey in India. *Iranian Journal of Medical Sciences, 37*(2), 92-99.

Schadt, J. C. & Ludbrook, J. (1991). Hemodynamic and neurohumoral responses to acute hypovolemia in conscious mammals. *American Journal of Physiology, 260* (2 Pt 2), H305-318.

Skoog, P., Mansson, J. & Thoren, P. (1985). Changes in renal sympathetic outflow during hypotensive haemorrhage in rats. *Acta Physiologica Scandinavica, 125*(4), 655-660.

Speerschneider, T. & Thomsen, M. B. (2013). Physiology and analysis of the electrocardiographic T wave in mice. *Acta Physiologica (Oxford), 209*(4), 262-271.

Stypmann, J. (2007). Doppler ultrasound in mice. Echocardiography, 24(1), 97-112.

Tan, T. P., Gao, X. M., Krawczyszyn, M., Feng, X., Kiriazis, H., Dart, A. M. & Du, X. J. (2003). Assessment of cardiac function by echocardiography in conscious and anesthetized mice: importance of the autonomic nervous system and disease state. *Journal of Cardiovascular Pharmacology, 42*(2), 182-190.

Xu, Q., Ming, Z., Dart, A. M. & Du, X. J. (2007). Optimizing dosage of ketamine and xylazine in murine echocardiography. *Clinical and Experimental Pharmacology & Physiology, 34*(5-6), 499-507.

Yan, G. X., Shimizu, W. & Antzelevitch, C. (1998). Characteristics and distribution of M cells in arterially perfused canine left ventricular wedge preparations. *Circulation, 98*(18), 1921-1927.

JIVS JOURNAL OF ISTANBUL VETERINARY SCIENCES

An uncommon case: feline tail post-traumatic osteomyelitis

Case Report
Volume: 2, Issue: 3
December 2018
Pages: 72-77

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ABSTRACT

Post-traumatic osteomyelitis (OM) is an uncommon event in cats, usually affecting distal phalanges of extremities. Tail injuries seldom cause bone infection, but often result in neural damage with subsequent tail paralysis, and occasionally in urinary/fecal incontinence. We present a case of old stray cat which developed post-traumatic tail OM, and endured it for years. It was an immuno-compromised, neglected, animal strongly infested with larvae of *Aelurostrongylus abs*trusus and *oocistae of Isospora felis.* Ultimately, it was treated by tail amputation, with without any health consequences. Relevance and novel information: This report describes the management and outcome of a rare and a life-threatening case of feline post-traumatic tail OM which was previously not reported in literature.

Article History
Received: 05.07.2018
Accepted: 05.10.2018
Available online: 09.10.2018

Keywords: Cat, osteomyelitis, tail, injury

DOI: 10.30704/http-www-jivs-net.438470

To cite this article: Katica, M., Stanić, Ž., Shafie, M., Obhođaš, M., Očuz, M., (2018). An uncommon case: feline tail post-traumatic osteomyelitis. *Journal of Istanbul Veterinary Sciences, 2*(3), 72-77. Abbreviated Title: *J Ist Vet Sci*

Introduction

When it comes to the indoor/house cats, tail trauma is usually caused by accidents with doors (their tail caught in the door), people stepping on them inadvertently or by having their tails hardly pulled. In outdoor cats, tail trauma occurs frequently by scratches and bites from other animals, or, unfortunately, by malicious reasons (Šehić, 2000). Any soft tissue damage can lead to adjacent bone infection. Paralyzed tail is often soiled with urine and feces promoting the infection if the open wound is present. If the wound is located more towards the end of the tail, it is not exposed that much to urine and feces contamination so it usually heals spontaneously, particularly if the injury is at the very end of the tail, on the last vertebrae. (Simonds, 2014). Posttraumatic OM is the most common type of OM in small animals. It is usually caused by bacteria, but fungal and viral etiology have also been described (Bubenik, 2005). Its pathogenesis involves interaction of an infected wound, avascular bone, and suitable environment (Braden, 1991). Open reduction and internal fixation

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Journal homepage: www.jivs.net
http://dergipark.gov.tr/http-www-jivs-net

of fractures by veterinarians cause most cases of osteomyelitis (Griffiths and Bellenger, 1979). Contaminated wounds to the limbs of dogs and cats have the potential to cause osteomyelitis. Bite wounds account for a significant portion of these traumatic wounds, especially in cats (Bubenik, 2005). Often the wound appears as a few small skin punctures caused by the teeth. However, the canine teeth, by crushing and tearing action, damage a considerable amount of muscle, tendon and subcutaneous tissue. Bite wounds are contaminated by microorganisms from the attacking animal's oral flora, the victim's skin bacteria and often soil organisms. This combination of bacterial contamination, devitalized parosteal tissue and periosteum, dead tissue and inflammatory exudate very likely leads to the development of infection (Johnson et.al., 2006). Skin puncture wounds can heal but the underlying infection can continue to develop to an abscess, either under the periosteum or as an extension to the periosteum from the adjacent subcutaneous tissue. Neglected bite wounds and abscesses, periodontal infection and chronic paronychia are common initiating cause of OM (Sia and Berbari, 2006). Normal, healthy bone in an immune-competent animal is highly resistant to infection, but injury and infection of adjacent soft tissue may induce a posttraumatic OM subsequent to a bite or scratch (Viskjer and Rapp, 2016). However, if the immune-competence of an injured animal is further compromised, as it is with many outdoor cats, the infection spreads easily affecting bone structures underneath inflamated soft tissue. Massive infestation with lung and bowel parasites like *Aelurostrongylus abstrusus* and *Isospora felis*, which were abundantly isolated from our patient, exhausts the immunity of the host , therefore facilitating development of OM (Traversa and Di Cesare, 2016). Most cases of osteomyelitis in dogs and cats are chronic at the time of diagnosis (Bubenik, 2005).

The main intention of this case report is to present the successful, routine surgical treatment of OM on the cat's tail, what is yet to be described in the literature.

Case

According to oral cavity inspection, an 10-11 years old, 5 kg of body mass, male, domestic short-haired, cat was brought due to sanguinolent-purulent discarge of the tail (Figure 1 and Figure 2).

It has been stated that the cat has been in this condition for at least three to four years. Firstly the cat was admited to a private veterinary outpatient office, where osteomyelitis was diagnosed in the tail. The patient had no fever and the general condition was in good state. It was frightened and agressive, but with good appetite, and no substantial difficulties on physical examination. The open wound with substantial soft tissue defect was dirty, covered with pus and producing unpleasant smell. There was no obvious tail fracture. The perianal and periurethral area were unaffected with trauma. The sensory as much as urinary/fecal continence were preserved.

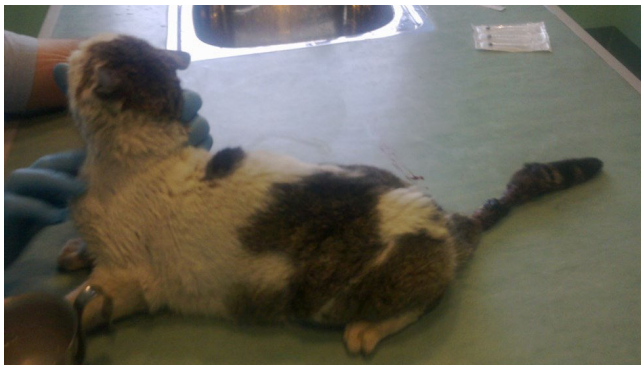

Figure 1. Street, short-haired cat with obvious tail injuries.

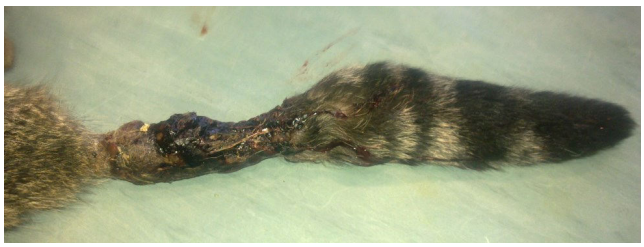

Figure 2. Aspect of long-lasting, untreated sanguinolent-purulent discarge of the tail.

Leukogram showed signs of leukocytosis, neutropenia, monocytosis, basophilia and eosinophilia (Table 1). The animal was immunocompromized due to long-term chronical infectious disease.Coproculture revealed high degree

Table 1. Some hematological parameters of the cat

Parameters	Measured values	Reference values*
RBC (1×10^{12}/L)	9. 98	5 - 10
WBC (1×10^{9}/L)	29.7	5.5 - 19.5
Platelets (1×10^{9}/L)	-	300- 800
Hematocrit (%)	39	30 - 45
Hemoglobin (g/L)	137	80 - 150
Neutrophil (%)	34	35 - 37
Lymphocyte (%)	39	20 - 50
Monocyte (%)	7	1 - 4
Eozinophil (%)	18	2 - 12
Basophil (%)	2	0 - 0.5

RBC = Red blood cell; WBC = White blood cell; * Blood & Studdert (1988); O'Brien et. al. (1998).

of infestation with larvae of *Aelurostrongylus abstrusus* and oocistae of *Isospora felis*. The wound was initially cleaned following the principles of management of dirty surgical wounds.

The cat was premedicated with medetomidine (Domitor; Orion Pharma Animal Health) 0.08 mg/kg SC, meloxicam (Metacam; Boehringer Ingelheim Vetmedica) 0.2 mg/kg SC and methadone (Metadon Recip; Meda) 0.3 mg/kg. Ketamine (Ketaminol; Intervet) 5 mg/kg was administered intramuscularly to maintain a dissociative anesthesia. Oxygen was delivered by flow-by with the mask during the procedure. With the cat positioned in right lateral recumbency and the tail directed to the surgeon, the surgical site was lavaged with saline respecting the principles of sepsis and antisepsis. Before incising the skin the intervertebral spaces in front and after the chosen tail vertebra for transection were identified by digital palpation. Two v-shaped skin incisions are made at each side of the tail over the middle region of the vertebra with the tip of the v to the direction of the head of the animal and both dividing branches caudally one to the dorsum of the tail and the other to the ventral part (Figure 3).

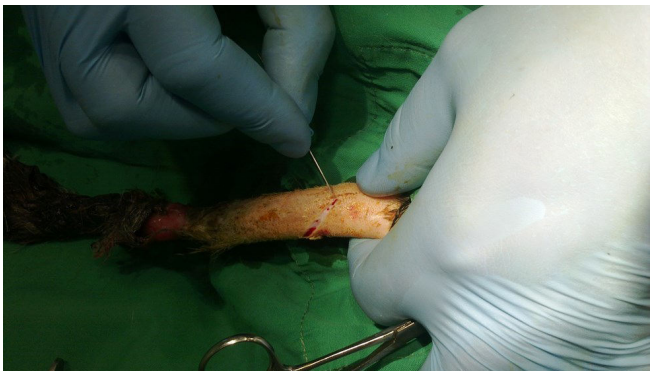

Figure 3. The initial steps of the amputation of the tail, the aspect of the two cuts in the „V“ shape.

This pattern of incision created two skin flaps, one dorsal and the other ventral. The dorsal one was slightly bigger than the ventral one. The suture line passed more ventrally for better protection and coverage of vertebra by one single flap. Therefore, no suture line was directly over the cut surface. The major vessels were ligated and the skin of both flaps was slightly reflected cranially by gentle blunt preparation with scissors (Figure 4). The muscles of the tail was sharply incised with the scalpel blade by a circular incision all around the vertebral body at the chosen place for transection. After bleeding vessels were occluded by cautery or ligation, the coccygeal vertebra was transected with bone scissors.

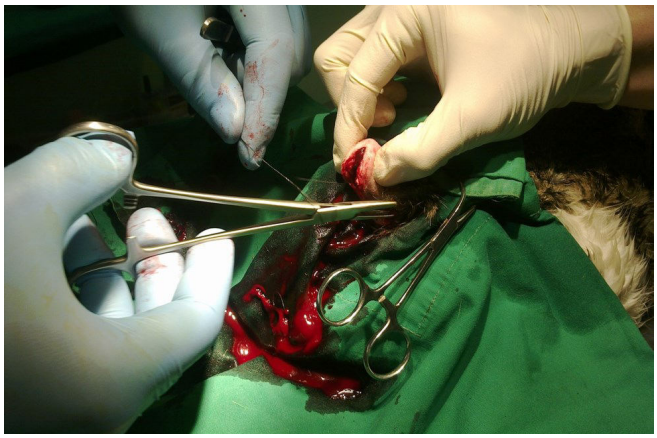

Figure 4. The final surgical procedures to the rest of the tail after a successful amputation.

The skin flaps were then apposed over the exposed bone with single subcutaneous suture by using absorbable suture material. stitches with small, (Wicrosorb, sterile synthetic absorbable suture 3/0, 75 mm) absorbable suture material and at last the skin was sutured with fine non-absorbable sutures. Postoperatively a wound dressing for two to three days was applied to repel dirt from the environment and an Elizabethan collar to hinder the animal from licking the wound. The cat received meloxicam 0.05 mg/kg administered orally q24h for 5 days after surgery. Treatment with a first-generation cephalosporin cefadroxil (Cefadroxil; Mylan), oral suspension 20 mg/ kg q12h, was initiated 24 hours after the surgery. The cat was cleaned of intestinal and skin parasites, and adopted by his carrier. Temporary adaptor was interviewed by telephone and reported that the cat was acting completely normal and had no detectable swelling on the tail. The control examination after 6 months revealed no signs of relapse, or signs of other diseases.

Discussion and Conclusion

This report describes the diagnosis, treatment and outcome of a severe tail OM in an old, male, outdoor domestic shorthair cat. To our knowledge this is the first reported case of feline tail OM. The literature describing OM in small animals is limited (Griffits and Bellenger, 1979). Most of it report the OM cases in dogs (Dunn et.al., 1992), occasionally including cats, and seldom other species. Beside well described cases of OM in dogs and cats located in distal phalanges of the extremities (Šehić, 2000), there are occasional reports of OM in other rare locations like scapula (Viskjer and Rapp, 2016), jaw (Rodrigues de Farias, 2012), nasal bone (Johnson et.al., 2006), or pelvis (Doolitle, 2017). We found no report about tail OM

either in dogs or cats. There are anecdotal reports about tail OM in cow (Nuss and Feist, 2011), and wild lioness (Eyarefe et al., 2015) developed after self-tail mutilation of a wild animal kept in captivity.

In its essence OM can be either hematogenous, or posttraumatic. In the latter inoculation of bacteria can occur from an exposure of the bone via propagation of infection from the infected adjacent soft tissue, from the open fracture or through a surgical intervention. The localization of OM in small animals seems to be connected with its etiology and spreading path. According to Griffiths and Bellenger, (1979), the most common source of OM in dogs was open reduction of closed fractures, while in the cat, the most common source of infection was an extension from soft tissue infection. However, it is used to believe that the most common localization of OM in small animals is within the long bones of the appendicular skeleton both for haematogenous and post-traumatic infections. Other skeletal involvement such as vertebral infections and discospondylitis are most often caused by a haematogenous spread from elsewhere in the body whereas OM of the jaw in cats is often associated with periodontal disease and caused by a broad range of bacteria, most of which are normal oral flora (Johnson et al., 2006). Haematogenous OM is uncommon in dogs and cats, and typically affects young animals (Viskjer and Rapp, 2016). Posttraumatic OM presents as spread of unrestricted infection from the infected soft tissue to the periostea, epiphysis, metaphysis and bone marrow. The process is promoted by creation of bacterial nests built of necrotic, avascular bone fragments. Disruption of the blood supply with thrombotic episodes further promotes bacterial growth and bone necrosis (Stead, 1984). OM is generally categorized as acute or chronic based on histopathologic findings, rather than duration of the infection. Acute OM is associated with inflammatory bone changes caused by pathogenic bacteria, and symptoms typically present within two weeks after infection. Necrotic bone is present in chronic OM, and symptoms may not occur until six weeks after the onset of infection. Chronic OM is generally secondary to open fractures, bacteriemia, or contiguous soft issue infection (Hatzenbuehler and Pulling, 2011).

Bacterial OM may be caused by any pathogenic bacteria and occasionally by bacteria not regarded as pathogenic (Johnson et al., 2006). The literature seems to suggest that similar organisms cause OM in both dogs and humans. Often cultures may contain more than one organism, and the presence of a gram-positive and gram-negative organism is quite common (Hatzenbuehler and Pulling, 2011). *Staphylococcus* is the most commonly cultured organism, isolated in up to 74% of bone infections reported in small animals (Bubenik, 2005), followed by Streptococcus, *Escherichia coli*, and Proteus species (Viskjer and Rapp, 2016).

Although gram-positive microorganisms are the most frequently cultivated, infections with a mixed bacterial flora, including aerobic and anaerobic combinations, are common. There are reports of OM cases in small animals caused by *Actinomyces viscosus*, *Fusobacterium nucleatum*, *Bacteroides spp*, *Clostridium villosum*, *Peptostreptococcus anaerobius*, *Wolinella recta* and *Bacteroides gingivalis* (Johnson et al., 2006), *Serratia marcescens* (Armstrong, 1984), *Listeria monocytogenes* (Doolitle, 2017), *Bartonella vinsonii* (Varana et al., 2009) or *Nocardia Africana* (Rodrigues de Farias et al., 2012).

The diagnosis of chronic OM is usually not difficult. Pain, disuse atrophy, tenderness, and drainage from the area constitute the hallmarks of chronic OM. At any stage in the natural history of chronic OM an acute exacerbation may occur that mimics the signs of acute OM, with systemic effects as well as local ones (Kahn, 1973). Usually chronic OM is limited to local effects, and the patient is not systemically ill, as it was with our patient.

Unfortunately, for economic reasons (outdoor cat without an owner) expensive diagnostic methods like tail X-Ray, bacterial culture, feline leukemia virus/ feline immunodeficiency virus detection, or histopathologic diagnostics were not available. However, the diagnostic value of mentioned tests when evaluating a localized OM is debated, and the authors do not believe that their results would have changed the treatment plan or the outcome in this case.

The OM treatment should include surgical debridement and administration of appropriate antimicrobial drugs (Sia and Berbari, 2006). The choice of antibiotic is related to the hospital environment and the known susceptibility of the most commonly isolated organism in that environment; usually one of the cephalosporins, sometimes in combination with an aminoglycoside. Except localized OM of the single digit or tail which may be cured by simple tail or digit amputation surgical therapy of OM is challenging. Surgical debridement must include all fragments of the dead bone. Any remaining sequestrum may act as a nidus for further infection, yet too vigorous removal of bone may lead to fracture and instability.

This represents one of the major problems in dealing with chronic OM. The greatest chance for the healing of the infection is through massive removal of dead bone; the greatest chance for nonunion or refracture is also related to massive removal of bone. Fortunately, to our patient, surgical treatment was quite simple.

Parasitary infestation reduces the body's healing response and contributes to chronically open wounds and subsequent soft tissue infection what is supported by frequent presence of eosinophilia in cases with OM developed from neglected fight wounds (Dehghani and Hajighahramani, 2005). These conditions may act synergistically to significantly increase the risk of OM in these patients (Hatzenbuehler and Pulling, 2011). The true prevalence of *A. abstrusus* infection is unknown. One study in California reported a prevalence of 1.9%, and a second study in Alabama reported a prevalence of 18.5% among stray cats euthanized at a humane shelter (Ellis, 2010). A study in Croatia reported prevalence's, based on histopathologic diagnosis, ranging from 3.9% to 22%, depending on geographic region (Grabarević, 1999). The most frequent signs of Aelurostrongylosis are mild to intense coughing, wheezing, sneezing, nasal discharge, dyspnea and tachypnea. Generalized signs such as lethargy and weight loss have also been described. Severe signs such as open mouth abdominal breathing, tachycardia and even death may occur in young, debilitated and/or immunosuppressed cats. Aelurostrongylosis has furthermore been implicated in cases of anesthetic-associated deaths, likely due to the decreased surface area for gas exchange and a combination of impaired lung perfusion and ventilation, hypoxia and systemic hypotension, culminating in cardiovascular collapse (Traversa and Di Cesare, 2016).

In severely infected, and especially if additionally fractured tails in small animals, it is often recommended to amputate the tail to ultimately cure the locus of permanent infection, and to prevent further injury to the nerves that supply the urethra and anus. After traumatic tail injury, as much as after tail amputation the cat will need to rest in a crate or cage. Cats use their tails for balance and navigation, but with a little adjustment they can learn to manage without their tails, and to function without major handicappe (Simonds, 2014).

The causal agent of the injury in this case was not defined. The presence of numerous population of stray dogs in this area (Katica et al., 2017), who present primary predators for the outdoor cats could be the answer, as well as territorial conflict with other stray cats. Malicious events with people or motor vehicle accidents could also play a role in this injury. The patient was successfully treated with simple tail amputation with full recovery.

References

Armstrong, P. J. (1984). Systemic *Serratia marcescens* infections in a dog and a cat. *Journal of the American Veterinary Medical Association,184*(9), 1154-1158.

Blood, D. C. & Studdert, V. P. (1988). *Bailliere's Comprehensive Veterinary Dictionary*. London, UK: Bailliere Tindall.

Braden, T. D. (1991). Posttraumatic osteomyelitis. *Vetinary Clinics of North America: Small Anim Practice, 21*(4), 781 -811.

Bubenik, L. J. (2005). Infections of the skeletal system *Vetinary Clinics of North America: Small Anim Practice*, 35, 1039–1049.

De Farias, M. R., Werner, J., Ribeiro, M. G., Rodigheri, S. M., Cavalcante, C. Z., Chi, K. D., Condas, L. A. Z., Gonoi, T., Matsuzama, T. & Yazama, K. (2012). Uncommon mandibular osteomyelitis in cat caused by Nocardia Africana. *BMC Veterinary Research,* 8, 239.

Dehghani, S. N. & Hajighahramani, S. (2005). Eosinophilia due to osteomyelitis in a dog. *Journal of Veterinary Science, 6*(3), 255–257.

Doolitle, J. (2017). Osteomyelitis and septicemia secondary to *Listeria Monocytogenes* in a young dog. Journal *of Dairy, Veterinary & Animal Research, 6*(1), 00170.

Dunn, J. K., Dennis, R. & Houlton, J. E. F. (1992). Successful treatment of two cases of metaphyseal osteomyelitis in the dog. *Journal of Smal Animal Practice, 33*(2), 85-89.

Ellis, A. E, Brown, C. A. & Yabsley, M. J. (2010). Aelurostrongylus abstrusus larvae in the colon of two cats. *Journal of Veterinary Diagnostic Investigation, 22* (4), 652-655.

Eyarefe, O. D., Oguntoye, Olusa T. A. & Morenikeji, O. A. (2015). Lifesaving tail amputation in an African lioness (*Panthera leo L*) in captivity. *Sokoto Journal of Veterinary Sciences,* 13(2), 61-64.

Grabarevic, Z., Curic, S., Tustonja, A., Artukovic, B., Šimec, Z., Ramadan, K. & Zivicnjak T.(1999). Incidence and regional distribution of the lungworm *Aelurostrongylus abstrusus* in cats in Croatia. *Veterinarski Arhiv, 69*, 279–287.

Griffiths, G. L. & Bellenger, C. R. (1979). A retrospective study of osteomyelitis in dogs and cats. *Australian Veterinary Journal, 55*(12), 587-591.

Hatzenbuehler, J. & Pulling, T. J. (2011). Diagnosis and management of osteomyelitis. *American Family*

Johnson, K. A., Lomas, G. R. & Wood, A. K. W. (2006). Osteomyelitis in dogs and cats caused by anaerobic bacteria. *Australian Veterinary Journal, 61*, 57-61.

Kahn, D. S. & Pritzker, K. P. H. (1973). The pathophysiology of bone infection. *Clinical Orthopaedics and Related Research*, 96, 12.

Katica, M., Obradovic, Z., Gradascevic, N., Hadzimusic, N., Mujkanovic, R., Mestric, E., Bašić, B., Toplaović, B., Smajkić, R., Dupovac, D. & Celebicic, M. (2017). Assessment of the effect of stray dogs as a risk factor for the health of population in certain areas of Bosnia and Herzegovina. *European Journal of Biomedical and Pharmaceutical Sciences, 4*(9), 107-111.

Nuss, K. & Feist, M. (2011). Tail amputation for treatment of osteomyelitis of the first and second coccygeal vertebrae in a cow. *Tierarztliche Praxis, 39*(3), 176-178.

O'Brien, M., Murphy, M. G. & Lowe, J. A. (1998). Hematology and clinical chemistry parameters in the cat (*Felis domesticus*). *Journal of Nutrition, 128*, 2678S–2679S.

Šehić, M. (2000). *Osteoarthropatije u domaćih životinja*. Zagreb, Hrvatska: Skaner Studio.

Sia, I. G. & Berbari, E. F. (2006). Infection and musculoskeletal conditions: Osteomyelitis. *Best Practice & Research: Clinical Rheumatology, 20*(6), 1065-1081.

Simons, M. C., Ben-Amotz, R. & Popovitch, C. (2014). Post-operative complications and owner satisfaction following partial caudectomies: 22 cases (2008 to 2013). *Journal of Small Animal Practice, 55*(10), 509-514.

Stead, A. C. (1984). Osteomyelitis in the dog and cat. *Journal of Small Animal Practice,* 25, 1-13.

Traversa, D. & Di Cesare, A. (2016). Diagnosis and management of lungworm infections in cats: Cornerstones, dilemmas and new avenues. *Journal of Feline Medicine & Surgery,* 18, 7-20.

Varanat, M., Travis, A., Lee, W., Maggi, R. G., Bissett, S. A., Linder, K. E. & Breitschwerdt E. B. (2009). Recurrent osteomyelitis in a cat due to infection with *Bartonella Vinsonii subsp*. *Berkhoffii genotype* II. *Journal of Veterinary Internal Medicine, 23*(6), 1273-1277.

Viskjer, S. & Rapp, M. (2016). Scapular osteomyelitis in an immature domestic shorthair cat. *Journal of feline medicine and surgery open reports, 2*(2), 2055116916668199.

JIVS JOURNAL OF ISTANBUL VETERINARY SCIENCES

Effects of L-NAME, DEXA, and L-NAME+DEXA on systemic blood pressure of hypertensive pregnant and non-pregnant Wistar Albino rats

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Research Article
Volume: 2, Issue: 3
December 2018
Pages: 78-85

ABSTRACT

Objective: NO-inhibition has no effect on blood pressure (BP) of some spontaneous hypertensive animals, but when combined with dexamethasone (DEXA), it increases BP. The study compared effects of L-NAME and/or DEXA on systemic BP of spontaneously hypertensive pregnant and non-pregnant Wistar albino rats. ***Method***: In two simultaneous experiments 62 female rats were used. All animals were mated for 7 days. Sperm positive (n1 = 33) and negative (n2 = 29) animals were each divided randomly into 4 groups. BPs were recorded in both experiments on the 15th day from tails indirectly, and then animals were given Physiologic Saline (Controls), L-NAME (150mg/kg/day), DEXA (100µg/kgBW/day) or L-NAME+DEXA (150mg and 100-µg per kg BW/day) for consequent 5 days. At 19th day, BPs were measured again, before applications. Then, animals put into individual metabolic cages for 24-h urine collection. Thereafter, blood was collected under ether anesthesia, animals were euthanized and necropsied. Weights of animals (BWs), left kidneys, adrenal glands, and fetuses; food consumptions; 24-h urine volume; urinary proteins, blood glucose, and fetus numbers were determined. Data were analyzed by ANOVA and ANOVA for repeated measures. ***Results***: In pregnant animals, L-NAME had higher BWs than DEXA and L-NAME+DEXA (P = 0.021 and P = 0.012, respectively). In non-pregnant animals, DEXA reduced BWs significantly compared with controls (P=0.042). Interventions influenced only the diastolic blood pressure of pregnant animals (P = 0.043). The difference between DEXA and L-NAME+DEXA was significant (P = 0.044). The effects of interventions on other variables varied according to whether animals are pregnant or not. ***Conclusion***: L-NAME and/or DEXA did not influence BP in hypertensive rats.

Article History
Received: 17.09.2018
Accepted: 22.10.2018
Available online: 22.10.2018

Keywords: Wistar albino rats, L-NAME, dexamethasone

DOI: 10.30704/http-www-jivs-net.460833

To cite this article: Bacak, G. & Balkaya, M. **(2018). Effects of L-NAME, DEXA, and L-NAME+DEXA on systemic blood pressure of hypertensive pregnant and non-pregnant Wistar Albino rats.** ***Journal.of Istanbul Veterinary Sciences. 2*****(3), 78-85. Abbreviated Title:** ***J Ist Vet Sci***

Introduction

Hypertension, characterized by a systolic blood pressure of ≥140 mmHg and diastolic blood pressure of ≥90 mmHg, is one of the most important reasons of premature deaths, and the most important risk factor for cardiovascular diseases in men worldwide (Baker et al., 2007). It may be primary, developing because of environmental or genetic factors, or secondary, with multiple etiologies including vascular, renal, nervous, endocrine and nutritional causes. Consequently, there are many risk factors implicating in the genesis of hypertension (Zhang et al. 2013). In general, they include genetic and environmental factors. More than 90-95% of cases are essential hypertension with yet unknown cause(s), but there are strong shreds of evidence for a possible familial background of the condition. Therefore, several studies targeting genes aimed to explain the genetic roots of various types of hypertension (Bernatova, 2014). However, none of

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Journal homepage: www.jivs.net
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the determined genetic abnormalities yet found to be responsible alone for a significant proportion of hypertension occurring in the general population.

Many other well-known pathophysiological risk factors of the hypertension include: overactivity of the sympathetic nervous system; immune reactivity and inflammation; overproduction of sodium-retaining hormones and vasoconstrictors; long-term dietary high sodium intake as well as low potassium and calcium intakes; disordered (high or inappropriate) renin secretion with consequent high production of angiotensin II and aldosterone; deficiencies in vasodilators including impairments of the nitric oxide (NO) system, prostacyclins and natriuretic peptide; increased secretion and/or activity of vascular growth factors; derangements in expressions of the kinin-kallikrein system involving in renal salt handling and vascular tone; alterations in cardiac adrenergic receptors and inotropic properties of the heart and systematic vascular tone; altered ion transport at the cellular level; abnormalities or lesions of vasculature due to various reasons including insulin resistance, diabetes and obesity; chronic kidney and lung diseases and activation of circulating proteins interfering with angiogenesis (Török, 2008; Bernatova, 2014).

There are also many pregnancy-related multifactorial disorders with different etiologies and characterized under others by hypertension with or without concomitant proteinuria (WHO, 2005). Preeclampsia and its severe form, eclampsia, are the most important conditions among these.

As for all other conditions and diseases of the men, research animals prove as a valuable tool also to understand the physiopathological mechanisms of hypertension. Because of the dramatic consequences of their implications on mother and their infants and so on the general population, numerous animal models described gaining insight into possible physiopathological mechanisms and treatment options of preeclampsia and eclampsia. The common models use mainly placental oxygen dysregulation, abnormal trophoblast invasion, maternal vascular damage and abnormal maternal-fetal immune interactions (Pennington et al., 2012).

Vascular endothelium produces various vasoactive factors including most potent vasoconstrictors angiotensin II (Ang II) and its mediator endothelin 1 (ET-1) (Cediel et al., 2002). On the other site, endothelium-derived NO is also the known most potent endogenous vasodilator and a local regulator of the vascular system. Increased production of NO during late gestation decreases systemic vascular resistance under normal conditions (Nathan et al. 1995). Thus, any decrease or failure of the NO contributes to the development of hypertension (Rafikov et al., 2011). Therefore, animal models of preeclampsia *via* nitric oxide synthase (NOS) inhibition are often preferred as an alternative approach which aims to target mechanisms at the level of the endothelium instead trying to reproduce entire disease (Podjarny et al., 2004). The effects of the L-NAME on the uterine vasculature, however, may differ depending on whether animals are pregnant or not (Osol et al., 2009). Púzserová et al. (2007) have also been reported a significant relationship between BP and L-NAME-sensitive component of relaxation of the femoral artery.

Increased production of endogenous glucocorticoids and their parenteral applications or applications of their synthetic analogs (e.g., dexamethasone) results also in hypertension. A recent study demonstrated that the hypertensive effect of glucocorticoids is a consequence of their direct effects on blood vessels (Goodwin et al., 2011). There is also evidence of multiple interactions between NO-system and corticosteroids (Yallampalli et al., 1994; Changbin and Baylis, 2000). Dexamethasone is able to modify α-adrenoreceptor-mediated effects of L-NAME on vascular smooth muscles (Adeagbo and Triggle, 1993). However, there is no comparative information about the interactions between L-NAME and corticosteroids regarding systemic blood pressure in pregnant and non-pregnant rats.

This study aimed to determine the effects of NOS-inhibition (L-NAME), dexamethasone (DEXA) and the combination of L-NAME and DEXA on blood pressure of both pregnant and non-pregnant Wistar albino rats with a high blood pressure of dietary origin.

Materials and methods

Study conditions: Institutional Animal Ethics Committee has approved the study (#2012/105). The study has been carried out in a semi-climatized experimental room with a microenvironment of 23±1° C temperature, 50-70% relative humidity and 12:12 h light:dark cycle, which was similar to their rearing conditions. A total of 62 female Wistar rats (33 pregnant and 29 non-pregnant) were used in two different experiments, which were carried out simultaneously to avoid possible effects of time. Animals were allowed an adaptation period for ten days. Then, all animals were mated on the following 9 days in the harem system. Pregnancy was detected by daily vaginal sperm controls where the sperm [+] day recorded as the beginning of pregnancy (day 0) for given animal. During the mating period 33 animals were sperm [+], and 29 sperm [-].

The animals received pelleted diet for rats and mice and daily fresh top water throughout the study (including adaptation and mating periods) *ad libitum*.

L-NAME was given to animals by oral gavage and DEXA by intraperitoneal injections.

Experiment I: In this experiment, 33 pregnant rats were used. Animals were divided randomly into four groups. The animals in Group I served as controls. Animals in other groups were given L-NAME (150 mg/kg/d) (Group II), DEXA (100 µg/kg/d) (Group III) or L-NAME + DEXA(150 mg/kg/d + 100 µg/kg/d) (Group IV) for 5 days starting at the 15th day of their pregnancy. Animals in the control group were given equal volumes of physiological saline on the same days.

Experiment II: In this experiment 29 non-pregnant animals were divided randomly into four groups as in Experiment I. The animals in Group I served as controls. Animals in other groups were given same amounts of physiological saline, L-NAME (Group II), DEXA (Group III) or L-NAME + DEXA (Group IV) for 5 days; exactly, on the same last 5 days of the experiment, as in Experiment I.

Data collection: The body weights of animals were recorded at the beginning (day 0) and 15th and 19th days of the experiments. Blood pressures were recorded *via* non-invasive method from tails of animals on days 15 and 19 of the experiment before substance applications. On day 19, animals were put into individual metabolic cages for 24 h after blood pressure measurements, and 24 h-urine samples were collected on day 20 of the experiment. Thereafter, 4-5 ml blood samples were collected by hearth punctures under ether anesthesia, and then the animals were euthanized. Body weights, and weights of the suprarenal glands and left kidneys and fetuses' numbers (in Experiment I) were recorded from necropsied animals.

Laboratory analysis: Protein concentrations were determined *ad modum* Esbach in urine samples, and glucose concentrations were determined *via* glucometer in blood samples, both by using strips.

Blood pressures measurements: Systolic and diastolic blood pressures were recorded from animals by non-invasive, indirect method (NIBP 200-A, Commat®, Ankara, Turkey). For this reason, animals were transported in pre-warmed recording room 2 hours before the measurements. Then, shortly before measurements they held for 15-20 minutes in a heated measurement box with inner temperature of 33-34 °C. Measurements were carried out when animals were relaxed and regular waves occurred. Three subsequent measurements were made from each animal.

Data were recorded, stored and evaluated by Biopac Version 3.7.2 Program (BIOPAC Systems, Inc. Aero Camino, USA).

Statistical analyses: SPSS for Windows® Version 21 was used to store and analyze the data gathered. Data were analyzed *via* ANOVA and ANOVA for repeated measures in factor time. Tukey test was used as *post hoc*. P≤ 0.05 was accepted as significant.

Results

Body weights: The body weights changes of the female Wistar rats are summarized in Table 1. There was no significant difference in mean body weights of both pregnant and non-pregnant animals at the beginning of the experiments (P>0.05).

During the 3-weeks experimental period, body weights of pregnant rats showed remarkable changes. The effects of interventions on the body weights of pregnant rats were significant (P=0.006). Paired comparisons for groups revealed significant differences for the groups given L-NAME, DEXA, and L-NAME + DEXA. Namely, the average body weight of the group given L-NAME was higher than those given DEXA and L-NAME + DEXA (P=0.025 and P=0.014 respectively). Analysis of variance for repeated

Table 1. Body weights of animals [g]

Groups	Pregnant rats $\bar{X} \mp Sx$			Non-pregnant rats $\bar{X} \mp Sx$		
	Day 0	Day 15	Day 20	Day 0	Day 15	Day 20
Control	214 ± 19	294 ± 22	317 ± 30	217 ± 08	268 ± 42	273 ± 44*
L-NAME	227 ± 21	313 ± 20*	315 ± 27	215 ± 50	255 ± 36	236 ± 29
DEXA	201 ± 07	274 ± 12$^{\Omega}$	286 ± 16	202 ± 22	223 ± 23	209 ± 18$^{\Omega}$
L-NAME + DEXA	213 ± 22	282 ± 34$^{\Omega}$	269 ± 32	215 ± 13	258 ± 36	216 ± 28

In the same colon* - Ω: P<0.05

Table 2: Blood Pressures of Rats [mmHg]

Groups	Pregnant rats		Non-pregnant rats	
	15th day	19th day	15th day	19th day
	Systolic blood pressures			
Control	165.38 ± 18.84	151.57 ± 10.26	148.29 ± 16.70	135.57 ± 20.07
L-NAME	144.67 ± 11.09	142.50 ± 18.15	146.29 ± 10.08	153.43 ± 12.31
DEXA	150.0 ± 19.28	143.78 ± 15.92	133.14 ± 07.03	150.86 ± 10.89
L-NAME + DEXA	154.56 ± 20.43	161.22 ± 18.38	122.25 ± 19.64	151.14 ± 22.70
	Diastolic blood pressures			
Control	134.50 ± 18.45	123.29 ± 05.47	122.57 ± 14.55	115.57 ± 15.97
L-NAME	118.33 ± 13.80	117.38 ± 14.61	117.71 ± 08.12	132.29 ± 13.09
DEXA	118.0 ± 18.29	115.44 ± 11.18	109.0 ± 09.13	119.29 ± 10.08
L-NAME + DEXA	125.67 ± 14.77	132.22 ± 16.09	104.38 ± 14.46	135.43 ± 22.84

measures in factor time revealed that the effect of time was significant, and there was a significant interaction between the time and interventions (P=0.000). Paired comparisons for time revealed significant differences between the 1st and 2nd and 1st and 3rd measurement points (P=0.000). However, the differences between the 2nd and 3rd measurements were not confirmed statistically (P=0.058, %95 CI: -12.157 - .150).

However, the effects of the interventions on the body weights of non-pregnant animals could not be confirmed statistically (P=0.066, F=2,729). Also, paired comparisons for groups did not confirm the difference in mean body weights of controls and DEXA (P=0.054, %95 CI: -.494 - 83,065). In contrast, the effect of time on the body weights of non-pregnant animals and the interaction between time and interventions proved to be significant (P=0.000). Paired comparisons for time revealed significant differences between the 1st and 2nd, 1st and 3rd and 2nd and 3rd measurements (P=0.000, P=0.007 and P=0.0000 respectively).

Systolic and diastolic blood pressures: Mean blood pressure values are presented in Table 2.

In pregnant rats, there was no significant difference in mean systolic blood pressure values, when recorded at day 15 before interventions. Statistical analysis did also not confirm the effects of interventions on systolic blood pressures recorded at day 19 (P=0.083, F= 2.456). The effect of time and interactions between time and interventions were also not significant (P>0.05).

There were significant differences in mean systolic blood pressure values of the non-pregnant Wistar albino rats at day 15 of the experiment, also before interventions. *Post hocs* revealed lower mean systolic blood pressure of the L-NAME + DEXA group when compared with control and DEXA groups (P=0.010 and P=0.018 respectively). However, no significant

Table 3. Other Variables

Variables	Control	L-NAME	DEXA	L-NAME+DEXA
		Pregnant rats		
Glucose (mEq/L)	134.83 ± 38.45	93.13 ± 37.78	86.88 ± 12.63	111.50 ± 46.64
Abs. Left Kid. Wt (g)	0.96 ± 0.12	0.89 ± 0.13	0.80 ± 0.04	0.87 ± 0.13
Rel. Left Kid. Wt. (%)	0.0030 ± 0.00027	0.0028 ± 0.00047	0.0028 ± 0.00021	0.0033 ± 0.00047
Adrenal Glands Wt. (g)	0.08 ± 0.02	0.10 ± 0.02	0.09 ± 0.01	0.07 ± 0.01
Urine Vol. (mL/24h)	2.59 ± 3.48	5.25 ± 7.31	4.83 ± 5.86	3.56 ± 1.74
Urinary Protein (g/dL)	0.80 ± 0.84	1.00 ± 0.00	0.33 ± 0.71	1.75 ± 0.89
Numbers of Fetuses	7.57 ± 3.60	10.00 ± 3.07	13.22 ± 1.86	10.22 ± 2.95
Total Wt of Fetuses (g)	43.96 ± 19.54	50.75 ± 14.66	58.14 ± 15.04	41.96 ± 15.34

Kid; Kidney, Abs: absolute, Rel: relative, Vol: volume, Wt: weight

difference was found for systolic blood pressure at day 19. There was a significant effect of the time on systolic blood pressure of animals (P=0.011), and a significant interaction has occurred between time and interventions in this respect (P=0.005).

No significant difference was in mean diastolic blood pressure values of pregnant Wistar albino rats when measured before intervention at day 15. *Post hocs* did also not confirm the difference between mean diastolic blood pressures of control and L-NAME groups (P=0.098, %95 CI: -2.716166 – 44.132833). However, the diastolic blood pressure values at 19th day of the experiment revealed significant differences between groups, indicating important effects of the interventions (P=0.043). *Post hocs* revealed that the difference between DEXA and L-NAME + DEXA groups is significant (P=0.044). Time had no important effect, and no significant interaction could be detected between time and interventions in this respect.

Mean diastolic blood pressure values of non-pregnant Wistar albino rats showed significant differences before interventions at day 15 of the experiment. Post hocs confirmed the difference between mean diastolic blood pressure values of the control and L-NAME + DEXA groups (P=0.035). In contrast, the differences between the mean diastolic blood pressure values at day 19 was not confirmed statistically (P=0.083, F= 2,506). However, ANOVA for repeated measures in factor time confirmed the effect of time (P=0.001) and its interactions with interventions (P=0.005).

Blood glucose concentrations: The mean blood glucose concentrations of animals are seen in Table 3. Statistical analyses did not confirm the effects of interventions on blood glucose concentrations of the pregnant Wistar albino rats (P=0.088, F=2.424). Similarly, multiple comparisons did not confirm the difference between control and DEXA groups (P=0.090, %95 CI: -5.403682 – 101.320349).

In contrast, the interventions had significant effects on blood glucose concentrations of non-pregnant Wistar albino rats (P=0.008). Mean blood glucose concentrations of control and L-NAME groups was higher than that of the DEXA group (P=0.017 and P=0.025, respectively).

Kidney weights: Only the left kidney's weights were evaluated (Table 3). Statistical analyses indicated significant effects of the interventions on kidney weights of pregnant Wistar albino rats (P=0.05, F=2.929). *Post hocs* revealed higher mean kidney weight of controls than that of DEXA given group (P=0.032).

In contrast, no significant effect of interventions on kidney weights of non-regnant rats could be detected.

Weights of Adrenal Glands: The mean adrenal gland weights of animals are summarized in Table 3.

Statistical analyses showed that the weights of adrenal glands of the pregnant Wistar albino rats were influenced by interventions (P=0.000). *Post hocs* showed that the mean weight of the adrenal glands of L-NAME given group was higher than those of control and L-NAME + DEXA given groups (P=0.038 and P=0.000, respectively). Similarly, the mean weight of the adrenal glands of the DEXA group was higher than that of animals given L- NAME + DEXA (P=0.002). However, interventions had no significant effect on mean adrenal gland weights of the non-pregnant animals.

Urine volumes and urinary protein concentrations: Average urine volumes and protein concentrations in the urine of pregnant and non-pregnant Wistar albino rats are shown in Table 3.

Statistical analyses of the data showed that interventions had no significant effect on 24-h urine volumes of pregnant Wistar albino rats, but their urinary protein excretions were influenced (P=0.010). *Post hocs* revealed significantly lower urinary protein concentrations of DEXA than that of L-NAME + DEXA group (P=0.005).

In non-pregnant rats, neither 24-h urine volumes nor urinary protein excretions were affected by interventions (P>0.05).

Numbers and weights of the fetuses: The mean numbers of fetuses of pregnant Wistar rats and their total weights are seen in Table 3. ANOVA revealed significant effects of interventions (P=0.005). The difference between control and DEXA groups was confirmed in *post hocs* (P=0.003). However, total fetal weighs did not differ among groups.

Discussion

In studies using L-NAME interventions, the body weight changes of animals were rarely assessed. Available two studies indicate possible effects of L-NAME on body weights. Osol et al. (2009) reported that L-NAME given in drinking water (0.5g/L) for 10 days had no effect on body weights of non-pregnant rats, but body weights of pregnant animals receiving L -NAME were in mean 5% lower than those of pregnant controls. Ribeiro et al. (1992) observed also a slowed growth of the rats with chronic L-NAME application. In contrast, short or long-term DEXA causes a decrease in body weight of animals (Beatty et al., 1971; Tonolo et al., 1988; Michel and Cabanac, 1999; Franco-Colin et al., 2006). Tonolo et al. (1988)

reported 10 g weight loss per week when rats were given 10 µg DEXA per day for 4 weeks. Motta et al. (2015) reported that DEXA decreased the body weights of non-pregnant animals significantly, and it abolished the weight gain of pregnant animals. Also, dexamethasone administered by continuous infusions (16 µg/kg/h/sc) to pregnant rats from day 16 of gestation resulted in a 30 g cumulative weight gain from day 15 to 21 of gestation while the cumulative weight gains of controls was 80 g in this time-period (Mostello et al. 1981).

The findings of this study reveal that the effects of L-NAME, DEXA, and L-NAME + DEXA combination depend on the physiological conditions (being pregnant or not pregnant) of female Wistar rats. Compared to controls, L-NAME and DEXA resulted in a slower weight gain while L-NAME + DEXA caused a slight decrease in body weights of pregnant animals within five days. However, all of these interventions caused decreases in mean body weights of non-pregnant rats (Table 1). Thus, these findings indicate that DEXA with or without L-NAME has a significant negative effect on body weights of the rats, in general.

Findings of previous studies suggested also that the applications of L-NAME and DEXA result in significant increases in systolic and diastolic blood pressures of pregnant and non-pregnant female rats (Kemse et al., 2014; Tain et al., 2014; Safaeian et al., 2015). Initially, the mechanism of hypertension by the L-NAME application has been explained *via* NOS inhibition. Findings from recent studies, however, suggested the involvement of a very complex regulation network including several factors in the development of hypertension by L-NAME. These include among others the renal angiotensin-converting enzyme (Giani et al., 2014), thromboxane (Francois et al., 2008), peroxisome proliferator-activated receptor gamma (PPAR-γ) (Kriska et al., 2014) and glucocorticoids (Wallerath et al., 1999).

Observations of modified effects of L-NAME in stress situations with high levels of endogenous glucocorticosteroids or by applications of synthetic glucocorticoids (Li et al., 1992; Wen et al., 2000) indicated possible interactions between L-NAME and glucocorticoids regarding the development of hypertension. Various studies revealed that these interactions have both synergistic and antagonistic properties (Changbin and Baylis, 2000; Li et al., 1992; Wen et al., 2000). Accordingly, as many other effects, the hypertensive effects of glucocorticoids are also mediated partly *via* suppression of the endothelial nitric oxide synthetase (eNOS) expression (Liu et al., 2009). Also, pregnancy seemingly alters the sensibility of the organism to L-NAME and DEXA (Nathan et al., 1995; Li et al., 2003; Losonczy et al., 1996; Troiano et al., 2016). Consequently, the effects of these substances on the cardiovascular system of pregnant and non-pregnant animals may differ. The findings of this study, however, did not support the findings of other studies, which clearly demonstrated that both L-NAME and DEXA applications induce hypertension, and, they have additive or synergic effects when used in combination in this respect (Changbin and Baylis, 2000; Wen et al., 2000; Lou et al., 2001).

The findings are also consistent with previous evidence gathered from human studies that L-NAME significantly increases blood pressure in healthy individuals, but not in hypertensive patients (Calver et al., 1992). However, the finding of this study did not support the hypertensive effects of L-NAME and glucocorticoids in this case DEXA in rats, and so far did not support preliminary studies cited above. The reasons may be multiple. First of all, basal blood pressures of animals in both control groups of this study are relatively high and indicate a possible spontaneous hypertension in the colony. The reason of this in the colony is not known exactly, but, basing to our recent experiences, it is assumed to be a dietary orgin, because of the blood pressure in the colony is normalized by changing diet. There is evidence that the cardiovascular system of diet-induced hypertensive animals is relatively resistant to NOS inhibition (Roberts et al. 2000).

These findings give also evidence that the effects of L-NAME and/or DEXA are modifiable by physiologic condition, in this case, the pregnancy. A finding, which is in accordance with preliminary studies, cited above. Tong et al. (1997) found significant increases in blood glucose concentrations of normotensive and spontaneously hypertensive Wistar-Kyoto rats given 20 mg/kg BW L-NAME for 4 consecutive days. DEXA also induces a significant increase in blood glucose concentrations of rats (Koricanac et al., 2006; Sood and Ismail-Beigi, 2010). However, Motta et al. (2015) reported a significant decrease in fasting glycaemia of pregnant animals which was absent on non-pregnant animals. Also in this study, DEXA failed to increase blood glucose concentrations, but the findings indicated that DEXA might work differently depending on if animals are pregnant or not.

No information was available about the effects of L-NAME on kidney weights. Jennings and Ferguson (Jennings and Ferguson, 1984) could not observe any effect of DEXA on kidney weights in rats, whereas

Rooman et al. (1999) reported a dose-dependent decrease in mice. In this study, only the average kidney weight of pregnant animals was lower than that of controls.

Also, no information was available about the effects of L-NAME on weights of adrenal glands in rats. Lesniewska et al. (1992) applied 15, 30 or 60 mg DEXA per 100 g BW of rats for 7 days and observed a remarkable decrease in their adrenal gland weights. This effect was reversible and resolved within 7 days after the last application. The findings of this study showed that when compared to the control group, a significant increase in adrenal gland weight was seen only in pregnant rats by giving L-NAME. However, the addition of DEXA had an adverse effect to L-NAME alone.

A common finding of L-NAME-induced hypertension is the proteinuria. Rats given L-NAME in drinking water caused a decrease in glomerular filtration rate and a progressive increase in protein excretion in 24-h urine samples (Qiu et al., 1998). In this study, the lowest and highest urinary protein excretions were recorded in DEXA and L-NAME + DEXA given pregnant rats, respectively.

Conclusion

This study is designed to evaluate the effects of L-NAME and DEXA, which were used routinely to produce hypertension in animal models, on blood pressure of female Wistar rats from a breeding colony with higher blood pressures seemingly due to dietary factors. The findings of this study indicate that the interventions caused no further increase in blood pressure in previously hypertensive animals and that the animals used are relatively resistant not only to L-NAME induced hypertension but also to DEXA and to a combination thereof. Thus, assessment of possible mechanisms would be important in explaining the ethiopathologies of resistances and predispositions for the NO- and DEXA-mediated hypertensions or hypertensions with multiple ethiopatogenesis.

References

Adeagbo, A. S., & Triggle, C. R. (1993). Interactions of nitric oxide synthase inhibitors and dexamethasone with alpha-adrenoceptor-mediated responses in rat aorta. British *Journal of Pharmacology, 109*(2), 495-501.

Baker, M., Rahman, T., Hall, D., Avery, P. J., Mayosi, B. M., Cornell, J. M., Farall, M., Watkins, H., & Keavney, B. (2007). The C-532T polymorphism of the angiotensinogen gene is associated with pulse pressure: a possible explanation for heterogeneity in genetic association studies of AGT and hypertension. *International Journal of Epidemiology, 36*(6), 1356-1362.

Beatty, W. W., Scouten, C. W., & Beatty, P. A. (1971). Differential effects of dexamethasone and body weight loss on two measures of activity. *Physiology and Behavior, 7*(6), 869-871.

Bernatova, I. (2014). Endothelial dysfunction in experimental models of arterial hypertension: cause or consequence? *BioMed Research International*, 2014:598271. doi: 10.1155/2014/598271.

Calver, A., Collier, J., Moncada, S., & Vallance, P. (1992). Effect of local intra-arterial NG-monomethyl l-arginine in patients with hypertension: the nitric oxide dilator mechanisms appears abnormal. *Journal of Hypertension, 10*, 1025-1031.

Cediel, E., Vazquez-Cruz, B., Navarro-Cid, J., de las Heras, N., Sanz-Rosa, D., Cachofeiro, V., & Lahera, V. (2002). Role of endothelin -1 and thromboxane A2 in renal vasoconstriction induced by angiotensin II in diabetes and hypertension. *Kidney International. Supplement, 62*(82), 2-7.

Changbin, Q., & Baylis, C. (2000). Dexamethasone worsens nitric oxide inhibition-induced hypertension and renal dysfunction. *American Journal of Hypertension, 13*(10), 1097–2002.

Franco-Colin, M., Villanueva, I., Pinon, M., & Racotta, R. (2006). The effects of sympathectomy and dexamethasone in rats ingesting sucrose. International *Journal of Biological Sciences, 2*(1), 17-22.

Francois, H., Makhanova, N., Ruiz, P., Ellison, J., Mao, L., Rockman, H. A, & Coffman, T. M. (2008). A role for the thromboxane receptor in l-NAME hypertension. *American journal of physiology. Renal Physiology, 295*(4), 1096–1102.

Giani, J. F., Janjulia, T., Kamat, N, Seth, D. M, Blackwell, L. B. W, Shah, K. H, Shen, X. Z, Fuchs, S, Delpire, E, Toblli, J. E, Bernstein, K. E, McDonough, A. A, & Gonzalez-Villalobos, R. A. (2014). Renal angiotensin-converting enzyme is essential for the hypertension induced by nitric oxide synthesis inhibition. *Journal of the American Society of Nephrology, 25*(12), 2752-2763.

Goodwin, J. E, Zhang, J, Gonzalez, D, Albinsson, S, Geller, D. S. (2011). Knockout of the vascular endothelial glucocorticoid receptor abrogates dexamethasone-induced hypertension. *Jornal of Hypertension, 29*(7), 1347-1356.

Jennings, A. S, Ferguson, D. C. (1984). Effect of dexamethasone on triiodothyronine production in the perfused rat liver and kidney. *Endocrinology, 114*(1), 31-36.

Kemse, N. G, Kale, A. A, & Joshi, S. R. (2014). A combined supplementation of omega-3 fatty acids and micro nutrients (folic acid, vitamin B12) reduces oxidative stress markers in a rat model of pregnancy induced hypertension. *PLoS One, 9(*11), 111902.

Koricanac, G, Isenovic, E, Stojanovic-Susulic, V, Miskovic, D, Zakula, Z., & Ribarac-Stepic, N. (2006). Time dependent effects of dexamethasone on serum insulin level and insulin receptors in rat liver and erythrocytes. General Physiology and Biophysics, 25(1), 11-24.

Kriska, T, Cepura, C, Gauthier, K. M, Campbell, W. B. (2014). Role of macrophage PPARγ in experimental hypertension. American journal of physiology. *Heart and Circulatory Physiology, 306*(1), 26-32.

Lesniewska, B, Nowak, K. W, Malendowicz, L. K. (1992). Dexamethasone-induced adrenal cortex atrophy and recovery of the gland from partial, steroid-induced atrophy. *Experimental and Clinical Endocrinology, 100*(3), 133-139.

Li, L., Storey, P., Kim, D., Li, W., & Prasad, P. (2003). Kidneys in hypertensive rats show reduced response to nitric oxide synthase inhibition as evaluated by BOLD MRI. *Journal of Magnetic Resonance Imaging, 17*(6), 671-675.

Li, M., Dusting, G. J., & Whitworth, J. A. (1992). Inhibition of NO synthesis has an additive effect on hypertension induced by ACTH in conscious rats. *Clinical and Experimental Pharmacology and Physiology, 19*(10), 675-681.

Liu, Y., Mladinov, D., Pietrusz, J. L., Usa, K., & Liang, M. (2009). Glucocorticoid response elements and11β-hydroxysteroid dehydrogenases in the regulation of endothelial nitric oxide synthase expression. *Cardiovascular Research Journal, 81*(1), 140-147.

Losonczy, G., Mucha, I., Müller, V., Kriston, T., Ungvari, Z., Tornóci, L., Rosivall, L., & Venuto, R. (1996). The vasoconstrictor effects of L-NAME, a nitric oxide synthase inhibitor, in pregnant rabbits. *British Journal of Pharmacology, 118*(4), 1012-1018.

Lou, Y. K., Wen, C., Li M., Adams, D. J., Wang, M. X., Yang, F., Morris, B. J., & Whitworth, J. A. (2001). Decreased renal expression of nitricoxide synthase isoforms in adrenocorticotropin-induced and corticosterone-induced hypertension. *Hypertension, 37*(4), 1164-1170.

Michel, C., & Cabanac, M. (1999). Effects of dexamethasone on the body weight set point of rats. *Physiology and Behavior, 68*(1), 145–150.

Mostello, D. J., Hamosh, M., & Hamosh, P. (1981). Effect of dexamethasone on lipoprotein lipase activity of fetal rat lung. *Biolology of the Neonate, 40*, 121-128.

Motta, K, Ruiz, M. F., Bordin, S., & Rafacho, A. (2015). Evaluation of lipid homeostasis in the late gestational period of rats exposed to dexamethasone. *Diabetology and Metabolic Syndrome, 7*(1), 74.

Nathan, L., Cuevas, J., & Chaudhuri, G. (1995). The role of nitric oxide in the altered vascular reactivity of pregnancy in the rat. *British Journal of Pharmacology, 114*(5), 955-960.

Osol, G., Barron, C., Gokina, N., & Mandala, M. (2009). Inhibition of nitric oxide synthases abrogates pregnancy-induced uterine vascular expansive remodeling. *Journal of Vascular Research, 46*(5), 478-486.

Pennington, K. A, Schlitt, J. M, Jackson, D. L, Schulz, L. C, & Schust, D. J. (2012). Preeclampsia: multiple approaches for a multifactorial disease. *Disese Models and Mechanisms, 5*(1), 9–18.

Podjarny, E., Losonczy, G., & Baylis, C. (2004). Animal models in preeclampsia. *Seminars in Nephrology, 24*(6), 596-606.

Púzserová, A., Csizmadiová, Z., & Bernátová, I. (2007). Effect of blood pressure on L-NAME-sensitive component of vasorelaxation in adult rats. *Physiological Research, 56* (Suppl. 2), S77-S84.

Qiu, C., Muchant, D., Beierwaltes, W. H., Racusen, L., Baylis, C. (1998). Evolution of chronic nitricoxide inhibition hypertension: relationship to renal function. *Hypertension, 31*(1), 21-26.

Rafikov, R., Fonseca, F. V., Kumar, S., Pardo, D., Darragh, C., Elms, S., Fulton, D., & Black, S. M. (2011). eNOS activation and NO function: structural motifs responsible for the post translational control of endothelial nitric oxide synthase activity. *Journal of Endocrinology, 210*(3), 271-284.

Ribeiro, M. O., Antunes, E., de Nucci, G., Lovisolo, S. M., Zatz, R. (1992). Chronic inhibition of nitric oxide synthesis. A new model of arterial hypertension. *Hypertension, 20*(3), 298-303.

Roberts, C. K, Vaziri, N. D, Wang, X. Q, & Barnard, R. J. (2000). Enhanced NO inactivation and hypertension induced by a high-fat, refined-carbohydrate diet. *Hypertension, 36*, 423-429.

Rooman, R., Koster, G., Bloemen, R., Gresnigt, R., & van Buul -Offers, S. C. (1999). The effect of dexamethasone on body and or gan growth of normal and IGF-II-transgenic mice. *Journal of Endocrinology, 163*(3), 543-552.

Safaeian, L., Ghannadi, A., Javanmard, S. H., Vahidian, M. H. (2015). The effect of hydroalcoholic extract of ferula foetida stems on blood pressure and oxidative stress in dexamethasone-induced hypertensive rats. *Research in Pharmaceutical Sciences, 10*(4), 326-334.

Sood, A., & Ismail-Beigi, F. (2010). Effect of dexamethasone on insulin secretion: examination of under lying mechanisms. *Endocrine Practice, 16*(5), 763-769.

Tain, Y. L, Chen, C. C, Sheen, J. M, Yu, H. R, Tiao, M. M, Kuo, H. C., & Huang, L. T. (2014). Melatonin attenuates prenatal dexamethasone-induced blood pressure increase in a rat model. *Journal of the American Society of Hypertension, 8*(4), 216-226.

Tong, Y. C., Wang, C. J., & Cheng, J. T. (1997). The role of nitric oxide in the control of plasma glucoseconcentration in spontaneously hypertensive rats. *Neuroscience Letters, 233*(2-3), 93-96.

Tonolo, G., Fraser, R., Connell, J. M., & Kenyon, C. J. (1988). Chronic low-dose infusions of dexamethasone in rats: effects on blood pressure, body weight and plasma atrial natriuretic peptide. *Jornal of Hypertension, 6*(1), 25-31.

Török, J. (2008). Participation of nitric oxide in different models of experimental hypertension. *Physiological Research, 57*(6), 813-825.

Troiano, J. A., Potje, S. R., Graton, M. E., Cavalari, P., Pereira, A. A., Vale, G. T., Nakamune, A. C., Sumida, D. H., Tirapelli, C. R., Antonialli, C. (2016). Decreased reactive oxygen species production and NOX1, NOX2, NOX4 expression contribute to hyporeactivity to phenylephrine in aortas of pregnant SHR. *Life Sciences Journal, 144*, 178 -184.

Wallerath, T, Witte, K, Schafer, S. C, Schwarz, P. M, Prellwitz, W., Wohlfart, P., Kleinert, H., Lehr, H. A., Lemmer, B., & Förstermann, U. (1999). Down-regulation of the expression of endothelial NO synthase is likely to contribute to glucocorticoid-mediated hypertension. *Proceedings of the National Academy of Sciences of the United States of America, 96*(23), 13357-13362.

Wen, C., Li, M., & Whitworth, J. A. (2000). Role of nitric oxide in adrenocorticotrophin-induced hypertension: L-arginine effects reversed by N-nitro-L-arginine. *Clinical and Experimental Pharmacology and Physiology, 27*(11), 887-890.

WHO. (2005). Risking death to give life. World Health Organization, Geneva, Switzerland.

Yallampalli, C., Byam-Smith, M, Nelson, S, Garfield, R. E. (1994). Steroid hormones modulate the production of nitric oxide and cGMP in the rat uterus. *Endocrinology, 134*, 1971-1974.

Zhang, L. N., Ji, L. D., Fei, L. J., Yuan, F., Zhang, Y. M., Xu, J. (2013). Association between polymorphisms of alpha-adducin gene and essential hypertension in Chinese population. *BioMed Research International, 2013*:451094. doi: 10.1155/2013/451094.

JIVS JOURNAL OF ISTANBUL VETERINARY SCIENCES

Comparison various body measurements of Aksaray Malakli and Kangal Dogs

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Research Article

Volume: 2, Issue: 3
December 2018
Pages: 86-91

ABSTRACT

This study was conducted to compare and evaluate some body measurements of Kangal Dog and Aksaray Malakli Dog breeds. The study group consist of dogs with an age range from 2 to 5 years. Samples for Kangal dogs were obtained from Sivas and for Aksaray Malakli dogs from Aksaray province. Observations from ten dogs from both species (5 male and 5 female), in total 20 adult dogs were used for this study. Some of the morphological characteristics as black mask around the head, cream fur colour and holding spiral tail were found evident for Kangal dogs while in all Aksaray Malakli dogs the head and body size, thimbleful black mask around the head, and 6th nail existence were determined as descriptive differences between the genotypes. While the effect of gender on muzzle length, body index and bone index was not found to be significant, it was found significant for other body measurements. The rump lengths in male Aksaray Malakli dogs were significantly larger than male Kangal dogs (P<0.001). However, this trait was not significant for female dogs. This can be associated with the significant interaction between breed and gender (P<0.01). Body index also showed the same trend. Also, withers height and head circumference traits were found significant (P<0.001) for male and female Aksaray Malakli dogs and for male and female Kangal dogs (P<0.01). This can be a reason for the significant interaction between breed and gender. As a conclusion, although there are some phenotypic similarities between Kangal and Aksaray Malakli dogs, obtained body measurements showed significant differences. Furthermore, Aksaray Malakli dogs tend to have bigger body structure than Kangal dogs.

Article History

Received: 21.09.2018
Accepted: 01.11.2018
Available online: 12.11.2018

Keywords: Kangal Dog, Aksaray Malakli Dog, Turkish Shepherd Dog, body measurements

DOI: 10.30704/http-www-jivs-net.462546

To cite this article: Oğrak, Y. Z., Öztürk, N., Akın, D., & Özcan, M. **(2018). Comparison various body measurements of Aksaray Malakli and Kangal Dogs.** ***Journal of Istanbul Veterinary Sciences. 2*****(3), 86-91. Abbreviated Title:** ***J Ist Vet Sci***

Introduction

Genetic information of the last a hundred thousand years reveals that a large number of dog breeds with different behavioural traits and working purposes, which are morphologically and physiologically different, have been formed by genetic and environmental factors. *The Federation Cynologique Internationale (FCI)* recognises 346 different dog, which are grouped in ten categories whose specific breed characteristics have been defined (Anonymous (b), 2018; Oğrak, 2009). However, it can be safely argued that the number of dog genotypes worldwide is much more than those recognized breeds.

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Journal homepage: www.jivs.net
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Dog Breeds and Cynology Federation of Turkey (KIF); the only full member of FCI from Turkey, describes the Kangal dogs as a separate breed. Besides, Kangal Dogs are officially recognized by FCI as the only Turkish dog breed since 15th June 2018. Kangal dogs are specific for Turkey and the recognisability of Kangal dogs has risen continuously due to their morphological and behavioural characteristics. Their success in the protection of livestock against attacks of wild animal, their loyalty towards their owners, their adaptability to the geography and local climate conditions increase Kangal dog's recognisability throughout the world. Kangal dogs have been taken to almost all continents of the world and they successfully adapted to the regions where they were taken. This dogs are mainly bred in order to protect the livestock against wild animal attacks. The breeders' associations/clubs related with Kangal dogs established in many countries, make important contributions to the recognition of this dog breed via their activities (Bruseke, 2003; Kocher, 2003; Marker, 2005; Reed, 2003; Anonymous (b), 2018).

It has been found that Kangal dogs do their herd guarding duties perfectly even against wild predators, thus the breeders can prevent animal losses without harming wildlife in various European countries, USA, Australia as well as some African countries (Marker, 2005).

Kangal dogs are reported to be loyal and affectionate to their owners, compassionate to their family but wary of strangers, stable against threats, brave and confident, at the same time naturally independent while on duty. Furthermore, they are characterised by black masks covering the muzzle on a dun to light grey coloured coat, medium size droopy ears, and when alert the tail is known to be curled over the back (Özcan et al., 2005; Oğrak, 2009).

Academic studies which focus on Kangal dogs in Turkey are becoming increasingly important since the early 1990s. Those studies often aim to reveal the relationship between morphological, physiological and behavioural traits and genetic structure of the dog.

A significant number of these academic studies collected information from Kangal dogs of different regions of Turkey and mostly from dog breeding farms (Atasoy et al., 2005; Daşkıran, 2007; Özbeyaz, 1994; Özcan and Altınel, 1997; Tepeli and Çetin, 2003). However, observations from Kangal dogs grown in Sivas region was so far limited.

KIF and FCI accept the height at the withers 72-78 ± 2 cm for male and 65-73 ± 2 cm for female dogs (Anonymous (b), 2018; Anonymous (c), 2018). Various studies reported that the height at withers varies between 62.4-75.69 cm, the body length between 66.2-75.67 cm, the chest circumference between 18.51-23.20 cm, the chest width 23.87 cm, the front carpus circumference between 12.12-14.00 cm, the back carpus circumference 13.37 cm, the head length between 23.8-29.0 cm, the face length between 10.8-14.0 cm for Kangal dogs in a private dog breeding farm (Atasoy et al., 2005; Daşkıran, 2007; Özbeyaz, 1994; Özcan and Altınel, 1997; Tepeli and Çetin, 2000). Another study by Urosevic et al. (2012) reported the height at withers as varying between 72.54 and 68.60 cm, and body length between 82.11 and 78.50 cm for the 51 male and 34 female Kangal dogs, 85 in total, based on the observations coming from villages in Sivas.

Aksaray Malakli dogs are guarding dogs that is grown in the Aksaray province in the Central Anatolia Region. Although Aksaray Malakli dogs show similarities to the Kangal dogs, it is distinguished from Kangal dogs of Sivas origin with the higher body weights and the body size and it is considered as a different dog genotype. Although the origin of both dogs are from the same genetic pool, the crossbreeding and selection methods, which was done by the owners, considering the body size resulted in some similarities in terms of the morphological traits such as coat colour, spots on head and on body. However, Aksaray Malakli dogs are usually associated with higher live body weight, larger body and head structure, droopy lips, eye lid and cheek, strong muscular body and excessive aggression. As such, they did not qualify as a genotype in terms of long-term follow-up and protection of domestic herds. In order to determine the various traits for Aksaray Malakli dogs and to register this breed as a special breed for Aksaray, Aksaray governor's office, Aksaray municipality, some universities and Dog Breeds and Cynology Federation are cooperating (Aslım and Sinmez, 2017; Anonymous (a), 2018).

A study investigating Aksaray Malakli dogs reported the height at withers to vary between 78.36-72.98 cm, the rump height between 78.65-72.87 cm, the body length between 82.68-79.02 cm, the girth circumference between 89.89-84.47 cm, the front carpus circumference between 15.76-14.71 cm, leg length between 32.98-30.92 cm, face length between 12.55-11.80 cm for male and female dogs, respectively (Atasoy et al., 2014). In the same study, the breed characteristics of Aksaray Malakli dogs were reported as having a black mask, large forehead and body, drooping lips, large ears, short hairy and non-curled tail.

The present study was carried out to determine the body measurements and ratios of Kangal dogs in Sivas province and Aksaray Malakli dogs in Aksaray province, which were grown in different commercial animal breed farms and do not originate from a specific dog breeding farm, and also to compare these two breeds/genotypes.

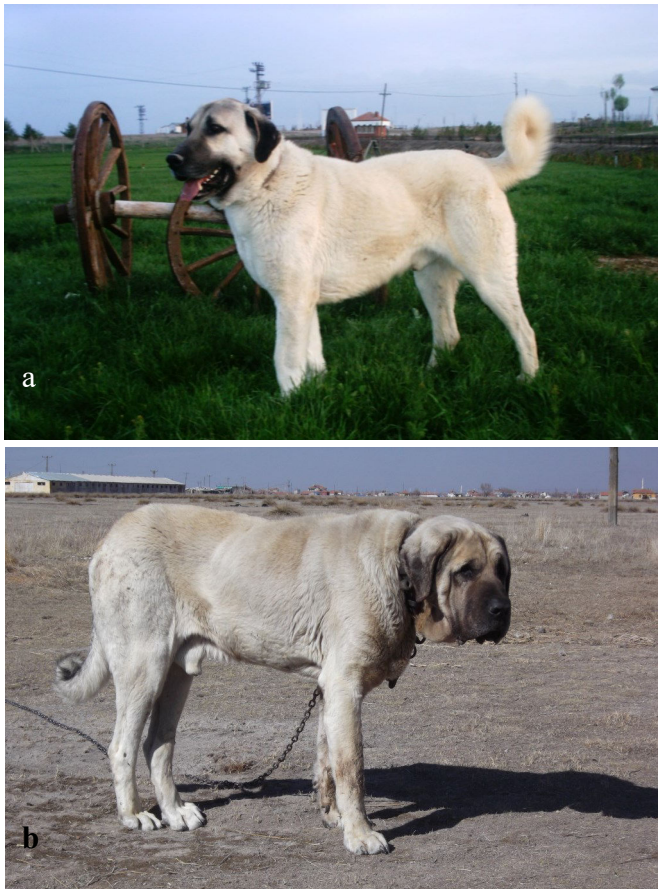

Figure 1. Kangal dog (a) and Aksaray Malakli dog (b).

Materials and methods

The material of this study was formed by 10 dogs from each breed (5 male, 5 female), 20 adult dogs in total. Kangal dogs were from Sivas and Aksaray Malakli dogs were from Aksaray, both breeds were obtained from the villages and not from the commercial dog breeding farms.

In order to determine and compare the body measurements of dogs, 12 different parameters were measured. The wither height, rump height and body length measurements were obtained using a measuring stick, the chest girth, front carpus circumference, head length, head circumference, muzzle length and muzzle circumference measurements were obtained using a tape measure. General views of dogs were evaluated and recorded.

The measurement localizations described below (Drobnjak et al., 2010; Oğrak et al., 2014):

***Wither height*:** The distance between ground to the highest point of wither (cm),

Rump Height: The distance between ground to the highest point of sacrum (cm),

Body length: The distance between Caput humeri and ischii (cm),

Chest girth: Measurement from the back of the scapula perpendicular to the body axis all the way around costa (cm),

Front carpus circumference: Perimeter from the narrowest point of Metacarpus (cm),

Head length: The distance between Crista occipitalis and end of the incisivum (cm),

Head circumference: The circumference from the widest part of right and left arcus zygomaticus (cm),

Muzzle (Face) Length: The distance between tip of the nose and eye's base (cm),

Figure 2. Head of Kangal dog (a) and head of Aksaray Malakli dog (b).

Muzzle Circumference: The circumference of the nose below the eyes (cm).

The body index values obtained by using the averages of the measurements and the formulas used in the calculation given below (Oğrak et al., 2014):

Body Index: Body length / Withers Height x 100 (%),

Massiveness Index: Chest girth / Withers Height x 100 (%),

Bone Index: Front carpus circumference / Withers height x 100 (%).

The least squares mean of the data which was obtained from measurements of the dogs, the effect of breed and gender and their interactions on these variables were analyzed by GLM procedure using SPSS (IBM SPSS Statistics, Version 23.00 IBM Corporation, USA) package program.

Results

Means of various body measurements of Kangal dogs and Aksaray Malakli dogs and the effects of genotype and sex on these parameters were given in Table 1.

The effect of genotype on body measurements were found significant for Aksaray Malakli dogs. On the other hand, the effect of sex was found significant for male dogs except muzzle length. When the effect of genotype x sex interaction on various body measurements were investigated, its effect on withers height, rump height and head circumference were found to be significant. Various body measurements were used in calculation of body, massiveness and bone index values. In addition to this, genotype and the two-way interaction had significant effect on body index, while sex had an effect on massiveness index (P<0.05).

Discussion

When the measured parameters in the study are evaluated, Aksaray Malakli dogs appear to have a significantly larger body conformation compared to Kangal dogs. Even though the morphological breed/ genotype definitions were similar, having a larger body conformation can be considered as an important differentiation point. As the body size in dogs can be evaluated as a criterion in the definition of breed/ genotype, as time passes, it may lead to differentiation in the behaviour and sense of duty of these dogs. Pinscher breeds can be an example to this situation, with common features in terms of morphological parameters (bristle length, post colour, marks, etc.). Miniature Pinscher and Doberman Pinscher are accepted as two different breeds based on body size, and duty and behaviour differences (Anonymous (b), 2018). Miniature Pinscher is defined as belonging to "Toy Groups" while Doberman Pinscher belongs to "Working Groups" amongst the dog breeds (Anonymous (d), 2018; Anonymous (e), 2018). A similar differentiation and identification can also be made for Aksaray Malakli dogs and Kangal dogs.

Table 1. Some body measurements of Kangal Dog and Aksaray Malakli Dog.

Traits	Genotype		Gender			Significance		
	Kangal Dog	Aksaray Malakli Dog	Female	Male	SEM	Genotype	Gender	Genotype X Gender
Withers Height	70.75	80.75	71.40	80.10	0.479	***	***	**
Rump Height	71.85	78.50	71.45	78.90	0.598	***	***	**
Body Length	82.90	99.60	87.10	95.40	1.090	***	***	N.S
Girth Circumference	94.00	104.20	96.40	101.80	0.862	***	**	N.S
Frontal corpus circum-ference	17.30	19.00	16.90	19.40	0.180	***	***	N.S
Head Length	32.80	35.20	31.50	36.50	0.468	*	***	N.S
Head Circumference	55.70	65.90	59.50	65.10	0.549	***	***	*
Muzzle Length	13.00	15.40	13.90	14.50	0.255	***	N.S	N.S
Muzzle Circumference	30.30	41.60	34.60	37.30	0.411	***	**	N.S
Body Index	117.14	123.63	121.71	119.06	1.181	*	N.S	*
Massiveness Index	132.95	129.63	135.13	127.44	1.348	N.S	*	N.S
Bone Index	22.45	23.53	23.68	24.29	0.230	N.S	N.S	N.S

*P<0.05, **P<0.01, ***P<0.001, N.S. : Non significant.

When the obtained body measurements of the Kangal dogs were compared with the values obtained from other studies, it was observed that there were similarities between Kangal dogs of this study and Kangal dogs from villages and dog breeding farms in Sivas in terms of the body measurements (Uroseviç et al., 2012; Daşkıran 2007). However, even though the withers height was found to be similar for Kangal dogs from this study and Kangal dogs from other regions, the values for the dogs from other regions was higher for body length, chest structure, carpus width and especially the head traits (Atasoy et al., 2005; Özbeyaz, 1994; Özcan and Altınel, 1997; Tepeli and Çetin, 2000).

These results imply that there might be regional differences in terms of the body size, possibly related with the different adaptation, management nutrition and utility traits of the dogs, also due to the differences between dog owners' preferences in crossbreeding and selection criteria. Although Kangal dogs that are bred in different regions have same and/ or similar origin, changes in selection criteria and inbreeding options might be the reason of the differences obtained in this study. The differences in head traits may also show that Kangal dogs from other regions have the tendency of disintegration from their genetic codes. The withers height reported by KIF (or FCI) was higher than the values obtained in this study. The higher withers height as was reported by KIF in order to define the Kangal breed could be related with the quality of the material. KIF evaluated the Kangal dogs from Sivas and Ankara provinces of Turkey in order to identify breed standards for Kangal dogs having common morphological traits.

The measurement results of Aksaray Malakli dogs were found higher than those by Atasoy et al. (2014). Limited number of dogs included in that study and the material of Aksaray Malakli dogs, which were the popular dogs of well-known people may have led to these differences. Only the effect of breed on body index score was found significant among all indexes, and this might be explained by the limited number of observations.

The higher mean scores of the body and bone index of Aksaray Malakli dogs shows that the shape is closer to a rectangle and bone and/or skeleton structure is proportionally heavier. However, in terms of the massiveness index, which points out the chest capacity, the higher values of Kangal dogs were in contrast with other body measurement and ratios. The fact that Kangal dogs are always used for herding purposes while Aksaray Malakli dogs are used for guarding purposes due to their massive and bulky bodies could explain this.

Conclusions

In conclusion, it was observed that Aksaray Malakli dogs, which have similarities to Kangal dogs in terms of the morphological traits, have more massive body structure than Kangals. The body index scores and the assumption of limited movement ability and durability of Aksaray Malakli dogs is related to the different utility purposes and behavioural traits of these dogs.

Detecting different traits of Aksaray Malakli dogs is important in terms of defining Aksaray Malakli as a different breed. In future, it will be useful to study the different aspects of dogs via new comparative studies, to investigate the genetic structure and disposition of the dogs, to determine the breed standards properly in terms of the official formations, to reveal the breed specific selection and pedigree breeding models, and to determine and describe Turkish dog breeds and protecting local genetic sources.

Acknowlegments

Authors would like to thank to Yunus Toker for his contributions to this study. Also the authors would like to thank to Muammer Tıpırdamaz, the president of the Dog Breed Association of Aksaray Malakli dog.

References

Anonymous (a). (2018). Retrieved from: http://www.aksaraykulturturizm.com/tr/haberler-duyurular/aksaray-malaklisi-irk-tespit-olcum-calismasi-yapildi_15-05-2016

Anonymous (b). (2018). Retrieved from: http://www.fci.be/en/Nomenclature/

Anonymous (c) (2018). Kangal çoban köpeği. Retrieved from: http://www.kif.org.tr/yerli-irklar/kangal.html

Anonymous (d). (2018). Doberman-pinschers. Retrieved from: https://www.akc.org/dog-breeds/doberman-pinscher/

Anonymous (e) (2018). Miniature-pinschers. Retrieved from: https://www.akc.org/dog-breeds/miniature-pinscher/

Aslım, G., & Sinmez, Ç. Ç. (2017). Aksaray ili folklorunda Aksaray Malaklısı yetiştiriciliği. *Eurasian Journal of Veterinary Sciences*, *33*(3), 148-157.

Atasoy, F., Ünal, N., Kanlı, O., & Yakan, A. (2005). Damızlık Kangal köpeklerinde canlı ağırlık ve bazı vücut ölçüleri. Lalahan Hayvancılık Araştırma Enstitüsü Dergisi, 45(1), 25-29.

Atasoy, F., Erdoğan, M., Özarslan, B., Yüceer, B., Kocakaya, A., & Akçapınar, H. (2014). Malakli Karabaş köpeklerde bazı morfolojik ve genetik özellikler, Ankara Ünversitesi Veteteriner Fakültesi Dergisi, 61, 125-132.

Bruseke, W. (2003, July 8). *The situation of the Kangal Dog in Germany*. Paper presented at the 1. International Kangal Dog Symposium, Sivas, Turkey.

Drobnjak, D., Matić, V., Miliyević, D., (2010). *Eksterijer pasa osnove procene*, Beogard, Serbia:Tipo Štampa.

Daşkıran, İ. (2007). Body weight and some morphological characteristics of Kangal dogs. *Journal of Animal and Veterinary Advances, 6*(3), 368-370.

Kocher, S. (2003, July 8). *Stewardship of the Kangal Dog Breed: Problems and progress*. Paper presented at the 1. International Kangal Dog Symposium. Sivas, Turkey.

Marker, L. L. (2005, July 8). *Evaluating the effectiveness of livestock guarding dogs as a method of conflict resolution*. Paper presented at the 2nd International Kangal Dog Symposium. Sivas, Turkey.

Oğrak, Y. Z. (2009). Kangal köpeği ve yetiştiriciliği. *Uzunyayla Dergisi, 1*(1), 13-16.

Oğrak, Y. Z., Yoldaş, A., Uroševic, M., & Drobnjak, D. (2014). Some morphological traits of Tarsus Çatalburun breed of Turkish hunting dog. *Eurasian Journal of Veterinary Sciences,*1, 25-29.

Özbeyaz, C., (1994). Kangal köpeklerinde bazı morfolojik özellikler. *Lalahan Hayvancılık Araştırma Enstitüsü Dergisi, 34*(1-2), 38-46.

Özcan, M., & Altınel, A. (1997). Kangal köpeği ve Alman çoban köpeği ırklarının bazı morfolojik özellikleri. *İstanbul Üniversitesi Veteriner Fakültesi Dergisi, 23* (2), 413-422.

Özcan, M., Yılmaz, A., & Oğrak, Y. Z. (2005, July 8). *The breed standart of Kangal Dog.* Paper presented at the 2nd International Kangal Dog Symposium. Sivas, Turkey.

Reed, S., (2003, July 8). *Turkish Dogs in U.K. & Europe.* Paper presented at the 1. International Kangal Dog Symposium. Sivas, Turkey.

Tepeli, C., & Çetin, O. (2000). Kangal Irkı Türk Çoban Köpeklerinde büyüme, bazı vücut ölçüleri ve döl verimi özelliklerinin belirlenmesi. 1. Büyüme ve bazı vücut ölçüleri. *Veteriner.Bilimleri Dergisi, 16*(1), 5-16.

Tepeli, C., Çetin, O. (2003, July 8). *A Study on head measurements of Kangal and Akbaş Turkish Shepherd Dogs*. Paper presented at the 1. International Kangal Dog Symposium. Sivas, Turkey.

Urosevic, M., Drobnjak, D., & Ograk Y. (2012). Body format of the Kangal Turkish shepherd dog. *Agro-knowledge journal, 13*(2), 209-216.

JIVS JOURNAL OF ISTANBUL VETERINARY SCIENCES

Prevalence and potential risk factors of bovine fasciolosis in Gurage Zone, Abeshege district, Southern Ethiopia

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Research Article

Volume: 2, Issue: 3
December 2018
Pages: 92-96

ABSTRACT

A cross-sectional study aimed at estimating the prevalence and assessing the potential risk factors of bovine fasciolosis was carried out from November, 2011 to April, 2012 in Abeshege district of Gurage Zone. A total of 288 faecal samples were collected directly from the rectum of cattle and examination using sedimentation technique was performed. Overall 142 (49.3%) cattle were found positive for faecal fluke egg detections. No significant variation was observed with feeding system, body condition, breed, sex, age, peasant association (PA) and herd size considered as potential risk factors (P>0.05). However, water source was the only factor found to be significantly associated (P<0.05) with occurrence of infections where cattle drinking water form rivers had 54.55% higher than those cattle getting water from other sources. The present study suggests that Fasciola infections in cattle is high and represent one of the constraints to livestock development in the study area. Hence, good management practice including provision of properly dried hay for those zero grazing cattle, an alternative use of lands of pasture, fluke elimination by regular treatment, and further detailed study could provide valuable information that foster local planning and implementation of a more sustainable fasciolosis control strategy for the district.

Article History

Received: 08.10.2018
Accepted: 13.11.2018
Available online: 14.11.2018

Keywords: Abeshege, bovine, fasciolosis, Gurage, prevalence, risk factors

DOI: 10.30704/http-www-jivs-net.467948

To cite this article: Dilbato, T. & Bekele, J. **(2018). Prevalence and potential risk factors of bovine fasciolosis in Gurage Zone, Abeshege district, Southern Ethiopia.** ***Journal of Istanbul Veterinary Sciences. 2*****(3), 92-96.**
Abbreviated Title: ***J Ist Vet Sci***

Introduction

Ethiopia has high livestock population, but productivity is very low as a result of disease, malnutrition and other management problems. Fasciolosis is one of major parasitic diseases contributing to loss in productivity (Abebe, 1992; FAO, 1993). Fasciolosis is caused by a leaf-shaped flukes whose anterior end is usually prolonged to the shape of cone. They are responsible for widespread morbidity and mortality in sheep and cattle characterized by weight loss, anemia and hypoproteinemia. The two most important species are Fasciola hepatica which has worldwide distribution but mostly found in temperate and cooler areas of high altitude in the tropics and sub tropics while Fasciola gigantica is predominant in tropical areas including parts of Africa and Asia (Urquhart et al., 1996; Radostatis et al., 2007).

The prevalence of fasciolosis due to F. hepatica and F. gigantica in Ethiopia has long been known and its prevalence has been reported by Dejene (1987), Mitiku (1988) and Abebe (1990). Bovine fasciolosis is economically very important in that causing direct and indirect loss. The direct losses due to fasciolosis are

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Journal homepage: www.jivs.net
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host mortality and liver condemnation, whereas indirect losses may occur in from of losses in body weight and reduced weight of lambs from infected ewes and decreased wool production, increased susceptibility to secondary infections and the expense of control measures (Jobre et al., 1998: Asrat et al., 2007).

The parasite lives part of its life in aquatic snails which acts as an intermediate host and found in and around wet areas such as waterholes and marshy. Farm animals are likely to pick up the infective stage of the parasite (metacercariae) if they drink from these sources (Okwole et al., 2000). Factors of stability are longevity of infection, chronic egg production of adult flukes (some 20000 egg or more/fluke /day) in sheep, availability of moisture (number of fluke larvae and snails a mean day and weight) temperature of at least 10 0C is the factor of instability (Kassai, 1999).

No study and published reports are available in some parts of the country especially Gurage zone, Southern Nations, Nationalities and Peoples Regional State (SNNPRS) where the ecological conditions are likely to serve as favorable for the intermediate host and hence dissemination of infections to ruminants. Therefore, the main objective of this research is: To estimate the prevalence of fasciolosis in cattle in Abeshege district of Gurage zone. And to assess the potential risk factors associated with the occurrence of the disease.

Materials and methods

Description of study area: The study was conducted in Abeshege district of Gurage zone. Abeshege district is one of the 13 districts of Gurage zone, located in the Southern Nations Nationalities and People Region of Ethiopia, 150 km away from Addis Ababa at 080 17' 251'N latitude and 0370 46` 023`` E longitude. Abeshege is bordered on the south by the Wabe River which separates it from Cheha district, on the west and north by the Oromia Region and on the east by Kebena district. The altitude of the district ranges from 1500-1850 meter above sea level with 75% Woyna dega and 25% kola agro-ecological zones. The annual temperature and rainfall ranges are 11-220C and 800-1200 mm, respectively. The society based on mixed farming system which include both crops and livestock production. The livestock population includes: 40.204 bovine, 4.217 equine, and 7.624 caprine, 1.825 ovine and 35.680 poultry. The most important food crops produced in the area are maize, sorghum, pepper, teff, haricot bean, and soybean (ADADO, 2004). The district (Abeshege) has 64,251 inhabitants (33.983 men and 30.268 women) with an area of 559 km2 with density of 114.9 inhabitants per km2 (ADADO, 2004).

Study animals: The study animals were different breed of cattle in Abeshege district. The animals in the study period were kept under different agro-ecologies, age groups and body conditions were recorded. The study involved 288 cattle from selected peasant associations (PA) of Abeshege district.

Study design: The cross- sectional study design type was used from November, 2011 to April, 2012 to estimate the prevalence and potential risk factors of bovine fasciolosis in Abeshege district.

Sample size and sampling procedures: Animals included in the study were selected with simple random sampling technique. The total number of cattle required for the research was calculated based on the formula given by Thrusfield (1995). For this study, 25% prevalence reported by Mulugeta (2008) in Hawassa was used to calculate the sample size using the following formula.

$$N = \frac{1.96^2(Pex)(1-Pex)}{d^2}$$

Where, N = Sample size
Pex = Expected prevalence
d = Desired level of precision (5%)
1.96 = Value of Z at 95% confidence interval

$$\text{Then, } N = \frac{1.96^2(0.25)(1-0.25)}{(0.05)^2} = 288$$

The expected prevalence of fasciolosis in Hawassa was 25% (Mulugeta. 2008).

Study methodology:

Coprological examination: Faecal samples were collected directly from the rectum of randomly selected animals and examination carried out in the field as much as possible. The samples were collected in tightly closed universal bottles and examined for presence of Fasciola eggs. Faecal examinations for fluke eggs usually require use of faecal sedimentation (Kassai, 1999). Accordingly, a total of 288 faecal samples were collected and processed.

Sedimentation procedures concentrate both eggs and faeces at the bottom of a liquid medium (water) and help to detect fluke eggs that have too high specific gravity (Hendrix, 1998). Therefore, the samples were analyzed using sedimentation procedure to demonstrate liver fluke eggs which are large, golden, and yellow in color. Briefly, about 3 grams of faeces as weighed and 42ml of tap water was poured to it. It was thoroughly mixed with a stirring device. The suspension was then filtered through a tea strainer. The filtered suspension was poured in to test tube which stood in the rack. The test tubes with filtered material was put in centrifuge

and centrifuged for three minutes for 1200 rpm. The supernatant was carefully discarded after centrifuged. The resulting sediment was stained by adding two drops of methylene blue and shacked carefully. A small drop of the stained sediment was transferred to a microscope slide using a pipette. Cover slip was put to over the droplet. It was then examined under microscope at 10 x objective.

Data on hypothesized risk factors like epidemiological area (peasant associations), breed, sex, age, body condition, watering source, feeding system and herd size of the study cattle were recorded. These data were collected by asking the owner on feeding system, water source, herd size and age of the animal and body condition. By visual examination the breed and sex of animal were registered as being local and cross breed: and male and female. According to Dabas et al., (2007), the age was categorized in to calf (0-1 year), yearling (1-3 year), and adults (>3 year) based on information from the owner, dentition and observation of the horn of study animals.

Data management and analysis: The findings from coprological examinations and the epidemiological data were recorded in Microsoft Excel spreadsheet and all analysis was conducted using STATA 11. Chi-square (x2) test was used to assess the association between prevalence and associated risk factors. In all cases of the analysis the confidence interval was set at 95% and P values less than 0.05 was considered significant.

Results and Discussion

Prevalence and Potential risk factors: Out of the total 288 faecal samples examined, 142 (49.3%) were found positive for Fasciola eggs. There was no significant variation (x2=0.63: P>0.05) observed in prevalence between the different (PAs) found in the study district as the prevalence only ranged from 46.43% at Laygnaw Garaba to 52.12% in Ambelta (Table 1).

Table 1. Prevalence of bovine fasciolosis in different Kebele of Abeshege district.

Kebele	Examined	Positive	Prevalence (%)
Ambelta	46	24	52.12
Mammedie	58	29	50
Laygnaw Garaba	56	26	46.43
Tachegnaw Garaba	62	29	46.77
Tatesa	66	34	51.5
Total	288	142	49.3

$X^2 = 0.63$; $P = 0.96$

Moreover, there was no significant variation (P>0.05) observed in terms of breed, sex and age of animals basis of faecal egg findings (Table 2).

Table 2. Prevalence of bovine fasciolosis based on breed, sex and age as risk factors.

Risk Factors		Examined	Positive	Prevalence	X^2	*P*-value
Breed	Local	182	91	50	0.09	0.75
	Cross	106	51	48.11		
	Total	288	142	49.3		
Sex	Male	119	59	49.58	0.006	0.94
	Female	169	83	49.11		
	Total	288	142	49.3		
Age	Calves	28	11	39.3	1.29	0.52
	Young	87	43	49.4		
	Adult	173	88	50.86		
	Total	288	142	49.3		

And again there was no significant variation (P>0.05) revealed in terms body, feeding and herd size whereas significant difference was noted with regard to water source (Table 3).

Table 3. Prevalence of bovine fasciolosis based on body condition, feeding, herd size and water source.

Risk factors	Examined	Positive	Prevalence	X^2	*P*-value
Body Condition					
Poor	45	27	60		
Medium	99	47	47.47	2.44	0.295
Good	144	68	47.22		
Total	288	142	49.3		
Feeding					
Grazing	206	109	52.91		
Zero grazing	37	15	40.54	3.76	0.15
Tethering	45	18	40		
Total	288	142	49.3		
Water source					
River	187	102	54.55	13.61	0.001
Pond	61	31	50.82		
Pipe	40	9	22.50		
Total	288	142	49.3		
Herd size					
<10	48	20	41.67		
10-30	101	46	45.54	3.29	0.192
>30	139	76	54.68		
Total	288	142	49.3		

The overall prevalence of bovine fasciolosis using coprological examination in the current study area is 49.3%. This finding shows a lower prevalence as compared to the results of other workers in other parts of the country such as, 80-89% in Debre Berhan (Dagne, 1994) cited in (Yilma and Mesfin, 2000), 82.5% in Western Shewa (Beyene, 1994), and moderately

higher as compared to 25% by Mulugeta (2008) in Hawassa, 28% recorded by Melaku (1991) in Chilalo. However, this finding was comparable to the prevalence of 53.72% reported by Abebe (1990) at Arsi administrative region. The differences observed between this study and the other works might be due to differences in ecological and climatic conditions which may vary from place to place and also within the same area from year to year.

The prevalence of fasciolosis in the district only varied from 46.43% in Laygnaw Garaba to 52.12% in Ambelta (PA). Such little ranges between PAs emerged to reveal no significant variations between them in detections of faecal eggs. This might be due to little variation in the ecological conditions in the district as well as access of metacercariae to cattle of all PA with the pasture harvested from the border of the rivers and marshy areas and/ or by grazing or watering in the shore of rivers as well as marshy areas, There was no significant variation between local and cross bred cattle that the prevalence in local breed was 50.0% while 48.11% in cross breed cattle. This might be due to an access of gaining metacercariae to both breed equally in either grazing or zero grazing with hay having sufficient moisture content for the survival of metacercariae to infect animals.

In the present study, there was no statistically significant association (P>0.05) in prevalence between female (49.11%) and male (57.98%) cattle. This finding showed that both sexes are equally susceptible and exposed to the infection and this might be due to grazing of both sexes in metacercariae contaminated pasture and swampy or marshy area. The current work indicated that there was no statistically significant association between different age groups of the study animals with prevalence of fasciolosis. It was 39.3% in calves, 49.4% yearlings and 50.86% in adults. Since most of the time cattle of all ages access the same pasture zones, infection is picked up without regard to age with young cattle getting infected almost immediately they are introduced to full grazing (Phiri et al., 2005).

Our findings also showed that there was no statistically significant difference (P>0.05) in prevalence among different body condition groups of study animals. It was 60% in poor, 47.47% in medium and 47.22% in good body condition animals. The prevalence was moderately high in poor body condition animals and similar in medium and good body condition animals. This might be due to animals with chronic liver fluke disease are often in poor body condition (Mc Gavin and Zachary, 2007).

In the present study, there was no significant association between prevalence and the feeding system of the study animals. This might be due to lack of wide ranging disparity among the feeding system in terms of ecological factors and difference in microclimate for the snail intermediate host and the parasite Fasciola. In the same way, presence of annual over flooding during the rainy season leaving pockets of water bodies, presence of marshy area for long period during the dry season, slowly river and swampy parts of the lakes and the river borders are contributory. It is a clear practice that people in this area usually graze their animals, particularly cattle, in swampy area and similar ecosystem as there is shortage of pasture mainly during drier months of the year. Such ecological conditions are considered favourable for breeding and survival of the intermediate host snail and the parasite (Urquhart et al., 1996).

Statistical analysis indicated that there was significant difference between prevalence and different water source for study animals (P<0.05). The high prevalence (56.04%) was recorded in those cattle drinking water from the river and this might be due to the chance of getting metacercariae during grazing on the border of the river and drinking slowly flowing river. The prevalence was low (22.5%) in cattle getting water from the pipe. In this case the source of metacercariae could only be from feed such as hay harvested from swampy areas having sufficient moisture content for survival of metacercariae. Furthermore, there was no significant variation among the herd size (P>0.05). The reason might be due to lack of ecological variability though it was anticipated that concentration of animals in one place facilitates the development of the parasite. The concentration (high herd size) is conductive to heavy contamination and distribution of the herd (low herd size) over a large area, decreased probability of infection, especially in herd grazing around a water hole (Fischer and Say, 1989).

Conclusion and Recommendations

The present study demonstrated that bovine fasciolosis is common in Abeshege district of Gurage zone, Southern Nations, Nationalities and People regional State (SNNPRS). No variability between the potential risk factors is suggestive of favorable environmental conditions with little range of variation. Hence, it would be essential to exercise the following strategies to alleviate the problem in the current area.

Create awareness among farmers about the disease to enable them to avoid local practices which

could expose their animals to infection and to help them identify animals suffering from the disease and present them to treatment.

Strategic use of anthelmintic combined with improved pasture management.

Zero grazing animals should be provided with dried hay rather than hay having sufficient moisture for the survival of metacercariae.

Comprehensive study should be carried out involving year round schedule and Gurage zone in general to determine the factors which contribute to the high prevalence of bovine fasciolosis in the area and information used to develop control strategies.

Acknowledgments

Our deepest gratitude is extending to Wolaita Soddo Regional Veterinary Laboratory Parasitological Department experts especially, Mr. Tekle Alaro and Mrs. Mulualem Zaza for their help in the field as well as in the laboratory activities.

References

Abebe, R. (1992). Fasciolosis Clinical occurrence, Coprological abattoir and snail survey in around Wolliso, *DVM thesis*, Addis Ababa University, Faculty of Veterinary Medicine Debre Zeit, Ethiopia.

Abebe, W. (1990). Prevalence of Bovine Fasciolosis in Arsi Administrative Region. *DVM thesis*, Addis Ababa University. Faculty of Veterinary Medicine, Debre Zeit, Ethiopia.

ADADO, Abeshege District Agriculture Development Office, (2004). Unpublished socio-economic data.

Asrat, M., Peden, D., Jobre, Y., Girma, Tadesse, G., Abebe, G., & Gideyelew, T. (2007). Evaluation of Spatial distribution of F. hepatica and F. gigantica using geographic information system (GIS) in the Nile River Basin. *Ethiopian Veterinary Journal*, *11*(2), 41-57.

Beyene, Y. (1994). Epidemiology of Bovine and Ovine Fasciolosis and Distribution of its Snail Intermediates Host in Western Shewa, *DVM thesis*, Addis Ababa University. Faculty of Veterinary Medicine Debre Zeit, Ethiopia.

Dabas, V. P., Saxena, O. P. & Dabas, R. (2007). *Veterinary jurisprudence and postmortem, 3rd ed*. IBDC (International) Book Distributing Co.

Dagne, M. (1994). Survey on Prevalence and Economic Significance of Bovine Fasciolosis in Debre Berhan Region, *DVM thesis*, Faculty of Veterinary Medicine, Addis Ababa University, Debre Zeit, Ethiopia.

Dejene, G. (1987). Incidence and Economic Significance of Fasciolosis in Wolaita Awuraja, Sidama, Ethiopia *DVM thesis* Addis Ababa University Faculty of Veterinary Medicine, Debre Zeit, Ethiopia.

FAO, (1993). Ethiopia livestock sector development project Preparation report volume 1, FAO. Rome Italy. 51.

Fischer, M. S. & Say, R. (1989). *Manual Tropical Veterinary Parasitological* UK: CAB International.

Hendrix, C. M. (1998). *Diagnostic Veterinary Parasitology 2nd ed*. Auburn University Alabama: Mosby.

Jobre, Y. M., Malone, J. B., Gommes, R., Hansen, J., Shinegbrg, J., Snijders, F., Nachet, O. F. & Ataman, E. (1998). A geographic information system on the potential distribution and abundance of F. hepatica and F. gigantica in East Africa based on Food Agriculture Organization data bases. *Veterinary Parasitology 78*, 87-102.

Kassai, T. (1999). *Veterinary Helminthology 1st ed*. Development of Parasitology and Zoology, University of Veterinary Science. Budapest: Hungary.

McGavin, M. D. & Zachary, J. F. (2007). *Pathologic basis of veterinary disease: 4th ed*. Phledelphia, US: Mosby Elsevier.

Melaku, Z. (1991). Prevalence of Ovine Gastrointestinal Helminthes of cattle Chilalo Awuraja. Arsi Admistrative Region. *DVM thesis*, Addis Ababa University, Faculty of Veterinary Medicine, Debre Zeit, Ethiopia.

Mitiku, A. (1988). Prevalence of Bovine Fasciolosis and its Economic Significance at Nekemite. *DVM thesis*, Faculty of Veterinary Medicine, Addis Ababa University, Debre Zeit, Ethiopia.

Mulugeta, B. H. (2008). Prevalence of Fasciola in Hawassa determined on the basis of Abattoir examination. *DVM thesis*, Faculty of Veterinary Medicine, Jimma, Ethiopia.

Okewole, E. A., Ogundipe, G. A. T., Adejinmi, J. O. & Olaniyan, A. O. (2000). Clinical Evaluation of the three chemoprophylactics regimes against ovine heliminthosis in a fasciola endemic farm in Ibadan, Nigeria. *Israel Journal of Veterinary Medicine, 56*(1), 15-28.

Phiri, A. M, Phiri, I. K., Siziya, S., Sikasunge, C. S., Chembensofu, M., & Monrad, J. (2005). Seasonal Pattern of Bovine Fasciolosis in the Kafue and Zambezi catchment areas of Zambia. Zambia, University of Zambia. School of Veterinary Medicine. Department of Clinical Studies. Lusaka. Zambia. *Veterinary Parasitology, 134*(1-2), 87-92.

Radostatis, O. M., Gay, C. C., Hichcliff, K. W. & Constable, P. O. (2007). *Veterinary medicine. A Text book of the diagnosis of cattle, horses, sheep, pigs. 10th ed* .London, UK: Saunders Ltd.

Thrusfield, M. (1995). *Veterinary Epidemiology. Department of Veterinary Clinical Study. 2nd ed*. Ames, Iowa, US: Blackwell Science.

Urquhart, G. M., Armour, J., Duncan, J. L., Dunn, A. M., Jennings, F. W. (1996). *Veterinary Parasitology. 2nd ed*. Scotland, UK: Blackwell Science.

Yilma, J. M. & Mesfin, A. (2000). Dry season bovine fasciolosis in Northwestern part of Ethiopia. *Revue de Médecine Vétérinaire, 151*(6), 493-500.