ISSN: 2146-7269
e-ISSN: 2687-5330

ANIMAL HEALTH, PRODUCTION AND HYGIENE

Volume 10, Issue 2 July-December 2021 Page: 39 - 77

www.aduveterinaryjournal.org

ANIMAL HEALTH, PRODUCTION AND HYGIENE

ANIMAL HEALTH, PRODUCTION AND HYGIENE
www.aduveterinaryjournal.org

Animal Health, Production and Hygiene (Animal Health, Prod and Hyg) is publication of Faculty of Veterinary Medicine, University of Aydın Adnan Menderes University. The journal publishes original researches and reviews on all aspects of veterinary science involving farm and pet animals, laboratory, marine and exotic/wild animals, zoonozes and public health.

Owner on behalf of the Faculty of Veterinary Medicine (İmtiyaz Sahibi)
Cavit Kum (Dean)

Address
Animal Health, Production and Hygiene, Faculty of Veterinary Medicine, Aydın Adnan Menderes University, West Campus, 09016, Efeler, Aydın, Türkiye
Tel : +90 256 247 07 00/357 Fax :+90 256 247 07 20 E-mail : animalhealth@adu.edu.tr
Dergipark: https://dergipark.org.tr/tr/pub/aduveterinary

Press
Berke Ofset Matbaacılık, Sanayi Caddesi No.30 Bornova, İzmir
Tel:232 449 77 47-449 77 14-4499797 Fax: 449 70 97 E-mail:info@berkeofset.com.tr

ANIMAL HEALTH, PRODUCTION AND HYGIENE

ANIMAL HEALTH, PRODUCTION AND HYGIENE

CONTENTS

ANIMAL HEALTH, PRODUCTION AND HYGIENE

İÇİNDEKİLER

Animal Health Prod and Hyg (2021) 10 (2) : 39 - 44

Animal Health, Production and Hygiene

www.aduveterinaryjournal.com

Research Article

Evaluation of Morphological Variations in Tympanic Portion of Temporal Bone in Dogs

Semra ÇINAR[1*], Figen SEVİL-KİLİMCİ[1], Mehmet Erkut KARA[1]

[1]*Department of Anatomy, Faculty of Veterinary Medicine, Aydin Adnan Menderes University, Aydin, Turkey*

ABSTRACT

In this study, it was aimed to examine the relationship between the morphometric-geometric features of the external acoustic meatus, tympanic bulla, and auditory tube and the cranium shape, age, gender, and race characteristics in the os temporale region in terms of the clinical-anatomical approach to the outer ear and middle ear regions of the dog's skull. A total of 110 dogs' skulls were used. The bones were photographed in three different views with the camera. The morphometric data on the skull, angle measurements, and index values related to them were calculated in obtained images. Auditory tube angle and external acoustic meatus angle were found to differ statistically among the age groups. In addition, it was determined that the index values of bulla tympanica, except for the height index, showed statistical differences among age groups. When the angle measurements and index values of the skulls of male and female animals were examined, it was determined that other index values did not differ between female and male dogs, except for the external acoustic meatus angle. As a result, it can be said that age and skull type is more effective than gender in the positioning of the tympanic region in dogs. The results of this research are supportive information that can be used in both clinical and zooarchaeological studies on the tympanic bulla region on dogs.

Keywords: Bulla tympanica, dog, morphometry, temporal bone, tuba auditiva

Köpeklerde Temporal Kemiğin Pars Tympanica'sındaki Morfolojik Varyasyonların Değerlendirilmesi

ÖZET

Bu çalışmada, köpeklerin kafatasının dış kulak ve orta kulak bölgelerine anatomik yaklaşım ve klinik açıdan meatus acusticus externus, bulla timpanica ve tuba auditiva'nın morfometrik-geometrik özellikleri ile os temporale bölgesindeki kafatası şekli, yaş, cinsiyet ve ırk özellikleri arasındaki ilişkinin incelenmesi amaçlanmıştır. Çalışmada toplam 110 adet köpek kafatası üzerinde çalışılmıştır. Kemiklerin üç farklı yönde fotoğrafları çekildi. Elde edilen görüntülerde kafa ile ilgili bazı morfometrik veriler, açı ölçümleri ve bunlara ilişkin indeks değerleri hesaplandı. Tuba auditiva açısı ve meatus acusticus externus açısı yaş grupları arasında istatistiksel olarak farklılık görüldü. Ayrıca bulla timpanika indeks değerlerinin boy indeksi dışında yaş grupları arasında istatistiksel olarak farklılık gösterdiği belirlendi. Dişi ve erkek hayvanların kafataslarının açı ölçümleri ve indeks değerleri incelendiğinde, meatus acusticus externus açısı dışındaki diğer indeks değerlerinin dişi ve erkek hayvanlar arasında farklılık göstermediği belirlendi. Sonuç olarak köpeklerde timpanik bölgenin pozisyonunda, yaş ve kafa tipinin cinsiyetten daha etkili olduğu söylenebilir. Bu araştırmanın sonuçları köpeklerde bulla timpanica bölgesi ile ilgili hem klinik hem de zooarkeolojik çalışmalarda kullanılabilecek destekleyici bilgilerdir.

Anahtar kelimeler: Bulla tympanica, köpek, morphometi, temporal kemik, tuba auditiva

***Corresponding Author:** Semra ÇINAR, Department of Anatomy, Faculty of Veterinary Medicine, Aydin Adnan Menderes University, Aydin, Turkey. e-mail: veterinariansemra@gmail.com

Received Date: 05.10.2021 Accepted Date: 24.11.2021

DOI: 10.53913/aduveterinary.1005009

Introduction

Morphometric studies of the skull can be done to determine species, race or sex characteristics (Onar, 1999; Onar and Gunes, 2003; Schmidt et al., 2011), as well as to form the basis of clinical approaches in various regions (Fox, 1964; Schmidt et al., 2011; Schmidt et al., 2013) is important. One of the most common ailments of dogs seen in veterinary medicine is ear disease. About 15%-20% of all canine patients have ear problems, which can range from mild erythema to severe otitis media (Louis, 2004). The temporal bone, which participates in the formation of a part of the lateral and base wall of the cavum cranii, is an important bone in terms of clinical applications, as some important vessels and nerves pass through it, articulates with the mandible, and forms the bony roof of the ear. In the middle of pars tympanica of the os temporale external hole of the meatus acoustics externus osseous is located. External acoustic meatus (external ear canal) is a tubular path which it is extending from concha auriculare to membrana tympanica and obliquely in dog (Evans, 1993; König, 2007; Njaa et al., 2012). The membrana tympanica at the end of external acoustic meatus is located at an angle of approximately 45 degrees to the horizontal central axis of the external acoustic meatus in dogs, however, this angle may differ according to dog breeds (Njaa et al., 2012). External acoustic meatus is closed in newborn kittens and puppies. For this reason, although newborn puppies can perceive some sounds, they cannot hear them properly. Generally, the external ear canal opens between the 6th and 14th days and the puppies begin to hear in the 3rd week after they are born (Samsar and Akın, 2006). Bony enlargement called tympanic bulla located on the ventral aspect of the pars tympanica is a structure that contains the middle ear (Bahadır and Yıldız, 2004). The space in the middle ear is called tympanic cavity and this space is in conjunction with the pharynx through the auditory tube (Eustachian tube). Since the position of the osseous roof of the external auditory canal, mastoid part of middle ear, and auditory tube, which is the functional unit of the ear, is related to various clinical approaches. Many studies are conducted on the morphometric and geometric features of the region (Mann et al., 1979; Albiin, 1984; Djeric and Savic, 1985; Sadler-Kimes et al., 1989; Kemaloğlu et al., 1996; Judkins and Li, 1997; Sırıkçı et al., 2001). In clinical applications, position of the external auditory canal for otoscope application and location of the tympanic membrane during cleaning of the ear canal must be taken into account (Njaa et al., 2012). For example, the dorso-rostral border of the external acoustic meatus and the shape of the zygomatic process of the os temporale and the angle of the skull to the long axis may show variations in different skull types of dogs. This is important in terms of placing the autoendoscope deeper during video otoscope use in procedures such as myringotomy (Njaa et al., 2012). In addition, regarding the angle of the external auditory canal with the tympanic annulus; provides an advantage in washing the external ear, inserting and passing the catheter into the horizontal ear canal, and collecting the washing solution and serum physiology without damaging the tympanic membrane (Njaa et al., 2012). Auditory tubeposition is considered to be the main cause of middle ear inflammations (Mawson, 1974; Kemaloğlu et al., 1996). The po sition of the Tympanic bulla and auditory tube changes depending on age (Takeuchi ve ark., 1980; Bluestone and Doyle, 1988; Kemaloğlu et al., 1996)

Due to the common middle ear inflammations in children, there are many studies on this subject both in humans and animals (Sadler-Kimes, 1989; Judkins and Li, 1997). For this reason, os temporale measurements and their ratios to ossa cranii measurements have gained importance. Considering the changes in development process of the studied measurements, it is seen that the os temporale continues to develop together with the ossa faciei until it reaches maturity (Takeuchi ve ark., 1980) and it has been revealed that the growth process of the skull significantly affects function of the auditory tube (Mann et al., 1979). In these studies, change in the shape, size and position of the auditory tube with age was also investigated (Mann et al., 1979; Takeuchi et al., 1980; Todd and Martin, 1988).

In literature review on the subject, detailed data on various ear diseases in dogs and temporal bone morphometry, which is important in terms of examination, and variations of this region depending on race, age, gender, and skull shape were not found. In this study, it was aimed to examine the relationship between the morphometric and geometric features of the external acoustic meatus, tympanic bulla and auditory tube and the cranium shape, age, and gender characteristics in the os temporale region in terms of the clinical-anatomical approaches to the outer ear and middle ear regions of the dog's skull.

Materials and Methods

In this study, dog skull bones obtained from the archive of Adnan Menderes University Veterinary Faculty Anatomy Department Osteometry Laboratory were used. A total of 110 dogs (48 female, 59 male, 3 non-gendered) were studied. In the absence of age records of the animals (n:16), the approximate age of the animals was determined according to the condition of their teeth and sutures (Dyce et al. 2002; Thrall and Robertson, 2011; Mihelic et al., 2013; Schmidt et al., 2013). Accordingly, animals with an approximate age range of 1.5 months to 168 months were used.

In order to demonstrate the reliability of the method, the cranium of a randomly selected dog was photographed five times and all measurements were taken repeatedly on these five images of the same skull to calculate the coefficients of variation (Özdamar, 2004).

After checking the validity and reliability of the measurement method, photographic images of the bones, which were placed on a flat platform with the

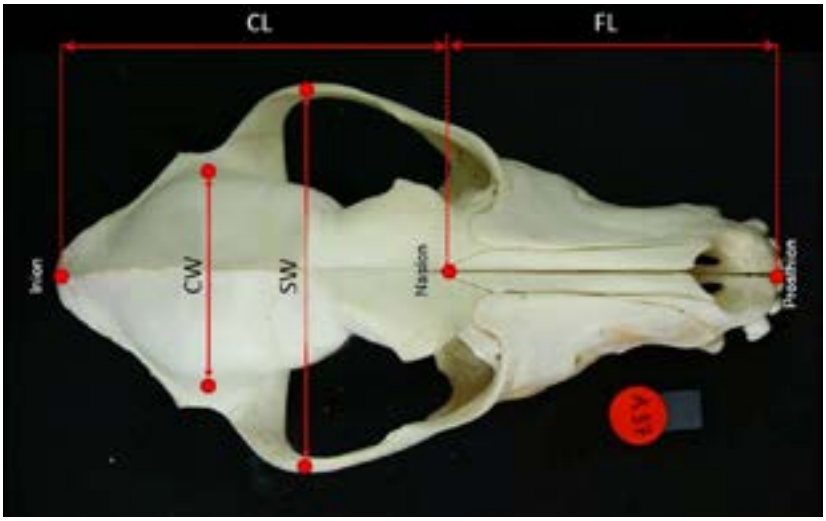


Figure 1. Dorsal view of the skull and the measurements

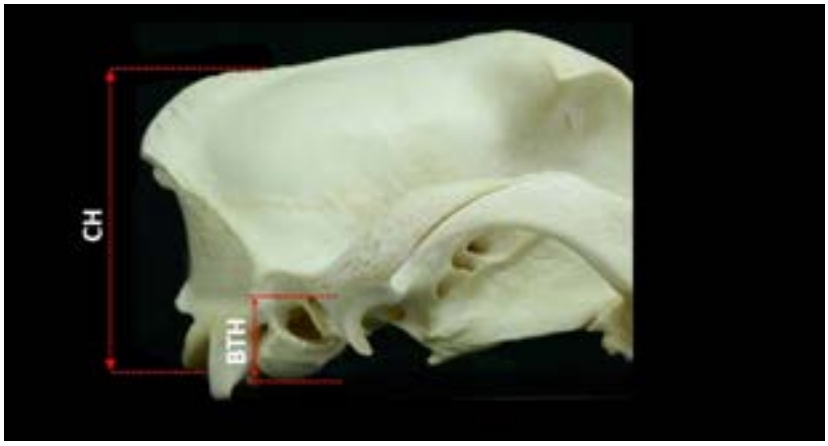


Figure 2. Lateral view of the skull and the measurements

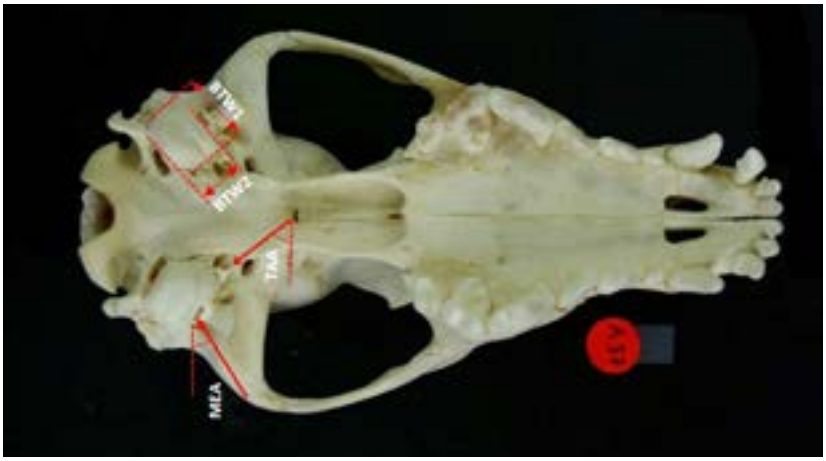


Figure 3. Basal view of the skull and the measurements

help of play dough, were taken from the dorsal, lateral and basal face with a Canon EOS 350D camera. A ruler was placed during photographing for calibration of the images. Photographs were taken perpendicular to the bone and focusing on the midpoint of the bone for all positions. All obtained photos were transferred to the computer in ".jpeg" format. After calibration of the transferred images in the "ImageJ" program, all the measurements of skull bones were taken using necessary commands in the "ImageJ" program", whose reference points were already defined. (Figure 1-3, Table 1).

Statistical analyzes were performed with the SPSS 19.0 program, and normal distribution of the data was checked with the "Shapiro-Wilk test". In age and gender groups, an intergroup comparison was checked for independent variables by t-test. The results were checked with the Mann-Whitney test, which is a non-parametric test for parameters that did not show normal distribution, and the significant (p) values were written according to the results of this test. Pearson correlation analysis method was used to determine whether there was a correlation between the cranium index values in the bones used and the index and angle values obtained from the pars tympanica region the data were presented as the number of animals in the group (n), mean value (MV), and standard deviation (SD) in tables. The significance level in the study was accepted as $p<0.05$. Checking the reliability of the measurement method, the coefficient of variation (%CV) of measurements was calculated using the formula, "(standard deviation/mean value) x 100" (Özdamar, 2004).

Results

When the coefficients of variation calculated for the measurement method in the study were examined, the lowest coefficient of variation was found to be 0.18% in facial length and skull width measurements, and the highest coefficient of variation was 1.96% in the measurement of auditory tube angle.

Angle measurements and index values of the young and adult age groups are presented in Table 2. Auditory tube angle and external acoustic meatus angle were found to differ statistically between two groups. In addition, it was determined that the index values of bulla tympanica, except for the height index, showed statistical differences among age groups.

Angle measurements and index values of the skulls of male and female animals are presented in Table 3. According to these results, it was determined that other index values did not differ between female and male animals, except for the external acoustic meatus angle.

Table 1. Identification of abbreviations in the study

	Parameter		Method	Source
Lenght Values (mm)	Facial length	FL	Between nasion and prosthion	EVANS (1993)
	Cranial length	CL	Between Inion and nasion	EVANS (1993)
	Cranial width	CW	The widest interparietal width	EVANS (1993)
	Cranial height	CH	Lateral side from base of occipital condyle to highest point of head	EVANS (1993)
	Skull width	SW	The widest interzygomatic distance	EVANS (1993)
	Skull length	SL	FL+CL	EVANS (1993)
	Width of Bulla tympanica 1	BTW1	The largest diamater of the Bulla tympanica	DRIESCH (1976)
	Width of Bulla tympanica 2	BTW2	The narrowest diamater of Bulla tympanica	DRIESCH (1976)
	Heigth of Bulla tympanica	BTH	The distance between the upper border of the porus acusticus externus and the ground contacting part of the bulla tympanica	-
Angle Values (°)	Angle of tuba auditiva	TAA	Angle of tuba auditiva with transversal plane	-
	Angle of meatus acusticus externus	MEA	The angle between ventral border of porus acusticus externus and the transversal axis	ALBIIN (1984)
Index Values (%)	Skull index	SI	SW x 100 /SL	EVANS (1993)
	Cranial index	CI	CW x 100 /CL	EVANS (1993)
	Facial index	FI	SW x 100 /FL	EVANS (1993)
	Width of Bulla tympanica 1 index	BWI1	BTW1x100/SL	-
	Width of Bulla tympanica 2 index	BWI2	BTW2x100/SW	-
	Heigth of Bulla tympanica index	BHI	BTHx100/CH	-

Table 2: Angular measurements and calculated index values of the skulls of animals in different age groups

	Young		Adult		p
Angular values (°)	**N**	**MV±SD**	**N**	**MV±SD**	
Angle of tuba auditiva	39	43.81±7.38	69	53.50±5.65	**0.000*** **
Angle of meatus acusticus externus	39	48.34±5.32	69	45.75±5.86	**0.021***
Index values (%)					
Skull index	41	57.90±4.09	69	56.38±9.51	**0.004****
Cranial index	41	75.69±10.02	69	57.90±10.67	**0.000*** **
Facial index	41	156.67±27.05	69	126.69±26.80	**0.000*** **
Width of bulla tympanica 1 index	41	16.86±4.411	69	12.28±1.34	**0.000*** **
Width of bulla tympanica 2 index	41	13.44±4.02	69	8.93±1.10	**0.000*** **
Heigth of bulla tympanica index	41	19.17±4.76	69	20.92±2.09	0.114

p<0.05 * , p<0.01 , p<0.001*****

The correlation coefficients between cranial index values and angle values measured from the pars tympanica region are presented in Table 4. In particular, it was observed that there was a correlation between the angle of the auditory tube and the all skull indices. External acoustic meatus angle was found to have correlation only with bulla tympanica indices

Discussion

Before starting the study, five images were taken from the skull of an animal to the demonstrate reliability of the method used and the measurements taken, and all measurements were taken again from these images. It is seen that the highest coefficient of variation was 1.96% in the angle of Tuba auditiva. According to these data, since all coefficients were below 5% value, it was seen that the measurement method used in the study was reliable (Özdamar 2004).

When index values of the tympanic bulla measurements were examined, it was noted that the width and length of the bulla were larger compared to the skull size in young animals, but the height ratio did not change with age. In addition, it was determined that the angle of the auditory

Table 3: Angular measurements and calculated index values of the skulls of animals of different sexes

	FEMALE		MALE		
Angular values (o)	**N**	**MV±SD**	**N**	**MV±SD**	**p**
Angle of tuba auditiva	48	49.83±6.79	57	50.03±8.80	0.616
Angle of meatus acusticus externus	48	45.48±4.73	57	47.77±6.51	**0.040***
Index values (%)					
Skull index	48	57.22±10.94	59	56.77±4.63	0.608
Cranial index	48	66.57±15.02	59	62.92±12.37	0.201
Facial index	48	137.43±31.1	59	138.95±30.36	0.735
Width of bulla tympanica 1 index	48	14.21±2.51	59	13.87±4.41	0.066
Width of bulla tympanica 2 index	48	10.83±2.62	59	10.47±3.98	0.146
Heigth of bulla tympanica index	48	20.78±3.23	59	19.83±3.63	0.176

p<0.05 *

Table 4: Correlation coefficients between cranial index values and the angle values on the pars tympanica

	SI	CI	FI	BWI1	BWI2	BHI
TAA	**-0,273****	**-0,581****	**-0,524****	**-0,454****	**-0,528****	**0,265****
MEA	-0,120	0,127	0,061	**0,220***	**0,212***	-0,110

p<0.01**

tube with the transversal plane was approximately 18% higher in adults than in young animals. The angle that the porus acusticus externus makes with the transversal axis is approximately 5% greater in young animals than in adults. In this case, it is seen that the long axis of the tympanic bulla rotates more rostro-medially in the horizontal plane depending on age. While the suturae-shaped sections of the occipital bone, which largely surround the tympanic bone sections in the skull posteriorly and laterally, are mostly closed by the age of six months, the sphenoid bone and associated synchondrosis type junctions anteriorly close after one year of age (Evans, 1993; Thrall and Robertson, 2011; Schmidt et al., 2013). Since the facial bones continue to grow at the front during these periods, it can be said that the position of the tympanic bone may show age-related changes in this way.

Studies of sexual dimorphism in the closure of skull sutures have shown that differences in suture closure can be seen in both male and female (Sahni et al., 2005; Vijay et al., 2013; Alhadi et al., 2019). Since differences between males and females occur at different points in the life cycle, a gender-related difference in the position of tympanic region can also be expected due to differences in suture closure. However, according to the results of this study, it was determined that other angle and index values did not differ between female and male animals, except for a small difference in the external acoustic meatus angle in the dog.

It has been stated that the closure time of synchondrosis type unions, which has the most important effect on the longitudinal growth of the skull-shape, may vary according to the skull-shape type or race (Schmidt et al., 2013). The auditory tube angle measurement is approximately the angle of the tympanic bulla long axis with the transversal plane. It can be said that the long axis of tympanic bulla may be more medially oriented in dogs with longer head type, as this angle has negative correlations with skull indices. In addition, the medial position of the tympanic bulla increases as the bulla height increases.

The most important limitation of this study is the insufficient exact age information of the animals. For this reason, animals estimated under one year of age, which are considered to be young, could not be divided into subgroups. The neurocranium (cranium) region of the skull, in which it is located in the temporal bone, has largely completed its development and this region changes less. However, the splanchnocranium (facies) region adjacent to the temporal bone develops and changes much more rapidly until about one year of age (Evans, 1993; Thrall and Robertson, 2011; Schmidt et al., 2011). Therefore, if the younger group could be further divided into subgroups, age-related changes could be seen more prominently. In addition, due to the lack of pedigree records of all dogs used, breed-related evaluations could not be made. Instead, skull indices were used to evaluate head type-related changes.

In conclusion, it can be said that age and skull type is more effective than gender in the position of the tympanic region in dogs. The results of this research are supportive information that can be used in both clinical and zooarchaeological studies on the tympanic bulla region in dogs.

Acknowledgement

This study is a part of the project supported by TUBITAK (2209-University Students Research Projects Support

Program/1919B011301511)

The first results of this study XVII. Presented at the International Veterinary Medicine Students Scientific Research Congress (Istanbul).

Conflict of interest

The authors declare that they have no conflict of interest in this study.

References

Albiin, N. (1984). The anatomy of the eustachian tube. *Acta-Otolaryngologica* (Stockh), suppl. 414, 34-37. https://doi.org/10.3109/00016488409122879

Alhadi, A., Issrani, R., Prabhu, N., & Alhadi, M. (2019). Observation on the closure of lambdoid suture in relation to age, sex and population variations using a novel radiographic technique – a prospective study. *Acta Odontologica Scandinavica*, 77(1), 61-65. https://doi.org/10.1080/00016357.2018.1503710

Bahadır, A., Yıldız, H. (2004). Veteriner Anatomi I (Hareket Sistemi). Ezgi Kitabevi.

Bluestone, C.D., Doyle, W.J. (1988). Anatomy and physiology of eustachian tube and middle ear related to otitis media. *Journal of Allergy and Clinical Immunology*, May; 81, 997-1003. https://doi.org/10.1016/0091-6749(88)90168-6. PMID: 3286738

Djeric, D., Savic D. (1985). Anatomical variations and relations of the bony portion of the eustachian tube. *Acta Oto-Laryngologica*, 99, 543-550. https://doi.org/10.3109/00016488509182259

Driesch, A.V.D. (1976). Measurement of the Mammalian Skeleton in: A Guide to The Measurement of Animal Bones from Archaeological Sites. Peabody Museum of Archaelogy and Ethnology Harvard University.

Dyce, K.M., Sack, W.O., Wensing, C.J.G. (2002). Textbook of Veterinary Anatomy (3rd Ed). Elsevier, USA,

Evans, H.E. (1993). The skeleton in: Millers Anatomy of the Dog. Ed: Evans H.E., W.B. Saunders Comp. Philadelphia. Pp.130-133

Fox, W.M. (1964). Anatomy of the Canine Skull in Low-grade Otocephaly. *Canadian Journal of Comparative Medicine Veterinary Science*, 28,105-107.

Judkins, R.F, Li, H. (1997). Surgical anatomy of the rat middle ear. *Official Journal of American Academy of Otolaryngology-Head and Neck Surgery*, 117, 438-47. https://doi.org/10.1016/s0194-5998(97)70011-1

Kemaloğlu, Y.K., Göksu. N., Öcal, K., Karademir, N., Hazıroğlu, R., İnal, E. (1996). The eustachian tube caliber and craniofacial skeleton in guinea pigs. *International journal of Pediatric Otorhinolaryngology,* 38, 53-63. https://doi.org/10.1016/S0165-5876(96)01419-X

König, H.E. (2007). The vestibulocochlear organ in: Veterinary Anatomy of Domestic Mammals Ed: Liebich H-G, Schattauer GmbH, Stuttgard \ Newyork.

Louis, G., (2004). Small Animal Ear Diseases (2nd ed.). Chapter 4 digital imaging of the ear, pp24.

Mann, W., Jonas, I., Münker G. (1979). Growth influence on tubal function. *Acta Oto-Laryngologica*, 87, 451-457. https://doi.org/10.3109/00016487909126450

Mawson SR. Middle ear effusions: therapy and clinical results. *Annals of Otology, Rhinology & Laryngology*. 1974 Mar-Apr;83: Suppl 11:71-2. PMID: 4819101.

Mihelic, D., Pavic, M., Hincak, Z. A. (2013). Craniometrical Analysis of the Early Bronze Age Dogs from Vucedol Site (East Slavonia, Croatia). *Collegium Antropologicum*, 37(1), 239–244. PMID: 23697279

Njaa, B.L., Cole, L.K., Tabacca, N. (2012). Practical otic anatomy and physiology of the dog and cat. *The Veterinary Clinics of North America Small Animal Practice*, 42, 1109-1126. https://doi.org/10.1016/j.cvsm.2012.08.011

Onar, V., (1999). Morphometric analysis of the foramen magnum in German Shepherd dogs (Alsatians). *Annals of Anatomy*, 179, 563-568, https://doi.org/10.1016/S0940-602(97)80022-

Onar, V., Güneş H., (2003). On the variability of skull shape in German Shepherd (Alsatian) puppies. *The Anatomical Record Part A,* 272A, 460-466. https://doi.org/10.1002/ar.a.10052

Özdamar, K., (2004). Paket programları ile istatistiksel veri analizi -1. MINITAB, NCSS, SPSS. Kaan kitabevi, Eskişehir,

Sadler-Kimes, D., Siegel, M.I., Todhunter, J.S. (1989). Age-related morphologic differences in the components of the eustachian tube\middle ear system. *The Annals of Otology, Rhinology and, Laryngology*, 98(11), 854-858. https://doi.org/10.1177%2F000348948909801104

Sahni, D., Jit, I., Neelam, Sanjeev. (2005). Time of closure of cranial sutures in northwest Indian adults. *Forensic Science International*, 148(2-3), 199-205. https://doi.org/10.1016/j.forsciint.2004.06.002

Samsar, E., Akın, F., (2006). Özel cerrahi, Medipress, Malatya

Schmidt, M.J., Neumann A.C., Amort, K.H., Failing, K., Kramer M. (2011). Cephalometric measurements and determination of general skull type of cavalier king charles spaniels. *Veterinary Radiology & Ultrasound,* 52(4), 436–440. https://doi.org/10.1111/j.1740-8261.2011.01825.x

Schmidt, M.J., Volk, H., Klingler, M., Failing K., Kramer K., Ondreka N. (2013). Comparison of closure times for cranial base Synchondroses ın mesaticephalıc, brachycephalıc, and cavalier kingcharles spaniel dogs. *Veterinary Radiology & Ultrasound*, 54 (5), 497-503. https://doi.org/10.1111/vru.12072

Sırıkçı, A., Bayazıt, Y.A., Bayram, M., Kanlıkama, M. (2001). Significance of the auditory tube and mastoid size in chronic ear disease. *Surgical and Radiologic Anatomy*, 23, 91-95. https://doi.org/10.1007/s00276-001-0091-1

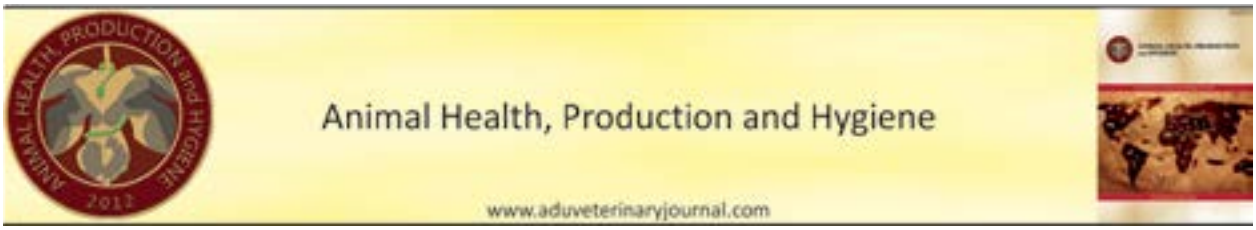
Takeuchi, Y., Savara, B.S., Shadel, R.J. (1980). Biennial size norms of eight measures of the temporal bone from four to twenty years of age. *The Angle Orthodontist*, 50(2), 107-113. https://doi.org/10.1043/0003-3219(1980)050%3C0107:bsnoem%3E2.0.co;2.

Thrall, D.E., Robertson, I.D. (2008). Atlas of Normal Radiographic Anatomy and Anatomic Variants in the Dog and Cat. St. Louis, Missourio: Elsevier/Saunders; 2011. p. 10

Todd, N.W., Martin, W.S., (1988). Relationship of eustachian tube bony landmarks and temporal bone pneumatization. *The Annals of Otology, Rhinology and, Laryngology*, 97(3 Pt 1), 277-80. https://doi.org/10.1177%2F000348948809700313

Vijay, K.A.G., Agarwal, S.S., Bastia, B.K., Shivaramu M.G., (2013). Sexual Variation in Cranial Suture Closure-A Cross Sectional Post Mortem Study Done on Tertiary Care Hospital. *Open Access Scientific Reports,* 2, 611. https://doi:10.4172/scientificreports.611

Animal Health Prod and Hyg (2021) 10 (2) : 45 - 51



Research Article

Investigation of Canine Distemper Virus Infection in Dogs in the Antalya Province

Yusuf SAYIN[1], Nural EROL[1*]
[1]*Department of Virology, Faculty of Veterinary Medicine, Aydin Adnan Menderes University, Aydin, Turkey*

A B S T R A C T

Canine distemper virus (CDV) is one of the most prevalent infectious agents causing severe clinical symptoms among canids. Communal life-habitat, for example, clinics, dog-shelters, or rural areas, is critical in terms of the transmission dynamics of CDV. In this study, the blood serum samples from 92 dogs, which were brought to the rehabilitation center and private veterinary clinics in Antalya with various internal medical problems, were examined for CDV infection. The sampled dogs were unvaccinated and aged 2 to 12 months. Samples were tested using a commercial immunochromatographic rapid test for detection of CDV-antigens and Enzyme Linked Immunosorbent Assays (ELISA) for detection of CDV-specific Immunoglobulin G (IgG) and Immunoglobulin M (IgM).

The most common clinical findings in the 92 dogs sampled were mucopurulent discharge in the eye (45.65%), nasal hyperkeratosis (35.87%), nasal mucopurulent discharge (25%), cough (13.04%), diarrhea (8.70%), and fatigue (6.52%). It was observed that clinical findings were more intense in the early reconvalescent period. 5.43% (5/92) of the samples examined by the immunochromatographic rapid test were positive. The positivity rates of IgG and IgM antibodies by ELISA were 80.43% (74/92) and 94.56% (87/92), respectively. Totally, 91 (98.91%) of the 92 dogs tested by ELISA for IgG and IgM antibodies were positive for one or both antibodies, and one (1.09%) was negative for both antibodies. In conclusion, it was determined that CDV infection is actively circulating in the Antalya province and poses a risk to unvaccinated dogs in the region.

Keywords: Antibody, Antigen, Canine distemper virus, ELISA

Antalya'daki Köpeklerde Canine Distemper Virus (CDV) Enfeksiyonunun Araştırılması

Ö Z E T

Canine distemper virusu (CDV), köpek türleri arasında ciddi klinik semptomlara neden olan en yaygın enfeksiyöz ajanlardan biridir. Klinikler, köpek barınakları veya kırsal alanlar gibi ortak yaşam alanları, CDV'nin bulaşma dinamikleri açısından kritik öneme sahiptir. Bu araştırmada Antalya ilindeki Rehabilitasyon merkezine ve özel veteriner kliniklerine çeşitli dahili sağlık problemleriyle getirilen, toplam 92 adet köpekten alınan kan örnekleri CDV enfeksiyonu yönünden incelendi. Köpekler, 2-12 aylık ve aşısızlardı. Örnekler CDV-antijenlerinin saptanması için ticari immunokromatografik hızlı test kiti ile, CDV spesifik immunoglobulin G (IgG) ve immunoglobulin M (IgM) antikorların saptanması için ise ticari Enzyme Linked Immunosorbent Assay (ELISA) kitleri ile test edildi.

Örneklenen 92 köpekte en fazla gözlenen klinik bulgular sırasıyla gözde mukopurulent karakterde akıntı (%45,65), nasal hiperkeratoz (%35,87), nasal mukopurulent akıntı (%25), öksürük (%13,04), ishal (%8,70), halsizlik (%6,52) olarak kaydedildi. Klinik bulguların erken rekonvalesan dönemde daha yoğun olduğu gözlendi. İmmunokromatografik hızlı test ile incelenen numunelerden %5,43 (5/92)'ü pozitif bulundu. ELISA ile IgG antikorları yönünden pozitiflik oranı %80,43 (74/92) olarak bulundu. ELISA ile IgM antikorları yönünden pozitiflik oranı ise %94,56 (87/92) olarak tespit edildi. Genel olarak, IgG ve IgM antikorlarının tespiti için ELISA ile test edilen 92 köpekten 91 tanesi (%98,91) IgG veya IgM antikorlarından biri veya her ikisi yönünden pozitif, 1 (%1,09) tanesi ise her iki antikor yönünden negatif bulundu. Sonuç olarak, Antalya ilinde CDV enfeksiyonunun yaygın bir şekilde dolaşımda olduğu ve bölgedeki aşısız köpekler için risk oluşturduğu belirlendi.

Anahtar kelimeler: Antijen, Antikor, Canine distemper virus, ELISA

Corresponding Author: Nural EROL, Department of Virology, Faculty of Veterinary Medicine, Aydin Adnan Menderes University, Aydin, Turkey.
e-mail: nuralerol@adu.edu.tr
Received Date: 24.09.2021 – Accepted Date: 29.11.2021
DOI: 10.53913/aduveterinary.1000408

Introduction

Canine Distemper Virus (CDV) is a negative single-stranded and non-segmented RNA virus belonging to the species Canine Morbillivirus, the genus Morbillivirus, the subfamily Orthoparamyxovirinae, and the family Paramyxoviridae (ICTV, 2020). CDV, which is one of the most important viruses for dogs, causes respiratory, digestive, and nervous system disorders and death in young dogs. Besides being a multisystemic infection, diagnosis and treatment of the disease can be difficult because it causes severe immunosuppression and prepares the ground for other diseases (Elia et al., 2015; Sabatino, 2016).

Distemper infection is endemic in many parts of the world. The incidence of the disease is very low in developed countries (Sabatino, 2016). CDV can infect dogs of all ages, but puppies aged 0–6 months are especially susceptible (Çalışkan, 2007; Latha et al., 2007b).

For the control and prevention of infection, it is necessary to know the incidence, prevalence rates, and risk factors in the region. The most basic preventive measure against CDV is vaccination. Since the early 1960s, the routine use of modified live virus vaccines has been done to struggle against the virus. Vaccination programs are important in order to prevent the transmission of infection and to ensure adequate immunity (Budaszewski et al., 2016).

Numerous clinical parameters and laboratory analyses are recommended for the definitive diagnosis of distemper. However, the unpredictable and variable disease course, such as duration of viremia, clinical findings, and deficient or delayed humoral or cellular immune responses, hinders the correct diagnosis of distemper and makes the collection of appropriate samples for laboratory confirmation very important. Meanwhile, given that the infection is highly contagious, detection of CDV from different biological samples is essential for determining the correct treatment and necessary precautions (Elia et al., 2015; Sabatino, 2016). It has been reported that the infection causes high mortality and morbidity, especially in unvaccinated puppies, and poses a serious threat to all unvaccinated house dogs and stray dogs (Elia et al., 2015; Zacharias et al., 2016).

According to studies conducted in Turkey, canine distemper virus infection is a disease that constantly increases and becomes widespread over the years (Çalışkan and Burgu, 2007; Esin, 2013; Saltık, 2018). In this study, it was aimed to determine the current status of CDV infection, which seriously threatens the health of dogs, both owned and stray dogs brought to private veterinary clinics and the rehabilitation center in Antalya province. In the study, clinical findings in dogs were monitored and evaluated with laboratory (test for the presence of antigen, IgG and IgM) results.

Materials and Methods

For this study, blood serum samples taken for diagnostic purposes from a total of 92 unvaccinated dogs under the age of one with various internal health problems in the province of Antalya in May 2019 were used. 28 of these animals were brought to private veterinary clinics, and 64 of them were dogs, either brought to a street animal rehabilitation center in Muratpaşa-Antalya or housed there due to health problems. It was known that all of the animals sampled had a connection with the street and could roam freely. Information about the sampled dogs (age, sex, health status, clinical findings, lifestyle, etc.) was recorded. Three of the animals ranged from 2–6 months, and 89 of them were between 6–12 months. It was recorded that 30 of the dogs were males and 62 of them were females. The sampled animals were randomly selected. Permission was obtained from the Akdeniz University Animal Experiments Local Ethics Committee for the study (Protocol no: 2017.08.005 Decision No: 74).

In order to be tested for CDV-antigen and IgG and IgM antibodies against CDV from blood samples taken into kaolin tubes by the veterinarian under appropriate conditions in the clinical environment, their serums were separated and stored at -20 °C.

The 92 blood serums from dogs were tested for the CDV antigen by a commercially available rapid test kit with an immunochromatography principle (CDV Ag-Antigen Rapid Test kit®, BioNote, Inc., Republic of Korea). The CDV specific IgG and IgM antibodies in all serums were investigated by commercially available indirect ELISA kits (Distemper IgG and IgM Ab ELISA, Biopronix Agrolabo, Italy). All the tests were performed according to the manufacturer's instructions.

Results

Dogs sampled in the study had clinically suspicious or significant signs of CDV infection. The clinical findings recorded in dogs are listed in Table 1.

The rates of clinical findings observed in dogs sampled in the study are as follows: 45.65% (42/92) eye mucopurulent discharge, 35.87% (33/92) nasal hyperkeratosis, 25% (23/92) nasal mucopurulent discharge, 13.04% (12/92) cough, 8.70% (8/92) diarrhea, 6.52% (6/92) fatigue, 1.09% (1/92) skin scaling, dermatitis around the eyes, anorexia, vomiting, myoclonus and hair loss (Table 1). While only 1 of the above-mentioned clinical findings was observed in 57 dogs, 26 dogs had two clinical findings and 9 had three of the above-mentioned clinical findings.

In the study, CDV antigen was investigated with a commercial immunochromatographic rapid test kit and CDV specific IgG and IgM antibodies were investigated with commercial ELISA kits in 92 blood serums taken from dogs for diagnostic purposes. Whereas the antigen positivity rate was 5.43% (5/92), the seropositivity rates for IgG and IgM antibodies were 80.43% (74/92) and

Table 1. The positivity rates of IgG and IgM antibodies according to the clinical findings of the dogs

Clinical Finding		IgG		IgM	
	N *(%)	IgG positive animals	**%	IgM positive animals	**%
Eye Mucopurulent Discharge	42 (45.65)	31	73.81	39	92.86
Nasal Hyperkeratosis	33 (35.87)	30	90.91	32	96.97
Nasal Mucopurulent Discharge	23 (25)	17	73.91	22	95.65
Cough	12 (13.04)	11	91.67	12	100
Diarrhea	8 (8.70)	7	87.5	7	87.5
Weakness	6 (6.52)	5	83.33	6	100
Cachexia	5 (5.43)	4	80	4	80
Flaking of the Skin	1 (1.1)	0	0	1	100
Myoclonus	1 (1.09)	1	100	1	100
Hair Loss	1 (1.09)	1	100	1	100
Dermatitis Around the Eyes	1 (1.09)	1	100	1	100
Anorexia	1 (1.09)	1	100	1	100
Vomiting	1 (1.09)	1	100	1	100

N: Number of animals showing clinical signs out of 92 dogs
*%: Percentage of animals with each observed clinical signs
**%: Percentage rates of IgG or IgM positivity in animals for each clinical signs

Table 2. Distribution of IgG and IgM antibodies in sampled dogs

		IgG		Total
		Positive (%)	Negative (%)	
IgM	Negative (%)	1 (%18.48)	4 (%4.35)	**5 (%5.43)**
	Positive (%)	17 (18.48)	70 (%76.09)	**87(%94.56)**
Total		**18 (%19.56)**	**74(%80.43)**	**92**

94.56% (87/92), respectively.

Of the samples tested by ELISA for the detection of IgG and IgM antibodies, 98.91% (91/92) were positive for one or both IgG and IgM antibodies, and 1.09% (1/92) were negative for both antibodies. It was determined that 76.09% (70/92) of these dogs were positive for both antibodies (IgM and IgG antibodies). While 18.48% (17/92) of the sampled dogs were positive only for IgM antibodies (negative for IgG), 4.35% (4/92) were found to be positive only for IgG antibodies (negative for IgM) (Table 2).

According to the method based on the presence of IgG and IgM antibodies defined by Richmann et al. (2017) and used for CDV infection by Saltik and Kale (2020), clinical periods of infection were evaluated as acute (IgG-, IgM+), early convalescent (IgG+, IgM+), late convalescent (IgG+, IgM-), and no infection (IgG-, IgM-). Table 3 shows the number of animals based on their infection status. In animals, clinical symptoms were most common in the early convalescent period (Table 4).

In the samples taken from the owned dogs brought to the private clinic, the positivity rates for IgM and IgG antibodies were detected at 89.29% (25/28) and 75% (21/28), respectively. In these dogs, antigen positivity was found at a rate of 3.57% (1/28), in the test performed with the rapid test kit. In 64 dogs from the rehabilitation center, a positivity rate of 96.87% (62/64) was found for IgM antibodies, whereas it was found to be 82.81% (53/64) positive in terms of IgG antibodies. Antigen positivity was detected in 6.25% (4/64) of 64 dogs examined with the rapid test kit.

Discussion

This study was carried out to determine the status of CDV infection in stray dogs in the rehabilitation center in Antalya and in owned dogs brought to private veterinary clinics. In the study, the clinical findings in dogs and the test results for the presence of antigen, IgG, and IgM were evaluated.

The detection of specific IgM antibodies has an important place in the diagnosis of CDV infections (Latha et al., 2007a), and it seems to be the ideal method for the serological diagnosis of acute distemper infections (von Messling et al., 1999). Saltık and Kale (2020) argue that the diagnosis gives highly reliable results by

Table 3. Number of animals in infection periods according to the presence of IgG and IgM antibodies against CDV

Infection periods	IgG	IgM	Animal / n	%
Acute	-	+	17/92	18.74
Early Convalescent	+	+	70/92	76.097
Late Convalescent	+	-	4/92	4.35
No Infection	-	-	1/92	1.09

testing IgG and IgM antibodies at the same time. Their study showed that detection of the canine distemper virus specific antibodies by the ELISA is quick and safe in naturally infected dogs. Consequently, this method is very useful for the pre-diagnosis of the disease when evaluated together with the clinical symptoms. In their research on 50 dogs, they found positivity rates for IgG and IgM antibodies of 94% (47/50) and 58% (29/50), respectively. Latha et al. (2007a) detected seropositivity for the presence of IgM antibodies by ELISA in 34 of the blood sera of 70 vaccinated dogs. Blixenkrone-Møller et al. (1993) collected blood serum from 66 vaccinated and unvaccinated dogs that were clinically suspicious and found 74% (49/66) IgM antibody positivity in these serums by ELISA. In the samples collected from the conjunctiva, they obtained antigen-positive results with a rate of 42% (27/65) by indirect immunofluorescence test. In this study, it was found to be 94.6% (87/92) positive for the presence of IgM antibodies in ELISA, whereas antigenpositivity was 5.43% (5/92) in the rapid test.

Çalışkan and Burgu (2007) found IgG-positivity at 41%. Esin (2013) reported that 70 of 116 (60.34%) dogs were IgG-positive in the ELISA. Saltık (2018) found 94% positive for IgG antibodies in his research. In this study, it was determined that 80.43% (74/92) of 92 dogs were positive for IgG antibodies in the ELISA.

In this study, 91 (98.91%) of the 92 samples tested with ELISA for detection of CDV-specific IgG and IgM antibodies were positive for either or both of these IgG or IgM antibodies, and 1 sample (1.09%) for both antibodies was found negative. This means that 98.91% of dogs are newly or previously infected with CDV. The reason for the high rate of up to 99% in the presented study can be shown that the sampling was done only in dogs with clinical findings. However, the fact that 99% of the animals brought to the clinic and rehabilitation center within 1 month show antibody positivity for distemper gives information that the prevalence of the infection has reached serious levels when the results of this study are compared with previous studies. It should also be noted that the dogs sampled in the study were chosen randomly from among dogs with internal health complaints, not because they were particularly suspicious of CDV. This shows that CDV infection is seriously prevalent in the region.

Depending on the severity of the infection and the viral strain, the virus-specific IgMs could be found from the 6-8th day up to the 3rd month following the infection (Appel and Summers, 1999; Barrett, 1999). Based on the literature information and the data obtained in the study, it is thought that acute CDV-infection is not short-lived in Antalya, but is widespread and effective for more than 3 months, and even endemic.

Richmann et al. (2017) reported the infection process as acute, early reconvalescent, and late reconvalescent periods based on the findings of IgG and IgM antibodies for viral infections such as measles and hepatitis A. Saltık (2018) determined the periods of CDV infection in line with the antibody findings using the same method in his study. In this study, 17 (18.48%) of the dogs sampled were positive only for IgM, 70 dogs (76.09%) were positive for both antibodies (IgG and IgM antibodies), and four dogs (4.35%) were found to be positive only for IgG antibodies. Based on these data, according to the evaluation method reported by Saltık (2020) and Richmann et al. (2017), 18.48% (17/92) of the animals were in the acute phase, and 76.09% (70/92) were in the early convalescent period. It was determined that 4.35% (4/92) of them were in the late reconvalescent period, that is, in the last period of the disease or had the disease. However, IgM-negative and IgG-positive results may also indicate chronic infection (Esin, 2013; Saltık, 2018).

The findings that only one dog did not have CDV infection and the high persentage rate of IgM antibodies indicate that the CDV infection is acutely intense in the region and that the dogs in the region are at a serious risk of distemper. Despite the inadequacy of the study in terms of epizootiology and the lack of epizootiological data, the high number of animals infected with CDV infection that were brought to clinics and a rehabilitation center within 1 month of sampling suggests that the viral load in Antalya is high. Consequently, the incidence may be high.

In some studies conducted in Turkey, it was reported that the rate of positivity was higher in dogs with respiratory and digestive system symptoms together (Çalışkan, 2007; Saltık and Kale, 2020). Blixenkrone-Møller et al. (1993) reported that they found the most (31/46) CDV positivity in terms of conjunctivitis, respiratory and gastro-intestinal symptoms. In this study, 45.65% (42/92) of the dogs sampled had mucopurulent discharge in the eye, 53.87% (33/92) nasal hyperkeratosis, 25% (23/92)

Table 4. Frequency of clinical findings during CDV infection periods

Clinical Finding		Acute IgG-/IgM+		Early Convalescent IgG+/IgM+		Late Convalescent IgG+/IgM-		No Infection IgG-/IgM-	
	N	n	%	n	%	n	%	n	%
Eye Mucopurulent Discharge	**42**	10	23.81	29	69.05	2	4.76	1	2.38
Nasal Hyperkeratosis	**33**	3	9.09	29	87.88	1	3.03	0	0
Nasal Mucopurulent Discharge	**23**	6	26.09	16	69.56	1	4.35	0	0
Cough	**12**	1	8.33	11	91.67	0	0	0	0
Diarrhea	**8**	1	12.50	6	75.00	1	12.50	0	0
Weakness	**6**	1	1.67	5	83.33	0	0	0	0
Cachexia	**5**	1	20	3	60	1	20	0	0
Flaking of the Skin	**1**	1	100	0	0	0	0	0	0
Myoclonus	**1**	0	0	1	100	0	0	0	0
Hair Loss	**1**	0	0	1	100	0	0	0	0
Dermatitis Around the Eyes	**1**	0	0	1	100	0	0	0	0
Anorexia	**1**	0	0	1	100	0	0	0	0
Vomiting	**1**	1	100	0	0	0	0	0	0

N: Number of animals showing clinical signs out of 92 dogs. n: Positive animals with clinical signs

nasal mucopurulent discharge, 13,04% (12/92) had cough, 8.70% (8/92) diarrhea, 6.52% (6/92) fatigue, 1.09% (1/92) had flaking of the skin, dermatitis around the eyes, loss of appetite, vomiting, myoclonus and hair loss were observed. It was observed that the period with the highest clinical symptoms was the early convalescent period (see Table 4). It can be said that the probable cause of most of the clinical findings seen in the late convalescent period is immunosuppression due to infection and secondary infections (Saltık, 2018).

Blixenkrone-Møller et al. (1993) found positive results in 50 out of 66 specimens, and they reported that 48 of those specimens were under the age of two. In this study, it was observed that all animals brought to the rehabilitation center and clinic were aged between 2 and 12 months. Of the 92 samples collected, 3.30% (3/92) positive for antibodies were aged 2–6 months, and 96.70% (88/92) were aged 6–12 months. It was determined that 32.97% (30/92) of the dogs were male and 67.03% (61/92) were female. Based on previous studies (Esin, 2013; Wang et al., 2018; Dorji et al., 2020), although no difference in susceptibility according to gender was reported in the disease, in this study, the number of female dogs with infection was twice as high as that of males. Dorji et al. (2020) reported that there were no significant differences in the seroprevalence of CDV among different sexes, breeds, age classes, pet and stray dogs, and between the two study sites in Western Bhutan.

In the samples taken from the owned dogs brought to the private clinic, antigen positivity was found in 3.6% (1/28), positive in terms of IgM antibodies in 89.3% (25/28) and IgG antibodies in 75% (21/28). While antigen positivity was found at a rate of 6.25% (4/64) in 64 dogs in the rehabilitation center, it was determined that they were 96.88% (62/64) positive for IgM antibodies and 82.81% (53/64) for IgG antibodies. The spread of CDV is primarily shaped by inhalation (Benieke et al., 2009). The severity of the disease, mortality and morbidity rates vary depending on the body's immune system and secondary infections. Especially in animal shelters with collective living conditions, a more suitable environment is created for the spread of the disease (Şahna et al., 2008). In the study, it is seen that the infection is quite intense in animals in shelters and street conditions. Since animals kept in rehabilitation centers and animal shelters are exposed to infection more frequently, they have an increased epizootiological risk as a source of viruses for both stray animals and domestic dogs after they are released into their natural environment. These centers play an important role in the spread of infectious diseases among animals. Animals with acute infections have a very high risk of transmitting the virus through saliva, feces, or aerosols. In the study, it can be said that stray and owned animals are at the same risk. Pets are always in contact with the street, and there is a possibility of contact with stray animals in order to meet the need for walking and toileting on the street. Therefore, there is always the risk of getting the virus. Since stray animals are unvaccinated and the virus is constantly circulating in them, they are in the position of a reservoir and pose

a potential risk to pets. For this reason, it is thought that establishing control programs against CDV and regularly vaccinating all dogs with and without owners living in city centers can minimize distemper-related losses. However, it is not currently feasible, either logistically or economically, to vaccinate all stray dogs. Therefore, it may be advisable to keep dogs at home, especially in the first phase, and only release them after they have been vaccinated against CDV. In addition, in order to prevent the spread of infectious diseases, it is necessary to develop control programs such as adopting stray dogs, neutering them, and improving the hygiene and management of shelters.

Due to their easy application in veterinary medicine and rapid results, immunochromatographic imaging systems, also called rapid tests, have become a highly preferred method. It is very practical and useful because the treatment protocol is quickly formed after simple and fast results are obtained and the diagnosis is made. Although the sensitivity and specificity of the rapid test are low, this test can be performed quickly before performing RT-PCR, electron microscopy, virus isolation, immunofluorescence, and ELISA tests due to its practicality (Elia et al., 2006). But in this study, the rate of antigen positivity with rapid testing was found to be very low (5.43%).

Wang et al. (2018) tested 32 nasal swab samples from clinically suspicious dogs with a rapid test kit and found 62% positivity. They also obtained the same results in their comparison with RT-PCR. Esin (2013) reported that 45 of the eye swabs collected from a total of 116 clinically suspicious animals were positive. Vivaldo (2019) reported that 54% positivity in ocular fluids, 51% in urine, and 46% in blood were observed in 141 dogs in his study. He reported that positivity was 62% by ELISA, 46% by the immunochromatographic method, and 95% by RT-PCR. In this study, positive results were obtained in 5/92 (5.4%) of the rapid antigen tests performed on blood serum. The reason for the low rates in the rapid antigen test may be the use of a serum sample instead of a conjunctival swab sample and the low amount of antigen in the circulation due to the formed antibodies. It is thought that this rate may be higher if it is done with tear samples. Due to the results obtained in this study, the availability of immunochromatographic rapid test methods in CDV diagnosis using different diagnostic materials (blood, serum, tears, saliva, etc.) should be investigated. In addition, it may be recommended to check the specificity and sensitivity of the rapid tests at periodic intervals, considering the antigenic variability of the virus, time, and geographical location.

Conclusion

As a result of this study, it was observed that an acute CDV infection was common in dogs in Antalya when the sampling was done, and an probably intense virus load was circulating in the region. It has been concluded that the infection poses the same risk to free-running dogs on the street, both stray and owned dogs. It has been observed that the rate of infected female animals is twice that of males, and it is frequently encountered in animals under one year old. Based on the results of this study, although epidemiological data is scarce, it is estimated that the incidence and prevalence of CDV infection may be high. In addition, it was observed that clinical findings of CDV infection were more intense in the early reconvalescent period.

Consequently, study data suggests that strategies to combat CDV infections need to be developed and strictly implemented. For this, more research on CDV's epizootiology (incidence, prevalence, host characteristics) and virological properties (genetic variability of the virus, the success of antibodies against vaccinia virus in protecting against existing strains in the region, the relationship between pathogenesis and field isolates, etc.) is needed.

Acknowledgements

This study was supported by Foundation of Scientific Research Projects of the Aydin Adnan Menderes University with project number VTF-18020. The paper was summarized from a Master's thesis of the first author.

Conflict of interest

The authors declare that they have no conflict of interest in this study.

References

Appel, M.J.G., & Summers, B.A. (1999). Canine distemper: Current Status. *Recent Advances in Canine Infectious Diseases*. https://www.ivis.com.

Barrett, T. (1999). Morbillivirus infections with special emphassis on morbilliviruses of carnivores. *Veterinary Microbiology*, 69, 3-13. https://doi.org.10.1016/s0378-1135(99)00080-2

Blixenkrone-Møller, M., Svansson, V., Have, P., Orvell, C., Appel, M., Rode Pedersen, I., & Henriksen, P. (1993). Studies on manifestations of canine distemper virus infection in an urban dog population. *Veterinary Microbiology*, 37(1-2), 163–173.

Çalışkan, E. (2007). Köpek Gençlik Hastalığı Virus İzolasyonu, H Proteini kodlayan gen bölgesinin karakterizasyonu ve seroepidemiyolojisi, Doktora Tezi, Ankara Üniversitesi, Sağlık Bilimleri Enstitüsü, Ankara.

Çalışkan, E., & Burgu, I. (2007). Köpek gençlik hastalığı virusunun prevalansı ve seroepidemiyolojisi. *Veterinary Microbiology*, 18, 5-10.

Dorji, T., Tenzin, T., K. Tenzin, K., Tshering, D., Rinzin, K., Phimpraphai, W., & de Garine-Wichatitsky, M. (2020). Seroprevalence and risk factors of canine distemper virus in the pet and stray dogs in Haa, western Bhutan. *BMC Veterinary Research*, 16, 135. https://doi.org/10.1186/s12917-020-02355-x.

Elia, G., Camero, M., Losurdo, M., Lucente, M. S., Larocca, V., Martella, V., Decaro, N., & Buonavoglia, C. (2015). Virological and serological findings in dogs with naturally occurring distemper. Journal of *Virological Methods*. 213, 127-130.

Esin, E. (2013). Köpeklerde Canine Distemper Virus Enfeksiyonun Araştırılması, Doktora Tezi, Konya Selçuk Üniversitesi, Sağlık Bilimleri Enstitüsü, Konya.

ICTV (2020). International Committee on Taxonomy of Viruses. *https://talk.ictvonline.org/taxonomy/* (accessed 20 August 2021).

Latha D., Geetha M., Ramadass., P., & Narayanan, R.B. (2007a). Development of recombinant nucleocapsid protein based IgM-ELISA for the early detection of distemper infection in dogs.

Veterinary Immunology and Immunopathology, 119, 278-286. https://doi.org.10.1016/j.vetimm.2007.06.006

Latha, D., Srinivasan, S.R., Thirunavukkarasu, P.S., Gunaselan, L., Ramadass, P., Narayanan, R.B. (2007b). Assessment of canine distemper virus infection in vaccinated and unvaccinated dogs. *Indian Journal of Biotechnology*, 6, 35-40.

Richmann, D.D., Whitley R.J., & Hayden, F.G. (2017). *Clinical virology*, 4th edition, American Society for Microbiology. Washington, 1489.

Sabatino, D. (2016). Lethal distemper in badgers (Meles meles) following epidemic in dogs and wolves, Infection, *Genetics and Evolution*, 46,130-137.

Saltık, H.S.(2018). Klinik semptomlu köpeklerin kan, idrar, rektal sürüntü, nasal ve oküler akıntı örneklerinde canine distemper virus enfeksiyonun real time RT-PCR ile tespit edilmesi. Doktora Tezi, Mehmet Akif Ersoy Üniversitesi, Sağlık Bilimleri Enstitüsü, Burdur.

Saltık, H.S., & Kale, M. (2020). Evaluation of infection with N protein-specific Immunoglobulin M and G in naturally occurring distemper in dogs. Veterinarni Medicina, 65, 2020 (04), 168–173. https://doi.org/10.17221/31/2019-VETMED.

Şahna, K.C., Gençay, A., & Atalay, O. (2008). Viral etilogy of diarrhoea in puppies from a same shelter in turkey, presence of mixed infections. *Revue de Médecine Vétérinaire*, 159, 345-347.

Von Messling, V., Harder, T.C., Moennig, V., Rautenberg, P., Nolte, I., & Haas, L. (1999). Rapid and sensitive detection of ımmunoglobulin M (IgM) and IgG antibodies against canine distemper virus by a new recombinant nucleocapsid protein-based enzyme-linked immunosorbent assay. *Journal of Clinical Microbiology*, 37(4), 1049–1056.

Wang, J., Li, R., Shi, R., Liu, L., & Yuan, W. (2018). Evaluation of an incubation instrument-free reverse transcription recombinase polymerase amplification assay for rapid and point-of-need detection of canine distemper virus. Journal of Virological Methods, 260, 56–61. https://doi.org.10.1016/jviromet.2018.07.007

Zacarias, J., Dimande, A., Achá, S., Dias, P.T., Leonel, E.M., Messa A., Macucule, B., Júnior, J.L., & Bila, C.G. (2016). Severe canine distemper outbreak in unvaccinated dogs in Mozambique. *Juornal of South African Veterinary Association*, 87(1), 1350. https://doi: 10.4102/jsava.v87i1.1350

Animal Health Prod and Hyg (2021) 10 (2) : 52 - 57

Animal Health, Production and Hygiene

www.aduveterinaryjournal.com

Research Article

The Effects of Footbath Management on Digital Dermatitis Distribution

Oğuzhan KALYONCU[1]*, Emre GÜRDAL[2], Yalçın Alper ÖZTURAN[3], İbrahim AKIN[3]

[1] Clinic for Cattle, University of Veterinary Medicine Hannover, Foundation, Hannover, Germany, [3] Department of Veterinary Surgery, Faculty of Veterinary Medicine, Adnan Menderes University, Aydin, Turkey, [2] Department of Food Hygiene and Technology, Faculty of Veterinary Medicine, Adnan Menderes University, Aydin, Turkey

ABSTRACT

Digital dermatitis (DD) is a common infectious disease that causes economic losses and lameness. In order to keep DD under control, the usage of footbath is very important in intensive dairy farms. It was aimed to determine the relationship between footbath use and the number of active lesions related to DD. In total of 1527 cattle from 6 dairy farms were included in the study to assess and correlate the prevalence of DD and footbath practices. Information about the farm structure and management practice of footbaths was obtained through a questionnaire. It was inspected all feet of cattle with DD lesions during the milking time and scored the lesions using six M-stages. Thereafter, the chi square test was performed to investigate the relation between digital dermatitis prevalence and footbath applications. DD lesions in 500 feet (8.2%) of 338 (22.1%) was observed in dairy cows. Farms using dry manure as a bedding material showed a higher DD prevalence (p<0.001). Farms using formalin footbath had the lowest DD lesions, whereas copper sulfate ($CuSO_4$) practice had the highest prevalence (p<0.001). Periodically renewed footbaths showed a statistically lower DD prevalence in farms (p<0.001). We concluded that the convenient design and management of footbath might help to reduce the prevalence of digital dermatitis in dairy farms.

Keywords: Cattle, copper sulfate, digital dermatitis, footbath, formalin

Ayak Banyosu Kullanımının Digital Dermatitis Dağılımına Etkileri

ÖZET

Digital dermatitis (DD); ekonomik kayıplara ve topallığa neden olan yaygın bir enfeksiyöz hastalıktır. Digital dermatitis'I lea ya altında tutmak için, entansif süt çiftliklerinde, ayak banyolarının kullanımı çok önemlidir. Bu çalışmanın amacı; ayak banyosu kullanımı ile DD'ye bağlı aktif lezyon sayısı arasındaki ilişkiyi araştırmaktı. DD lea yak banyosu uygulamalarının yaygınlığını değerlendirmek ve ilişkilendirmek için, 6 süt çiftliğinden toplam 1527 sığır çalışmaya dahil edildi. Çiftlik yapısı ve ayak banyolarının yönetim pratiği hakkındaki bilgiler, anket yoluyla elde edildi. İneklerin ayaklarındaki DD lezyonları, sağım periyodu sırasında altı M-aşaması kullanılarak skorlandı. Daha sonra DD prevalansı lea yak banyosu uygulamaları arasındaki ilişkiyi araştırmak için ki kare testi yapıldı. Çiftliklerdeki 338 (%22.1) süt ineğinin 500 adet ayağında (%8,2) DD lezyonları gözlemlendi. Altlık olarak kuru gübre kullanan çiftlikler, daha yüksek bir DD prevalansı gösterdi (p<0,001). Ayak banyosunda formalin kullanan çiftlikler en düşük DD lezyonlarına sahipken, bakır sülfat ($CuSO_4$) kullanan çiftlikler en yüksek prevalansa sahipti (p<0,001). Ayak banyolarını periyodik olarak yenileyen çiftlikler, istatistiksel olarak daha düşük bir DD prevalansı gösterdi (p<0,001). Yapılan çalışmalar ve elde edilen sonuçlar doğrultusunda, uygun ayak banyosu tasarımı ve yönetiminin süt çiftliklerinde DD prevalansının azaltılmasına yardımcı olabileceği sonucuna varıldı.

Anahtar Kelimeler: Ayak banyosu, bakır sülfat, digital dermatitis, formalin, sığır

*Corresponding Author: Oğuzhan KALYONCU: Clinic for Cattle, University of Veterinary Medicine Hannover, Foundation, Hannover, Germany, e-mail: Oguzhan.Kalyoncu@tiho-hannover.de

Received Date: 13.09.2021 – Accepted Date: 15.12.2021

DOI: 10.53913/aduveterinary.993485

Introduction

Digital dermatitis (DD) is a common infectious foot lesion in livestock animals typically in intensively managed dairy farming systems (Blowey, 2005; Cheli & Mortellaro, 1974; Cramer et al., 2008; Holzhauer et al., 2006; Read & Walker, 1998; Solano et al., 2016). DD is one of the most common causes of cattle lameness, which effects animal welfare and results with economic losses (Apley, 2015; Blowey & Sharp, 1988; Gomez et al., 2015). DD lesions can be painful and last for long terms (Bruijnis et al., 2012; Dopfer et al., 2012). Moreover, it can trigger disruptions in foot conformation that promote other infectious foot lesions (Gomez et al., 2015). In addition, DD has significant negative financial consequences associated with reduced milk production, reduced fertility rate, increased risk of culling, and increased cost of care (Akin & Akin, 2018; Bruijnis et al., 2010; Cha et al., 2010; Ettema et al., 2010; Holzhauer et al., 2008) due to its high incidence. Digital dermatitis is a multifactorial and a multi bacterial disease. *Treponema spp.* is frequently isolated from DD lesions (Döpfer et al., 2012; Gomez et al., 2015; Krull et al., 2016). This anaerobic spirochete bacteria can be identified in lesions (Blowey et al., 1994), indicating that they are invasive. Infected hoof trimming equipment and contact with manure are potential routes of transmission (Evans et al., 2012; Gillespie et al., 2020). Therefore, DD prevention techniques primarily involve the good hygiene practices and dry environment. To prevent the spreading of DD, host susceptibility (Scholey et al., 2010), inadequate hygiene and wet environments (Rodríguez-Lainz et al., 1996) should be eliminated and optimized.

Several control strategies have been recommended, including maintaining a clean, dry environment, individual topical treatment of affected cows, and herd-level strategies, including footbath usage (Dopfer et al., 2012; Laven & Logue, 2006; Nuss, 2006). Early intervention and effective topical treatments of active DD lesions increase cure rates and minimize spreading of the infection. In addition, an efficient footbath program can prevent active lesions from emerging (Dopfer et al., 2012). Footbaths are a common preventive method due to labor force needed to manage large numbers of sick cows, with strong evidence from intervention trials supporting the effectiveness of footbathing in reducing the incidence of DD (Fjeldaas et al., 2014; Laven & Logue, 2006; Relun et al., 2012; Speijers et al., 2010). The most common substances in footbath practice are $CuSO_4$ and formalin. Unfortunately, $CuSO_4$ has adverse environmental effects (Flemming & Trevors, 1989; Hoff et al., 1998) and formalin is carcinogenic (Doane & Sarenbo, 2014). These impacts show the need for more experimental research on new chemicals or discovering the most effective use of the existing products. It is important to determine how often footbaths should be performed based on the hygiene score (Reneau et al., 2005). In order to increase the effectiveness of footbaths, cleaning animal's feet before entering the bath solution is important. The passage of animals in footbaths must be comfortable and the possibility of slipping should be eliminated. Mismanagement of one or more of these key points may decrease efficacy regardless of which footbath is used. Nevertheless, there is a broad variety in on-farm practices related to footbath protocols (Cook et al., 2012; Relun et al., 2013; Solano et al., 2015).

It was aimed to determine the relationship between footbath use and the number of active lesions related to DD. The protocol based on current scientific literature including footbath chemicals and management practice.

Materials and Methods

Farm and Cow Selection

A total of 6 freestall dairy farms in Aydin province in Turkey were contacted by a phone call to be enrolled as part of a longitudinal study. We selected the farms that met the requirements of the study. The requirements of the farms were; to have a freestall housing with no access to pasture, the herd size should be more than 50 lactation cows, cows had to milk in a milking parlor and farms had to have a DD prevalence (regardless of six M-stage) of ≥ 10%. Six farms met all criteria and agreed to participate in the study from July 2016 to July 2017. The Turkish Scientific Council of Turkey (2209/A, Application Number- 1919B011602116) approved all of the methods.

Study Design

A longitudinal observational study was conducted on

Table 1. Detailed table about footbath application practice from six dairy farms in Aydin, Turkey

Farm	Bedding type	Footbath dimensions (length × width × height) (cm)	Active Component	The number of footbath application days per week *
1	Dry Manure	150 × 150 × 10	$CuSO_4$, Formalin	7d
2	Dry Manure	100 × 300 × 15	$CuSO_4$	2d
3	Dry Manure	90 × 300 × 20	$CuSO_4$	3d
4	Rubber Mattress	100 × 200 × 10	Formalin	5d
5	Straw Bedding	100 × 300 × 15	$CuSO_4$, Formalin	2d
6	Dry Manure	-	-	-

*All farms except farm 6, applied footbath practices after milking.

Table 2. Distribution and prevalence of digital dermatitis (DD) lesions in farms

Farm	Number of cattle affected by digital dermatitis/ total number of cattle (%)	Affected Legs*				Total number of legs affected by digital dermatitis, n (%)
		RF	LF	LH	RH	
1	39 / 160 (24.38)	2	-	28	23	53 (33.97)
2	97 / 203 (47.78)	8	11	67	78	164 (42.27)
3	81 / 224 (36.16)	-	1	57	55	113 (34.88)
4	62 / 543 (11.42)	43	-	39	-	82 (33.06)
5	49 / 272 (18.01)	1	1	31	38	71 (36.22)
6	10 / 125 (8.00)	1	1	9	6	17 (3.40)
Total, n (%)	338 / 1527 (22.13)	55	14	231	200	500 (8.18)

* RF: Right front leg, LF: Left front leg, LH: Left hind leg, RH: Right hind leg

the selected farms. All lactating cows were assessed for DD lesions in the milking parlor. The 6 farms were visited on predetermined days to gathering information about prevalence of DD, farm structure, footbath and management practice (Table 1). We inspected the entire lactating herd in the milking parlor. Farmers were encouraged to continue their regular individual treatments for DD.

General Management

A questionnaire was conducted in each farm. The questions were either open-ended (e.g.," What is the active ingredient used as a footbath?) (Table 1) or closed-ended (e.g.," Do you use a footbath on the farm?; response scale: yes or no). Specific information on the frequency of its use (how many times per week) and the renewing frequency of solutions also obtained from the questionnaire. In addition, the dimensions of footbaths measured (length, depth, and width) on each farm (Table 1).

Assessment of Digital Dermatitis Lesions

According to Döpfer et al. (1997), the DD lesions were scored by using the six M stages. The skin categorized as M5, if it was normal and without any DD-compatible lesions. Lesions were categorized as M1, if a small focal clinical lesion (< 2 cm in diameter) observed, with red-gray surface and small red foci dispersed (~ 1 mm in diameter), M2 if an ulcerative active lesion of around 2 cm in diameter observed, with extensively mottled a red-gray surface. M3 (healing stage) was determined if the lesion has a dry brown and scab-like tissue. This typically seen within a few days of topical treatment. Lesions were categorized as M4 (chronic stage) if a tan irregular hyperkeratotic surface was observed. M4.1 was chronic stage with a small active painful M1 focus. A professional observer completed all DD assessments throughout the presented study. Except M5, other M stages recorded as digital dermatitis.

Milking Parlor Inspection

Throughout the entire study, we visited each farm to obtain the correlation between footbath usage and its effects on the prevalence of digital dermatitis. During milking, we washed cow's hind and front feet with hose water before the examination to get a clear image of the lesion if present.

Statistical Analyses

We entered all gathered data into Excel (Microsoft Corp., Redmond, WA) and performed all statistical analyses using IBM SPSS Statistic 22.0® (IBM, Armonk, NY). A *p*-value of <0.05 considered significant for all analyses. The relationship between footbath use and the number of active lesions related to DD examined using the "Chi - Square" test.

Results

Average herd size was 254.5 lactating cows (range, 125 to 543). Footbaths had a median length of 108 cm (range, 90 to 150), width of 250 cm (range, 150 to 300) and a mean depth of 14 cm (range, 10 to 20). Two farms used a combination of two active chemicals ($CuSO_4$ and Formalin). All farms had an individual DD treatment protocol in place. Farm number 3 and 4 renewed their footbath after milking, whereas farm number 1, 2 and 5 did not renew it regularly.

Digital Dermatitis Prevalence

A total of 6108 observations on 1527 lactating cows (6108 feet) in 6 farms were collected throughout the study period. As a result, Digital Dermatitis observed in 500 feet (8.2%) of 338 (22.1%) dairy cows in total. 5608 feet classified as healthy (no DD lesions) (91.8%) (Table 2).

Associations Between Digital Dermatitis and Footbath Managements

The existence of digital dermatitis cases was statistically significant between dry manure (farm no: 1, 2, 3, and 6) (farm no: 1, 2, 3, and 6) with rubber mattress bedding (farm no: 4) practices (p<0.001). There was also a statistically significant difference in the presence of digital dermatitis between straw (farm no: 5) with dry manure bedding (farm no: 1, 2, 3, and 6) practices (p<0.001). There were statistically significant differences between $CuSO_4$ (farm no: 4) and $CuSO_4$+formalin (farm no: 1 and 5) farms on the relationship between the use

Table 3. Digital dermatitis (DD) lesion distribution and their association with management factors

		Number of DD Lesions (n)		Farm Number	P -value
		Exist	Non-Exist		
Bedding material	Dry Manure	227	485	(1, 2, 3, 6)[a]	<0.001
	Straw Bedding	49	223	(5)[b]	
	Rubber Mattress	62	481	(4)[b]	
Active chemical	$CuSO_4$	178	249	(2, 3)[a]	<0.001
	Formalin	62	481	(4)[a]	
	CuSO4, Formalin	88	344	(1, 5)[b]	
	Not Used	10	115	(6)[b]	
Frequency of footbath application per week?	2	155	320	(2, 5)[a]	<0.001
	3	81	143	(3)[a]	
	5	62	481	(4)[a]	
	7	39	121	(1)[b]	
	Not Used	10	115	(6)[a]	

* Different superscript letters indicate statistical significance.

and types of active chemicals and the existence of digital dermatitis (p<0.001). The existence of digital dermatitis lesions was statistically significant (p<0.001) on farms with footbath use with $CuSO_4$+Formalin (farm no: 1 and 5) and no footbath used dairy farms (farm no: 6). There was a statistically significant difference in digital dermatitis existence between farms that used formalin as an active chemical on footbath (farm no: 4) and farms that did not use footbath practice (farm no: 6) (p<0.001). According to the frequency of footbath use, digital dermatitis lesion cases were statistically higher (p<0.001) in farms (farm numbers 2 and 3,4, 5) that did not renew or did not implement any footbath in their farm (farm no: 6).

Discussion

In this study, the prevalence of DD lesions in herds was associated with footbath management. As an interpretation, footbath practices among producers remain empirical as it relies on mouth-to-mouth advice from other farmers leading non-standardized managements (Relun et al., 2013).

It is widely known that inadequate hygiene is leading to digital dermatitis (Potterton et al., 2012). Dairy management practices such as floor scrapping, bedding material, and cow's diet may directly affect environmental hygiene and lead to contaminated footbaths. The defecation rates inside of the footbath were mentioned in previous studies (Fjeldaas et al., 2014). A previous study claimed that cows can defecate in the alleys between the milking parlor and footbath after adaptation of frequent renewal of footbaths (Ariza et al., 2019). The renewal rate of footbath can affect the defecation rate inside the footbath therefore decrease the contamination of footbath solution especially for the farms with a long interval (Ariza et al., 2019). Also, a large volume of footbaths and less frequency of renewal might lead footbath solutions to a slurry include manure, urine, and dirt (Holzhauer et al., 2006). The close and frequent contact of the feet with slurry might alter the skin permeability, and increase the risk of infection (Palmer et al., 2013). Solano et al. (2017) investigated the influence of literature guidelines concerning footbath dimensions and protocols using a footbath protocol of 5% $CuSO_4$ for 4 consecutive milking (2 days), with a limitation of 200 total passing cows. They assumed that footbath dimensions and changing frequency might have an effect in the prevalence of DD lesions. In our present findings, average value of footbath dimensions was 108 cm × 250 cm × 14 cm, whereas just two of the farms renewed their footbaths regularly after milking. There were statistically significant differences between footbath used and not used dairy farms (p<0.001). Interestingly, there was no statistically significant difference in between farms with footbaths in terms of frequency of footbath use (Table 3).

The presence of feces creates a suitable environment for DD factors (Sullivan et al., 2015). Similarly, in the present study, the prevalence of DD lesions was more common in farms using dry manure as a bedding material. The possible reason for this is that rain and constant urination are unable to avoid. If not managed properly, it should be assumed that the causative source could be the bedding material used, and it should assess that DD lesions may be more prominent. In the present study, there were statistically significant differences on cases of digital dermatitis lesions between dry manure bedding with straw or rubber mattress used dairy farms as a bedding material (p<0.001). This result may be related with the use of dry manure which led an environment for DD lesions on dairy farms.

In the present findings, there were statistically significant differences on DD lesions cases with footbath practices and active chemical used in footbaths (Table 3, p<0.001).

Despite the widespread use and proven effectiveness of footbaths (Laven & Logue, 2006), footbathing is an expensive practice primarily due to labor costs (Bruijnis et al., 2013). On the other hand, the advantages of providing clear and accurate footbath procedures may lead to a decreased incidence of DD and, therefore, lower costs associated with hoof trimming, treatments, and a high benefit to welfare. However, in the present study, there was no footbath in Farm No: 6, and Digital Dermatitis lesions were detected in 8.0% of the population. As a limitation, the concentration of used footbath chemicals, renew rate of the farm number 1, 2 and 5, the chemical amount used in footbaths, hygiene of the walking alleys or claws were not taken into account in the present study due to limited visit days to the farms and the willingness of not intervene any protocol in farms. This may be related to the present study's finding such as there was no statistically significant relationship between the use of combined use of $CuSO_4$+formalin and digital dermatitis prevalence (Table 3). It may be more beneficial not to use a footbath at all, than if it is not prepared properly.

Conclusion

The use of footbath is very important in terms of the prevalence of digital dermatitis. Mentioning these results in farms that exist or will be built in similar climatic geographic regions may help to decrease the prevalence of digital dermatitis. Furthermore, instead of inappropriate usage on fields, it might be best not to use footbath at all.

Acknowledgement

Authors would like to thanks to The Scientific and Technological Research Council of Turkey for funding the research project 2209/ A, Application Number-1919B011602116 and Aykut Göktürk Üner for their contributions to statistical analysis.

Conflict of Interest

The authors declare that they have no conflicts of interest.

References

Akin, I., & Akin, T. (2018). Economic impact of digital dermatitis treatment on a dairy farm: an application of the break-even analysis. *Ciência Rural*, 48(8). 10.1590/01038478cr20170791.

Apley, M. D. (2015). Clinical evidence for individual animal therapy for papillomatous digital dermatitis (hairy heel wart) and infectious bovine pododermatitis (foot rot). *Veterinary Clinics of North America: Food Animal Practice*, 31(1), 81-95. 10.1016/j.cvfa.2014.11.009.

Ariza, J. M., Bareille, N., Oberle, K., & Guatteo, R. (2019). Current recommendations for footbath solutions renewal rates in dairy cattle: the need for adaptation? *Animal*, 13(6), 1319-1325. 10.1017/S1751731118002847.

Blowey, R. (2005). Factors associated with lameness in dairy cattle. *In Practice*, 27(3), 154-162. 10.1136/inpract.27.3.154.

Blowey, R. W., & Sharp, M.W. (1988). Digital dermatitis in dairy cattle. *Veterinary Record*, 122(21), 505-508.-10.1136/vr.122.21.505.

Blowey, R.W., Carter, S.D., White, A. G., & Barnes, A. (1994). Borrelia burgdorferi infections in UK cattle: a possible association with digital dermatitis. *Veterinary Record*, 135(24), 577-578.

Bruijnis, M. R., Beerda, B., Hogeveen, H., & Stassen, E. N. (2012). Assessing the welfare impact of foot disorders in dairy cattle by a modeling approach. *Animal*, 6(6), 962-970. 10.1017/S1751731111002606.

Bruijnis, M. R., Hogeveen, H., & Stassen, E. N. (2013). Measures to improve dairy cow foot health: consequences for farmer income and dairy cow welfare. *Animal*, 7(1), 167-175. 10.1017/S1751731112001383.

Bruijnis, M.R., Hogeveen, H., & Stassen, E.N. (2010). Assessing economic consequences of foot disorders in dairy cattle using a dynamic stochastic simulation model. *Journal of Dairy Science*, 93(6), 2419-2432. 10.3168/jds.2009-2721.

Cha, E., Hertl, J.A., Bar, D., & Grohn, Y.T. (2010). The cost of different types of lameness in dairy cows calculated by dynamic programming. *Preventive Veterinary Medicine*, 97(1), 1-8. 10.1016/j.prevetmed.2010.07.011.

Cheli, R., & Mortellaro, C. (1974). La dermatite digitale del bovino. *In* Proceedings of the 8th International Conference on Diseases of Cattle, Piacenza, Milan, pp.208-213.

Cook, N.B., Rieman, J., Gomez, A., & Burgi, K. (2012). Observations on the design and use of footbaths for the control of infectious hoof disease in dairy cattle. *The Veterinary Journal*, 193(3), 669-673. 10.1016/j.tvjl.2012.06.051.

Cramer, G., Lissemore, K.D., Guard, C.L., Leslie, K.E., & Kelton, D.F. (2008). Herd- and cow-level prevalence of foot lesions in Ontario dairy cattle. *Journal of Dairy Science*, 91(10), 3888-3895. 10.3168/jds.2008-1135.

Doane, M., & Sarenbo, S. (2014). Exposure of farm laborers and dairy cattle to formaldehyde from footbath use at a dairy farm in New York State. *Science of the Total Environment*, 487, 65-71. 10.1016/j.scitotenv.2014.04.007.

Döpfer, D., Anklam, K., Mikheil, D., & Ladell, P. (2012). Growth curves and morphology of three Treponema subtypes isolated from digital dermatitis in cattle. *The Veterinary Journal*, 193(3), 685-693. 10.1016/j.tvjl.2012.06.054.

Döpfer, D., Holzhauer, M., & Boven, M. (2012). The dynamics of digital dermatitis in populations of dairy cattle: model-based estimates of transition rates and implications for control. *The Veterinary Journal*, 193(3), 648-653. 10.1016/j.tvjl.2012.06.047.

Döpfer, D., Koopmans, A., Meijer, F. A., Szakáll, I., Schukken, Y. H., Klee, W., Bosma, R. B., Cornelisse, J. L., van Asten, A. J., & ter Huurne, A. A. (1997). Histological and bacteriological evaluation of digital dermatitis in cattle, with special reference to spirochaetes and Campylobacter faecalis. *The Veterinary record*, 140(24), 620–623. 10.1136/vr.140.24.620

Ettema, J., Ostergaard, S., & Kristensen, A.R. (2010). Modelling the economic impact of three lameness causing diseases using herd and cow level evidence. *Preventive Veterinary Medicine*, 95(1-2), 64-73. 10.1016/j.prevetmed.2010.03.001.

Evans, N.J., Timofte, D., Isherwood, D.R., Brown, J.M., Williams, J.M., Sherlock, K., & Carter, S.D. (2012). Host and environmental reservoirs of infection for bovine digital dermatitis treponemes. *Veterinary Microbiology*, 156(1-2), 102-109. 10.1016/j.vetmic.2011.09.029.

Fjeldaas, T., Knappe-Poindecker, M., Boe, K.E., & Larssen, R.B. (2014). Water footbath, automatic flushing, and disinfection to improve the health of bovine feet. *Journal of Dairy Science*, 97(5), 2835-2846. 10.3168/jds.2013-7531.

Flemming, C.A., & Trevors, J.T. (1989). Copper toxicity and chemistry in the environment: a review. *Water, Air, and Soil Pollution*, 44(1), 143-158. 10.1007/BF00228784.

Gillespie, A.V., Carter, S.D., Blowey, R.W., Staton, G.J., & Evans, N.J. (2020). Removal of bovine digital dermatitis-associated treponemes from hoof knives after foot-trimming: a disinfection field study. *BMC Veterinary Research*, 16(1), 330. 10.1186/s12917-020-02552-8.

Gomez, A., Cook, N.B., Rieman, J., Dunbar, K. A., Cooley, K.E., Socha, M.T., & Dopfer, D. (2015). The effect of digital dermatitis on hoof conformation. *Journal of Dairy Science*, 98(2), 927-936. 10.3168/jds.2014-8483.

Hoff, B., Boermans, H.J., & Baird, J.D. (1998). Retrospective study of toxic metal analyses requested at a veterinary diagnostic toxicology

laboratory in Ontario (1990-1995). *Canadian Veterinary Journal*, 39(1), 39-43.

Holzhauer, M., Döpfer, D., de Boer, J., & van Schaik, G. (2008). Effects of different intervention strategies on the incidence of papillomatous digital dermatitis in dairy cows. *Veterinary Record*, 162(2), 41-46. 10.1136/vr.162.2.41.

Holzhauer, M., Hardenberg, C., Bartels, C.J., & Frankena, K. (2006). Herd- and cow-level prevalence of digital dermatitis in the Netherlands and associated risk factors. *Journal of Dairy Science*, 89(2), 580-588. 10.3168/jds.S0022-0302(06)72121-X.

Krull, A.C., Cooper, V.L., Coatney, J.W., Shearer, J.K., Gorden, P.J., & Plummer, P.J. (2016). A Highly Effective Protocol for the Rapid and Consistent Induction of Digital Dermatitis in Holstein Calves. *PLOS ONE*, 11(4), e0154481. 10.1371/journal.pone.0154481.

Laven, R.A., & Logue, D.N. (2006). Treatment strategies for digital dermatitis for the UK. *The Veterinary Journal*, 171(1), 79-88. 10.1016/j.tvjl.2004.08.009.

Nuss, K. (2006). Footbaths: the solution to digital dermatitis? *The Veterinary Journal*, 171(1), 11-13. 10.1016/j.tvjl.2005.02.010.

Palmer, M.A., Donnelly, R.F., Garland, M.J., Majithiya, R., & O'Connell, N.E. (2013). The effect of slurry on skin permeability to methylene blue dye in dairy cows with and without a history of digital dermatitis. *Animal*, 7(10), 1731-1737. 10.1017/s1751731113001274.

Potterton, S.L., Bell, N.J., Whay, H.R., Berry, E.A., Atkinson, O.C., Dean, R.S., & Huxley, J.N. (2012). A descriptive review of the peer and non-peer reviewed literature on the treatment and prevention of foot lameness in cattle published between 2000 and 2011. *The Veterinary Journal*, 193(3), 612-616. 10.1016/j.tvjl.2012.06.040.

Read, D.H., & Walker, R.L. (1998). Papillomatous digital dermatitis (footwarts) in California dairy cattle: clinical and gross pathologic findings. *Journal of Veterinary Diagnostic Investigation*, 10(1), 67-76. 10.1177/104063879801000112.

Relun, A., Guatteo, R., Auzanneau, M.M., & Bareille, N. (2013). Farmers' practices, motivators and barriers for adoption of treatments of digital dermatitis in dairy farms. *Animal*, 7(9), 1542-1550. 10.1017/s1751731113000803.

Relun, A., Lehebel, A., Bareille, N., & Guatteo, R. (2012). Effectiveness of different regimens of a collective topical treatment using a solution of copper and zinc chelates in the cure of digital dermatitis in dairy farms under field conditions. *Journal of Dairy Science*, 95(7), 3722-3735. 10.3168/jds.2011-4983.

Reneau, J.K., Seykora, A.J., Heins, B.J., Endres, M.I., Farnsworth, R.J., & Bey, R.F. (2005). Association between hygiene scores and somatic cell scores in dairy cattle. *Journal of the American Veterinary Medical Association*, 227(8), 1297-1301. 10.2460/javma.2005.227.1297.

Rodríguez-Lainz, A., Hird, D.W., Walker, R.L., & Read, D.H. (1996). Papillomatous digital dermatitis in 458 dairies. *Journal of the American Veterinary Medical Association*, 209(8), 1464-1467.

Scholey, R., Ollier, W., Blowey, R., Murray, R., & Carter, S. (2010). Determining host genetic susceptibility or resistance to bovine digital dermatitis in cattle. *Advances in Animal Biosciences*, 1(1), 2-2.

Solano, L., Barkema, H.W., Mason, S., Pajor, E.A., LeBlanc, S.J., & Orsel, K. (2016). Prevalence and distribution of foot lesions in dairy cattle in Alberta, Canada. *Journal Dairy Science*, 99(8), 6828-6841. 10.3168/jds.2016-10941.

Solano, L., Barkema, H.W., Pajor, E.A., Mason, S., LeBlanc, S.J., Zaffino Heyerhoff, J.C., & Orsel, K. (2015). Prevalence of lameness and associated risk factors in Canadian Holstein-Friesian cows housed in freestall barns. *Journal of Dairy Science*, 98(10), 6978-6991. 10.3168/jds.2015-9652.

Solano, L., Barkema, H.W., Pickel, C., & Orsel, K. (2017). Effectiveness of a standardized footbath protocol for prevention of digital dermatitis. *Journal of Dairy Science*, 100(2), 1295-1307. 10.3168/jds.2016-11464.

Speijers, M.H., Baird, L.G., Finney, G.A., McBride, J., Kilpatrick, D.J., Logue, D.N., & O'Connell, N.E. (2010). Effectiveness of different footbath solutions in the treatment of digital dermatitis in dairy cows. *Journal of Dairy Science*, 93(12), 5782-5791. 10.3168/jds.2010-3468.

Sullivan, L.E., Evans, N.J., Blowey, R.W., Grove-White, D.H., Clegg, S.R., Duncan, J. S., & Carter, S.D. (2015). A molecular epidemiology of treponemes in beef cattle digital dermatitis lesions and comparative analyses with sheep contagious ovine digital dermatitis and dairy cattle digital dermatitis lesions. *Veterinary Microbiology*, 178(1-2), 77-87. 10.1016/j.vetmic.2015.04.011.

Animal Health Prod and Hyg (2021) 10 (2) : 58 - 65

Animal Health, Production and Hygiene

www.aduveterinaryjournal.com

Review

miRNA and Biogenesis

Beyza SUVARIKLI ALAN[1*], Mehmet NİZAMLIOĞLU[1], Zafer BULUT[1]

[1] *Selçuk University, Faculty of Veterinary Medicine, Department of Biochemistry, 42031 Konya, Turkey*

ABSTRACT

The number of uncoded (non-coding; nc) RNAs with known functions is increasing each day. Since their first detections in 1993, micro RNA of ncRNAs have become very important as shown by many studies. In fact, their importance has been well understood and their relations with diseases are well documented that they can be used in the diagnosis of most diseases in the future. In this review, topics such as general properties, structure and biogenesis of miRNAs were discussed.

Keywords: miRNA, Drosha, Dicer

miRNA ve Biyogenezi

ÖZET

Fonksiyonu belirlenen kodlanmamış (non-coding; nc) RNA'ların sayısı her geçen gün artmaktadır. İlk olarak 1993 yılında bulunmasından sonra çok sayıda araştırma ile ncRNA'lardan olan miRNA'lar çok önemli yere sahip olmuşturlar. Hatta önemi her geçen gün daha çok anlaşılmakta ve hastalıklar ile ilişkileri daha çok belirlenmekte, gelecekte de çoğu hastalığın teşhisinde kullanılabilecekleri açıktır. Bu derlemede, miRNA'ların genel özellikleri, yapısı, biyogenezi gibi konular ele alınmıştır.

Anahtar kelimeler: miRNA, Drosha, Dicer

Corresponding Author: Beyza SUVARIKLI ALAN, Selçuk University, Faculty of Veterinary Medicine, Department of Biochemistry, 42031 Konya, Turkey e-mail: beyza.alan@selcuk.edu.tr
Received Date: 11.10.2021 – Accepted Date: 01.12.2021
DOI Number: 10.53913/aduveterinary.1008317

Introduction

Gene editing is focused on genes that encode proteins through DNA-mRNA-protein dogma. Cellular functions are regulated by the expression of protein encoding or non-coding (nc) RNAs that have important functions in both nucleus and cytoplasm (Williams et al., 2018). Therefore, it is important to know the RNA structure and to understand the functions of all RNAs. In addition, RNAs are also critical because of their role in the regulation of gene expression (Mortimer et al., 2014). RNA is a biopolymer consisting of organic bases (adenine, uracil, guanine, cytosine), ribosine sugar, and a phosphate group. These monomers combine between the 3′ end of one sugar molecule and 5' carbon atom of the other by having a phosphodiester bond. All prokaryote and eukaryote cells have three main types of RNA such as messenger RNA (mRNA), transfer RNA (tRNA) and ribosomal RNA (rRNA) (Jedrzejczyk et al., 2017). A wide range of small RNAs have been found and recent developments have led to the discovery of small RNAs in eukaryotic cells. Noncoding RNAs from these RNAs have been examined in three different categories based on biogenesis, their mechanism and the type of Argounaute protein they interact. Of these, small noncoding RNAs are microRNAs (miRNA), small interfering RNAs (siRNA), and Piwi-interacting RNAs (piRNA) (Kim et al., 2009). The discovery of miRNAs, the subject of this review, is very important in modern molecular biology and genetics. A large number of cell pathways, which are important in both disease and health condition, appear to be regulated by miRNAs (Shukla et al., 2011). The number of miRNAs defined by the development and use of highly efficient sequencing techniques is abundant these days (Olena and Patton, 2010). miRNAs, which target most protein-coding transcripts, play a role in almost all developmental and pathological events in eukaryotes. It is reportedly associated with many human diseases, including cancer and neurodevelopmental disorders (Ha and Kim, 2014).

Classification of Non-Coding RNAs

ncRNAs are usually distinguished by their length; Short non-coding RNAs have a length of <30 nucleotides while long non-coding RNAs have a nucleotide count of more than 200. In addition, these RNAs are molecules acting as negative regulators of gene expression in the RNA interference (RNAi) mechanism (Santosh et al., 2014).

Short non-coding RNAs are divided into three main groups such as miRNA, piRNA, and siRNA. These RNAs are quite difficult to distinguish from each other (Carthew and Sontheimer, 2009). Obviously, biochemically and functionally, miRNA and siRNA are indistinguishable. These molecules can be distinguished by their origin.

When these three RNA types are examined in terms of common and different characteristics:

1-While siRNA and miRNA bind to argonoute proteins, piRNAs bind to Piwi members (Carthew and Sontheimer, 2009).

2- miRNAs and siRNAs are both approximately 19-20 nucleotide lengths.

3- They combine with the RISC complex in silencing gene expression (MacFarlane and Murphy, 2010).

4- The cellular origins of miRNA and siRNA are slightly different; miRNAs are endogenous products of an organism's genome while siRNAs are mostly caused by exogens derived from viruses and similar organisms (Carthew and Sontheimer, 2009; Wilson and Doudna, 2013).

5- siRNAs are complementary to their targets whereas miRNAs (those found in animals) demonstrate limited complementariness (Shabalina and Koonin, 2008).

6- miRNA sequences are almost always preserved in the relevant organism whereas siRNA sequences are rarely preserved (Bartel, 2004).

7- siRNAs are found in plants, animals and fungi. It provides antiviral defense in plants and animals. By contrast, miRNAs are found only in terrestrial plants, single-celled green *algae Chlamydomonas reinhardtii* and metazoan animals. They are not found in single cell choanoflagellates and fungi (Ghildiyal and Zamore, 2009).

History of miRNA

In 1993, Lee and his *colleagues* first discovered a miRNA called linage-4 (lin-4), which was 22 nt (nucleotide) long and did not encode a protein in *Caenorhabditis elegans*. In the study conducted by Wightman et al. (1993) on C. elegans, it was shown that lin-4 acts as a negative regulator of the lin-14 gene. Reinhart et al. (2000) discovered a miRNA, called lethal-7 (let-7), a 22 nt long in *C. elegans* that has been shown to control the transition from larvae to adulthood. It was observed that transition from the larval period to the adult period was impired when there was a loss in the activation of Let-7. After the discovery of Let-7, the short RNA's were called micro RNA (miRNA) (Lucas and Raikhel, 2013; Bartel, 2004; Wienholds and Plasterk, 2005; Carthew and Sontheimer, 2009; Tetreault and Guire, 2013).

Small RNAs do not encode any proteins, but rather terminate the functions of protein-coding mRNAs. Due to these functions, they are known as the negative control mechanism of gene expression (Carthew and Sontheimer, 2009). These RNA molecules are present in precursor forms with no functions when they are first produced. They are then processed and acquire their functional structures (Kim et al., 2009). First discovered in *Caenorhabditis elegans*, miRNAs were later shown to exist in many eukaryotes, including humans (MacFarlane and Murphy, 2010). Complex genomes are known to encode hundreds of miRNA genes (Graves and Zeng, 2012). It is thought that about 1000 miRNAs in the human genome are encoded (Cowland et al., 2007). Most miRNAs are expressed from genome regions that

differ from known protein encoding sequences (Du and Zamore, 2005). In other words, most miRNAs are produced within the cell by non-encoding gene regions (Cai et al., 2004). The coding loci of some miRNAs are located separately from other miRNAs and indicate that they create their own transcription units; others cluster and share similar patterns of expression, which indicates that they are transcribed as polycistronic transcripts (Du and Zamore, 2005). About half of the known mammalian miRNAs are found in introns of protein-coding genes or introns or exons of non-encoding RNAs (Du and Zamore, 2005).

Biogenesis and processing of Non-Coding RNAs

The biogenesis of miRNAs is a highly complex, tightly regulated multi-step transcription that begins in the nucleus of the cell and continues throughout the cytoplasm through which mature miRNA completes its main function (Melo and Melo, 2013). Since miRNA biogenesis contains multiple steps, the abnormalities that occurs at any step of the process can prevent effective miRNA maturation (Williams et al., 2018). miRNAs are non-encoded RNAs, which means that they are transcribed but do not yield protein synthesis. However, this does not imply that these RNAs do not contain information and have no functions (Mattick and Makunin, 2006). Mature miRNA molecules are thought to be partially complementary with one or more mRNA. Their main function is to regulate downstream gene expression (Pillai, 2005).

miRNAs are produced with the action of two RNase-type proteins (Dicer and Drosha). Then they are connected with Ago proteins, the main component of the RISC complex. In this way, they perform their functions as post-transcription regulators (Kim et al., 2009). In miRNA biogenesis, miRNAs are first copied to the primary miRNA (pri-miRNA). These pri-miRNAs have a 7-methylguanosine cap at the 5' end, such as mRNAs. At the 3' end is the poly A tail (MacFarlane and Murphy, 2010). pri-miRNAs consist of a single chain. They curl up on themselves to form hairpin structures (Melo and Melo, 2013). Then, the pri-miRNA is cleaved by the enzyme Drosha in the nucleus. That is, they cleave the hairpin structure of the pri-miRNA. Enzyme-cut miRNAs turn into pre-miRNA (Alvarez-Garcia and Miska, 2005). Exportin-5 (EXP-5) in the nucleus mediate the transport of pre-miRNAs to the cytoplasm. Exportin-5 (EXP5) is a Ran-GTP-bound nuclear transport receptor (Wahid et al., 2010). They mediate the transfer of Ran-GTP RNA from the cell nucleus (Lei and Silver, 2002). EXP5, which binds to pre-miRNA, allows pre-miRNA to be removed from the nucleus as a result of the hydrolysis of GTP (with GDP formation). pre-miRNAs in the cytoplasm are processed by Dicer (protein) to become mature miRNAs (Wahid et al., 2010).

During the encounter of the miRNA and mRNA target sequences, it was found that the 2-8-based nucleotides of miRNA were bound to an excellent complementary recognition sequence on Mrna. Located at the 5' end of miRNAs, complementary 2-8 basis to the 3' UTR (untranslated region) region of the target mRNA is also called the seed region (Jansson and Lund, 2012). The comprehensive match between the miRNA seed region and mRNA is one of the main criteria used to identify mRNA targets (Olena and Patton, 2010). The central part (typically 10-11 nt) of miRNA usually lacks complementariness to mRNA. However, the 3'-region of miRNA is more or less specifically connected to mRNA and partly contributes to the specificity and efficacy of the miRNA:mRNA complex (Jansson and Lund, 2012). It has been discovered that most of these miRNAs are located in intergenic regions and some in intronic regions (Wahid et al., 2010).

The main component of the RISC (RNA-induced silencing complex) complex is argonaute proteins. The RISC complex ensures the selection and removal of the RNA strand with the lowest thermodynamic stability at the 5' end (Tetreault and De Guire, 2013). A strand of double-strand miRNAs binds to the Argonuate-2 (Ago-2) connected to the RISC complex, binding to the promoter region, 3'UTR, and 5'UTR of the target mRNA. Then they act as the regulator of gene expression (Melo and Melo, 2013; Bartel, 2009). This is done in two ways: the first is the inhibition of the initiation of translation, and the second is the degradation of mRNA (Betel et al., 2007).

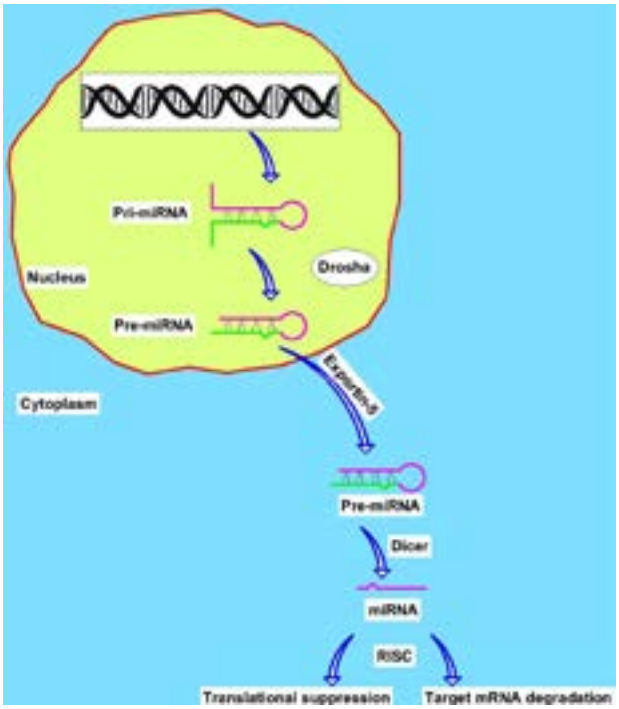


Figure 1. General microRNA pathway

miRNA: microRNA, Drosha: RNAse III enzyme, RISC: RNA-induced silencing complex, primiRNA: primary microRNA.

Structure and Properties of miRNAs

miRNAs play a crucial role in post-transcription gene

regulation in mammals through mRNA degradation and translational suppression (Williams et al., 2018). miRNAs are small, regulatory, and non-encoding RNA molecules. In general, they control the expression of the target mRNA through 3'UTR binding (Shukla et al., 2011). 3-UTR of the RNA transcripts located between the protein coding region of mRNA and the poly (A) tail (Jansson and Lund, 2012). miRNAs are non-encoded RNAs approximately 22 nt long and have their own hairpin structure. To create this, they are derived from self-folding transcripts (Bartel, 2009). miRNAs are initially copied into long chain structures, then go through stages to produce mature miRNAs (Graves and Zeng, 2012). A single miRNA can affect hundreds of targets, i.e. mRNA (Wienholds and Plasterk, 2005). In the earlier studies, the miRNA profile was performed on samples extracted from tissues, and subsequent studies found that there were miRNAs in bodily fluids such as serum, plasma, urine and saliva (MacFarlane and Murphy, 2010).

Naming and classifying of miRNAs

The first miRNAs discovered were named according to their phenotypes (let-7, lin-4 vb.) (Ha and Kim, 2014). Later, miRNAs are named by pre-appendix "mir " or "miR". Pre-mir refer to the pre-miRNA form while miR refer to the mature miRNA form (Hydbring and Badalian-Very, 2013). Under normal circumstances, pre-miRNAs and mature miRNAs are exactly the same.

Since they are located in other locations in the genome, pre-fix and numerical naming are also needed to indicate this. In addition, triple pre-fixes are used to indicate the type in which miRNA is present (e.g. rno-mir-34a rno=*Rattus norvegicus*). In the example of miR-15a and miR-15b; they both have the same 5' ends, but differ in four nucleotides in the 3' regions (Ambros et al., 2003; Hydbring and Badalian-Very, 2013).

UTR Structure

As well as the target sequence, the UTR structure is also important in connecting miRNA to the target mRNA (Ha and Kim, 2014). Mature and functional ncRNAs are connected to their target molecules, RNAs, from the 3'UTR region to control the expression of target mRNAs (Bartel, 2009). The most common method used to verify the target region of miRNA is to clone the predicted target mRNA's UTR to the luciferase reporter. By linking the target UTR to the lusiferase, it will demonstrate whether the change in lusiferase binds to UTR and regulates the expression of the gene at the mRNA or protein level (Cowland et al., 2007). The UTR region is known to have many functions in mRNA metabolism, such as transport, localization, translation efficiency, and stability (Krol et al., 2010).

Drosha

Since their first discovery in *Escherichia coli*, RIIID (RNase III domain) has been found in various proteins of different sizes from ~140 (mini-III) to ~1,900 (Dicer) amino acid residues. It is stated that proteins with RNAse activity are also generally protected in all bacteria and eukaryote species. The RNase III family have RNase III of bacterial origin and Rnt1p, Drosha, and Dicer of eukaryotic; among these *E. coli* RNase III is the most extensively investiaged member (Court et al., 2013). The bacterial RNAse III carries a single catalytic RNase III domain and a dsRNA binding domain (dsRBD) in terminal C. The enzyme drosha nuclease carries two catalytic RNase III domains and dsRNA binding domain (dsRBD) (Kwon et al., 2016).

It is known that the stages of microRNA maturation are initiated by Drosha, an RNase III (Kwon et al., 2016). This protein has about 160 kDa (Ha and Kim, 2014). A large protein, Drosha, has multiple domains and it increaes the overall production rate of miRNA by producing 3' protruding ends effectively recognized by Exp5 and Dicer (Han et al., 2004). Drosha is very similar in structure to Dicer, but compared to Dicer Drosha uses a rather complex mechanism. The structural comparison reveals some common characteristics between Drosha and Dicer. The first similarity is the length and conformation of the connector helix. Both Dicer and Drosha were found to share a proline, which causes bending in the binding helix. Despite this general similarity, there are very pronounced differences between Drosha and Dicer. Drosha is larger than Giardia Dicer and human Dicer, and contains an additional β sheet, at least one more α helix, and two ZnF motifs (Kwon et al., 2016).

Correct pri-miRNA process by Drosha requires dsRBD protein known as Pasha in Drosphila, *Pash-1 in C. elegans*, and DCGR8 in mammals (Du and Zamore, 2005*).* The DCGR8 gene was initially detected in the "DiGeorge syndrome chromosome region (DGCR)" of the human genome (Han et al., 2004).

Dicer

Dicer is a protein that has proven to exist in almost all eukaryotes (Vermeulen et al., 2005). This protein weighs about 200 kDa (Ha and Kim, 2014). It was called Dicer because it cleaves double-chain RNAs into small RNAs of equal size (Bernstein et al., 2001). Dicer was initially described in Drosophila and was later reported in humans, plants, and fungi (Singh et al., 2008). It was first recognized for its role in the production of small interfering RNAs that mediated the RNA interference (Bartel, 2004). It is a multi-domain enzyme of the RNase III family. It is stated that it plays a role in the degradation of double helix siRNA and miRNA (Singh et al., 2008). Dicer also initiates the formation of RISC which is complex for silencing of genes caused by miRNA expression and RNA interference (Betancur and Tomari, 2012). Although the Dicer protein differs in animal and plant species, it is characterized by the presence of N-terminal helicase domain, PAZ domain, dsRNA binding domain in C-terminal, and two catalytic RNAase III domains (MacRae and Doudna, 2007). The dicer enzyme C terminal, just like the Drosha protein, creates an intra-molecular dimer to form a catalytic center. The N-terminal helicase domain makes it easier

to recognize pre-miRNA and increases the functions of miRNAs (Ha and Kim, 2014). The presence of DUF 283 domain, whose function is still unclear, has also been shown. In addition, Dicer is a special ribonuclease which cleaves RNAi double-strand RNA (dsRNA) into small RNA fragments about 21-27 nt long (Tijsterman et al., 2004).

The Dicer's crystal structure was reported from the *G. intestinalis* parasite. Dicer of Giardia is smaller than eukaryotic Dicer enzymes because an amino-terminal helicase and carboxy-terminal dsRNA-binding domain (dsRBD) were found to be missing (Macrae et al., 2006). Giardia Dicer is a molecule of about 100 Å length and 30–50 Å width. Dicer recognizes the PAZ domain located at the end of a dsRNA. It then cleaves the dsRNA from RNase III domains. The distance between the PAZ and RNase III domains controls the length of small RNAs produced by Dicer. In Giardia dicer, this distance is about 65 Å and it generates small RRNAs with a length of 25 nt (Macrae et al., 2006). Paz in humans is a large protein containing dsRNA binding (dsRBD) and helicase fields, as well as two RNase III-like domain (Vermeulen et al., 2005).

RISC Complex and Ago Proteins

They use protein-RNA complexes called RISC for sequence-specific gene silencing through translational inhibition, mRNA destruction, and heterochromatin formation (Macrae et al., 2006). Depending on the complementarity of miRNA-mRNA, the RISC complex can be connected to 3'UTR portions of the target mRNA (Singh et al., 2008). Argonaute proteins function as the core components of RISC (Macrae et al., 2006) and various Argonaute proteins are isolated.

Argonaute proteins are proteins that exist in eukaryotes and prokaryotes. Ago proteins in eukaryotes are well known for their role in silencing RNA, while in prokaryotes their function is not fully known, but they are predicted to perform tasks similar to those in eukaryotes (Hall, 2005). Ago proteins are divided into 3 basic families: the first is known as AGO found in mammals, and the second is known as WAGO, which is found only in nematodes (worms). Ago proteins have four main domains: N-terminal, PAZ, Mid, and PIWI. In eukaryotes, in gene-regulating mechanisms mediated by small RNAs, AGO proteins are always divided into these areas (Hutvagner et al., 2008). The PAZ domain is defined as an area of 110 amino acids found in both dicer and ago proteins (Collins et al., 2005). Structural studies show that PAZ domain is connected to RNAs at the 5' end of the PIWI domain while PAZ domain is connected to 3' ends of single-strand RNAs, and PIWI structure is very similar to RNase H, and biochemical analyses indicate that AGO is the endonuclease of RISC (Du and Zamore, 2005). All hypotheses are based on the Drosophila model (MacFarlane and Murphy, 2010).

Ago2 is the only Argonaute with endonuclease activity in mammalian cells (Karginov et al., 2010). For example, dAgo1 and dAgo2 are found in separate complexes that contain siRNAs or miRNAs, respectively (Denli and Hannon, 2003). A study in human cells also demonstrated that AGO proteins bind to 3'UTR regions of mRNAs and stimulate translation, and it was suggested that these proteins are released during suppression and activation (Höck et al., 2008).

RNA Interference (RNAi)

The RNAi pathway was first demonstrated when trying to increase the expression of the chalcone synthase (CHS) gene in the Petunia plant. In this study, marbling petunia was obtained while trying to obtain dark purple petunia. As a result, it was stated that it can be a gene silencing mechanism following transcription (Napoli et al.,1990; Agrawal et al., 2003). It was later described as a response to long double-chain RNA (dsRNA) exogenously inserted to *C. elegans* (Fire et al., 1998; Doench et al., 2018). Fire and Mello (1998) won the Nobel Prize in 2006 for their work on RNAi, which silenced gene expression with siRNA and miRNA (Lucas and Raikhel, 2013). RNAi is a post-transcription gene silencing mechanism (Kim and Rossi, 2008) that causes the double-strand RNA to break down when it enters the cell. The key points of the RNAi mechanism are miRNAs and siRNAs (Jedrzejczyk et al., 2017).

The RNAi mechanism is a natural process and the initial molecule is mostly hairpin and matched parts in the intron regions of the primary mRNA are synthesized by double-chain RNA or RNA polymerase II. RNA-bound RNA polymerase synthesizes these using endogenous or exogenous-derived RNA (Bartel, 2004). With the discovery that siRNAs were made from exogenous dsRNA through the RNAi mechanism, it is realized that miRNAs and endogenous siRNAs regulate gene expression. SiRNAs, 21-23 nt long, are endogenous and exogenous and serve as guide RNA for proper mRNA degradation (Tomari and Zamore, 2018, Doench et al., 2018). Although chemically similar to miRNAs, siRNAs are generated by dicer by dividing long, double-strand RNAs. siRNAs are 2 nt shorter at the 5' phosphate end and 2 nt long at the 3' hydroxyl end. siRNA and RISC complex hybridize with target mRNA according to the principle of Watson-Crick base mapping. The RISC complex contains complementary sequences to 10 nt at 5' ends of single-strand siRNA. Endonucleases in the RISC complex degrade mRNA. In other words, siRNAs function in RNAi within an RNA-bound silencer complex (RISC) (Carrington et al., 2003).

RNA initiates the first step of interference and the Dicer, which belongs to the RNAse III ribonuclease enzyme family, allows double-strand RNA to be cleaved into small silencing (siRNA) RNAs (Song and Rossi, 2017). These RNAs are then transferred to the RISC complex in an ATP dependent manner. RISC is an RNA-multiprotein complex with nuclease activity, binds to siRNAs, and is directed to mRNA accompanied by the appropriate siRNA chain (Doench et al., 2018). The interaction of siRNA or miRNA with mRNA also occurs within the RISC complex. siRNA

matches the target mRNA sequence one-on-one, and the mRNA molecule is cleaved from the matching regions with endonucleases and removed. If the substrate is miRNA, the target can be matched (i.e., partially) with certain nucleotides in the 3'-non-translational region of mRNA. The most critical match is seen in the approximately 8 nt at the 5' end of miRNA. When paired with the appropriate mRNA chain, mRNA degradation occurs, thereby silencing the gene. In addition to being a natural process in the living organism, RNA interference suppresses the expression of endogenic genes by using siRNAs in *in vitro* conditions and this suppression is important in the research of gene functions. It provides genome identification in species and help determine the presence of many unknown genes. The RNAi mechanism is an ideal technique in genome research. Suppression of gene expression is very important in researching gene function and gene therapy (Zamore P, 2000; Raja et al., 2019; Xu et al., 2019).

miRNA Uses

miRNAs are associated with post-transcriptional regulation of gene transcription, differentiation, control of growth, apoptosis, and oncogenes. It was found to play a role tumorigenesis and in the development of the organisms (Melo and Melo, 2013). It is also reported to play a role in a wide range of developmental processes, including cell-division, cell proliferation, cell differentiation, apoptosis, development, and neuronal regeneration and differentiation. In addition, it is reported that upregulated miRNA expression results in oncogenesis as well as downregulated expression may cause tumor suppression, thereby regulating various characteristics of cancer such as angiogenesis, apoptosis, cell proliferation, differentiation, etc. miRNAs are involved in regulating heart function and in the functioning of the cardiovascular system of mammals. It has been found that the miRNAs have important roles in every step of development of the central nervous system (CNS) neurogenesis and are important in brain development, as well as the pathogenesis of neuronal diseases (Dwivedi et al., 2019). In humans, miRNAs were found to exist in all chromosomes except the Y chromosome (Melo and Melo, 2013). In general, some miRNAs have an obvious role in vertebrate development, while some miRNAs are effective in physiological and cellular processes (Wienholds and Plasterk, 2005).

Conclusion

The importance of miRNAs in proliferation and cell differentiation, as well as abnormal conditions such as cardiovascular diseases, diabetes, and cancer is indicated. Recently, there have been great advances in the number of studies and the use of more precise techniques to determine the relationship of miRNAs with targeted genes and diseases. Further understanding of the molecular pathways of miRNA is thought to be necessary in advancing therapeutic treatments for other diseases, especially cancer. Together with all this information, the importance of miRNAs has become indisputable.

Acknowledgements

We would like to thank Architect Büşra SUVARIKLI SOLAK for her help in the drawing the figure in this review.

Conflict of Interest

The authors declare that they have no conflict of interests.

References

Agrawal, N., Dasaradhi, P.V., Mohmmed, A., Malhotra, P., Bhatnagar, R.K., & Mukherjee, S.K. (2003). RNA interference: biology, mechanism, and applications. *Microbiology and Molecular Biology Reviews,* 67 (4), 657-85. doi: 10.1128/MMBR.67.4.657-685.2003

Alvarez-Garcia, I., & Miska, E.A. (2005). MicroRNA functions in animal development and human disease. *Development,* 132 (21), 4653-62. doi: 10.1242/dev.02073

Ambros, V., Bartel, B., Bartel, D.P., Burge, C.B., Carrington, J.C., Chen, X., Dreyfuss, G., Eddy, S.R., Griffiths-Jones, S., Marshall, M., Matzke, M., Ruvkun, G., & Tuschl, T. (2003). A uniform system for microRNA annotation. *RNA*, 9 (3), 277-9. doi: 10.1261/rna.2183803

Bartel, D.P. (2004). MicroRNAs: genomics, biogenesis, mechanism, and function. *Cell*, 116 (2), 281-97. doi: 10.1016/s0092-8674(04)00045-5

Bartel, D.P. (2009). MicroRNAs: target recognition and regulatory functions. *Cell*, 136 (2), 215-33. doi: 10.1016/j.cell.2009.01.002

Bernstein, E., Caudy, A.A., Hammond, S.M., & Hannon, G.H. (2001). Role For A Bidentate Ribonuclease in The Initation Step of RNA Interference. *Nature*, 409, 363- 366. doi: 10.1038/35053110

Betancur, J.G., & Tomari, Y. (2012). Dicer is dispensable for asymmetric RISC loading in mammals. *RNA*, 18 (1), 24-30. doi: 10.1261/rna.029785.111

Betel, D., Wilson, M., Gabow, A., Marks, D.S., & Sander, C. (2007). The microRNA.org resource: targets and expression. *Nucleic Acids Research*, 36, 149-153. doi: 10.1093/nar/gkm995

Cai, X., Hagedorn, C.H., & Cullen, B.R. (2004). Human microRNAs are processed from capped, polyadenylated transcripts that can also function as mRNAs. *RNA*, 10 (12), 1957-66. doi: 10.1261/rna.7135204

Carrington, J.C., & Ambros, V. (2003). Role of microRNAs in plant and animal development. *Science*, 301**,** 336–338. doi: 10.1126/science.1085242

Carthew, R.W., & Sontheimer, E.J. (2009). Origins and Mechanisms of miRNAs and siRNAs. *Cell*, 136 (4), 642-55. doi: 10.1016/j.cell.2009.01.035

Collins, R.E., & Cheng, X. (2005). Structural domains in RNAi. *FEBS Letters*, 579 (26), 5841-9. doi.org/10.1016/j.febslet.2005.07.072

Court, D.L., Gan, J., Liang, Y.H., Shaw, G.X., Tropea, J.E., Costantino, N., Waugh, D.S., & Ji, X. (2013). RNase III: Genetics and function; structure and mechanism. *Annual Review of Genetics,* 47, 405-31. doi: 10.1146/annurev-genet-110711-155618

Cowland, J.B., Hother, C., & Gronbaek, K. (2007). MicroRNAs and cancer. *APMIS*, 115 (10), 1090-106. doi: 10.1111/j.1600-0463.2007.apm_775.xml.x

Denli, A.M., & Hannon, G.J. (2003). RNAi: an ever-growing puzzle. *Trends in Biochemical Sciences,* 28 (4), 196-201. doi: 10.1016/S0968-0004(03)00058-6

Doench, J.G., Petersen, C.P., & Sharp, P.A. (2018). siRNAs can function as miRNAs. *Genes & Development*, 17 (4), 438–42. doi: 10.1101/gad.1064703

Du, T., & Zamore, P.D. (2005). microPrimer: the biogenesis and function of microRNA. *Development*, 132 (21), 4645-52. doi.org/10.1242/dev.02070

Dwivedi, S., Purohit, P., & Sharma, P. (2019). MicroRNAs and Diseases: Promising Biomarkers for Diagnosis and Therapeutics. *Indian Journal of Clinical Biochemistry*, 34 (3), 243-5. doi: 10.1007/s12291-019-00844-x

Ghildiyal, M., & Zamore, P.D. (2009). Small silencing RNAs: an expanding universe. *Nature Reviews Genetics,* 10 (2), 94-108. doi: 10.1038/nrg2504

Graves, P., & Zeng, Y. (2012). Biogenesis of Mammalian MicroRNAs: A Global View. *Genomics Proteomics Bioinformatics.* 10 (5), 239–245. doi: 10.1016/j.gpb.2012.06.004

Fire, A., Xu, S.Q., Montgomery, M.K., Kostas, S.A., Driver, S.E., & Mello, C.C. (1998). Potent and specific genetic interference by double-stranded RNA in Caenorhabditis elegans. *Nature,* 391, 806-811. doi: 10.1038/35888

Ha, M., & Kim, V.N. (2014). Regulation of microRNA biogenesis. *Nature Reviews Molecular Cell Biology,* 15 (8), 509-524. doi:10.1038/nrm3838

Hall, T.M. (2005). Structure and function of argonaute proteins. *Structure,* 13 (10), 1403-1408. doi: 10.1016/j.str.2005.08.005

Han, J., Lee, Y., Yeom, K.H., Kim, Y.K., Jin, H., & Kim, V.N. (2004). The Drosha-DGCR8 complex in primary microRNA processing. *Genes & Development,* 18 (24), 3016-3027. doi: 10.1101/gad.1262504

Höck, J., & Meister, G. (2008). Protein family review The Argonaute protein family. *Genome Biology,* 9 (2),210, doi: 10.1186/gb-2008-9-2-210

Hutvagner, G., & Simard, M.J. (2008). Argonaute proteins: key players in RNA silencing. *Nature Reviews Molecular Cell Biology*, 9 (1), 22-32. doi: 10.1038/nrm2321

Hydbring, P., & Badalian-Very, G. (2013). Clinical applications of microRNAs. *F1000 Research*, 6 (2), 136. doi: 10.12688/f1000research.2-136.v3

Jansson, M.D., & Lund, A.H. (2012). MicroRNA and cancer. *Molecular Oncology,* 6 (6), 590-610. doi.org/10.1016/j.molonc.2012.09.006

Jedrzejczyk, D., Gendaszewska-Darmach, E., Pawlowska, R., & Chworos, A. (2017). Designing synthetic RNA for delivery by nanoparticles. *Journal of Physics: Condensed Matter,* 29 (12), 123001. doi: 10.1088/1361-648X/aa5561

Karginov, F.V., Cheloufi, S., Chong, M.M., Stark, A., Smith, A.D., & Hannon, G.J. (2010). Diverse endonucleolytic cleavage sites in the mammalian transcriptome depend upon microRNAs, Drosha, and additional nucleases. *Molecular Cell*, 38 (6), 781-788. doi: 10.1016/j.molcel.2010.06.001

Kim, D., & Rossi, J. (2008). RNAi mechanisms and applications. *Biotechniques,* 44 (5), 613-616. doi: 10.2144/000112792

Kim, V.N., Han, J., & Siomi, M.C. (2009). Biogenesis of small RNAs in animals. *Nature Reviews Molecular Cell Biology*, 10 (2), 126-139. doi: 10.1038/nrm2632

Krol, J., Loedige, I., & Filipowicz, W. (2010). The widespread regulation of microRNA biogenesis, function and decay. *Nature Reviews Genetics,* 11 (9), 597-610. doi: 10.1038/nrg2843

Kwon, S.C., Nguyen, T.A., Choi, Y.G., Jo, M.H., Hohng, S., Kim, V.N., & Woo, J.S. (2016). Structure of Human DROSHA. *Cell,* 164 (1-2), 81-90. doi: 10.1016/j.cell.2015.12.019

Lee, R.C., Feinbaum, R.L., & Ambros, V. (1993). The C. elegans heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14. *Cell,* 75 (5), 843–854. doi: 10.1016/0092-8674(93)90529-y

Lei, E.P., & Silver, P.A. (2002). Protein and RNA export from the nucleus. *Developmental Cell*, 2 (3), 261-272. doi.org/10.1016/S1534-5807(02)00134-X

Lucas, K., & Raikhel, A.S. (2013). Insect microRNAs: biogenesis, expression profiling and biological functions. *Insect Biochemistry and Molecular Biology*, 43 (1), 24-38. doi: 10.1016/j.ibmb.2012.10.009

MacFarlane, L., & Murphy, P.R. (2010). MicroRNA: Biogenesis, Function and Role in Cancer. *Current Genomics,* 11 (7), 537-561. doi: 10.2174/138920210793175895

MacRae, I.J., Zhou, K., Li, F., Repic, A., Brooks, A.N., Cande, W.Z., Adams, P.D., & Doudna, J.A. (2006). Structural basis for double-stranded RNA processing by Dicer. *Science,* 311 (5758), 195-198. doi: 10.1126/science.1121638

Macrae, I.J., Li, F., Zhou, K., Cande, W.Z., & Doudna, J.A. (2006). Structure of Dicer and mechanistic implications for RNAi. *Cold Spring Harbor Symposia on Quantitative Biology,* 71 (71), 73-80. doi: 10.1101/sqb.2006.71.042

MacRae, I.J., & Doudna, J.A. (2007). Ribonuclease revisited: structural insights into ribonuclease III family enzymes. *Current Opinion in Structural Biology*, 17 (1), 138-145. doi: 10.1016/j.sbi.2006.12.002

Mattick, J.S., & Makunin, I.V. (2006). Non-coding RNA. *Human Molecular Genetics,* 15 (1), 17-29. doi.org/10.1093/hmg/ddl046

Melo, C.A., & Melo, S.A. (2013). Biogenesis and Physiology of MicroRNAs. *Non-coding RNAs and Cancer*, 5-24. doi: 10.1007/978-1-4614-8444-8_2

Mortimer, S.A., Kidwell, M.A., & Doudna, J.A. (2014). Insights into RNA structure and function from genome-wide studies. *Nature Reviews Genetics,* 15 (7), 469-79. doi: 10.1038/nrg3681

Napoli, C., Lemieux, C., & Jorgensen, R. (1990). Introduction of a chimeric chalcone synthase gene into Petunia result in supression of homologous revesible co-supression of homologous genes in trans. *The Plant Cell,* 2 (4), 279-289. doi: 10.1105/tpc.2.4.279

Olena, A.F., & Patton, J.G. (2010). Genomic organization of microRNAs. *Journal of Cellular Physiology,* 222 (3), 540-545. doi: 10.1002/jcp.21993

Pillai, R.S. (2005). MicroRNA function: multiple mechanisms for a tiny RNA?, *RNA*, 11 (12), 1753-1761. doi: 10.1261/rna.2248605

Raja, M.A.G., Katas, H., & Amjad, M.W. (2019). Design, mechanism, delivery and therapeutics of canonical and Dicer-substrate siRNA. *Asian Journal of Pharmaceutics Science,* 14 (5), 497-510. doi.org/10.1016/j.ajps.2018.12.005

Santosh, B., Varshney, A., & Yadava, P.K. (2014). Non-coding RNAs: biological functions and applications. *Cell Biochemistry & Function,* 33 (1), 14-22. doi: 10.1002/cbf.3079

Shabalina, S.A., & Koonin, E.V. (2008). Origins and evolution of eukaryotic RNA interference. *Trends Ecology Evolution,* 23 (10), 578-587. doi: 10.1016/j.tree.2008.06.005

Shukla, G.C., Singh, J., & Barik, S. (2011). MicroRNAs: Processing, Maturation, Target Recognition and Regulatory Functions. *Molecular And Cellular Pharmacology,* 3 (3), 83-92. doi: 10.4255/mcpharmacol.11.13

Singh, S.K., Pal Bhadra, M., Girschick, H.J., & Bhadra, U. (2008). MicroRNAs--micro in size but macro in function. *The FEBS Journal,* 275 (20), 4929-4944. doi: 10.1111/j.1742-4658.2008.06624.x

Song, M.S., & Rossi, J.J. (2017). Molecular mechanisms of Dicer: endonuclease and enzymatic activity. *Biochemical Journal,* 474 (10), 1603-18. doi: 10.1042/BCJ20160759

Tetreault, N., & De Guire, V. (2013). miRNAs: their discovery, biogenesis and mechanism of action. *Clinical Biochemistry*, 46 (10-11), 842-845. doi: 10.1016/j.clinbiochem.2013.02.009

Tijsterman, M., & Plasterk, P.H. (2004). Dicers at RISC; the mechanism of RNAi, *Cell,* 117 (1), 1-3. doi: 10.1016/s0092-8674(04)00293-4

Tomari, Y., & Zamore, P.D. (2018). Perspective: machines for RNAi. *Genes & Development,* 19 (5), 517–529. doi: 10.1101/gad.1284105

Vermeulen, A., Behlen, L., Reynolds, A., Wolfson, A., Marshall, W.S., Karpilow, J., & Khvorova, A. (2005). The contributions of dsRNA structure to Dicer specificity and efficiency. *RNA*, 11 (5), 674-682. doi: 10.1261/rna.7272305

Wahid, F., Shehzad, A., Khan, T., & Kim, Y.Y. (2010). MicroRNAs: synthesis, mechanism, function, and recent clinical trials. *Biochimica et Biophysica Acta*, 1803 (11), 1231-43. doi: 10.1016/j.bbamcr.2010.06.013

Wienholds, E., & Plasterk, R.H. (2005). MicroRNA function in animal development. *FEBS Letters*, 579 (26), 5911-5922. doi: 10.1016/j.febslet.2005.07.070

Williams, T., Ngo, L.H., & Wickramasinghe, V.O. (2018). Nuclear export of RNA: Different sizes, shapes and functions. *Seminars in Cell and Developmental Biology,* 75, 70-77. doi: 10.1016/j.semcdb.2017.08.054

Wilson, R.C., & Doudna, J.A. (2013). Molecular mechanisms of RNA interference. *Annual Review of Biophysics,* 42 (1), 217-239. doi: 10.1146/annurev-biophys-083012-130404

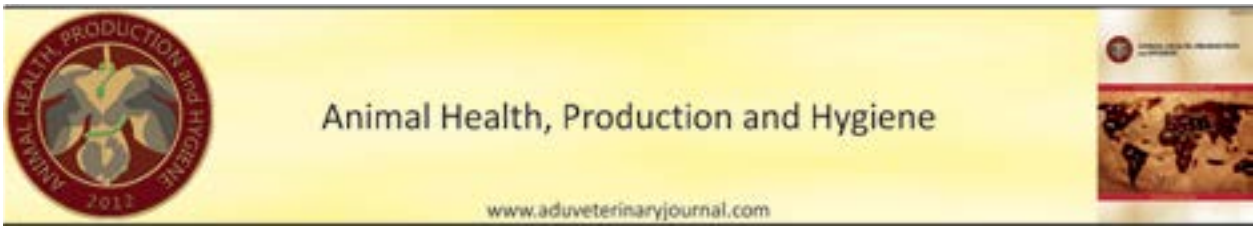
Wightman, B., & Ha, I.G.R. (1993). Posttranscriptional Regulation of the Heterochronic Gene lin-14 by W-4 Mediates Temporal Pattern Formation in C. elegans. *Cell*, 75 (5), 855-862. doi: 10.1016/0092-8674(93)90530-4

Xu, W., Jiang, X., & Huang, L. (2019). RNA Interference Technology. In:

Comprehensive Biotechnology, 560-575. doi: 10.1016/B978-0-444-64046-8.00282-2

Zamore, P.D., Tuschl, T., Sharp, P.A, & Bartel, D.P. (2000). RNAi: double-stranded RNA directs the ATP-dependent cleavage of mRNA at 21 to 23 nucleotide intervals. *Cell,* 101 (1), 25-33. doi: 10.1016/S0092-8674(00)80620-0

Animal Health Prod and Hyg (2021) 10 (2) : 66 - 73



Review

An Alternative Treatment Method for Poisoning in Veterinary Medicine: Intravenous Lipid Emulsion

Büşra ASLAN AKYOL[1*], Cengiz GÖKBULUT[2]

[1] *Department of Pharmacology and Toxicology, CoHE 100/2000 Scholarship Holder, Faculty of Veterinary Medicine, Balikesir University,10145, Balikesir, Turkey,* [2] *Department of Medical Pharmacology, Faculty of Medicine, Balikesir University,10145, Balikesir, Turkey*

ABSTRACT

Animal poison control centers receive numerous complaints about possible ingestion of substances that can cause deadly toxicities in the home. In recent years, over-the-counter medications such as ibuprofen, acetaminophen, and herbal supplements are the most common toxic substances ingested by pets. Removal of the toxin and supportive treatment is recommended in case of exposure to a toxin that does not have a known antidote. There have been many studies in both human and veterinary medicine that supporting the use of intravenous lipid emulsions in the treatment of intoxications. Intravenous lipid emulsion (ILE) is an oil-in-water emulsion that consists of egg yolk phospholipids, water, glycerin and various oils such as soybean, fish, coconut and olive oil. It is defined as a microemulsion with a long history of use as a parenteral nutrition formulation in both adult and pediatric patients. Also used as a drug carrier in addition to parenteral nutrition. In recent years, it has been used as an effective antidote for the treatment of intoxications caused by compounds with high oil solubility in both human and veterinary medicine. The first efficacy of the use of intravenous lipid emulsions in treatments was demonstrated in the systemic toxicity of local anesthetics and nowadays it comes to the fore in the poisoning of various drugs and compounds. However, it can also be used as an antidote in various intoxication cases caused by different chemicals that do not have any known antidote. Although clinically positive responses are received, more research is needed to more clearly understand the effect of intravenous lipid emulsion.

Keywords: Antidote, intravenous lipid emulsion, poisoning, toxicity

Veteriner Hekimlikteki Zehirlenmelerde Alternatif Bir Tedavi Yöntemi: İntravenöz Lipit Emülsiyonu

ÖZET

Hayvanlardaki toksikasyon vakalarında aktif görev alan kontrol yardım hatları, evlerde ölümcül toksisitelere neden olabilecek maddelerin muhtemel alımıyla ilgili çok sayıda şikâyet almaktadır. Son yıllarda ibuprofen, asetominofen ve bitkisel takviyeler gibi tezgâh üstü ilaçlar evcil hayvanlar tarafından alınan en yaygın toksik maddelerdir. Bilinen bir antidotu olmayan toksinin alınmasında, mümkünse toksinin uzaklaştırılması ve destekleyici tedavinin yapılması önerilmektedir. Toksikasyonların önlenmesinde intravenöz lipit emülsiyonu kullanımını destekleyen hem beşerî hem de veteriner hekimliğinde birçok çalışma yapılmıştır. İntravenöz lipit emülsiyonu; yumurta fosfolipidleri, su, gliserin ve başta soya fasulyesi yağı olmak üzere balık, hindistan cevizi ile zeytin yağı gibi çeşitli bileşiklerden oluşan hem yetişkin hem de pediatrik hastalarda parenteral beslenme formülasyonu olarak uzun bir kullanım geçmişine sahip olan bir mikroemülsiyon olarak tanımlanır. Parenteral beslenmeye ek olarak ilaç taşıyıcısı olarak da kullanılmaktadır. Son yıllarda ise hem beşerî hem de veteriner hekimliğinde yağda çözünürlüğü yüksek olan bileşiklerin sebep olduğu toksikasyonlarda tedavi amacıyla etkin bir antidot olarak kullanılmaya başlanmıştır. Tedavilerdeki kullanımının ilk etkinliği lokal anesteziklerin sistemik toksisitesinde gösterilmiş olup, günümüzde çeşitli ilaç ve bileşiklerin sebep olduğu zehirlenmelerde ön plana çıkmaktadır. Bununla birlikte bilinen bir antidotu olmayan bileşiklerin neden olduğu zehirlenmelerde de antidot olarak kullanılabilmektedir. Her ne kadar klinik olarak olumlu yanıtlar alınıyor olsa da intravenöz lipit emülsiyon kullanımının etkisini daha net bir şekilde anlamak için daha fazla araştırmaya ihtiyaç vardır.

Anahtar kelimeler: Antidot, intravenöz lipit emülsiyonu, zehirlenme, toksisite

Corresponding Author: Büşra ASLAN AKYOL, Department of Pharmacology and Toxicology, Faculty of Veterinary Medicine, Balikesir University,10145, Balikesir, Turkey e-mail: busraslan26@gmail.com
Received Date: 31.07.2021 – Accepted Date: 01.12.2021
DOI: 10.53913/aduveterinary.992845

Introduction

Animal poison control centers (APCC) receive numerous calls about possible ingestion of substances that can cause deadly toxicities in the home. In 2020, over-the-counter medications such as ibuprofen, acetaminophen, and herbal supplements are the most common group of toxicants ingested by pets and 19.7% of incoming emergency calls. Also, human prescription drugs are in the second place with 17.2% of the cases. Other cases have been reported to be caused by foodstuffs such as onions and garlic, and substances such as rodenticide and insecticide/herbicide (ASPCA, 2021). Removal of the toxin and supportive treatment is recommended in case of exposure to a toxin that does not have a known antidote (Fernandez et al., 2011). In the last decade, there have been many studies in both human and veterinary fields supporting the administration of ILE (intravenous lipid emulsion) in the treatment of major intoxications (Fernandez et al., 2011; Rosenblatt et al., 2006; Weinberg et al., 1998).

Research on ILE, as a parenteral nutrition formulation, began in the 1970s (Krieglstein et al., 1974; Straathof et al., 1984). Its use in toxicology started with the treatment of experimental bupivacaine toxicity in the late 1990s (Weinberg et al., 1998). In cases of LAST (local anesthetic systemic toxicity) which standard resuscitation treatments were unsuccessful, positive responses could be obtained rapidly with the use of ILE (Weinberg et al., 2008; Weinberg, 2006). ILE, which is accepted as a treatment option for lipophilic drug toxicity in emergency departments and other critical care units, is also used for the treatment of LAST (Cave et al., 2010). However, it is also used as a treatment option for the toxicities of other lipophilic compounds, including herbicides and pesticides. The first use of ILE for therapeutic purposes in veterinary medicine was reported by Crandell and Weinberg (2009). A positive improvement was observed after the first application and a serious improvement was noted after the second application according to this study. However, it was reported that the neurological status returned to normal 6 hours after the application without recurrence (Crandell and Weinberg, 2009).

The aim of this review is to provide information to scientists and clinicians about the mechanism of action of ILE, dose recommendations in treatment protocols and potential side effects, by evaluating the existing experimental studies and case reports on the treatment of intoxications with ILE.

ILE Formulations

ILE is an oil-in-water emulsion which consists of egg yolk phospholipids, water, glycerin and various oils such as primarily soybean, fish, coconut and olive oil. 20% Intralipid emulsion, which is widely used in the treatment of lipophilic drug toxicity, consists of long-chain triglycerides originating from soybean oil (Charbonneau et al., 2009; Waitzberg et al., 2006). Emulsions containing soybean oil are the most commonly used lipid emulsions in parenteral nutrition and in the treatment of lipophilic drug toxicity, and they contain linoleic, oleic, palmitic, linolenic and stearic fatty acids. They also contain fish and evening primrose oil (Aljadani et al., 2020).

Commercially available ILE formulations (Intralipid, Liposyn III, Lipoplus, Lipoven, SMOFLipid, Lipofundin-MCT, Structolipid, Omegaven, Clinoleic) are listed in Table 1 (Fernandez et al., 2011). It consists of small liposome molecules, typically 80 nm in diameter. The liposome content is higher in a 10% emulsion compared to a 30% emulsion. This causes a higher level of emulsification of the oil rate. Due to the high liposome content, the catabolism of the liposomes is also high and causes the formation of lipoprotein-X. Intravascular accumulation of lipoprotein-X may cause hypercholesterolemia. The oil concentration of the emulsion and the rate of administration, as well as the time of treatment, cause the deposition of liposomes and thus cholesterol. The daily given total amount of lipid is much lower in toxicological applications than recommended maximum amount in parenteral nutrition. Rapid administration of ILE is recommended in intoxicated patients to rapidly increase energy production, alter toxin kinetics, or rapidly create a “lipid sink” compartment within the intravascular space (Fernandez et al., 2011; Waitzberg et al., 2006).

Mechanism of Action

The mechanism of action of the ILE in toxicities has not been fully revealed and some hypotheses have been put forward (Cave and Harvey, 2014).

Table 1. Commercial preparations of ILE and fat contents (Fernandez et al., 2011)

Preparation	Manufacturer	Fat Content
Intralipit	Fresenius Kabi, Uppsala, Sweden	Soybean (%100)
Liposyn III	Hospira, Lake Forest, IL, USA	Soybean (%100)
Lipoven	Fresenius Kabi, Uppsala, Sweden	Soybean (%100)
Lipofundin-MCT	B. Braun, Melsungen, Germany	Coconut (%50), Soybean (%50)
Structolipit	Fresenius Kabi, Uppsala, Sweden	Coconut (%36), Soybean (%64)
Omegaven	Fresenius Kabi, Uppsala, Sweden	Fish (%100)
Lipoplus	B. Braun, Melsungen, Germany	Coconut (%50), Soybean (%40), Fish (%10)
Clinoleic	Baxter, Deerfield, IL, USA	Olive (%80), Soybean (%20)
SMOFLipit	Fresenius Kabi, Uppsala, Sweden	Coconut (%30), Soybean (%30), Olive (%25), Fish (%15)

Table 2. Log P values of some lipophilic drugs that may cause toxicity (Fernandez et al., 2011)

Drug	Log P	Drug	Log P
Amlodipine	1.90	Lidocaine	2.26
Baclofen	1.30	Loratadine	5.20
Bupivacaine	3.64	Metoprolol	1.88
Bupropion	3.47	Moxidectin	4.10
Carbamazepine	2.30	Naproxen	3.18
Carprofen	4.13	Ivermectin	3.50
Chlorpheniramine	3.17	Nifedipine	3.22
Chlorpromazine	5.35	Promethazine	2.85

The lipid sink phenomenon: The "lipid sink" phenomenon, primarily reported by Weinberg et al. (1998), is the most widely accepted mechanism of action for ILE. Administration of ILE formed an expanded intravascular lipid sink leads to the equilibrium, the toxic compound passes from the tissue to the liquid plasma phase and then to the lipid phase. Thus, leading to decrease the concentration of unbound toxin in plasma and distribution from organ and tissues to the bloodstream. Although the exact mechanism of action is not known, the binding property of the emulsion is probably the key criterion (Mazoit et al., 2009). Infusion of emulsified lipid droplets, into an aqueous medium such as blood, form a lipid compartment into which degraded lipophilic substances are collected. In particular, lipophilic substances such as local anesthetics are drawn into the "lipid sink", and the concentration gradient occurs between the tissue and blood, which allows the transition of lipophilic substances from high concentration to lipid compartment (Fernandez et al., 2011). Based on this hypothesis, ILE can be used in the treatment of intoxication caused by any lipophilic drug. Due to the log P value and thus lipophilicity, drugs or toxic substances are drawn into lipid compartments. Reaching high concentrations of drugs or toxic substances in the plasma results in less free toxic substance transfer to the tissues and therefore decrease the intoxication effects (Cave and Harvey, 2009; Picard and Meek, 2006; Straathof et al., 1984; Weinberg, 2003). The lipid sink phenomenon can be effectively used in the intoxications of chlorpromazine, bupropion, bupivacaine and some other lipophilic drugs (log P>1.0) (Table 2) (Krieglstein et al., 1974; Litz et al., 2008; Sirianni et al., 2008; Weinberg et al., 1998, 2006).

Alternative Mechanisms: Under normal aerobic conditions, fatty acids are the preferred substrate for myocyte oxidative phosphorylation, and it is approximately 90% of cardiac ATP. Interruption of fatty acid transport reduces ATP production and leading to cardiac toxicity due to adversely affect myocyte survival (Collins-Nakai et al., 1994). Lipid emulsions contribute to improved ATP synthesis in cardiomyocytes by increasing the intracellular fatty acid content and inhibit the reduction of ATP synthesis resulting from local anesthetic blockade. Thus, myocardial ischemia can be prevented after ischemic reperfusion (Van de Velde et al., 1996). Inhibition of mitochondrial fatty acid metabolism induced by toxic compounds is inhibited by ILE due to increasing the free fatty acid compartments. Local anesthetics that can be caused intoxication block the transport of fatty acids to mitochondria by inhibiting carnitine acylcarnitine translocase (Turner-Lawrence and Kerns, 2008). Through competitive inhibition or an unknown mechanism, ILE invalidates this inhibition and increases ATP production with the use of free fatty acids (Cave and Harvey, 2009).

The myocardial performance-enhancing effects are shaped by the increase of intracellular calcium concentration by ILE. Increased free fatty acids (especially stearate, linolenate, palmitate and oleate) stimulate the activation of voltage-dependent calcium channels in the myocardium and increase cytosolic calcium concentrations with cardiac function. However, it has been reported that increased intracellular calcium concentrations may have harmful effects, but this increase may contribute to the improvement of cardiac function in cases of secondary myocardial dysfunction due to calcium channel blocker toxicity (Turner-Lawrence and Kerns, 2008).

Dosage Regimen Recommendations

The recommended dose and rate of lipid emulsions for parenteral nutrition should also be considered for ILE administration in intoxication cases (Fernandez et al., 2011). In human medicine, the recommended dose for ILE is 2 g/kg/day (Turner-Lawrence and Kerns, 2008). This dose can also be expressed by volume as 10 ml/kg/day with 20% ILE. Recommended doses in veterinary medicine are similar to those in human medicine, but the doses may be exceeded due to parenteral nutrition requirements (Fernandez et al., 2011). The dose may need to be adapted for each lipophilic compound individually, depending on the degree of lipid solubility of the toxic compound (Hiller et al., 2010; Weinberg, 2003). Although optimal treatment protocols may vary between toxic compounds and species, different ILE protocols commonly used in veterinary medicine are listed in Table 3 (Felice and Schumann, 2008).

Most treatment protocols use 20% lipid solutions. It is usually administered as a slow bolus over several minutes and then as an infusion over 30-60 minutes via a venous catheter. The infusion should be administered at

Table 3. Different ILE protocols commonly used in veterinary medicine

Bolus	Infusion	Notes
1.5 ml/kg 2-3 min	0.25 ml/kg/min over 30-60 min	The serum should be checked every 2 hours, repeated as needed, if there is no improvement after 3 doses, it should not be continued (Fernandez et al., 2011).
1.5 ml/kg 5-15 min	0.25 ml/kg/min over 1-2 hours	If the symptoms recur, ILE can be repeated within a few hours, but if the serum is lipemic, treatment should not be continued (Johnson, 2011).
-	1.5 mL/kg over 30 min	Used in lidocaine toxicity in a cat (O'Brien et al., 2010).
2 ml/kg	0.06 ml/kg/min over 4 hours, then 0.5 ml/kg/min over 30 mins	It has been used in moxidectin toxicity in a dog, with the second infusion given 11 hours after the first infusion (Crandell & Weinberg, 2009).

a dose of 0.11 g/kg/hour and should not be administered at faster doses to avoid side effects. Serum should be monitored every two hours, and additional infusions should be considered if the patient is still symptomatic and there is no lipemia in the serum. If the serum turns orange or yellow, or if no improvement is noted after 3 doses, treatment with ILE should not be continued (Felice and Schumann, 2008). However, 20% formulations are generally preferred because they contain higher free phospholipids than 10% formulations. The potential for side effects may increase due to interactions of free phospholipids with lipoprotein lipase activity, which reduces the clearance of ILE (Weinberg, 2006).

Side Effects

Although side effects are rare, they may occur due to direct reaction to the emulsion or contamination of the emulsion. The contamination is particularly important for nutrient-rich products such as lipid emulsions. The use of inappropriate and non-sterile techniques may lead to microbial contamination. In addition, local or systemic infection and venous irritation may be observed with thrombophlebitis. An acute counter-pyrogenic reaction or a colloid reaction may be observed in direct reactions to the emulsion (Turner-Lawrence and Kerns, 2008). Allergic reactions may also occur to egg yolk phospholipid or soybean oil of formulation. Although clinical reactions are rare, they may include anaphylactic reaction-like symptoms that may occur within 20 minutes after administration. Fever, nausea, vomiting, dyspnea, tachypnea, cyanosis, arrhythmia, hypotension and cardiovascular collapse are among these symptoms (Driscoll, 2006; Felice and Schumann, 2008).

Subacute reactions to emulsion use may also occur and are often referred to as FOS (fat overload syndrome). It occurs as a result of excess volume or high administration rates that predominate endogenous lipid clearance mechanisms. FOS may also occur in patients with reduced plasma clearance of lipids. It may result in fat embolism, hyperlipidemia, hepatomegaly, icterus, splenomegaly, thrombocytopenia, increased clotting times and hemolysis (Turner-Lawrence and Kerns, 2008). Neurological complications such as multifocal deficiencies and focal seizures may also be observed in patients with FOS due to chronic lipid administration as a result of pathologies occurring in the brain tissue (Schulz et al., 1994).

The use of ILE may have adverse effects on the functions of the lungs and, however, the physiological effects may vary. An increase in pulmonary arterial pressure, decrease in partial arterial oxygen pressure to inspired oxygen level, increase in alveolar/arterial partial oxygen pressure and intrapulmonary shunt can be observed and these changes can be prevented by discontinuing the use of ILE. Changes in lung function are due to lipid metabolism products, decreased diffusion capacity due to lipid particle deposition in the reticuloendothelial system, and changes in pulmonary vascular tone, and secondary reduction of arterial oxygen. A rapid infusion of ILE should be carefully considered in patients with potential lung disease (Hwang et al., 1990; Venus et al., 1989).

Hypertriglyceridemia and lipemia have been accepted as inevitable consequences of ILE use and have been associated with the risk of cardiovascular disease and pancreatitis in humans (Ng, 2001). In dogs, it is thought to predispose to the development of pancreatitis and seizures (Xenoulis et al., 2008). However, an increased incidence of pancreatitis has been observed in dogs with primary hyperlipidemia (Westropp et al., 2005).

Specific formulations of ILE may have different physiological effects. Compared to emulsions containing 20% Intralipid, 20% Medialipid and omega-3 polyunsaturated fatty acids, Intralipid causes a slight increase in heart rate and a transient decrease in arterial pH; others may lead to a decrease in myocardial contractile performance. For these reasons, care should also be taken in the selection of ILE formulations in animals with specific conditions (Van de Velde et al., 1998).

Use of ILE in Veterinary Medicine

It was first evaluated in vivo and in vitro with the chlorpromazine toxicity model in rabbits in 1974, and many experimental studies were conducted afterwards (Kaplan and Whelan, 2012; Krieglstein et al., 1974). The free chlorpromazine fraction was significantly reduced with the use of ILE in rabbits (Krieglstein et al., 1974). In addition, ILE co-administered with cyclosporine to rabbits reduced the total body clearance and volume of distribution of cyclosporine (Shah and Sawchuk, 1991).

Table 4. ILE use with positive results in various intoxication cases

Species	Compound	Symptoms	ILE	Dosage	Reference
Cat	Baclofen	Ataxia, Mydriasis	%20 Intralipid	2 ml/kg bolus then 15 minutes 0.05 ml/kg/min over 4 hours	(Cavana, 2020)
Cat	Carprofen	Hyperesthesia	%20 Intralipid	1.5 ml/kg bolus then 15 minutes 0.25 ml/kg/min over 1 hour	(Chumbler et al., 2020)
Dog	Permethrin	Tremor	%20 Intralipid	1.5 ml/kg bolus then 0.25 ml/kg/min over 1 hour	(Kopke & Yozova, 2020)
Dog	Chlorfenapyr	Hyperemic mucous membranes	%20 Intralipid	1.5 ml/kg bolus then 15 ml/kg over 1 hour	(Davy et al., 2019)
Dog	Emodepside, Praziquantel	Tremor, Ataxia	%20 Lipofundin	2 ml/kg bolus then next hour 210 ml	(Gaens et al., 2019)
Dog	Lamotrigine	Vomiting, Lethargy	%20 Intralipid	2 ml/kg bolus then 0.25 ml/kg/min over 1 hour	(Bellis & Gibeon, 2018)
Dog	Metaldehyde	Tremor, Loss of consciousness	%20 Lipofundin	4 ml/kg bolus then 15 mins later 0.25 ml/kg/min over 1 hour	(Lelescu et al., 2017)
Dog	Ivermectin	Mydriasis, Ataxia	%20 Intralipid	1.5 ml/kg bolus then 5 mins later 0.25 ml/kg/min over 30 mins	(Pollio et al., 2018)
Dog	Amplodipine	-	%20 Intralipid	1.5 ml/kg bolus then 5 mins later 0.25 ml/kg/min over 1 hour	(Becker & Young, 2017)
Dog	Loperamide	Ataxia, Salivation	%20 Intralipid	1.5 ml/kg bolus then 15 mins later 0.25 ml/kg/min over 2 hours	(Long et al., 2017)
Dog	Bromethaline	Hyperaesthesia	%20 Intralipid	1.5 ml/kg bolus then 20 mins later 0.38 ml/kg/min over 1 hour	(Heggem-Perry et al., 2016)
Dog	Moxidectin	Tremor, Pityalism	%10 Intralipid	3 ml/kg bolus then 10 mins later 0.5 ml/kg/min over 30 mins	(Sines, 2016)
Dog	Vitamin D_3	-	%20 Intralipid	1.5 ml/kg bolus then 15 mins later 0.5 ml/kg/min over 30 mins	(Perry et al., 2016)
Dog	Naproxen	-	%20 Intralipid	1.5 ml/kg bolus then 15 mins later 0.31ml/kg/min over 1 hour	(Herring et al., 2015)
Dog	Synthetic Cannabinoid	Aggression, Hyperaesthesia	%20 Intralipid	1.5 ml/kg bolus then 0.5 ml/kg/hour	(Williams et al., 2015)
Dog	Ibuprofen	Aggression, Salivation	%20 Intralipid	1.5 ml/kg bolus then 15 mins later 5 ml/kg/min over 2 hours	(Bolfer et al., 2014)
Cat	Baclofen	Miosis	%20 Intralipid	1.5 ml/kg bolus then 90 mins later 7.5 ml/kg/min over 30 mins (x2), 5 mins later same dosage infusion	(Edwards et al., 2014)
Cat	Permethrin	Tremor, Salivation	%20 Intralipid	1.5 ml/kg bolus then 0.25 ml/kg/min over 1 hour	(DeGroot, 2014)
Dog	Diltiazem	-	%20 Intralipid	1.5 ml/kg bolus (x2) then 0.25 ml/kg/hour over 1 hour and 0.4-0.5 ml/kg/hour over 16 hours	(Maton et al., 2013)
Horse	Ivermectin Praziquantel	Tremor, Nystagmus	%20 Intralipid	1.5 ml/kg bolus then 0.25 ml/kg/min over 30 mins	(Bruenisholz et al., 2012)
Cat	Permethrin	Tremor	%20 Ivelip	1.5 ml/kg bolus then 30 mins later 0.25 ml/kg/min over 45 mins	(Haworth & Smart, 2012)
Dog	Ivermectin	Ataxia, Depression, Tremor	%20 Liposyn II	1.5 ml/kg bolus then 0.25 ml/kg/hour over 14 hours, next day 7.5 ml/kg bolus (x2)	(Wright et al., 2011)
Dog	Ivermectin	Tremor, Miosis	%20 Liposyn II	1.5 ml/kg bolus then 0.5 ml/kg/min over 30 mins	(Wright et al., 2011)
Cat	Lidocaine	Lethargy	%20 Intralipid	3 ml/kg bolus	(O'Brien et al., 2010)
Dog	Moxidectin	Ataxia, Tremor	%20 Intralipid	6.5 ml bolus then 12 ml/hour over 4 hours	(Crandell & Weinberg, 2009)

ILE has been used effectively in LAST cases in animals (Hoegberg et al., 2016). Use of ILE, especially in bupivacaine-induced asystole; the LD_{50} value of bupivacaine can be increased by approximately 50% (Weinberg et al., 1998), a faster return to the spontaneous circulation can be achieved, and with positive progress in cardiac functions, bupivacaine in the heart tissue can be rapidly reduced at the same time (Weinberg et al., 2006). ILE can also be used in the treatment of bupivacaine-induced cardiotoxicity. It has been reported that all patients who underwent ILE survived, even in dogs who developed cardiac arrest, and that blood pressure and pulse returned to normal levels 30 minutes after the use of ILE (Weinberg, 2003).

In addition to local anesthetics, ILE can also be used in the intoxication of lipophilic compounds (Levine et al., 2016). The efficacy of the use of ILE in the treatment of clomipramine-induced hypotension in rabbits has been reported (Harvey and Cave, 2007). However, similar results have been reported in the evaluation of respiratory depression caused by thiopentone, cardiotoxicity of verapamil, and hypotension induced by propranolol (Cave et al., 2005; Harvey and Cave, 2008; Tebbutt, 2006).

A case report on the successful use of ILE in the treatment of moxidectin toxicity was first reported in 2009 (Crandell and Weinberg, 2009). Subsequently, many case reports of the use of ILE in the treatment of various types and intoxications have been published (Bischoff et al., 2014; Bruenisholz et al., 2012; Robben and Dijkman, 2017; Saqib et al., 2015; West and Rusbridge, 2021). According to these studies, the use of ILE can be success in most of the intoxications. Although positive responses have been reported, the use of ILE may fail in some disulfoton, ivermectin and bromethalin toxicity. It is thought that ABCB1 mutants (defective P-glycoprotein) may be responsible for this failure (Becker and Young, 2017; Wright et al., 2011).

The use of ILE in the treatment of various toxic substances (such as amlodipine, baclofen, diltiazem, lamotrigine, local anesthetics, loperamide, macrocyclic lactones, permethrin, synthetic cannabinoids, tremorgenic mycotoxins, Pieris Japonica) is summarized in Table 4. Experimental studies in animal and human clinical cases have shown that β-blockers, bupropion, bupivacaine, carbamazepine, clomipramine, doxepin, flecainide, hydroxychloroquine, and verapamil toxicity respond to the use of ILE (Fernandez et al., 2011). However, determining the responses of intoxications to the application is not as easy as determining the lipophilicity of the toxic substance. Because, some weak lipophilic toxic substances (baclofen, Log P 1.30) have been reported to respond well to the use of ILE, suggesting that other physicochemical factors such as electrostatic interactions may also be effective (Abdel-Hafez and Abdel-Wahab, 2008; Fettiplace and Weinberg, 2018). Failure of the use of antidotal ILE; It may be due to incompatibility between the toxic substance and ILE, insufficient dosing or infusion rates, other factors yet to be discovered, and its determination is difficult due to the lack of knowledge about the mechanism of action of ILE (Tampakis et al., 2020).

It has been reported that 20% of formulations are more effective than 10% ILE in most intoxication cases, however, 30% of formulations can provide a faster recovery in severe cardiotoxicity cases (Fettiplace et al., 2014). Infusion dose and rate differ in veterinary patients (Table 3,4). When adequate clinical improvement is observed following administration, patients should be monitored for at least 12 hours for return of clinical symptoms or delayed allergic responses (Robben and Dijkman, 2017).

Conclusion

In recent years, ILE has been used as an effective antidote for the treatment of intoxication cases of compounds with high oil solubility in both human and veterinary medicine. Administered ILE provides an extended intravascular lipid phase by sequestering lipid-soluble toxic compounds from target tissues, lowering free drug/toxin levels, thereby reducing toxic effects. As a result, it is thought that elimination is accelerated by drawing the drug from the target tissues into the systemic circulation through the lipid phase formed in the systemic circulation. Based on this hypothesis, ILE is considered a treatment option for any lipophilic compound toxicity. However, advantages such as clinical improvement, relatively easy administration, and low cost have led to increased off-label use of ILE. Although it is useful in the treatment of toxicities, the possible risks and side effects against its beneficial effects have not been fully defined. However, there is no safe dosage protocol due to off-label use. Therefore, a benefit-risk analysis should be performed for each patient before using ILE. In addition, the effect of ILE administration on the pharmacokinetic/toxicokinetic behavior of the toxic compounds has not been clearly demonstrated. Therefore, new studies are needed to determine the efficacy and safety of ILE, which is used as an antidote in the treatment of intoxications, as well as to establish an effective and safe dosing regimen in veterinary medicine.

Conflict of Interest

The authors declare that they have no conflicts of interest.

References

Abdel-Hafez, A. A., & Abdel-Wahab, B. A. (2008). 5-(4-Chlorophenyl)-5,6-dihydro-1,3-oxazepin-7(4H)-one derivatives as lipophilic cyclic analogues of baclofen: Design, synthesis, and neuropharmacological evaluation. *Bioorganic & Medicinal Chemistry*, 16(17), 7983–7991. https://doi.org/10.1016/j.bmc.2008.07.064

Aljadani, N. A., Elnaggar, M. H. R., & Assaggaff, A. I. (2020). The role of fish oil and evening primrose oil against the toxicity of fenitrothion pesticide in male rats. International *Journal of Pharmaceutical Research & Allied Sciences*, 9(2).

ASPCA. (2021). Top 10 pet poisons of the year. *American Society for the Prevention of Cruelty to Animals*. https://www.aspca.org/news/announcing-top-10-pet-toxins (accessed 3 September 2021).

Becker, M., & Young, B. (2017). Treatment of severe lipophilic intoxications with intravenous lipid emulsion: a case series (2011-2014). *Veterinary Medicine*: Research and Reports, 8, 77–85. https://doi.org/10.2147/VMRR.S129576

Bellis, T. J., & Gibeon, L. (2018). The use of intralipid emulsion therapy to treat severe cardiotoxicity secondary to lamotrigine ingestion in a dog. *Clinical Case Reports*, 6(10), 1982–1988. https://doi.org/10.1002/ccr3.1733

Bischoff, K., Smith, M. C., & Stump, S. (2014). Treatment of Pieris Ingestion in Goats with Intravenous Lipid Emulsion. *Journal of Medical Toxicology*, 10(4), 411–414. https://doi.org/10.1007/s13181-014-0426-z

Bolfer, L., McMichael, M., Ngwenyama, T. R., & O'Brien, M. A. (2014). Treatment of Ibuprofen Toxicosis in a Dog with IV Lipid Emulsion. *Journal of the American Animal Hospital Association*, 50(2), 136–140. https://doi.org/10.5326/JAAHA-MS-5979

Bruenisholz, H., Kupper, J., Muentener, C. R., Dally, A., Kraemer, T., Naegeli, H., & Schwarzwald, C. C. (2012). Treatment of Ivermectin Overdose in a Miniature Shetland Pony Using Intravenous Administration of a Lipid Emulsion. *Journal of Veterinary Internal Medicine*, 26(2), 407–411. https://doi.org/10.1111/j.1939-1676.2011.00865.x

Cavana, P. (2020). Use of Intravenously Lipid Emulsion for Treatment of Baclofen Toxicosis in a Cat. *Pakistan Veterinary Journal*, 40(1), 133–135. https://doi.org/10.29261/pakvetj/2019.123

Cave, G, Harrop Griffiths, W., Harvey, M., Meek, T., Picard, J., Short, T., & Weinberg, G. (2010). Safety Guideline, Management of Severe Local Anaesthetic Toxicity. https://anaesthetists.org/Portals/0/PDFs/Guidelines PDFs/Guideline_management_severe_local_anaesthetic_toxicity_v2_2010_final.pdf?ver=2018-07-11-163755-240&ver=2018-07-11-163755-240 (accessed 3 September 2021).

Cave, G. & Harvey, M. (2009). Intravenous Lipid Emulsion as Antidote Beyond Local Anesthetic Toxicity: A Systematic Review. *Academic Emergency Medicine*, 16(9), 815–824. https://doi.org/10.1111/j.1553-2712.2009.00499.x

Cave, G. & Harvey, M. G. (2014). Should we consider the infusion of lipid emulsion in the resuscitation of poisoned patients? *Critical Care*, 18(5), 457. https://doi.org/10.1186/s13054-014-0457-5

Cave, G., Harvey, M. G., & Castle, C. D. (2005). Intralipid ameliorates thiopentone induced respiratory depression in rats: Investigative pilot study. *Emergency Medicine Australasia*, 17(2), 180–181. https://doi.org/10.1111/j.1742-6723.2005.00714.x

Charbonneau, H., Marcou, T. A. P., Mazoit, J.-X., Zetlaoui, P. J., & Benhamou, D. (2009). Early Use of Lipid Emulsion To Treat Incipient Mepivacaine Intoxication. *Regional Anesthesia and Pain Medicine*, 34(3), 277–278. https://doi.org/10.1097/AAP.0b013e31819340be

Chumbler, N. S., Schildt, J. C., Mawby, D. I., & Papich, M. G. (2020). Use of intravenous lipid therapy in a cat with carprofen overdose. *Clinical Case Reports*, 8(4), 653–657. https://doi.org/10.1002/ccr3.2772

Collins-Nakai, R. L., Noseworthy, D., & Lopaschuk, G. D. (1994). Epinephrine increases ATP production in hearts by preferentially increasing glucose metabolism. *American Journal of Physiology-Heart and Circulatory Physiology*, 267(5), H1862–H1871. https://doi.org/10.1152/ajpheart.1994.267.5.H1862

Crandell, D. E., & Weinberg, G. L. (2009). Moxidectin toxicosis in a puppy successfully treated with intravenous lipids. *Journal of Veterinary Emergency and Critical Care*, 19(2), 181–186. https://doi.org/10.1111/j.1476-4431.2009.00402.x

Davy, R. B., Campos, S., & Lynch, A. M. (2019). Acute chlorfenapyr toxicity in 3 dogs from a single household. *Journal of Veterinary Emergency and Critical Care*, 29(6), 686–689. https://doi.org/10.1111/vec.12894

DeGroot, W. D. (2014). Intravenous lipid emulsion for treating permethrin toxicosis in a cat. *The Canadian Veterinary Journal*, 55(1), 1253.

Driscoll, D. F. (2006). Lipid Injectable Emulsions: Pharmacopeial and Safety Issues. *Pharmaceutical Research*, 23(9), 1959–1969. https://doi.org/10.1007/s11095-006-9092-4

Edwards, P., Shihab, N., & Scott, H. W. (2014). Treatment of a case of feline baclofen toxicosis with intravenous lipid therapy. *Veterinary Record Case Reports*, 2(1). https://doi.org/10.1136/vetreccr-2014-000059

Felice, K. L., & Schumann, H. M. (2008). Intravenous lipid emulsion for local anesthetic toxicity: A review of the literature. *Journal of Medical Toxicology*, 4(3), 184–191. https://doi.org/10.1007/BF03161199

Fernandez, A. L., Lee, J. A., Rahilly, L., Hovda, L., Brutlag, A. G., & Engebretsen, K. (2011). The use of intravenous lipid emulsion as an antidote in veterinary toxicology. *Journal of Veterinary Emergency and Critical Care*, 21(4), 309–320. https://doi.org/10.1111/j.1476-4431.2011.00657.x

Fettiplace, M. R., Akpa, B. S., Ripper, R., Zider, B., Lang, J., Rubinstein, I., & Weinberg, G. (2014). Resuscitation with lipid emulsion: dose-dependent recovery from cardiac pharmacotoxicity requires a cardiotonic effect. *Anesthesiology*, 120(4), 915–925. https://doi.org/10.1097/ALN.0000000000000142

Fettiplace, M. R., & Weinberg, G. (2018). The Mechanisms Underlying Lipid Resuscitation Therapy. *Regional Anesthesia and Pain Medicine*, 43(2), 138–149. https://doi.org/10.1097/AAP.0000000000000719

Gaens, D., Leithäuser, C., Hamann, M., & Geyer, J. (2019). Adverse Drug Reactions After Administration of Emodepside/Praziquantel (Profender®) in an MDR1-Mutant Australian Shepherd Dog: Case Report. *Frontiers in Veterinary Science*, 6, 296. https://doi.org/10.3389/fvets.2019.00296

Harvey, M., & Cave, G. (2007). Intralipid Outperforms Sodium Bicarbonate in a Rabbit Model of Clomipramine Toxicity. *Annals of Emergency Medicine*, 49(2), 178-185.e4. https://doi.org/10.1016/j.annemergmed.2006.07.016

Harvey, M. G., & Cave, G. R. (2008). Intralipid infusion ameliorates propranolol-induced hypotension in rabbits. *Journal of Medical Toxicology*, 4(2), 71–76. https://doi.org/10.1007/BF03160958

Haworth, M. D., & Smart, L. (2012). Use of intravenous lipid therapy in three cases of feline permethrin toxicosis. *Journal of Veterinary Emergency and Critical Care*, 22(6), 697–702. https://doi.org/10.1111/j.1476-4431.2012.00804.x

Heggem-Perry, B., McMichael, M., O'Brien, M., & Moran, C. (2016). Intravenous Lipid Emulsion Therapy for Bromethalin Toxicity in a Dog. *Journal of the American Animal Hospital Association*, 52(4), 265–268. https://doi.org/10.5326/JAAHA-MS-6396

Herring, J. M., McMichael, M. A., Corsi, R., & Wurlod, V. (2015). Intravenous lipid emulsion therapy in three cases of canine naproxen overdose. *Journal of Veterinary Emergency and Critical Care*, 25(5), 672–678. https://doi.org/10.1111/vec.12307

Hiller, D. B., Di Gregorio, G., Kelly, K., Ripper, R., Edelman, L., Boumendjel, R., Drasner, K., & Weinberg, G. L. (2010). Safety of High Volume Lipid Emulsion Infusion. *Regional Anesthesia and Pain Medicine,* 35(2), 140–144. https://doi.org/10.1097/AAP.0b013e3181c6f5aa

Hoegberg, L. C. G., Bania, T. C., Lavergne, V., Bailey, B., Turgeon, A. F., Thomas, S. H. L., Morris, M., Miller-Nesbitt, A., Mégarbane, B., Magder, S., & Gosselin, S. (2016). Systematic review of the effect of intravenous lipid emulsion therapy for local anesthetic toxicity. *Clinical Toxicology*, 54(3), 167–193. https://doi.org/10.3109/15563650.2015.1121270

Hwang, T.-L., Huang, S.-L., & Chen, M.-F. (1990). Effects of Intravenous Fat Emulsion on Respiratory Failure. *Chest*, 97(4), 934–938. https://doi.org/10.1378/chest.97.4.934

Johnson, T. (2011). Intravenous lipid emulsion (IVLE) therapy for selected toxicoses. *Proeedings of the Interntional Veterinary Emergency and Critical Care Society*, San Antonio, TX, USA.

Kaplan, A., & Whelan, M. (2012). The Use of IV Lipid Emulsion for Lipophilic Drug Toxicities. *Journal of the American Animal Hospital Association*, 48(4), 221–227. https://doi.org/10.5326/JAAHA-MS-5761

Kopke, M. A., & Yozova, I. D. (2020). Management of presumptive canine permethrin toxicosis using intravenous lipid emulsion as an adjunctive therapy. *Veterinary Record Case Reports*, 8(2), e001041. https://doi.org/10.1136/vetreccr-2019-001041

Krieglstein, J., Meffert, A., & Niemeyer, D. H. (1974). Influence of emulsified fat on chlorpromazine availability in rabbit blood. *Experientia*, 30(8), 924–926. https://doi.org/10.1007/BF01938365

Lelescu, C. A., Mureşan, C., Muste, A., Taulescu, M. A., Neagu, A. M., & Nagy, A. L. (2017). Successful treatment of metaldehyde toxicosis with intravenous lipid emulsion in a dog. *Acta Veterinaria Brno*, 86(4), 379–383. https://doi.org/10.2754/avb201786040379

Levine, M., Hoffman, R. S., Lavergne, V., Stork, C. M., Graudins, A., Chuang, R., Stellpflug, S. J., Morris, M., Miller-Nesbitt, A., &

Gosselin, S. (2016). Systematic review of the effect of intravenous lipid emulsion therapy for non-local anesthetics toxicity. *Clinical Toxicology*, 54(3), 194–221. https://doi.org/10.3109/15563650.2015.1126286

Litz, R. J., Roessel, T., Heller, A. R., & Stehr, S. N. (2008). Reversal of Central Nervous System and Cardiac Toxicity After Local Anesthetic Intoxication by Lipid Emulsion Injection. *Anesthesia & Analgesia*, 106(5), 1575–1577. https://doi.org/10.1213/ane.0b013e3181683dd7

Long, W. M., Sinnott, V. B., Bracker, K., & Thomas, D. (2017). Use of 20% intravenous lipid emulsion for the treatment of loperamide toxicosis in a Collie homozygous for the ABCB1-1Δ mutation. *Journal of Veterinary Emergency and Critical Care*, 27(3), 357–361. https://doi.org/10.1111/vec.12562

Maton, B. L., Simmonds, E. E., Lee, J. A., & Alwood, A. J. (2013). The use of high-dose insulin therapy and intravenous lipid emulsion to treat severe, refractory diltiazem toxicosis in a dog. *Journal of Veterinary Emergency and Critical Care*, 23(3), 321–327. https://doi.org/10.1111/vec.12053

Mazoit, J.-X., Le Guen, R., Beloeil, H., & Benhamou, D. (2009). Binding of Long-lasting Local Anesthetics to Lipid Emulsions. *Anesthesiology*, 110(2), 380–386. https://doi.org/10.1097/ALN.0b013e318194b252

Ng, D. (2001). Heparin in hypertriglyceridemia: Friend or foe? *Journal of Laboratory and Clinical Medicine*, 138(6), 356–358. https://doi.org/10.1067/mlc.2001.119433

O'Brien, T. Q., Clark-Price, S. C., Evans, E. E., Di Fazio, R., & McMichael, M. A. (2010). Infusion of a lipid emulsion to treat lidocaine intoxication in a cat. *Journal of the American Veterinary Medical Association,* 237(12), 1455–1458. https://doi.org/10.2460/javma.237.12.1455

Perry, B. H., McMichael, M., Rick, M., & Jewell, E. (2016). Reduction of serum 25-hydroxyvitamin D concentrations with intravenous lipid emulsion in a dog. *The Canadian Veterinary Journal*, 57(12), 1284.

Picard, J., & Meek, T. (2006). Lipid emulsion to treat overdose of local anaesthetic: the gift of the glob. *Anaesthesia*, 61(2), 107–109. https://doi.org/10.1111/j.1365-2044.2005.04494.x

Pollio, D., Michau, T. M., Weaver, E., & Kuebelbeck, K. L. (2018). Electroretinographic changes after intravenous lipid emulsion therapy in a dog and a foal with ivermectin toxicosis. *Veterinary Ophthalmology*, 21(1), 82–87. https://doi.org/10.1111/vop.12410

Robben, J. H., & Dijkman, M. A. (2017). Lipid Therapy for Intoxications. Veterinary Clinics of North America: Small Animal Practice, 47(2), 435–450. https://doi.org/10.1016/j.cvsm.2016.10.018

Rosenblatt, M. A., Abel, M., Fischer, G. W., Itzkovich, C. J., & Eisenkraft, J. B. (2006). Successful Use of a 20% Lipid Emulsion to Resuscitate a Patient after a Presumed Bupivacaine-related Cardiac Arrest. *Anesthesiology*, 105(1), 217–218. https://doi.org/10.1097/00000542-200607000-00033

Saqib, M., Abbas, G., & Mughal, M. N. (2015). Successful management of ivermectin-induced blindness in an African lion (Panthera leo) by intravenous administration of a lipid emulsion. *BMC Veterinary Research*, 11(1), 287. https://doi.org/10.1186/s12917-015-0603-6

Schulz, P. E., Weiner, S. P., Haber, L. M., Armstrong, D. D., & Fishman, M. A. (1994). Neurological complications from fat emulsion therapy. *Annals of Neurology*, 35(5), 628–630. https://doi.org/10.1002/ana.410350521

Shah, A. K., & Sawchuk, R. J. (1991). Effect of co-administration of intralipidTM on the pharmacokinetics of cyclosporine in the rabbit. *Biopharmaceutics & Drug Disposition*, 12(6), 457–466. https://doi.org/10.1002/bdd.2510120607

Sines, D. (2016). Moxidectin intoxication in a dog — a patient care report. *The Veterinary Nurse*, 7(3), 182–186. https://doi.org/10.12968/vetn.2016.7.3.182

Sirianni, A. J., Osterhoudt, K. C., Calello, D. P., Muller, A. A., Waterhouse, M. R., Goodkin, M. B., Weinberg, G. L., & Henretig, F. M. (2008). Use of Lipid Emulsion in the Resuscitation of a Patient With Prolonged Cardiovascular Collapse After Overdose of Bupropion and Lamotrigine. *Annals of Emergency Medicine*, 51(4), 412-415. e1. https://doi.org/10.1016/j.annemergmed.2007.06.004

Straathof, D. J., Driessen, O., Meijer, J. W., Van Rees, H., Vermeij, P., & Vermeij, T. A. (1984). Influence of Intralipid infusion on the elimination of phenytoin. *Archives Internationales de Pharmacodynamie et de Thérapie*, 267(2), 180–186.

Tampakis, K., Vogiatzakis, N., Kontogiannis, C., Spartalis, M., Ntalianis, A., Spartalis, E., Siafaka, I., Iacovidou, N., Chalkias, A., & Xanthos, T. (2020). Intravenous lipid emulsion as an antidote in clinical toxicology: a systematic review. *European Review for Medical and Pharmacological Sciences*, 24(12), 7138–7148. https://doi.org/10.26355/eurrev_202006_21708

Tebbutt, S. (2006). Intralipid Prolongs Survival in a Rat Model of Verapamil Toxicity. *Academic Emergency Medicine*, 13(2), 134–139. https://doi.org/10.1197/j.aem.2005.08.016

Turner-Lawrence, D. E., & Kerns, W. (2008). Intravenous fat emulsion: A potential novel antidote. *Journal of Medical Toxicology*, 4(2), 109–114. https://doi.org/10.1007/BF03160965

Van de Velde, M., Wouters, P. F., Rolf, N., Van Aken, H., & Vandermeersch, E. (1998). Comparative hemodynamic effects of three different parenterally administered lipid emulsions in conscious dogs. *Critical Care Medicine*, 26(1), 132–137. https://doi.org/10.1097/00003246-199801000-00028

Van de Velde, M., Wouters, P., Rolf, N., Van Aken, H., Flameng, W., & Vandermeersch, E. (1996). Long-chain triglycerides improve recovery from myocardial stunning in conscious dogs. *Cardiovascular Research*, 32(6), 1008–1015. https://doi.org/10.1016/S0008-6363(96)00165-4

Venus, B., Smith, R. A., Patel, C., & Sandoval, E. (1989). Hemodynamic and Gas Exchange Alterations during Intralipid Infusion in Patients with Adult Respiratory Distress Syndrome. *Chest*, 95(6), 1278–1281. https://doi.org/10.1378/chest.95.6.1278

Waitzberg, D. L., Torrinhas, R. S., & Jacintho, T. M. (2006). New Parenteral Lipid Emulsions for Clinical Use. *Journal of Parenteral and Enteral Nutrition*, 30(4), 351–367. https://doi.org/10.1177/0148607106030004351

Weinberg, G. L., Di Gregorio, G., Ripper, R., Kelly, K., Massad, M., Edelman, L., Schwartz, D., Shah, N., Zheng, S., & Feinstein, D. L. (2008). Resuscitation with Lipid versus Epinephrine in a Rat Model of Bupivacaine Overdose. *Anesthesiology*, 108(5), 907–913. https://doi.org/10.1097/ALN.0b013e31816d91d2

Weinberg, G, Ripper, R., Murphy, P., Edelman, L., Hoffman, W., Strichartz, G., & Feinstein, D. (2004). Lipid Infusion Accelerates Removal of Bupivacaine and Recovery From Bupivacaine Toxicity in the Isolated Rat Heart. *Regional Anesthesia and Pain Medicine,* 31(4), 296–303. https://doi.org/10.1016/j.rapm.2005.02.011

Weinberg, G. L., VadeBoncouer, T., Ramaraju, G. A., Garcia-Amaro, M. F., & Cwik, M. J. (1995). Pretreatment or Resuscitation with a Lipid Infusion Shifts the Dose-Response to Bupivacaine-induced Asystole in Rats. *Anesthesiology*, 88(4), 1071–1075. https://doi.org/10.1097/00000542-199804000-00028

Weinberg, Guy. (2003). Lipid emulsion infusion rescues dogs from bupivacaine-induced cardiac toxicity. *Regional Anesthesia and Pain Medicine*, 28(3), 198–202. https://doi.org/10.1053/rapm.2003.50041

Weinberg, Guy. (2006). Lipid Infusion Resuscitation for Local Anesthetic Toxicity: Proof of clinical efficacy. *Anesthesiology*, 105(1), 7–8. https://doi.org/10.1097/00000542-200607000-00005

West, N., & Rusbridge, C. (2021). Ethanol toxicity in a dog due to gin-soaked sloe berry (Prunus spinosa) ingestion. *Veterinary Record Case Reports*, 9(1), e12. https://doi.org/10.1002/vrc2.12

Westropp, J. L., Buffington, C. A. T., Chew, D., Ettinger, S. J., & Feldman, E. C. (2005). *Textbook of Veterinary Internal Medicine*. Elsevier Saunders Philadelphia.

Williams, K., Wells, R. J., & McLean, M. K. (2015). Suspected synthetic cannabinoid toxicosis in a dog. *Journal of Veterinary Emergency and Critical Care*, 25(6), 739–744. https://doi.org/10.1111/vec.12378

Wright, H. M., Chen, A. V, Talcott, P. A., Poppenga, R. H., & Mealey, K. L. (2011). Intravenous fat emulsion as treatment for ivermectin toxicosis in three dogs homozygous for the ABCB1-1Δ gene mutation. *Journal of Veterinary Emergency and Critical Care*, 21(6), 666–672. https://doi.org/10.1111/j.1476-4431.2011.00687.x

Xenoulis, P. G., Suchodolski, J. S., Levinski, M. D., & Steiner, J. M. (2008). Serum liver enzyme activities in healthy Miniature Schnauzers with and without hypertriglyceridemia. *Journal of the American Veterinary Medical Association*, 232(1), 63–67. https://doi.org/10.2460/javma.232.1.63

Animal Health Prod and Hyg (2021) 10 (2) : 74 - 78

Animal Health, Production and Hygiene

www.aduveterinaryjournal.com

Case Report

Swimmer Syndrome in Two Kittens

Rahime YAYGINGÜL[1]*, Gülten Emek TUNA[2]

[1]Aydın Adnan Menderes University, Veterinary Faculty, Department of Surgery, 09016, Aydin, Turkey;
[2]Aydın Adnan Menderes University, Veterinary Faculty, Department of Internal Medicine, 09016, Aydin, Turkey.

ABSTRACT

Swimmer syndrome is a rare developmental abnormality observed in neonatal dogs and cats. This study aimed to give information about the clinical finding and treatment outcomes of swimmer syndrome diagnosed in the two crossbreed kittens. Four weeks old two kittens with the opening of their posterior legs sideways and difficulty walking were brought to the clinic. Clinic examination revealed that the kittens could hold and use both anterior extremities in the normal position; however, both posterior extremities were laterally extended. The kittens had difficulty walking and could not bring their posterior extremities to a normal position. Neurologic and radiologic examinations of the animals were normal. Treatment involved bandaging the lower half of the tibias of the posterior legs with the legs in the normal anatomical posture position. Vitamins B and Vitamin D_3 were administered to the kittens during treatment as well. This case report describes the successful treatment of swimmer syndrome in two kittens.

Keywords: Cat, neonatal disease, swimmer syndrome, treatment

İki Yavru Kedide Yüzme Sendromu

ÖZET

Yüzme sendromu, yeni doğan köpek ve kedilerde nadir görülen gelişimsel bir anomalidir. Bu çalışmada iki melez yavru kedide teşhis edilen yüzme sendromunun klinik bulguları ve tedavi sonuçları hakkında bilgi verilmesi amaçlanmıştır. Dört haftalık iki yavru kedi arka bacakların yanlara doğru açılması ve yürüme güçlüğü şikayeti ile cerrahi kliniğine getirildi. Klinik muayenede yavru kedilerin her iki ön ekstremiteyi normal pozisyonda tutabildikleri ve kullanabildikleri; ancak her iki arka ekstremiteleri yanlara doğru açık bir durumda olduğu görüldü. Kedilerin yürümekte zorlandıkları ve arka ekstremitelerini normal pozisyona getiremedikleri saplandı. Hayvanların gerçekleştirilen nörolojik ve radyolojik muayenede herhangi bir anormallik gözlenmedi. Tedavilerinde bacaklar normal anatomik duruş pozisyonuna getirilerek tibianın alt yarımından bandaj uygulaması yapıldı. Tedavi sırasında yavrulara B grubu vitaminler ve D_3 vitamini uygulandı. Bu olgu sunumunda iki kedide görülen yüzme sendromu başarılı bir şekilde tedavi edilmiştir.

Anahtar kelimeler: Kedi, neonatal hastalık, yüzme sendromu, tedavi

Corresponding author: Rahime YAYGINGÜL, Aydın Adnan Menderes University, Veterinary Faculty, Department of Surgery, 09016, Aydin, Turkey
e-mail: ryaygingul@adu.eu.tr
Received Date: 31.07.2021 – Accepted Date: 17.09.2021
DOI: 10.53913/aduveterinary.977031

Introduction

Swimmer syndrome, also known as swimming puppy syndrome, flat pup syndrome, splay leg (paraparesis), splay weak (tetraparesis), and myofibrillar hypoplasia, is an uncommon developmental musculoskeletal disorder observed in neonatal dogs and cats (Harkness and McCormick, 1982; Hoskins, 2001; Madhu et al., 2015). During the first few weeks of life, affected newborn animals appear normal and gain weight quickly. Clinical symptoms become clear at 2-3 weeks when they try to walk. They cannot lift their bodies off the ground and try to move with their feet open sideways like a swimmer or frog. Affected kittens may have hyperextension at their knee and elbow joints, as well as the lateral opening of the extremities from the hip joints (Hosgood and Hoskins, 1998; Hoskins, 2001; Akgül et al., 2014; Mahesh, 2014). The aetiology of the condition is unclear and may well involve multiple causes. Current theories include hereditary, neurological, environmental, nutritional, obesity-related, maternal metabolic disorders, and musculoskeletal development problems (Hosgood and Hoskins, 1998; Hoskins, 2001; Dumon, 2005; Verhoeven et al., 2006; Bürger et al., 2007; Fossum, 2007; Yardımcı et al., 2009; Linde-Forsberg, 2010).

This disease has been reported in conjunction with deformities such as pectus excavatum thoracic dorsal deviation, dorsoventral compression, patellar dislocation (medial), genu recurvatum, and innocent heart murmur.

Figure 1. Pre-treatment image of cat with swimmer syndrome.

While a specific treatment protocol has not been documented in the literature (Verhoeven et al., 2006; Yardımcı et al., 2009; Nganvongpanit and Yano, 2013) based upon the suspicious aetiology, many treatment options have been reported, including anatomic immobilization of the affected limbs, using bandages made of eight-girdle or cuffs, physical therapy exercises, thermo- and hydro-therapy, massage for muscle strengthening, vitamin E, and selenium supplementation (Dumon, 2005; Verhoeven et al., 2006; Bürger et al., 2007; Fossum, 2007; Linde-Forsberg, 2010; Cardilli et al., 2013). Literature related to swimmer puppy syndrome in cats is limited. This report aims to describe the syndrome as it affected two kittens and evaluate the treatment results.

Case Description

Four weeks old two kittens with the opening of their posterior legs sideways and difficulty walking were brought to Adnan Menderes University, Faculty of Veterinary Medicine, Department of Surgery. The owner

Figure 2. a. Treatment with bandaging applied to the lower half of the tibia of the posterior legs, b. 10th-day post-treatment

of the kittens stated that the queen had six kittens, two of which were affected. One of the kittens was a 350 g female, and the other was a 400 g male. The mother was a British Shorthair, and the father was a British Longhair. The owner had observed no abnormalities during the birth of the kittens. Clinical examination revealed that the kittens could hold and use both anterior extremities in the normal position; however, both posterior extremities were laterally open an extension (Figure 1). The kittens had difficulty walking and could not bring their posterior extremities to a normal position. Neurologic examinations were normal. On presentation, all vital signs of both kittens were within normal limits, and they had good body condition. Radiologic examination of the thorax, sternum and pelvis were performed. This radiographs detected normal bone density and bone development. Swimmer syndrome was diagnosed based the animals' history and on clinical findings. Treatment included bandaging applied to the lower half of the tibia of the posterior legs. The leg openings were fixed in the standard anatomical position. The bandage was removed every 12 hours, and physiotherapy was applied to the posterior legs after which the bandaging was re-applied. The bandages were removed after 10 days. There were no bandage-related complications. During

the treatment, vitamins B (Vitamin B_1: 5mg/cat/day and vitamin B_{12}: 50 mcg/cat/day) and vitamin D_3 (0.03–0.06 µg/kg/day) were administered to the kittens as well. The kittens were able to hold their extremities in the normal position and could walk normally following treatment (Figure 2).

Discussion

Swimmer syndrome is a rare developmental anomaly in neonatal cats. While developmental abnormalities are present at birth, clinical signs become obvious within the first few days or months. In affected animals, limbs are maintained in an abducted position and attempts to walk resulted in paddling or walrus-swimming movements. In dogs, the problem is more common in brachiocephalic breeds (Harkness and McCormick, 1981; Hosgood and Hoskins, 1998; Yardımcı et al., 2009). In cats, this syndrome has been reported in a Devon Rex and crossbreed kitten (Verhoeven et al., 2006; Gomes et al., 2015). Our cases involved British crossbreeds.

Affected animals look normal in their first weeks. Clinical symptoms emerge at 2-3 weeks when they start to walk. They cannot lift their bodies off the ground and try to move with their feet open sideways like a swimmer (Hosgood and Hoskins, 1998; Hoskins, 2001; Yardımcı et al., 2009; Akgül et al., 2014; Mahesh, 2014). According to Nganvongpanit and Yano, 75% of posterior extremities, 15.38% of all four limbs, and 9.62% of anterior extremities were affected in dogs. Gomes et al., (2015) have reported that only the posterior extremities of cats were affected. In the present situation, clinical findings appeared at two weeks in the posterior extremities of two kittens.

Defects and deformities, such as pectus excavatum, thoracic dorsal deviation, dorsoventral compression, patellar luxation (mediale), genu recurvatum and the innocent heart murmur may arise simultaneously associated with the syndrome (Hosgood and Hoskins, 1998; Hoskins, 2001; Fossum, 2007; Rahal et al., 2008; Linde-Forsberg, 2010; Nelson and Couto, 2015). In this study, defects and deformities were not found in either of our cases.

The causes and pathology of this disease remain uncertain, although several aetiologies and theories have been proposed. Among these, hereditary, environmental (presence of a smooth floor), nutritional (excessive protein in the queen's diet), obesity, maternal metabolic disorders, musculoskeletal development problems, and neurological disorders have been considered (Hosgood and Hoskins, 1998; Hoskins, 2001; Dumon, 2005; Verhoeven et al., 2006; Bürger et al., 2007; Fossum, 2007; Linde-Forsberg, 2010). Based upon to the suspicious aetiology, many treatment options have been proposed including dietary modifications, relocation to a rough surface for motor stimulation, anatomical immobilization of the affected limbs, intensive physiotherapy, thermo/hydrotherapy and massage for muscle strengthening (Dumon, 2005; Verhoeven et al., 2006; Bürger, 2007; Fossum, 2007; Lind-Forsberg, 2010; Karcher et al., 2018). According to Hosgood and Hoskins (1998), affected animals have a favourable prognosis if treated early, because bones and articulations are pliable and easy to correct at this stage. In the present case, a bandage passing through the lower half of the tibia was applied. Vitamin supplementation was administered to the kittens as well. According to the literature, the duration of bandaging may vary between 1 week and 1 month, depending upon the animal's response to treatment. In the present cases, after the animals was re-evaluated, the bandage was removed after ten days.

In conclusion, the present report describes the successful management of swimmer syndrome in two kittens.

Conflict of Interest

The authors declared that there is no conflict of interest.

References

Akgül, B., Şen, İ.A., Çeşme, H., & Yanık, K. (2014). Yüzen köpek sendromu. *Uludağ Üniversitesi Veteriner Fakültesi Dergisi*, 33(1-2), 75-78. https://doi.org/10.30782/uluvfd.384904

Bürger, C.P., Silva, R.B., Canola, J.C., Padilla Filho J.G., & Oliveira, G.G.S. (2007). Swimmer puppy syndorm: case report. *Acta Scientiae Veterinariae*, 35 (Suppl 4), 1393-1394.

Cardilli, D.J., João, C.F., Voorwald, F.A., & Faria, J.L.M. (2013). Swimmer syndrome affecting an entire litter of kittens. *Arquivo Brasileiro Medicina Veterinaria e Zootecnia*, 65(3), 705-709. https://doi.org/10.1590/S0102-09352013000300014

Dumon, C. (2005). The musculoskeletal system: the first 15 days. *Neonatology and pediatrics canine and feline* (1st ed.) (pp. 126-151). Sao Ca-etano do Sul: Interbook.

Fossum, T.W. (2007). Surgery of the lower respiratory system: Lungs and thoracic wall. In T.W. Fossum, (Ed). *Small Animal Surgery* (3rd ed.) (pp. 867-895). St. Louis, Missouri: Mosby Elsevier.

Gomes, L.C.V.M., De Santana, V.L., & De Farias, L.A. (2015). Swimming puppy syndrome in cats. *Acta Scientia Veterinariae*, 43(1), 109.

Harkness, J.E., & McCormick, L.F. (1981). Swimming-puppy syndrome in a litter of German Shepherd pups. *Veterinary Medicine Small Animal Clinician*, 76(6), 817–821.

Hoskins, J.D. (2001). Swimmer puppies and kittens. In: J.D. Hoskins (Ed.): *Veterinary Pediatrics* (3rd ed.) (pp. 419-420). WB Saunders, Philadelphia.

Hosgood, G., & Hoskins, J.D. (1998). Musculoskeletal disorders. In G. Hosgood, & J.D Hoskins (Eds), *Small Animal Paediatric Medicine and Surgery* (pp. 242-303). Oxford, UK: Butterworth-Heinemann.

Karcher, D.E., Costa, R. C., Prada, T. C., Moraes, P. C. Ramon, L. A., Minto, B. W., & Dias, L.G.G.G. (2018). A modified technique for treating swimmer puppy syndrome. *Veterinarni Medicina*, 63(4): 161-167. https://doi.org/10.17221/140/2017-VETMED

Linde-Forsberg, C. (2010). Abnormalities in pregnancy, parturition, and the periparturient period. In: S.J. Ettinger; E.C. Feldman, (Eds). *Textbook of Veterinary Internal Medicine* (7th.ed.) (pp. 1890-1901). St Louis, Missouri: Elsevier Saunders.

Madhu, D.N., Sivanarayanan, T.B., & Kumar, R. (2015). Clinical management of swimming syndrome in a Pup. *Intas Polivet*, 16(1), 136-137.

Mahesh, V. (2014). Swimmer puppy syndrome in a neopolitan mastiff: A case report. *Indian Journal Canine Practice*, 6(2), 170.

Nelson, R.W., & Couto, C.G. (2015). *Small Animals Internal Medicine* (in Portuguese) (5th ed.) (pp. 1512). Elsevier, Rio de Janeiro.

Nganvongpanit, K., & Yano, T. (2013). Prevalence of swimming puppy syndrome in 2,443 Puppies during the Years 2006-2012 in Thailand. *Veterinary Medicine International*, 1-6.

Rahal, S.C., Morishin-Filho, M.M., Hatschbach, E., Machado, V.M.V., & Aptekmann, K.P. (2008). Pectus excavatum in two littermate dogs. *The Canadian Veterinary Journal*, 49, 880-884.

Verhoeven, G., De Rooster, H., Risselada, M., Wiemer, P., Scheire, L., & Van Bree, H. (2006). Swimmer syndrome in a devon rex kitten and an English bulldog puppy. *Journal Small Animal Practice*, 47(10), 615-619.

Yardımcı, C., Özak A., Nisbet, H.Ö., & Şirin, Y.S. (2009). Swimming syndrome in two Labrador Puppies. *Kafkas Üniversitesi Veteriner Fakültesi Dergisi*, 15 (4), 637-640. Doi:10.9775/kvfd.2009.110

ANIMAL HEALTH, PRODUCTION AND HYGIENE

Volume 10, Issue 2 July-December 2021 Page: 39 - 77
Aydın Adnan Menderes University, West Campus, 09016, Efeler, Aydın, Turkey
Tel: + 90 256 247 07 00 Fax: + 90 256 247 07 20

www.aduveterinaryjournal.org