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VETERINARY SCIENCE AND VETERINARY MEDICINE

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Prevalence and Risk Factors Associated with Dermatophytosis in the one Humped Camel (*Camelus Dromedarius*) Dairy Farms in Benadir Region, Somalia

By Shafii Abdullahi Mohamed, Ibrahim Omar Mohamed, Abdirashid Abdullahi Mohamed, Hamdi Hassan Ali & Mohamed Yonis Abdullahi

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Abstract- Dermatophytosis is zoonotic skin disease and one of the most frequently occurring in human and domesticated animals. It is estimated that 20% of the world population is affected by Dermatophytosis. Camel Dermatophytosis has great public health and economic impact that leads to low milk yield, meat production and poor hide quality. A cross-sectional study was carried out to determine the prevalence and risk factors associated with Dermatophytosis infection among One-humped camel (*Camelus dromedarius*) in Camel dairy farms of Benadir Region, Somalia. Three hundred eighty four camels randomly surveyed from camel dairy farms. During screening 54 camels (14%) were clinically suspected and samples of skin scraping were examined by using 20% KOH for detection of Dermatophytosis under the microscope. Among these 29 camels (7.5%) were infected with Ringworm. According to the age group the highest prevalence was recorded camels younger than three years.

Keywords: dermatophytosis, camel, public health, KOH.

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Prevalence and Risk Factors Associated with Dermatophytosis in the one Humped Camel (*Camelus Dromedarius*) Dairy Farms in Benadir Region, Somalia

Shafii Abdullahi Mohamed ^α, Ibrahim Omar Mohamed ^σ, Abdirashid Abdullahi Mohamed ^ρ,
Hamdi Hassan Ali ^ω & Mohamed Yonis Abdullahi [¥]

Abstract- Dermatophytosis is zoonotic skin disease and one of the most frequently occurring in human and domesticated animals. It is estimated that 20% of the world population is affected by Dermatophytosis. Camel Dermatophytosis has great public health and economic impact that leads to low milk yield, meat production and poor hide quality. A cross-sectional study was carried out to determine the prevalence and risk factors associated with Dermatophytosis infection among One-humped camel (*Camelus dromedarius*) in Camel dairy farms of Benadir Region, Somalia. Three hundred eighty four camels randomly surveyed from camel dairy farms. During screening 54 camels (14%) were clinically suspected and samples of skin scraping were examined by using 20% KOH for detection of Dermatophytosis under the microscope. Among these 29 camels (7.5%) were infected with Ringworm. According to the age group the highest prevalence was recorded in camels younger than three years.

There was significant difference in the prevalence of Dermatophytosis infection between male and female camels ($P \leq 0.05$), also there was significant difference ($P \leq 0.05$) the majority of Ringworm infection associated with appetite, body condition, rearing system, and type of lesion. The results obtained showed that camel Dermatophytosis infection poses a problem in camel dairy farms in Benadir Region. Direct examination based on KOH is rapid and reliable technique for diagnosis of Dermatophytosis infection. It could be recommended in a field test to control the prevalence and the impact of zoonotic Dermatophytosis on camel health, human health, welfare and production.

Keywords: dermatophytosis, camel, public health, KOH.

1. INTRODUCTION

The one-humped camel (*Camelus dromedarius*) or Arabian camel, commonly called the dromedary, is an important species uniquely adapted to hot and arid environments. The Genus *Camelus* was probably among the last of the major domestic species to be put to regular use by man. Since its domestication 3000-4000 years ago, the one-humped camel (*Camelus*

dromedarius) accompanied humans and provided many facilities in arid and semi-arid areas. It is an indispensable species of domestic animal. It had been exploited by man in Asia and Africa in arid and semi-arid areas often being the only supplier of food and transport for people. It is a multipurpose animal (Ahmed, 2011). That can be used for milk, meat, wool, hide, transport, races, tourism, agricultural work and beauty contests. No other domestic animal is able to provide as many variable services to humans. The dromedary one-humped camel (*Camelus dromedarius*) served the needs of people for thousands of years ago. They secured trade and communication through wide arid and semi-arid expanses. The majority of camels are kept by pastoralists in subsistence production systems (Ahmed, 2011). There are two species in the genus *Camelus*: the dromedary, or Arabian camel, (*Camelus dromedarius*) which gets its name from the Greek and *C. bactrianus*, the Bactrian camel named after the area of Bactria in Central Asia where it has been domesticated (Al-Ani, 1998) the ten first countries in the world according to their camel populations are Somalia, Sudan, Ethiopia, Niger, Mauritania, Chad, Kenya, Mali, Pakistan and India (Faye, 2011). Somalia has the largest number of one-humped camels (*Camelus dromedarius*) globally. The camel population of the country is 7.5 million tropical livestock units (TLUs). (FAO, 1978, 1984). And currently there is no information about camel populations.

Camels in the Somali are normally found in the southern, central and northern parts of the country. Past and present experiences proved that the dromedary camel is a very hardy animal and has very special anatomical characteristics, and many varied physiological mechanisms, which enable the animal to live, reproduce and produce milk and meat, and to work under extreme conditions of heat and aridness, even during periods of drought when cattle, sheep and goats barely survive. Nevertheless, they suffer from ectoparasitic disease which is the major constraints in the improvement of camel health. This disease causes substantial economic losses in terms of decrease in

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working capacity, growth and productivity (Parsani, 2008)

a) *Significance of the study*

Somalia is considered home of one-humped camels globally, mainly camel pastoralists and other livestock providing livelihoods for nearly 60% of the country. Camel hides are important products that fulfill many functions. The hides are used traditional purposes of pastoral communities. Some Dermatophytes have great zoonotic importance, where many of them occurring primarily in animals and can be transmitted from infected animals to man. The transmission of Ringworm is usually by direct contact with infected host (animals or humans) or asymptomatic carriers and/or indirect contact with infected exfoliated skin or hairs in premises and equipment. Recently there are semi-intensive camel dairy farms in Benadir region and there is need to determine the prevalence and risk factors associated Ringworms in camel farms so that to create appropriate measurement, improve the awareness of the camel farmers and reduce economic impact of Dermatophytosis.

II. MATERIALS AND METHODS

a) *Study Area*

The study was conducted mainly camel dairy farms at three districts in Benadir region, Somalia, and

$$n = \frac{(1.96)^2 p_{exp} (1 - p_{exp})}{d^2} = \frac{(1.96)^2 0.5(1 - 0.5)}{(0.05)^2}$$

Is as follows:

Where:

N = sample size required.

q = 1- pexp.

Pexp = expected prevalence

d = desired absolute precision

In this formula expected prevalence of 50 % and absolute precision of 95% were considered. The number of camels required to determine the prevalence will calculated to be 384.

e) *Risk factors*

Data on the risk factors were collected through a questionnaire addressing camel farm owners and were as follows: age, sex, body condition, appetite, rearing system and distribution and type of lesions.

f) *Camels Surveyed*

Camels were examined by visual inspection, and suspected animals with Dermatophytosis were carefully examined by skin the scraping technique and photographed. A questionnaire regarding the potential risk factors associated with ringworm infection (age, sex,

most of the farms were outskirts such as Deynile, Kaxda and Dharkenley districts. Benadir Region is the southern part of the country. It lies at the latitude 2.0469°N and longitude 45.31°E, the city is situated along side of the Indian with an average rain fall of 428 mm. The mean ambient temperature ranges from 24 °C to 30°C. Mean relative humidity stands at 80%. (Metz at.al, 1993)

b) *Study Design*

The study was a cross-sectional to establish the prevalence and risk factors associated with Dermatophytosis in One-humped Camel dairy farms, in Benadir Region, Somalia.

c) *Camels Examined*

The study was conducted in three hundred eighty four camels selected by the simple random sampling method.

d) *Sample Size*

The sample size was determined based on the formula recommended by (Thrusfield, 1995), and

body condition, appetite, rearing system and distribution and type of lesions) was completed.

g) *Collection and examination of skin scrapings*

Deep skin scrapings were collected from camels with ringworm. The skin scrapings were conducted until hyperemia without bleeding occurred.

h) *Laboratory investigation*

On a clean glass slide, a part of each specimen was placed, added to it few drops of 20% Potassium Hydroxide (KOH) to digest the keratin material, then covered with a clean glass and gently heated for one minute, the slide was microscopically examined used 10X objective lens for the presence of arthrospores.

i) *Statistical analysis*

The data obtained were statistically analyzed using Statistical Package of social science (SPSSv20).

III. RESULTS

a) *Dermatophytosis (Ringworm) in camels*

Skin scrapings examined in 20 % potassium hydroxide (KOH) showed arthrospores characteristic to

the Dermatophytes in 29 samples. The lesions of Ringworm appeared on the head especially round the eyelids and muzzle, neck, shoulders, abdomen, legs and tail. The lesions varied in size and characterized by their circular or semi-circular shape.

b) Prevalence of Dermatophytosis in the surveyed camels

Three-hundred and eighty-four camels were surveyed and 54 were clinically suspected with Ringworm infection. Among those suspected the prevalence of Ringworm was 14 %.

Table 3.2: Prevalence of Dermatophytosis of surveyed camels

Animals surveyed	No.	%
Infected	54	14.0
Not infected	330	85.9
Total	384	100

Table 3.3: Prevalence of Dermatophytosis infection in examined camels under Microscope

Microscopic examination	No.	% within the total number of animals
Ring worm infection	29	7.5%
Not infected	355	92.4%
Total	384	100%

c) Dermatophytosis and its associated potential Risk factors

i. Sex of the animals

Among the total number of camels surveyed (384 camels); 111 were males and 273 were females. Among male camels 13 (11.7%) were found infected, while among female camels 16 (5.8%) were infected.

ii. Age of the animals

Among the total number of camels surveyed (384 camels), 96 camels were young (1 to 3 years), 173 were adult animals (4 to 8 years) and 115 old animals (more than 8 years). Among young animals 14 (14.5%) were found infected and among adult animals 7(4.04%) animals were found infected, while in old animals 8 (6.95%) were infected.

a. Appetite of the animal

Among the total number of camels surveyed (384 camels), 48 animals showed inappetence and 336 animals had normal appetite. Among the camels that showed inappetence 25 (52%) were found infected, while only 4(1.19%) animals with normal appetite were found infected.

b. Body condition

Among the total number of camels surveyed (384 camels), 13 animals were found with poor body condition, Another 13 animals were in fair body condition, and 358 with good body condition. Among the camels showing poor body condition 17(76.4%) animals were found infected, 8 (61.5%) 28 animals with fair condition were found infected, while among animals showing good body condition only four (1.1%) were found infected.

c. Rearing system

Among the total number of camels surveyed (384 camels), 22 animals were kept under mixed rearing conditions, and 362 animals were kept under separate rearing system. Among camels kept under mixed

rearing 14 (63.6%) were found infected with ringworm; while those kept under separate rearing 15 (4.14%) were found infected.

d. Type of lesion

Among the total number of camels surveyed (384 camels), 32 animals showed scab covered lesions, 1 animal had oozing lesions and 351 animals were healthy. The animals that had scab covered lesions were 28 (90.3%) were found infected, while animals showing moist lesions were all (100%) infected.

IV. DISCUSSION

Dermatophytosis (syn. ringworm) is a zoonotic skin infection of the keratinized tissues caused by a specialized group of fungi named Dermatophytes. The disease can affect man, and all domestic animals, including camels (Almuzainia et al, 2016).

In this study the overall infection rate was 7.5% (29/384) verified by microscopic examination. The prevalence of Ringworm recorded in the present study was higher than the prevalence rate reported by (Agab and Abbas, 1992) in Sudan who reported sero-prevalence of 5.8 % (217/3681) of Ringworm in camels, and in Ethiopia by (Megersa, 2010) who reported prevalence of 8.3% (14/169) in camels. Ringworm was more prevalent in male camels than female. This finding was contrary to the findings of (Fadlelmula, 1994)), and (salim, 2010) who reported no significant difference between Ringworm infection and gender. The prevalence rate of Ringworm in young animals was higher than old and adult animals. This finding was in agreement with (Agab, 1993)) who reported that ringworm occurs in camels less than 3 years of age. This was probably due the weak immunity in young and old animals. The prevalence rate of Ringworm in animals showing in appetite was higher than animals with normal appetite. This result supported the findings of

(Saber, 2015). The most probable explanation to this finding is that animals showing in appetite become weak and are more susceptible to infection with Ringworm. The prevalence rate of Ringworm in animals with poor and fair body condition was higher than animals with good body condition. This result was in agreement with (Enany et al., 2013). This could probably be explained in terms of resistance to infection; animals with good body condition are more resistant to Ringworm infection than animals with fair or poor body condition. The prevalence rate of Ringworm in animals kept under mixed rearing was higher than animals kept under separate rearing system. Similar results were obtained by the (Center for Food Security and Public Health, 2013) who reported that Dermatophytes may be acquired from other species of animals grazing side by side with camels. Camels showing scab lesions (90.3%) were infected and (100%) of animals with moist lesions were infected. This finding authenticated the finding of (Fadlelmula and Shathele, 2010) who reported that the lesions start with thickening of skin, alopecia and scaliness involving small circular areas or become confluent covering extensive areas. The distribution of camel Ringworm infection in the body is shown above. Lesions of Ringworm initially involve the head, neck, abdomen and back with different ratios. Similarly, (Chermette et al, 2008) reported that the lesions of Ringworm initially involve the head, neck and shoulders, with a possible extension to the flanks and legs, leading sometimes to pyoderma and emaciation.

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Conflict of interest

The authors have declared no conflict of interest.

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A Comparative Study of the Production Performance and Management of Cobb 500 Broiler Parent Stock among Three Renowned Farms of Chattogram

By Shaolin Ferdouse, Md. Shahadat Hossain, Tanim Ferdous
& Yaing Nue Frue Marma

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Abstract- A study was carried out in three renowned breeder farms in Chattogram district of Bangladesh. The objectives of the study were to compare the management practices and production performances of parent breeder stock of about 29800 Cobb 500 strain reared in those farms (farm A, B and C). The duration of the study was August 10 to August 30, 2016 which was done on 18-50 weeks of ages of birds. Relevant information's related to management of birds (i.e. housing, feeding, lighting, vaccination schedule, temperature management, bio-security etc.) were recorded from register book of farms and compared with the standard. Data related to production performance (i.e. feed intake by male and females, live weight, egg production etc.) were also recorded and analyzed. It was revealed that farm A, B and C maintained standard management systems.

Keywords: Cobb 500, management, production performance.

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A Comparative Study of the Production Performance and Management of Cobb 500 Broiler Parent Stock among Three Renowned Farms of Chattogram

Shaolin Ferdouse ^α, Md. Shahadat Hossain ^ο, Tanim Ferdous ^ρ & Yaing Nue Frue Marma ^ω

Abstract- A study was carried out in three renowned breeder farms in Chattogram district of Bangladesh. The objectives of the study were to compare the management practices and production performances of parent breeder stock of about 29800 Cobb 500 strain reared in those farms (farm A, B and C). The duration of the study was August 10 to August 30, 2016 which was done on 18-50 weeks of ages of birds. Relevant information's related to management of birds (i.e. housing, feeding, lighting, vaccination schedule, temperature management, bio-security etc.) were recorded from register book of farms and compared with the standard. Data related to production performance (i.e. feed intake by male and females, live weight, egg production etc.) were also recorded and analyzed. It was revealed that farm A, B and C maintained standard management systems. There was no significant difference ($P>0.05$) in feed intake by female breeders of three farms though it differed significantly ($P<0.05$) in males and was higher in farm B and C in comparison with farm A. However, no significant differences ($P>0.05$) were observed among body weight of male and female birds of farm A, B and C. Egg production was insignificantly ($P>0.05$) higher in farm B compared to farm A and B. Peak egg production in farm A was observed at 30th weeks (90%), farm B at 31- 42th weeks (95%) and farm C at 32th weeks (91%). From the study it was evident that there were fewer differences in existing management systems of farm A, B and C. Cob 500 performed well in that system. Insignificant amount of differences were observed among the production data of three farms almost in all parameters. It was also noted that production performance in a breeder farm depends mainly on the management practices and there is a negative correlation between the feed intake and egg production of birds.

Keywords: Cobb 500, management, production performance.

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I. INTRODUCTION

Poultry is an integral part of the livestock sector. Approximately 40% of total animal protein is supplied by poultry meat in our country (Rahman et al., 1998) at present chicken contributes 56% of total meat production of the country through the share of broiler. Every person should consume 55gms of protein per day who weigh 150 pounds. According to DLS, (2007) meat requirement is about 120gms/day/head and 6.26 million metric ton/year. But our achievement is 20gms/day/head and 1.04 million metric ton/year. Poultry meats contribute approximately 37% of the total animal protein supplied in the country (Rahman et al., 1998). Bangladesh bureau of statistic (BBS, 2002) reported that about 89% of the rural household rear poultry and the average number of per household is 6.8. With a view to meet the protein gap in a shortest possible time, there has been a shift of policy emphasis on intensive poultry farming in recent years. Consequently a number of poultry farms have been established on commercial basis in an around the cities and towns. The demand of day old chicks is also increasing gradually. A number of breeder farms and hatcheries have been established by the private entrepreneurs for getting a higher profit within shorter possible time. Data revealed that total number of chicken in 2008 was about 118.7 million (Banglapedia, 2015). It has been found by the survey (ATDP/IFDC) that there are 63 hatchers at the ends of year 1999. Now the number of hatcheries in Bangladesh is 75% (Poultry khamarbichitra, 2010). Chowdhury et al., (2003) reported that exotic broiler parent stocks reared in open-sided house under Bangladesh conditions, in general, able to achieve expected body weight though they were found to be very sensitive to environmental stresses. Robinson and Wilson, (1999) showed that broiler breeder either fed ad-libitum or restricted to achieve typical industry target weight during 22 to 26 weeks of age. Ad-libitum fed hens weighed significantly heavier and produced fewer eggs than restricted fed hens.

Cobb 500 is an English strain which shows an excellent production and reproduction performance in

standard condition. It has a worldwide reputation for the lowest cost of producing chicken meat. Cobb geneticists have developed this breed by the research of more than 30 years progress using a combination of both traditional pedigree selection and new technology. They have developed a very high breeder performance of Cobb 500, such as Cobb 500 starts laying at 18-22 weeks of age. Age at 5% egg production is 24 weeks of age. At 65 weeks of age - total eggs/hen housed is 175, hatching eggs/hen housed is 170, peak hatchability 91 %, broiler chicks/hen housed 144 (*Cobb 500 breeder management guide*, 2009). For such high breeder performance different renowned farms in Bangladesh choose Cobb 500 as a broiler parent stock for rearing. Production performances in a poultry farm depend on largely on its management practice.

Therefore the present study was undertaken with the following objectives:

- To observe the comparative management practices, biosecurity of Cobb 500 broiler breeder parent stock of three different farms in Chattogram.
- To compare the production performance of the birds in different farms.

II. MATERIALS AND METHODS

a) Study area

The study was conducted within the facilities of comparative study of the performance of the Cobb 500 parent breeder. The study areas were three renowned breeder poultry farms of Chattogram, Bangladesh.

b) Study population

The study populations were 10,000 Cobb 500 parent breeder stock from farm A, 12000 from farm B

and 7800 from farm C with the grand total of 29800 birds.

c) Study period

The study carried out from August 10, 2016 to August 30, 2016 at the laying stage of birds.

d) Data collection and analysis

Data were collected from three renowned poultry farm of Chattogram, Bangladesh, including egg production, body weight gain along with other related data like housing, lighting, feeding, of the study batch. The birds which were selected for data ranged from 18-50 weeks.

e) Study design

This experiment was conducted by a completely randomized design (CRD). From each farm 40 samples were collected randomly.

f) Feeding in take management

The feed that are supplied to the male and female breeder in their laying period are produced by respective farms by their own. In the farm the male and female birds are supplied with different feeds in laying period. These feeds having the optimum level of nutrient required for the breeder. Feed was given once daily for short period, usually by the early morning of the day. There the chicks were offered ad- libitum feeding unto 2 weeks of age from 15 weeks up to stimulation usually reproductive organs develop so in this period the amount of feed were strictly maintained according to recommendation. After stimulation feed were increased according to the production percentage.

Table 1: Feed ingredients used for feed formulation

Ingredients	Starter	Grower	Layer	Male breeder feed
Maize	60	50	56	50
Soyameal	28	20	22	15
Full fat soybean	-	-	4	-
Rice polish	3	11.2	8	15.4
Calcium	2	8.3	8	3
DCP	2	1.8	2	1.6
DORB	-	12		15

Source: *Cobb 500 breeder management guide*, 2009

Table 2: Recommended minimum specifications of feed

	Starter	Grower	Layer	Male breeder
Crude protein (%)	21-22	19-20	18-19	17-18
Metabolizable energy (Kcal/kg)	3008	3086	3167	3191
Lysine (%)	1.32	1.19	1.05	1
Methionine (%)	0.50	0.48	0.43	0.41
Tryptophan (%)	0.20	0.19	0.19	0.18
Na (%)	0.16-0.23	0.16-0.23	0.15-0.23	0.15-0.23

Chloride (%)	0.17-0.35	0.16-0.35	0.15-0.35	0.15-0.35
Potassium (%)	0.63-0.95	0.60-0.85	0.60-0.80	0.60-0.80
Linoleic acid	1	1	1	1

Source: Cobb 500 breeder management guide, 2009

According to AOAC (Association of official analytical chemists) I have studied feed ingredients of all three farms and found all three farms maintain feed specifications as close as standards.

g) Lighting Management

Lighting is an important factor for the breeder at the period of laying specially. At the growing period there was no need of artificial light in open sided housing system other than daylight. But during production light should never be reduced in time on intensity. It is important to start the light stimulation in time, but not before the bird is ready. It means that, the lighting schedule should be started when the flock reached at right body weight as well as sexual development and fleshing should be sufficient. It is better to delay light stimulation slight than to stimulate too early. There are two factors influencing the intensity of light falling on birds - power of light source the amount of light given out by the bulb is directly proportional to its wattage and distance of surface from the light source the light intensity decreases, as the source of light is placed further away from the surface (Cobb 500 breeder management guide, 2009).

h) Body weight and uniformity monitoring of three farms

The body weights of 40 birds of each three farms were taken in a breeder farm once weekly at the weekend in empty stomach. The main objective in a breeder farm during rearing of birds is to reach the target body weight and uniform growth rate according to the standard of birds.

i) Housing and floor space

Under modern conditions the hen is required to lay many eggs throughout the year and this object can

best be achieved if a comfortable shed is provided for them. It is very important that chicks should be housed and cared for so as to provide an environment that will enable them to maintain their thermal balance. Because of being warm blooded they have the ability to maintain a rather uniform temperature of their internal organ (Cobb 500 breeder management guide, 2009). For economic production of laying hens it is always better to keep them in small units of 15-20 bird. This number can go up to a maximum limit of 250 birds. The house should be about 400 feet long to accommodate large number of birds. In farm A, B, and C the standard length of was maintained. Spray was also done as regular practice and the litter was scratched once a day. The litter was replaced by new litter $\frac{1}{2}$ (half) or $\frac{2}{3}$ (two third) if they become too damp.

j) Ventilation

Ventilation in the poultry house is necessary to provide the birds with fresh air and to carry off moisture since the birds are small animal with rapid metabolism. Air requirement per unit of body are high in comparison with that of other animals. Since CO₂ content of expired air is about 3.55.

k) Vaccination program

One needs to develop uniform disease resistance in parents and produce high levels of maternal antibodies that can be passed on to the chicks by the eggs. It has several methods - water vaccination, spray vaccination, intra-ocular vaccination, intranasal vaccination, subcutaneous injection system, intramuscular injection, wing web punching and vaccination through feed.

Table 3: Vaccination schedule for Cobb 500 broiler breeder

Age (day)	Age (Week)	Name of Vaccine	Route
4th	-	IBD Live (INTER)	Eye
5th	-	Cocci Vaccine	Water
6th	-	Debeaking	-
7th	1	MA5 Clone 30+ $\frac{1}{2}$ dose IBD Killed	Eye & S/C
9th	2	Reo live	S/C
12th	2	IBD live + $\frac{1}{2}$ dose ND killed	Eye & S/C
13th	2	IB 4/91	I/O
16th	2	ND Lasota	Eye
26th	3	IBD live	Eye
35th	5	Reo Live	S/C
42th	6	ND+IB Killed	S/C
45th	7	Fowl Pox	Wing Web
56th	8	Fowl Cholera (killed)	I/M or S/C

63th	9	Salmonella (killed)	S/C
70th	10	4/91 IB	I/O
80th	12	Coryza (Optional)	I/M or S/C
84th	12	Fowl cholera (killed)	I/M or S/C
91th	13	Salmonella (killed)	S/C
98th	14	AE + Pox	Wing Web
105th	15	Coryza(Optional)	I/M or S/C
112th	16	ND+IB killed MA5+(IB live)	S/C & Eye
126th	18	EDS Killed	S/C
147th	21	ND+IB+IBD+Reo killed	S/C

Source: Cobb 500 breeder management guide, 2009

l) Bio-security

Bio-security is necessary to prevent the introduction of disease organisms into the flock by any means. Some of the bio-security practice includes-

- Farm location and construction: It is best to build up the farm in an isolated area, at least 2 km distance from the nearest poultry farm. It should fence the perimeter of the farm to prevent unwanted visitors. The design and construction of the houses should be in a manner that does not provide openings for wild birds and animals to enter the buildings.
- Preventing disease transmitted by humans: Restriction of the movement of visitors to the poultry farms. If supervisory personnel must visit, they should make an effort to visit the youngest flock first then visits flock with disease problems last. All people entering the farm should follow a bio-security procedure. All workers and visitors should shower and use clean & calendared farm clothes.

- Preventing disease transmitted by animals

Whenever possible all in all out placement cycle of birds should be followed. A minimum downtime of two weeks between flock is recommended. It should provide an entry barrier to rodents and wild animal, keep wild birds out of all buildings and should maintain an effective rodent control program.

m) Statistical Analysis

All the data of male and female related to production performance of parent breeder stock (i.e. live weight of male and female, feed consumption by male and female, egg production) were entered into MS excel (Microsoft office excel, 2007, USA). Data management and data analysis were done by STATA version-12.1 (STATA corporation; college station, Texas). P value ≤ 0.05 was considered statically significant.

III. RESULT AND DISCUSSIONS

a) Management practices

i. Lighting

Table 4: Standard lighting schedule for broiler parent stock

Age in weeks	Duration of light (Hours	Intensity(Lux)
1 day	24	60
2 days	24	60
3 days	23	40
4 days	22	30
5 days	21	20
6 days	20	15
7 days	19	10
8 days	18	10
9 days	17	10
10 days	16	5
11 days	15	5
12 days	14	5
13 days	13	5
14 days	12	5
15 days	11	5
16 days	10	5

17 days	9	5
18 days-21 days	8	5
22 days	10	40 minimum
23 days	11	Do
24 days	12	Do
25 days	13	Do
26 days	14	Do
27 days	15	Do
28 - end	16	Do

Source: Cobb 500 breeder management guide, 2009

Farm A, B and C follow a lighting schedule of 16, 15 and 16 hours, respectively. For laying birds the minimum light stimulation should around 16-17 hours (Summer, 2009). Brooding temperature ranges from 98-101°F in all three farms which is close with several researcher *Meijerhof et al.*, (2004) stated that the brooding temperature must be constant between 100-100.5°F with a maximum of 101°F. Brooding period of three farms almost same on 7th or 8th day. All three farms check fertility by candling.

ii. Hatchability

Hatchability of farm A was 90%, farm B 88% and farm C 90%. It is revealed that Farm B has lower hatchability than other two farms. *Elbol et al.*, (2003) stated that hatchability of fertile eggs was significantly lower when there was no pre-warming period compared to having a pre-warming period of 10 hours or 18 hours. Doc mortality rate of A, Farm B and Farm C is 3%, 2% and 2% respectively.

iii. Male Female ratio

All these three farms follow same Female and male ratio 10:1. Although mating ratio of all three farms were same but mating ratio should be reviewed weekly (Ross, 2013). In all litter system of rearing the male and female bird are reared together. The male and female are reared separately at the early age. When they reached at 22 weeks, they are joined in the same shed. The male female ratio should 1:10 (Cobb 500 breeder guide management, 2009).

iv. Drinker and water management

It is desirable to supply an adequate amount of portable water for chicken considering the few points - should use a reliable water sanitizer such as chlorine or iodine, testing of the chlorine level of the drinker and testing of the water monthly to ensure acceptable coli form levels. In open drinking system gradually to move chick drinker towards the automatic drinkers. Until seven days of age, the top lip of the drinker should be set to the height of the average bird's back. After 7 days of age, the drinker should be gradually raised. The proper water depth is 1.9 cm and Drinker should be washed daily.

In nipple drinking system it should make sure that the nipple drinker lines and litter are level. Just prior

to pacing the birds on the nipple drinking system, triggering all of the nipples to check perfect flowing of water. The height of the water lines should adjust in such a way that the lines are at the bird's eye level for the first two days (Cobb 500 breeder management guide, 2009). Overall water management of all three farms was good. All three farms used nipple drinker and deep well as water source.

v. Vaccination and Anthelmintics

Vaccines commonly used in these three farms are Mareks vaccine, Ma5+clone 30 (IB% ND), D78 (IBD live), 228E (IBD live), ND Clone 30 (ND live), Corvac (Coryza), CIVAC ND IB EDS-K, Fowl cholera vaccine etc. In these three farms most of vaccines used were live vaccines. It may because of live vaccines have advantages of quick and easy administration, inexpensive and give almost immediate immunity (Summer, 2009). Anthelmintics that are used commonly in these three farms are piperazine, livamisole, albendazole.

vi. Routine Tests

Common routine tests (i.e. feed sample analysis, aflatoxin detection test and CS test) are performed in these three farms. It is cited that less than 1 ppm of aflatoxin may cause liver damage, reduced egg production and hatchability (Summer, 2009).

vii. Record Keeping

Records that maintained in these three farms include vaccination, feed and water consumption, egg production, mortality, body weight, egg weight, fertility percentage, hatchability percentage, Production cost etc. Record keeping is an essential aid to effective management. Records should include all factors related with rearing, laying, treatment and significant events and target performance (Ross, 2013).

b) Production Performances

i. Feed intake

Table 5: Feed intake of female breeders of different farms (gm/bird)

Farms	Mean	Standard deviation (SD)	Chi square value	P value
A	147.66	27.81	4.37	0.96
B	149.66	26.55		
C	149.15	37.12		

Table 5 represents that feed intake by female breeders was increased in farm B and C in comparison with farm A though the differences were not significant statistically ($P > 0.05$). Scott *et al.*, (1999) found that feed

restriction reduce feed intake, body weight and hen day egg production proportionately. As farm A followed restricted feeding what probably influenced on feed intake.

Table 6: Feed intake of male breeders of different farms (gm/bird)

Farms	Mean	Standard deviation (SD)	Chi square value	P value
A	132.06	11.64	1.57	0.45
B	137.93	14.09		
C	138.72	14.23		

Significant ($P < 0.05$) differences in feed intake of males were observed among difference farms. Feed consumption by male breeders of Farm B and C was higher than farm A (table 6). Leclercq and Whitehead, (2004) stated that male birds ate more than females and vary with breeding lines which may responsible for feed intake variability.

uniformity in the flock. When flock uniformity become low it is necessary to place those higher and heavier birds in separate pan. To correct poor uniformity flocks should be guarded early at 4 weeks but not later than 5 weeks. The lighter birds should give extra feed for achieving weight and the heavier birds should restrict the feeding till reduces the weight (Cobb 500 breeder farm management, 2009).

ii. Body weight

It is a great advantage to achieve a uniform flocks during laying. It is crucial to maintain the

Table 7: Body weight of females of different farms (gm/bird)

Farms	Mean	Standard deviation (SD)	Chi square value	P value
A	3419.18	561.95	0.007	0.96
B	3359.21	553.67		
C	3409.45	559.44		

There was no significant difference ($P > 0.96$) among body weight of breeder males of different farms (table 7). So females are more or less homogenous in weight. Hurwitz and Plavnik, (1998) concluded that the weight of egg is related to both age and body weight

during the onset of production. The egg weight and the body weight were significantly correlated for one year of production. As all the birds of these farms were at the same stage of production for that reason body weight of female birds got close enough.

Table 8: Body weight of males of different farms (gm/bird)

Farms	Mean	Standard deviation (SD)	Chi square value	P value
A	4043.03	524.41	1.01	0.68
B	4043.03	524.41		
C	3940.39	610.95		

From table 8 data indicating that male gain more body weight than female birds. Birds of farm C has lower body weight than farm A and B. Deschepper and Degroot, (1995) stated that body weight may be higher if birds were fed on low protein diet with supplementary synthetic amino acids, which might be a factors for higher body weight gain of farm A and B.

Table 9: Egg production in different farms

Farms	Mean	Standard deviation (SD)	Chi square value	P value
A	54.93	31.98	0.06	0.87
B	62.69	31.79		
C	50.93	30.70		

Apparently, egg production of farm B is higher than other two farms (A and C). However, the difference is not significant ($P > 0.05$) statistically. *Spratt and Leeson, (1987)* reported that the excess intake predominantly fat cause gradually results in increased body weight of female birds which was negatively correlated with hen day egg production. So higher fat intake might be responsible for lower egg production of farm A and C. Egg production before peak at farm A, B and C was 50% on 30th week, 22.1% on 16th week, and 50% on 26th weeks, respectively. Egg production at peak was 90% at 30th weeks in farm A, 95% at 31- 42th weeks in farm B and 91% at 32th weeks in farm was C.

IV. CONCLUSION

It can be concluded that the production performance of Cobb 500 broiler breeder parent stock is overall good in environmentally control house. The management practices in farm A, B and C were very close to the standard. However, the males gained more live weight than females in those farms. Egg production was highest in farm B than farm A and C. The difference was due to higher feed intake in farm A and C. So it can say that standard management practices should be followed strictly in a breeder farm to gain maximum production.

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Evaluation of Radiographically Evident Cardio-Pulmonary, Metastatic and Paraneoplastic Changes in Thorax Associated with Superficial and Mammary Neoplasms in Dogs

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Abstract- Nowadays, neoplasms have become the common surgical affections encountered in the pet population, especially in dogs. Skin neoplasms are the most common neoplasm observed in dogs, followed by mammary neoplasms. Most of the neoplasm may exhibit metastatic and paraneoplastic changes in distant sites other than primary tumor sites, mainly in the thorax. The present study investigated the evaluation of radiographically evident cardio-pulmonary, metastatic and paraneoplastic changes in the thorax of twenty- four clinical cases of dogs of either sex belonging to different breeds, and age, having superficial cutaneous and mammary neoplasm conditions.

Keywords: *pulmonary metastasis, computed thoracic radiography, atypical metastatic lesions.*

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Evaluation of Radiographically Evident Cardio-Pulmonary, Metastatic and Paraneoplastic Changes in Thorax Associated with Superficial and Mammary Neoplasms in Dogs

Lekshmi S.L ^α, Sudheesh S Nair ^σ, Sajitha I.S ^ρ, Narayanan M.K. ^ω & K.D. John Martin [¥]

Abstract- Nowadays, neoplasms have become the common surgical affections encountered in the pet population, especially in dogs. Skin neoplasms are the most common neoplasm observed in dogs, followed by mammary neoplasms. Most of the neoplasm may exhibit metastatic and paraneoplastic changes in distant sites other than primary tumor sites, mainly in the thorax. The present study investigated the evaluation of radiographically evident cardio-pulmonary, metastatic and paraneoplastic changes in the thorax of twenty-four clinical cases of dogs of either sex belonging to different breeds, and age, having superficial cutaneous and mammary neoplasm conditions. Fine Needle Aspiration Cytology (FNAC) of the primary tumour was performed on all of the dogs for neoplasm confirmation, and orthogonal thoracic radiography was used to assess each region of the thorax, including the pleura, pulmonary parenchyma, mediastinum, trachea, cardiovascular system, and extrathoracic structures. The size, morphology, and area of distribution of metastatic pulmonary lesions, as well as atypical metastatic lesions such as calcification, secondary pneumothorax, feeding vessel signs, cavitation, were assessed in three-view thoracic radiographs of both neoplasm conditions, and the results are described here.

Keywords: pulmonary metastasis, computed thoracic radiography, atypical metastatic lesions.

I. INTRODUCTION

Radiographic examination is usually considered as the first investigative modality, after the clinical examination and undisputedly most beneficial, being non-invasive which can provide rapid and valuable information in the diagnosis and treatment of a wide range of diseases (Rudorf *et al.*, 2008). Radiography remains a popular diagnostic screening tool for the thorax because it is time- and cost-efficient, uses readily available equipment, is relatively easy to perform, usually does not require general anesthesia, is non-invasive, and

causes relatively low stress to the patient, and is unrivalled by any other diagnostic test for these characteristics. The main advantage over other more recent techniques is the inherent revelation of the morphology of thoracic structures. Most neoplasms metastasize to the lung, particularly mammary neoplasms, with solitary or numerous pulmonary nodules, either benign or malignant, lymphangitic metastasis, and/or endobronchial metastasis being common lesions (Jung *et al.*, 2004). An awareness of the spectrum of radiology for indications in pulmonary metastases and pulmonary changes in paraneoplastic syndrome allows the distinction of metastases from other non-malignant pulmonary diseases. The evaluation of radiographically evident cardio-pulmonary, metastatic, and paraneoplastic changes in the thorax of twenty-four clinical cases of superficial and mammary neoplasms in dogs is documented in this work.

II. MATERIALS AND METHODS

The selected animals were grouped into two containing twelve animals each – group I with superficial neoplasm designated as A1 to A12 and group II with mammary neoplasm as B1 to B12. Fine Needle Aspiration Cytology (FNAC) of primary tumor mass was carried out in all dogs to confirm the malignancy before the imaging examination. A 200 mA X-ray machine with a computed radiography system was used to perform a three-way thoracic radiographic evaluation (right lateral, left lateral, and ventrodorsal views) on all cases. All animals were subjected to surgical management, and excisional biopsy of tumor samples were histopathologically analysed.

Tracheal Diameter: Tracheal Inlet (TD: TI) ratio was calculated by recording the diameter of the trachea at the level of the thoracic inlet and the inner diameter of the thoracic inlet (Fig 1). Cardiothoracic ratios (CTR) were calculated by dividing the largest width of the thorax by chest depth on radiographs (Fig 3). On a dorsoventral/ventrodorsal radiograph, the width of the thorax was measured as the distance between the medial borders of the eight ribs (Fig 4). The depth was

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assessed on the right lateral radiographic view from the dorsal margin of the xiphoid process to the ventral margin of the vertebral body, with the electronic calipers aligned perpendicular to the vertebral column. The Vertebral heart score was calculated according to Buchanan and Bücheler, 1995 (Fig 2).

Radiographical assessment of each region of thorax, including pleura, pulmonary parenchyma, cardiac silhouette, mediastinum and trachea, as well as extrathoracic structures, including sternum, ribs, vertebrae, thoracic wall, and diaphragm were performed.

The primary lesions studied were pleural effusion, pleural mass and change in radiodensity for pleura and pulmonary patterns (bronchial, interstitial, alveolar, vascular, and mixed pattern) and other pulmonary parenchymal lesions (peribronchial thickening, bronchial mineralization, lobar signs, consolidation, cavitary lesions, generalized increased lung opacity, and pneumothorax) for lung parenchyma. The presence of mediastinal masses and pneumomediastinum, as well as other radiographic aspects of the mediastinum, were investigated. Tracheal changes, such as tracheal mineralization, tracheal deviation, tracheal hypoplasia, etc., were also evaluated.

The cardiac silhouette features observed in orthogonal thoracic radiographs were categorized as positional shift, chamber enlargement (left and right atrial enlargement, left and right ventricular enlargement), pericardial effusion, and pulmonary arterial or venous changes.

The extrathoracic region including, sternum (sternal spondylosis and degenerative changes), ribs (costal cartilage mineralization, degenerative changes, and other lesions), vertebrae (vertebral spondylosis and intervertebral herniation), thoracic wall changes, and diaphragmatic abnormalities were all investigated.

This study also investigated at the size, morphology, and distribution area of pulmonary metastatic lesions as well as the atypical metastatic lesions such as calcification, secondary pneumothorax, feeding vessel signs, cavitation lesions, and so on.

III. RESULTS AND DISCUSSION

All the cases were confirmed as neoplasm by performing Fine Needle Aspiration Cytology (FNAC) of the primary tumor. The cytology of superficial neoplasms revealed two (16.67 percent) mesenchymal tumors, five (55.55 percent) epithelial tumors and, one (11.11 percent) round cell tumors (A3). Four (33.33 percent) out of 12 samples were classified as inadequate for diagnosis because of no cells ($n=2$) and the presence of a large number of red blood cells and inflammatory cells ($n=2$). The malignancy was indicated by the presence of clusters of cells with anisocytosis,

anisokaryosis, and hyperchromasia, as well as nuclear anomalies such as the double nucleus, giant nucleus, mitotic figures, and abnormal chromatin structures. Similar observations were also reported by Meinkoth *et al.* (2007), Yildirim and Gurel (2012), and Yumusak and Kutsal (2016). In mammary neoplasms, the common cytological characteristics observed were anisocytosis, anisokaryosis, enlarged nuclei, double nuclei, multiple nucleoli, and clumping of chromatin (hyperchromasia). These findings were as per Shafiee *et al.* (2013) and Kuppasamy *et al.* (2019).

Tracheal diameter: Thoracic inlet ratios was calculated from lateral radiographs in all animals by the method proposed by Harvey and Fink (1982). Three of the animals with mammary neoplasm had a ratio of less than 0.2, indicating tracheal hypoplasia, whereas the TD: TI ratio in superficial neoplasm affected animals was in the normal range, indicating normal tracheal confirmation. The relationship between TD: TI ratio and neoplasm conditions was not yet reported. The finding of tracheal hypoplasia in this study could be related to other physiological factors. The exact measurements of the tracheal diameter were challenging with radiography owing to the absence of sharp luminal borders due to overlying soft tissues as opined by Fingland *et al.* (1995).

From measurements of Vertebral Heart Score in both right lateral and left lateral thoracic radiographs, 33.33 percent of group I animals and 25 percent of group II animals were diagnosed as suffering from cardiomegaly. There was no significant difference between the groups. A VHS over 10.7 on the lateral radiograph was a moderately accurate sign of cardiac disease, as opined by Lamb *et al.* (2000). The highest vertebral heart score in the dogs might be due to the onset of mitral valve regurgitation following congestive heart failure, as documented by Lord (2010). The pulmonary metastases of malignancy might be lead to epicardial, myocardial and, endocardial involvement and further pathophysiological changes such as pericardial effusion, filling impairment, myocardial dysfunction, etc., as documented by Bussani *et al.* (2007).

Group I animals had a Cardio-thoracic ratio ranging from 0.73- 1.03, and Group II had a cardio-thoracic ratio ranging from 0.69-1.12. All the animals had CTR values in the normal range irrespective of type of the neoplasm.

Pleura was identified as very thin and silhouetted with adjacent soft tissue everywhere except in interlobar fissures, where it was in contact only with the lung. In the present study, normal pleura was not visible radiographically, but the pathology of pleura enhanced the radiographic diagnostic visibility as documented by Thrall (2018). Pleural effusion, change in radiodensity, and extrapleural lesions were the most

common radiographically visible pleural lesions observed in this study. Pleural effusion was observed in lateral thoracic radiographs of two cases of mammary neoplasm and four cases of superficial neoplasm. There was no significant difference in pleural lesions between the superficial and mammary neoplasm conditions. The most common cause of pleural effusion was identified as pleural metastasis by Noone (1985).

The typical pulmonary pattern observed in both superficial and mammary neoplasm conditions were interstitial nodular pattern followed by bronchial pattern, alveolar pattern, and vascular pattern. Similar pulmonary patterns were observed by Gupta *et al.* (2014) in thoracic radiograph of dogs affected with mammary neoplasm, which showed a nodular pattern, miliary pattern, alveolar pattern, doughnut pattern and mixed interstitial and bronchial pattern evident in most of the animals. Other pulmonary lesions such as bronchial mineralization, peribronchial thickening, consolidation, lobar symptoms, and pneumothorax were seen in both neoplasm groups with no significant differences.

Many of the mediastinal reflections can be appreciated at conventional radiography, and their presence or distortion could serve as a key to the interpretation of mediastinal abnormalities (Whitten *et al.* 2007). However, computed tomography is the most essential tool in evaluating the mediastinal masses (Takahashi and Al- Janabi, 2010). A mediastinal shift was observed in ventrodorsal radiographs of one animal having superficial neoplasm and two animals having mammary neoplasm. Mediastinal shift, cranioventral mediastinal mass, dorsal mediastinal mass, hilar mediastinal mass, caudoventral mediastinal mass, and pneumomediastinum were seen in both groups of neoplasms in this investigation. There was no significant differences observed between the groups.

The cranioventral aspect of the thorax was one of the most common locations for a mediastinal mass. In this study, cranioventral mass suspecting sternal lymphadenopathy was observed in two cases (A8 and A11) affected with squamous cell carcinoma (Fig 5), one case with liposarcoma (A7), one case with fibroma (A2), and two cases (B1 and B2) with mammary carcinoma . Similar observations were made by Baumann *et al.* (2004) during their study in metastatic disease of dogs affected with mammary gland tumors. Enlarged sternal lymph nodes may result from diseases of the peritoneum, the cranial abdominal organs, or the abdominal orthoracic wall.

The mammary gland complexes I, II, and III usually drain to the axillary lymph nodes and metastasize into the lungs with or without concurrent involvement of the intrathoracic lymph nodes (Brodey *et al.*, 1983 and Pereira *et al.*, 2003). Epithelial tumors, such as carcinoma, readily metastasize via lymphatics to the regional lymph nodes (Sorenmo, 2003). Hopper *et*

al. (2004) assumed that the sternal lymph node enlargement could be a sign of intrathoracic disease, but enlargement of the sternal lymph node is often secondary to abdominal diseases, such as peritonitis or peritoneal tumor seeding. We assume the cranial mediastinal lymphadenopathy or sternal lymphnode enlargement in these cases is due to metastatic disease. In the present study, pneumomediastinum was observed in two cases from both groups (A9 and B6). Park *et al.* (2003) opined that the pneumomediastinum was a part of the disease process, rather than a complication of pneumothorax, and the most likely cause of the pneumomediastinum found in their patient suffering from angiosarcoma would be cystic rupture into the interstitial space with subsequent tracking of air through the peribronchial space into the mediastinum.

The common radiographical features of the trachea observed in dogs with superficial and mammary neoplasms were mineralization of tracheal rings, tracheal displacement, and tracheal hypoplasia. There was no significant difference observed between these two groups. Similar observations were made by Gowthami (2017) in their study on geriatric dogs. The most commonly observed tracheal displacement in this study was dorsal deviation, which could be due to cranial mediastinal mass, neck positioning during radiography (at craniomediastinal region) or heart base mass, pulmonary artery enlargement, or right atrial enlargement (at heart base), or left atrial enlargement, tracheobronchial lymphadenomegaly or generalized cardiomegaly (at carina) (Thrall, 2013) (Fig 5).

The most common cardiac silhouette changes in this study were left atrial enlargement, left ventricle enlargement, right ventricle enlargement and pericardial effusion in both groups. There is no significant difference noticed between the groups. Tumors could spread to the heart by alternative pathways such as by direct extension, through the bloodstream, through the lymphatic system, and by intracavitary diffusion through either the inferior vena cava or the pulmonary veins (Virmani, 1995). This might lead to epicardial, myocardial, and endocardial involvement and further pathophysiological changes such as pericardial effusion, filling impairment, myocardial dysfunction etc., in humans (Bussani *et al.* 2007). When comparing dogs with mammary neoplasms to dogs with superficial neoplasms, pericardial effusion was found at a higher percentage. In the present study, peripheral artery and vein alterations were observed in lateral radiographs of both groups, more in mammary neoplasm condition.

The common extrathoracic lesions observed in this study were costal cartilage mineralization and degenerative changes in ribs, vertebral spondylosis, and change in opacity of thoracic wall, and presence of soft tissue tumors in the thoracic wall, which mainly observed in the group with superficial neoplasm. Other lesions

such as sternal spondylosis, sternal degenerative changes, diaphragmatic changes (change in position or shape) etc., were also observed in both groups. There is no significant difference noticed between these groups. Exostosis of the ribs (costal cartilage mineralization or calcification) and degenerative or lytic lesions were the prominent lesions observed in ribs of both groups, especially from lateral radiographs. This could be due to age-related changes or the spread of metastatic disease. Schiavon *et al.* (1993) observed that costal cartilage calcification was a common finding in old age, seen as small islands of compact bone tissue or as nodules, mistaken for solitary pulmonary nodules. Plasma cell tumors, as well as metastatic dissemination, can cause lytic or proliferative rib changes in felines, according to Adams *et al.* (2010). Larson (2020) also observed that the aggressive rib changes were metastatic lesions secondary to a diffuse pulmonary carcinoma with secondary pleural effusion.

Depending on the source and type of tumor, the pulmonary metastases could develop a wide range of radiographic appearance (Franquet *et al.* 2020). Pulmonary metastases typically appeared as peripheral, rounded nodules of variable size, scattered throughout both lungs and diffuse thickening of interstitium as per Seo *et al.* (2001). In this study, the categorization of the metastatic lesions noticed in the thoracic radiographs of the neoplasm-affected dogs were performed based on the size of metastatic lesions and distribution pattern or area. Based on the size of metastatic lesions, they were classified into military nodules (<2 mm), pulmonary micronodules (2-7 mm), pulmonary nodules (7-30mm), and pulmonary mass (>30mm) as per Weerakkody *et al.* (2019). The most commonly observed pulmonary metastatic lesions were pulmonary nodules followed by pulmonary micronodules, military nodules, and pulmonary mass in both groups in lateral thoracic radiographs. The most common pulmonary metastatic nodular pattern in the case of superficial neoplasm

cases were pulmonary micronodules and reticulonodular patterns (Fig 7 and Fig 8). Lung consolidation might lead to the formation of pulmonary masses (> 30 mm size), which were observed in both mammary neoplasm and superficial neoplasm in the present study (Fig 6). The similar unusual form of metastatic spread to the lung is characterized by lepidic growth of the tumor cells along the lining of the alveolar walls, resulting in a primary pulmonary adenocarcinoma-like appearance (Franquet *et al.* 2020). Most of the metastatic malignant nodules appeared as lobulated or with spiculations, as observed by Woodring (1990). The radiographic appearance of metastases from benign tumors included multiple peripherally located round variable-sized nodules and tended to remain stable and show prolonged growth (Franquet *et al.* 2020). In the present study, most of the metastatic lesions were located in the perihilar region, followed by the caudodorsal, midventral, and cranioventral area, and in maximum number of cases metastatic lesions were observed uniformly in all lung regions as reported by Gowthami (2017). Other metastatic lesions patterns, such as interstitial disseminated reticulonodular pattern and mixed disseminated alveolar interstitial pattern was observed in both groups. The atypical metastatic lesions such as calcification, secondary pneumothorax, cavitation signs, and feeding vessel signs were observed in this study. Calcification of a pulmonary nodule is usually suggestive of its benign nature but it can also occur in metastatic nodules from an osteosarcoma or chondrosarcoma (Seo *et al.* 2001). Pneumothorax was observed in three mammary neoplasms and one superficial neoplasm case in this study. Weerakkody and Niknejad (2019) reported a "feeding vessel sign," which consisted of a particular vessel leading directly to a nodule or a mass. Some of the malignant metastatic lesions in this study suspected this sign and can be confirmed by computed tomography.

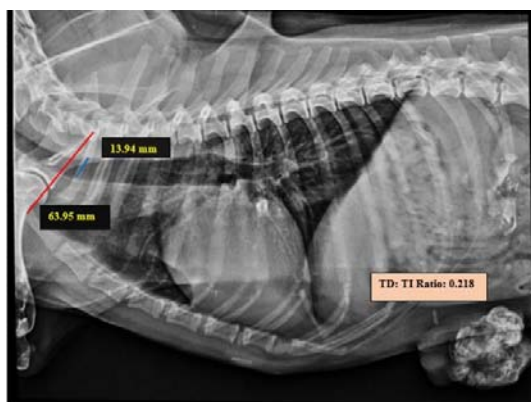


Fig.1: Skiagram showing measurement of TD: TI ratio from the right lateral view

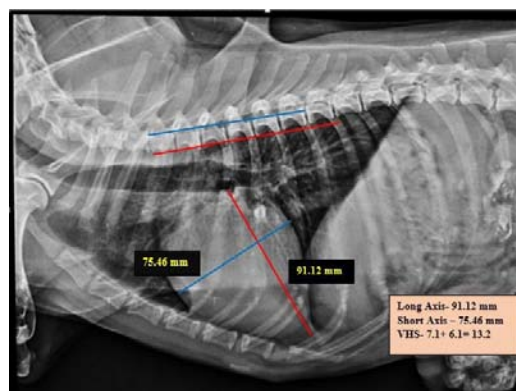


Fig. 2: Skiagram showing measurement of vertebral heart score from the right lateral view

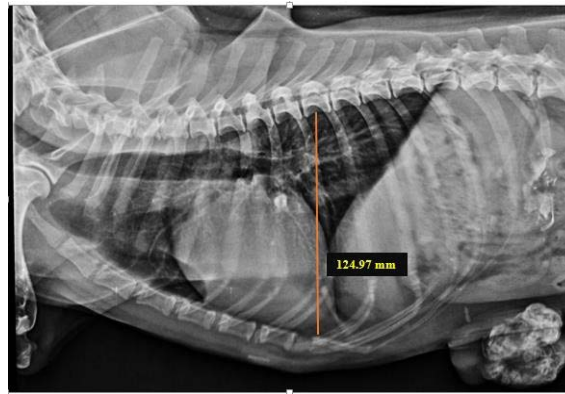


Fig. 3: Skiagram showing measurement of chest depth on right lateral view for assessment of the cardiothoracic ratio

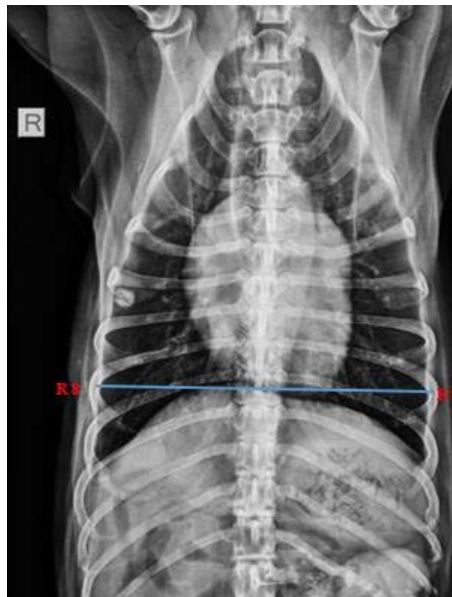


Fig. 4: Skiagram showing measurement of thoracic width on ventrodorsal view for assessment of the cardiothoracic ratio

Cardiothoracic ratio, CTR =
Largest width of the thorax / Chest
Depth = 0.89



Fig. 5: Skiagram of the thorax on the right lateral view showing cranioventral mediastinal mass (1), tracheal displacement (2), and suspecting tracheobronchial lymphadenopathy (3) in dog A11

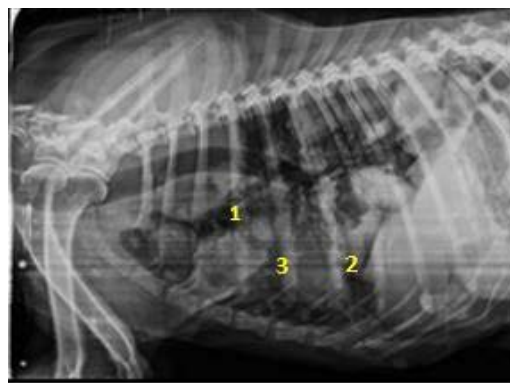


Fig. 6: Skiagram of the thorax on the right lateral view showing cavitory lesion (1), lobar signs (2), consolidation (3), and pulmonary metastatic lesions in dog B11

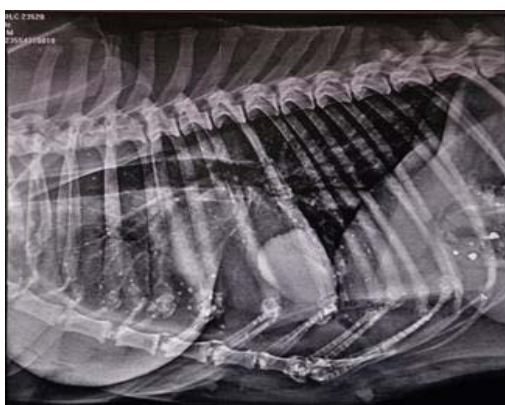


Fig. 7: Skiagram of the thorax showing pulmonary miliary nodules, pulmonary micronodules at the cranioventral region and pulmonary mass at caudoventral region of lung parenchyma and suspecting cavitation at the cranioventral area in right lateral view in dog A9



Fig. 8: Skiagrams of thorax showing Pulmonary mass (Size 34.21mm) at the midventral area and nodular lesion at the caudoventral area and suspecting feeding vessel sign in right lateral view in dog B1

IV. CONCLUSION

Thoracic radiography was found to be a reliable, cost and time-effective diagnostic procedure for the identification of initial or advanced stages of pulmonary metastasis and other paraneoplastic changes associated with neoplasm conditions such as superficial and mammary neoplasms. Even though thoracic radiography is the primary diagnostic choice for pulmonary metastasis, there are so many limitations for using this as a confirmatory diagnosis, such as soft tissue nodules of diameter less than 2mm could not be diagnosed by using radiography. Feeding vessel signs, cavitation, tumor embolism and hemorrhage around the nodule, etc., can be interpreted by other techniques such as CT or MRI.

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Adding Krill Oil and Low Molecular Weight Hyaluronic Acid to Movoflex® Soft Chews Helps Improve Dogs' Mobility

By Nicolas CS, Schreiber P, Rème CA, Lopez J & Ereau C

Abstract- Movoflex® Soft Chew (Virbac, USA) is a nutritional supplement to support dogs' mobility. It contains a unique combination of five ingredients: eggshell membrane, hyaluronic acid (HA) of high molecular weight, *Boswellia serrata* extract, astaxanthin, and vitamin D3. Krill oil and low molecular weight HA are now added to the supplement. The tolerance of this advanced formula was first evaluated in dogs. The aspect, palatability of the chew, and effectiveness on mobility criteria were then assessed by sixty-nine dog owners who were regular users of the current Movoflex® Soft Chews.

The chew was found to be well-tolerated and similar in terms of aspect and acceptability to the current one. All but one mobility criteria significantly improved after 30 days, including the ability to walk, climb, stand, interact with people, or the way of walking.

Keywords: movoflex; movoflex advanced; dogs' mobility support; tolerance in dogs; movoflex efficiency; dog mobility; krill; hyaluronic acid; owner perception; movoflex soft chew.

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Strictly as per the compliance and regulations of:



Adding Krill Oil and Low Molecular Weight Hyaluronic Acid to Movoflex® Soft Chews Helps Improve Dogs' Mobility

Movoflex® Advanced Soft Chews on Dogs' Mobility

Nicolas CS ^α, Schreiber P ^σ, Rème CA ^ρ, Lopez J ^ω & Ereau C [¥]

Abstract- Movoflex® Soft Chew (Virbac, USA) is a nutritional supplement to support dogs' mobility. It contains a unique combination of five ingredients: eggshell membrane, hyaluronic acid (HA) of high molecular weight, *Boswellia serrata* extract, astaxanthin, and vitamin D3. Krill oil and low molecular weight HA are now added to the supplement. The tolerance of this advanced formula was first evaluated in dogs. The aspect, palatability of the chew, and effectiveness on mobility criteria were then assessed by sixty-nine dog owners who were regular users of the current Movoflex® Soft Chews.

The chew was found to be well-tolerated and similar in terms of aspect and acceptability to the current one. All but one mobility criteria significantly improved after 30 days, including the ability to walk, climb, stand, interact with people, or the way of walking.

Therefore, the new Movoflex® Advanced Soft Chew (Virbac, USA) is well tolerated and more effective than the current formula.

Keywords: movoflex; movoflex advanced; dogs' mobility support; tolerance in dogs; movoflex efficiency; dog mobility; krill; hyaluronic acid; owner perception; movoflex soft chew.

1. INTRODUCTION

Osteoarthritis (OA) is a common disorder in humans and animals, and its prevalence increases with age. It can reach more than 80% of animals eight years of age or older [1]. It is a debilitating and painful disease, involving local tissue damage as well as local and systemic inflammatory responses. It leads to decreased mobility and impacts the quality of life of both dogs and owners [2–4]. Preventing cartilage damage and oxidative stress, and maintaining a balanced immune response, are therefore key to disease prevention and to slowing down its progression. Management of OA in dogs usually involves nonsteroidal anti-inflammatory drugs despite their side effects. Many efforts have been put over the last decades into finding new active ingredients and nutraceuticals to maintain joint health and mobility [3,5].

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Movoflex® Soft Chew (Virbac, USA) is a nutritional supplement that can help support dogs' mobility and joint health [2]. It contains a unique combination of ingredients that are well tolerated by dogs and that have shown effectiveness in supporting joint health, either individually or in combination: eggshell membrane (ESM), astaxanthin, hyaluronic acid (of high molecular weight), *Boswellia serrata* extract, and vitamin D3 [2]. Now, the advanced formula also contains hyaluronic acid (HA) of low molecular weight and krill oil as a source of omega-3 fatty acids.

Eggshell membrane contains different molecules found in joints, including glucosamine and glycosaminoglycans, elastin, collagen, and hyaluronic acid. It also contains different amino acids (mainly proline, glutamic acid, and glycine) that can help support protein synthesis [6]. Eggshell membrane has shown beneficial effects on joints and mobility, in humans with osteoarthritis [7–14] and in dogs [2,15,16].

Astaxanthin is a naturally occurring carotenoid with potent antioxidant properties (more powerful than other carotenoids and 500 times more powerful than vitamin E) [17–19]. Astaxanthin limits the production of mitochondrial reactive oxygen species (ROS) and modulates their effects, scavenging free radicals and decreasing the development of nitric oxide [18,19]. Astaxanthin has also been shown to stimulate the immune response in animals and humans [20,21]. The astaxanthin in Movoflex® Soft Chews comes from *Haematococcus pluvialis*, a unicellular microalga, one of the safest and richest sources of natural astaxanthin [18,19].

Boswellia serrata extracts, from the resin of the tree, have been used for joint health for a long time, notably in ayurvedic medicine. Several studies have shown its good tolerance and beneficial effects on joints in humans and animals [22,23], including in dogs [24]. Boswellic acids like 3-O-Acetyl-11-keto-beta-boswellic acid (AKBA) and 11-keto-b-boswellic acid (KBA) would indeed help control the immune system by inhibiting the 5-lipoxygenase (5-LOX) and would help improve pain and joint function [22,23]. Other mechanisms may also be involved.

Cholecalciferol is the inactive form of vitamin D3. It requires sequential hydroxylations in the liver and kidney to reach its physiologically active form (calcitriol or 1,25-dihydroxyvitamin D3). It is a steroidal hormone known to regulate calcium and phosphorus homeostasis in the body, as well as bone metabolism, among other properties [25,26]. The blood level of vitamin D is positively correlated with good musculoskeletal health and negatively correlated with OA or mobility reduction [25–29]. It has been shown in some studies that vitamin D supplementation can help patients with OA [25,30,31]. In dogs, vitamin D supplementation can help maintain bone health [26,29].

Hyaluronic acid (HA) is a large glycosaminoglycan consisting of repeating disaccharide units of N-acetylglucosamine and glucuronate. It is found in several tissues, including in the articular cartilage and synovial fluid. It is highly involved in the lubrication and viscoelasticity of the synovial fluid and plays a significant role in joint mobility [32,33]. Therefore, administering HA has shown beneficial effects in OA dogs [32,33]. Since OA is associated with HA degradation over time, the HA administered was, mainly, of high molecular weight (MW) and usually injected directly into the joint [33]. However, oral forms can also be used with good bioavailability and efficacy [34,35]. A study has shown that HA of high MW can be found in dogs' joints after oral administration [36]. On the other hand, lower MW HA, which should have a higher oral bioavailability than the high MW HA [34,37], could bring the elements required for the HA synthesis and help initiate the restorative processes [33,38]. A recent study in an animal model even showed that the lower molecular weight HA would be more effective than the higher molecular weight HA for cartilage repair [38].

Krill are shrimp-like crustaceans that are small (about the size of a paperclip) but form abundant and dense swarms in polar seas. The oil extracted from the main Antarctic species, *Euphausia superba*, is naturally rich in omega-3 polyunsaturated fatty acids (or PUFAs), mainly eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) [39]. Contrary to what is found in fish oil (mainly triacylglyceride-bound PUFAs), PUFAs from krill oil come primarily in the phospholipid (PLs)-bound form. Phosphatidylcholine is a major PL in krill oil. On top of bringing essential nutrients like choline, PLs also seem to be a more effective delivery form of PUFAs, with higher bioavailability [39,40]. The health benefits of krill oil have been demonstrated in several studies, including those focusing on joint health [39,41,42]. It has also been suggested that PLs, like those in krill oil, could increase the bioavailability of astaxanthin and hyaluronic acid [43,44]. Combining these three ingredients has shown beneficial effects on joint and inflammation in different studies [45,46] and is now commercially available for humans (FlexPro MD®, Valensa).

The krill used to make the oil in Movoflex® advanced Soft Chews (Virbac, USA) is fished using an eco-harvesting technology to harvest krill in a sustainable and environmentally friendly way. This technology reduces the risk of by-catch of other species to almost zero.

We hypothesized that adding krill oil and a lower molecular weight HA could increase the performance of Movoflex® Soft Chews in dogs and remain well tolerated. We first tested its tolerance, in kennel dogs, before testing its acceptability and perception of effectiveness on mobility, with dog owners already using the current Movoflex® Soft Chews.

II. MATERIAL AND METHODS

• Tolerance

The tolerance of the chew was tested in 16 beagle dogs (6 months to 2 years old, > 5 kg) receiving either one or five times the daily recommended number of chews, according to Virbac's standard procedures. The project was approved by the Virbac Ethical Committee and by French Authorities under authorization number 20210111113595530#28915.

Eight dogs received one Movoflex® Soft Chew size S per day (as recommended) for small dogs < 18 kg (1x group), and eight dogs received five chews per day (5x group), during 28 days. Another group of four dogs did not receive any chew (control group). A 14-day acclimation phase was included before administering the chews on Day 0. Dogs were housed in pairs and fed Veterinary HPM® Adult Neutered Dog (Virbac). They were observed daily, and a complete clinical examination, including body weight and body temperature measurements, was performed once a week from Day -14 to 28. Food consumption was checked daily. An adjustment of the food ration (-10g of kibbles daily) was made for the dogs receiving five chews per day (6.27 kcal/chew). Feces were examined and scored daily based on a simple scale (1- molded stools, 2- not molded stools, 3- liquid stools) during the whole study period. Blood samples were taken at the start and the end of the study for standard hematology [RBC, HGB, HCT, MCV, MCH, MCHC, RETIC, WBC, NEU, EOS, BASO, LYM, MONO and PLT - ProCyteDx Hematology Analyser (IDEXX)] and blood chemistry [BUN; CREA; TP; ALB; GLOB; GLU; ALKP; ALT; AST; Calcium; Chloride; Potassium; Magnesium; Sodium; Phosphate - Catalyst Chemistry Analyser (IDEXX)] analysis. Urine samples were also taken on fasted and sedated animals by ultrasound-guided cystocentesis in females and by vesical catheterization in males, when possible (no empty bladder) at the beginning and the end of the study for pH analysis. Urinary pH was measured with a pHmeter (Mettler Toledo Seven Multi pH meter) quickly after urine collection.

- Perceived effectiveness

Movoflex® Advanced Soft Chews were provided to 78 owners of dogs, in the USA, already using Movoflex® Soft Chews before the study. Selected dogs had to be five years or older and included 26 small dogs weighing less than 18 kg (<40 lbs), 26 medium dogs between 18 and 36 kg (40-80 lbs) and 26 large dogs weighing more than 36 kg (>80 lbs). The participants tested the chew over 30 days (1 chew per day - chew size according to body weight: 2g, 4g, or 6g, respectively). The participants had to fill out an online survey on Days 0, 7, and 30 to assess the product's physical properties (versus the current Movoflex® Soft Chews), palatability, reactions of the dog, and perception of effectiveness on their dog's mobility.

Every day, the owner had to score the reaction of the dog when given the chew, according to the following scale: 1- the dog ate the chew all at once; 2 - the dog ate part of it, stopped, then ate the rest; 3 - the dog ate some of it only; 4 - the dog played with it but did not eat; or 5 - the dog would not touch it spontaneously. To assess their dog's mobility, the owners had to describe five parameters:

- Dog's interaction with family and friends (1 - Happy and playful/Jumped around; 2 - Happy but reserved; 3 - Neutral; 4 - Sad, but reacted when called; 5 - Sad and didn't react much when called; 6 - Tended to isolate from others; 7 - Became aggressive when disturbed)
- Dog's ability to stand up after resting/lying down for some time (1 - Normal, stood up without difficulty; 2 - Slow, with some difficulty; 3 - Slow, with a lot of difficulty; 4 - Needed help getting up)
- Dog's ability to walk (1 - Normal, walks with ease; 2 - Normal, but needs to stop often; 3 - Difficult, needs to be encouraged; 4 - Difficult, refuses to walk)
- The way the dog walks (1 - Walks normally; 2 - Walks with stiffness; 3 - Unsteady when walking; 4 - Walks with lameness)
- Dog's ability to climb steps (1 - Normal; 2 - Slow; 3 - Made regular stops; 4 - Needed to be encouraged; 5 - Needed help/holding; 6 - Did not climb steps)

They also had to score from 0 (not difficult) to 10 (very difficult) the dog's ability/difficulty to walk, and the dog's ability/difficulty to stand up after some rest.

Statistics: Due to the ordinal nature of the data, Friedman's tests were used with the results obtained on Days 0, 7, and 30, for each mobility criteria. In case of significance ($p < 0.05$), Wilcoxon's signed-rank tests were used, using Bonferroni's correction to compare the data obtained on Day 0 versus Day 7 or Day 30 ($p < 0.025$ for significance).

III. RESULTS

- Tolerance

All dogs in the group receiving five chews per day (5x group) ate all their chews every day, while five dogs in the group receiving one chew per day (1x group) did not consume it every day (no forced administration). These same dogs also generally had a low food consumption, suggesting a general lack of appetite. Four of these dogs (who did not eat their chew more than 15 times) were excluded from the analysis while the other one was kept for analysis (only one day with no consumption). All dogs remained healthy throughout the study, and no product-related clinical signs were observed. Mild signs (ocular discharge, mild induced cough, licking granuloma) could be observed in some dogs, in all groups, before and after product administration, and were therefore unrelated to product administration. Digestive signs (vomiting or bloody stools) were observed twice in the 1x group animals during the administration period, with no link to product administration. Indeed, these signs can be observed sporadically in dogs with no clinical significance and were not observed in the 5x group.

The mean dog body weight and body temperature remained stable during the administration period (Supp. Table 1).

Concerning blood analysis (hematology, biochemistry and electrolytes), all mean values were within physiological ranges except for the globulin and MCHC, which were slightly lower than the reference range in all groups and at both time points (Supp. Table 2-5). All values remained pretty stable throughout the study. Some individual values could be slightly out of range but with no link to product administration (observed before and after the chew administration and in all groups).

Urine samples could be taken at both time points (Day -14 and Day 28) for the four control dogs, three dogs in the 1x group (excluding those who did not eat the chew every day), and six dogs in the 5x group. In all individuals, urine pH was between 5.11 and 6.82, with no relevant change between Day -14 and 28 (Supp. Table 6).

Therefore, no product-related clinical signs were observed during the study. Movoflex® Advanced Soft Chews are well tolerated by dogs, even when given five chews per day for 28 days, instead of one as recommended.

- Perceived effectiveness

Among the 78 owners recruited on Day 0, 69 completed the study by filling in the questionnaires on the three days (Days 0, 7, and 30). The 69 owners included 21 owners of small dogs (<18 kg or <40 lbs), 25 owners of medium dogs (18-36 kg, or 40-80 lbs), and 23 owners of large dogs (>36 kg or >80 lbs).

Most owners reported giving the chew by hand (57% and 51% on Day 7 and Day 30, respectively) or on the bowl with food (51% and 41%, respectively). The remaining owners mostly gave it on the floor (13% and 9%, respectively).

During the trial, depending on the days, between 90% and 96% of dogs completely ate the chew. In 89% of cases (over a total of 2070 chew presentations), the dogs ate the chew all at once, and in 4% of cases, the dog ate part of it at first and the rest later (total consumption in 93% of cases). The chew was partially taken in 1% of cases, and in 7% of cases the dog would not take the chew spontaneously. Five dogs (7%: three small dogs and two medium dogs) partially took the chew or refused to take the chew spontaneously more than 50% of the time and were excluded from the analysis on mobility criteria (but were kept for the general appreciation).

By Day 30, most owners thought the tested chew was as easy (83%), or easier (13%) to give as/

than the previous Movoflex® Soft Chew and that palatability was similar (75%) or even better (16%).

Most owners also found the size, smell, color or shape of the new test chew were similar to the previous product they used.

Concerning the dog's mobility, 63% of the 64 owners analyzed considered there was an improvement in their dog's ability to move around by Day 30 (34% on Day 7).

- Interaction with family and friends: on Day 0, 55% of owners considered their dogs happy but reserved (score 2), while 42% considered them happy and playful (score 1). On Day 30, this repartition changed to 34% and 61%, respectively (Figure 1). The appreciation (score) given for this parameter was significantly different by Day 30 compared to Day 0 ($p < 0.01$), with 23% of owners giving a better (lower) score at the end of the study.

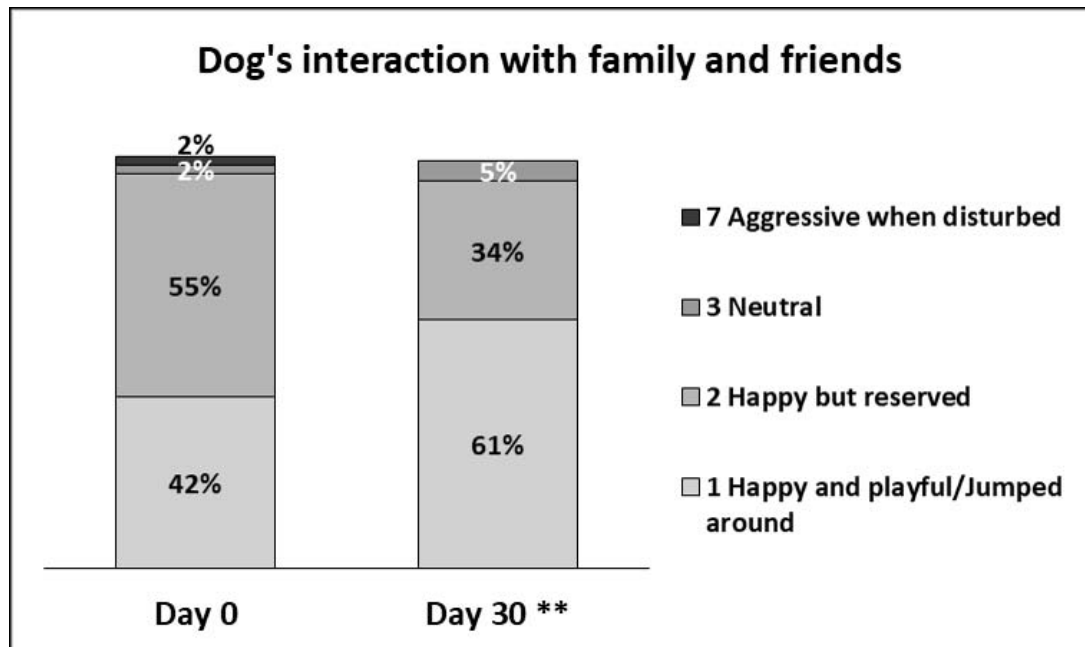


Figure 1: Dog's interaction with family and friends. The percentage of owners describing their dogs as: 1 - Happy and playful/Jumped around; 2 - Happy but reserved; 3 - Neutral; 4 - Sad, but reacted when called; 5 - Sad and didn't react much when called; 6 - Tended to isolate from others; or 7 - Became aggressive when disturbed, are depicted in this graph on day 0 (D0) and day 30 (D30). No owners reported their dogs to be 4, 5 or 6. A significant improvement was observed between Day 0 and Day 30 (**, $p < 0.01$, Wilcoxon Signed rank test, $n = 64$).

- Dogs walking ability
 - Dog's ability to walk: most owners found their dog's ability to walk normal and with ease on Day 0 (61%) while some said their dogs needed to stop often (38%). By Day 30, more owners said their dogs walked with ease (78%, Figure 2). The appreciation (score) given for this parameter significantly improved by Day 30 ($p < 0.01$), with 19% of owners giving a better appreciation by then.
 - The way of walking was described to involve stiffness by 55% of owners on Day 0, while only 39% said their dogs walked normally. By Day 30, more owners said their dogs walked normally (61%, Figure 2). The appreciation of this parameter significantly improved as of Day 7 ($p < 0.01$; 20% of owners gave a better appreciation) and was further improved by Day 30 (31% of owners gave a better appreciation).

- When asked to score their dog's ability/difficulty to walk from 0 (very easy) to 10 (very difficult), the median (Q1-Q3) score significantly decreased from 3 (1-4) to 2 (0-3) between Day 0 and Day 30 ($p < 0.01$, Figure 2).

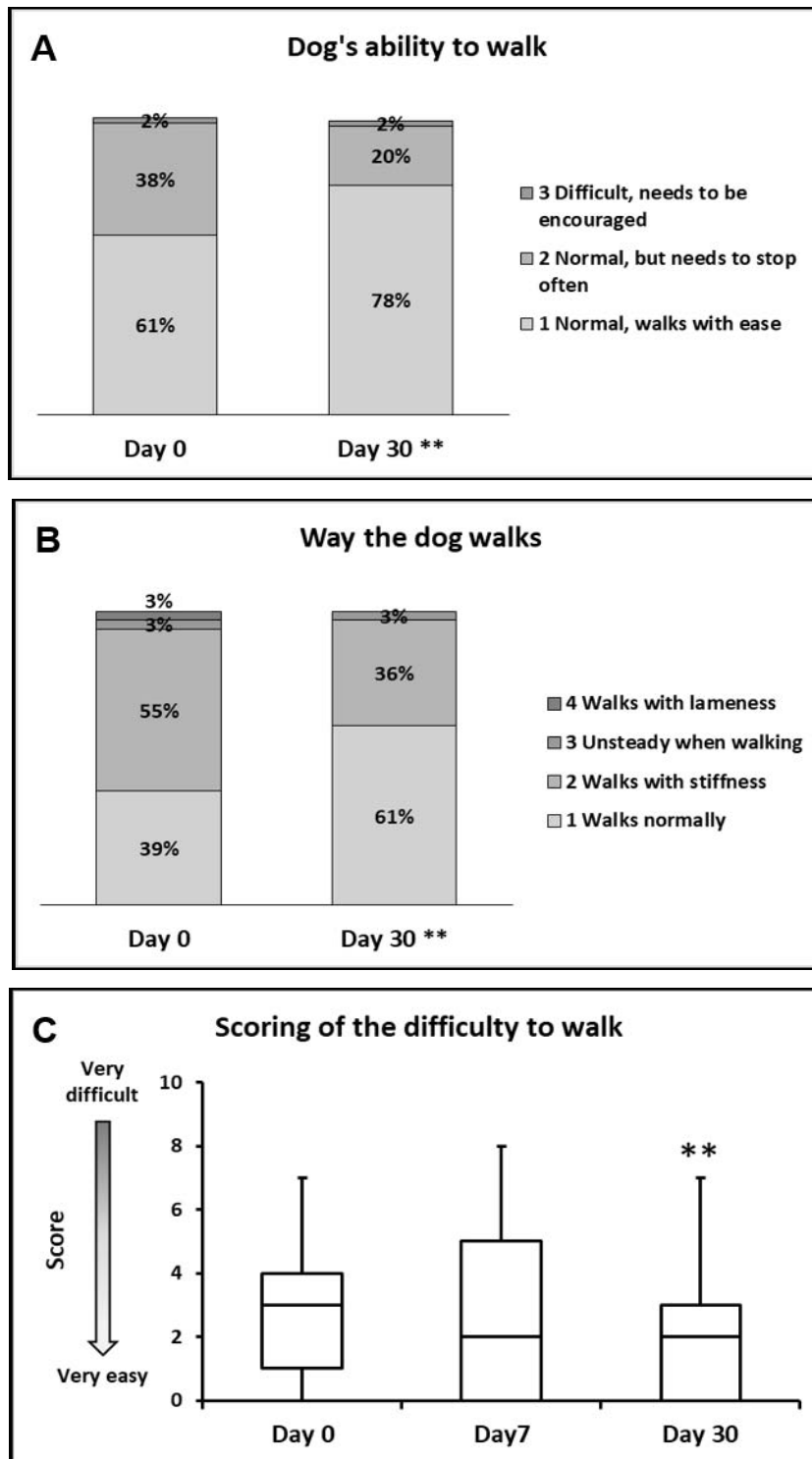


Figure 2: Dog's walking ability. A: Percentages of owners describing their dogs ability to walk as: 1 - Normal, walks with ease; 2 - Normal, but needs to stop often; 3 - Difficult, needs to be encouraged or 4 - Difficult, refuses to walk. B: Percentages of owners describing their dog's way of walking as: 1 - Walks normally; 2 - Walks with stiffness; 3 - Unsteady when walking or 4 - Walks with lameness. C: Box plot representing the median, Q1-Q3, min-max scores given by the owners for the dog's ability/difficulty to walk (on a scale from 0 - very easy to 10 - very difficult). For A,B,C: **, $p < 0.01$.

- Dog's ability to stand up
- The dog's ability to stand after resting/lying also improved significantly as of Day 7 ($p < 0.025$; 20% of owners gave a better appreciation) and was further improved by Day 30 (31% of owners gave a better appreciation. $p < 0.001$). Indeed, on Day 0, 66% of owners described their dog's ability to stand as slow, with some difficulty, while on Day 30, this number fell to 42%, and more owners felt their dogs could stand up with no difficulty (Figure 3)
- When asked to score their dog's ability/difficulty to stand from 0 (very easy) to 10 (very difficult), the median (Q1-Q3) scores given by owners significantly decreased from 4 (2-6) to 3 (2-5) and 2 (1-5) on Days 0, 7, and 30, respectively ($p < 0.01$, Figure 3).

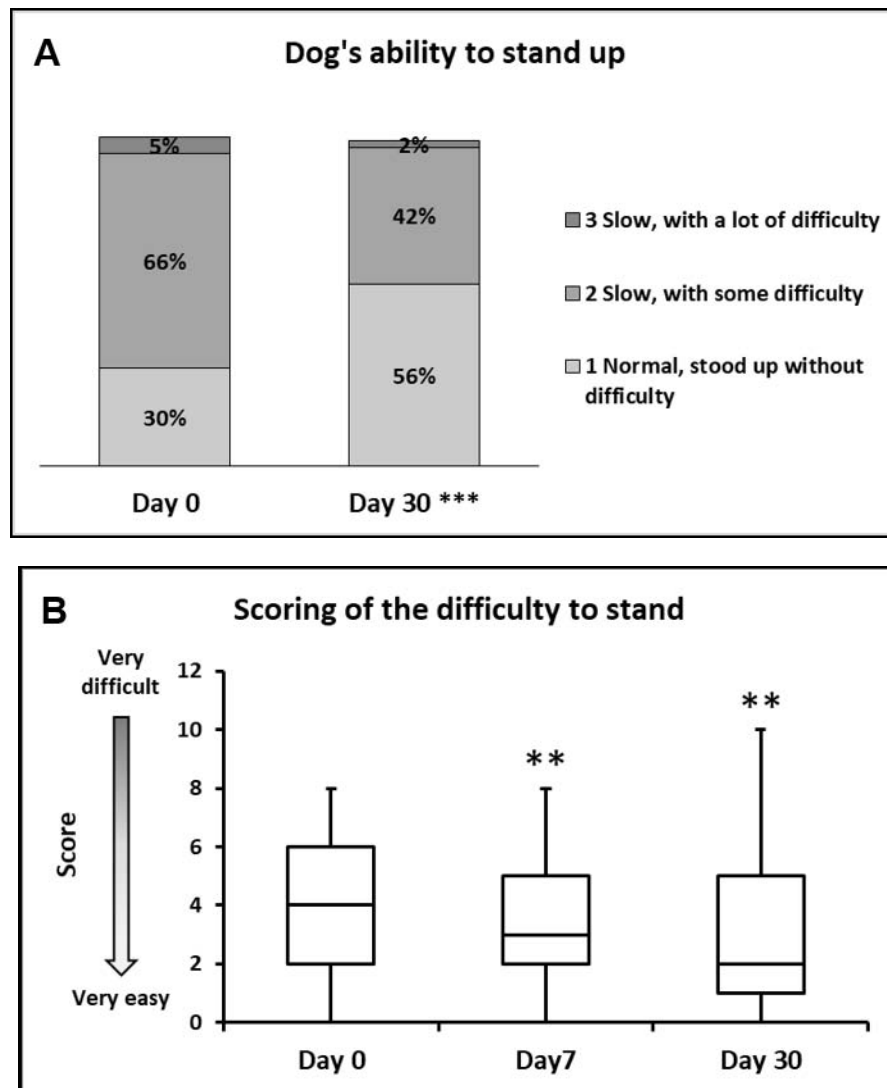


Figure 3: Dog's ability to stand up after resting/lying down for a period of time. A: Percentages of owners describing their dog's ability to stand as 1 - Normal, stood up without difficulty; 2 - Slow, with some difficulty; 3 - Slow, with a lot of difficulty; 4 - Needed help getting up. ***, $p < 0.001$ B: Box plot representing the median, Q1-Q3, min-max scores given by the owners for the dog's ability to stand up after resting (on a scale from 0 - very easy to 10 - very difficult). **, $p < 0.01$.

- Dog's ability to climb steps: no significant improvement was observed for this parameter, based on the owner's appreciation (score). However, most owners described their dogs as slow when climbing steps on Day 0 (43%), while on Day 30, most owners (51%) described their dog's ability to climb as normal (Figure 4).
- Finally, among the 69 owners who tested the new product, 87% said they would definitely (65%) or probably (22%) buy the new Movoflex® Advanced Soft Chews. This purchase intention rate increased to 90% (70% definitely and 20% probably) if it is recommended by a veterinarian.

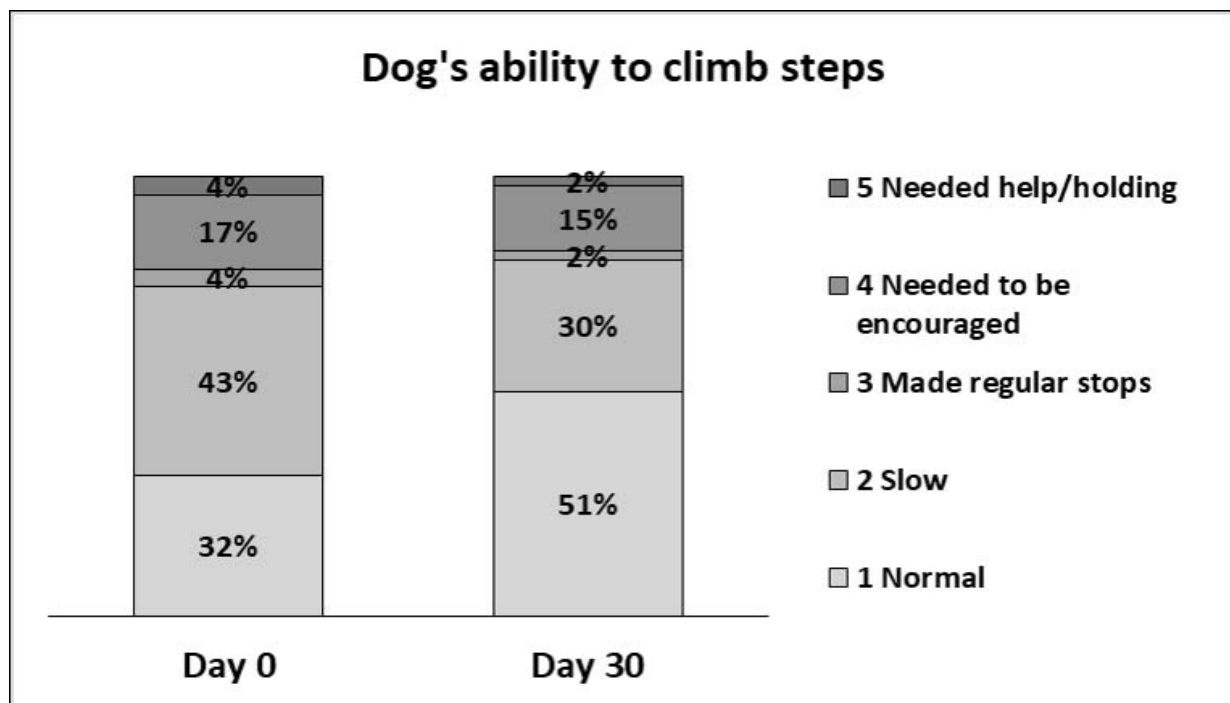


Figure 4: Dog's ability to climb steps. Percentages of owners describing their dog's ability to climb steps as 1 - Normal; 2 - Slow; 3 - Made regular stops; 4 - Needed to be encouraged; 5 - Needed help/holding; or 6 - Did not climb steps.

IV. DISCUSSION

The advanced formula of Movoflex® Soft Chews (Virbac, USA), with low molecular weight HA and krill oil, was tested for its tolerance, palatability, and perceived effectiveness in dogs.

The tolerance evaluation showed that the chew was well tolerated by healthy dogs, even with five chews per day for 28 days. The dogs remained in good health, and no clinical signs linked to the product were observed. There was also no change in blood parameters or urine pH.

When the new chew formula was given for one month to 69 dogs who were previously taking the current Movoflex® Soft Chews, it was completely consumed in 93% of cases, and 91% of owners found the acceptability of the new chew was similar or even better than the previous one. According to dog owners, no change in the shape, aspect, or odor was observed compared to the current Movoflex® Soft Chews.

Since the dogs who tried the new formula previously received Movoflex® Soft Chews regularly, their mobility issues were mild but could still be improved. After seven days of daily administration, some mobility criteria were already improved, like the way of walking, and the ability to stand after lying down for some time. At this stage (Day 7), 34% of owners considered their dog's mobility had improved. By Day 30, all mobility criteria but one (ability to climb steps) significantly improved, and 63% of owners noticed the improvement in their dog's mobility.

These data suggest that adding krill oil and hyaluronic acid (HA) of low molecular weight to a supplement already containing ESM, astaxanthin, vitamin D3, *Boswellia serrata* extract, and HA of high molecular weight (Movoflex® Soft Chew) can improve its efficiency.

This finding could be explained by the omega-3 PUFA found in krill oil that can improve the bioavailability of astaxanthin and HA [43,44], enhancing their effects, on top of the known beneficial effects of omega-3 PUFA on joint health [41,42,47].

Hyaluronic acid can be found in joints, particularly in the synovial fluid. It has been shown that the concentration and molecular weight (MW) of HA in animals with OA were decreasing and that administering HA could help in maintaining joint health [33]. The high MW HA would be of interest to preserve the viscoelasticity of the synovial fluid, and the lower MW HA could help modulate the inflammation process and participate in cartilage repair [33,38].

Owners' rating of improvement remains a subjective way to evaluate the efficacy of supplements, and the findings need to be further supported by proper clinical studies involving veterinarians. However, owners remain the best persons to appreciate the progress of their dog's mobility on a day-to-day basis. Such studies, with less limited inclusion criteria and constraints, can include more participants and are performed in circumstances of evaluation closer to what is happening in real life.

Despite these limitations, the data suggest that the complex blend of ingredients found in Movoflex® Advanced Soft Chews can help improve dogs' mobility. Adding low MW HA and krill oil to the original formula led to improved benefits.

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CONFLICT OF INTEREST

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- Abbreviations**
 AKBA: 3-O-Acetyl-11-keto-beta-boswellic acid
 BASO: Basophils
 DHA: Docosahexaenoic Acid
 EOS: Eosinophils
 EPA: Eicosapentaenoic Acid
 ESM: Eggshell Membrane
 HA: Hyaluronic Acid
 HCT: Hematocrit;
 HGB: Hemoglobin;
 KBA: 11-keto-b-boswellic acid
 LOX: Lipoxygenase
 LYM: Lymphocytes
 MCV: Mean corpuscular volume
 MCH: Mean corpuscular hemoglobin
 MCHC: Mean corpuscular hemoglobin concentration
 MONO: Monocytes
 MW: Molecular Weight
 NEU: Neutrophils
 OA: Osteoarthritis
 PL: Phospholipid
 PLT: Platelets
 PUFA: Polyunsaturated Fatty Acids
 RETIC: Reticulocytes
 ROS: Reactive Oxygen Species
 RBC: Red blood cells
 WBC: White blood cells

Supp. Table 1: Body weight and temperature (mean and SD are reported)

Group		Body weight (kg)		Body temperature	
		D0	D28	D0	D28
Control (n=8)	Mean	9,93	9,97	38,4	38,2
	SD	2,33	2,36	0,3	0,3
1x group (n=4)	Mean	11,52	11,53	38,1	38,3
	SD	3,31	3,26	0,2	0,2
5x group (n=8)	Mean	11,63	11,81	38,8	38,4
	SD	3,60	3,67	0,4	0,3

Supp. Table 2: Red blood cell count and parameters (mean and SD are reported).

Group		RBC (10x6.0/ μ l)		HGB (g/dl)		HCT (%)		MCV (fl)		MCH (pg)		MCHC (g/dl)		RETIC (/ μ l)		RETIC (%)	
		D-7	D28	D-7	D28	D-7	D28	D-7	D28	D-7	D28	D-7	D28	D-7	D28	D-7	D28
Control (n=8)	Mean	7.4	7.5	17.2	17.0	52.5	53.1	71.5	71.5	23.5	22.9	32.8	32.0	37325	35225	0.51	0.48
	SD	0.1	0.7	0.3	1.2	1.4	3.1	2.4	3.1	0.5	0.8	0.4	0.4	21255	2173	0.30	0.04
1x group (n=4)	Mean	7.5	7.4	17.5	17.2	53.7	53.2	72.1	71.8	23.5	23.2	32.6	32.3	70025	80125	0.93	1.06
	SD	0.6	0.6	1.5	1.3	3.9	3.6	2.3	2.2	1.3	1.1	0.8	0.6	26644	31916	0.33	0.34
5x group (n=8)	Mean	7.0	7.2	16.5	16.8	50.6	52.1	72.8	72.9	23.6	23.5	32.4	32.3	52400	50450	0.76	0.70
	SD	0.7	0.6	1.7	1.2	4.1	3.7	3.0	2.0	0.5	0.7	1.1	0.4	15737	18654	0.23	0.21
Ref. values		5.48.7		13.420.7		38.3 - 56.5		59 - 76		21.9 - 26.1		32.6 - 39.2					

RBC: Red blood cells; HGB: Hemoglobin; HCT: Hematocrit; MCV: Mean corpuscular volume; MCH: Mean corpuscular hemoglobin; MCHC: Mean corpuscular hemoglobin concentration; RETIC: Reticulocytes.

Supp. Table 3: White blood cell count (mean and SD are reported).

Group		WBC (1000/ μ l)		NEU (/ μ l)		NEU (%)		EOS (/ μ l)		EOS (%)		BASO (/ μ l)		BASO (%)		LYM (/ μ l)		LYM (%)		MONO (/ μ l)		MONO (%)		PLT (1000/ μ l)	
		D-7	D28	D-7	D28	D-7	D28	D-7	D28	D-7	D28	D-7	D28	D-7	D28	D-7	D28	D-7	D28	D-7	D28	D-7	D28	D-7	D28
Control (n=8)	Mean	9,7	8,9	7593	5551	79	64	290	420	3	4	0	0	0	0	1353	2416	14	27	400	496	4	5	252	198
	SD	2,5	2,3	1931	874	3	9	247	332	2	3	0	0	0	0	524	1083	3	6	171	297	2	2	79	34
1x group (n=4)	Mean	8,4	9,0	5407	5635	66	63	437	790	5	9	0	0	0	0	2032	2079	23	23	505	449	7	5	272	247
	SD	2,3	2,2	1001	1292	10	4	409	334	3	3	0	0	0	0	1435	779	9	5	116	57	2	2	50	31
5x group (n=8)	Mean	10,5	11,7	7184	7786	69	66	285	363	3	3	0	0	0	0	2504	3028	23	27	472	477	5	4	293	277
	SD	1,6	3,2	844	3011	8	7	223	100	2	1	0	0	0	0	1149	768	7	6	158	147	2	1	105	101
Ref. values		4,9-17,6		2940-12670				70-1490				0- 100				1060-4950				130-1150				143 -448	

WBC: White blood cells; NEU: Neutrophils; EOS: Eosinophils; BASO: Basophils; LYM: Lymphocytes; MONO: Monocytes; PLT: Platelets

Supp. Table 4: Blood chemistry parameters (mean and SD are reported).

Group		BUN (mmol/L)		CREA (mmol/L)		TP (g/L)		ALB (g/L)		GLOB (g/L)		GLU (mmol/L)		ALKP (IU/L)		ALT (IU/L)		AST (IU/L)	
		D-7	D28	D-7	D28	D-7	D28	D-7	D28	D-7	D28	D-7	D28	D-7	D28	D-7	D28	D-7	D28
Control (n=8)	Mean	5,1	5,1	54	53	55	55	33	32	22	23	5,2	5,1	33	33	29	31	22	27
	SD	0,6	0,6	11	8	3	3	1	1	2	2	0,9	0,8	2	6	13	17	3	6
1x group (n=4)	Mean	5,3	5,9	59	60	53	54	32	32	21	22	5,4	5,7	35	47	21	22	22	23
	SD	0,3	0,6	9	7	6	6	6	5	1	1	0,5	0,5	14	21	7	6	3	5
5x group (n=8)	Mean	5,2	5,5	57	57	55	55	32	31	23	23	5,7	5,5	39	39	33	34	24	27
	SD	0,8	0,8	13	10	3	3	2	2	3	2	0,3	0,4	16	13	12	14	5	6
Ref. values		3,2 -10,3		44- 103		54-76		28-43		24 -43		3,2 -7,0		14 - 147		25-122		14-59	

BUN: Blood ureanitrogen; CREA: Creatinine; TP: Total protein; ALB: Albumin; GLOB: Globulin; GLU: Glucose; ALKP: Alkaline Phosphatase; ALT: Alanine Aminotransferase; AST: Aspartate Aminotransferase

Supp. Table 5: Blood electrolytes (mean and SD are reported).

Group		Ca (mmol/L)		Cl (mmol/L)		K (mmol/L)		Mg (mmol/L)		Na (mmol/L)		Phos (mmol/L)	
		D-7	D28	D-7	D28	D-7	D28	D-7	D28	D-7	D28	D-7	D28
Control (n=8)	Mean	2,5	2,5	114	112	4,2	4,0	0,8	0,8	148	148	1,2	1,3
	SD	0,1	0,1	2	1	0,3	0,1	0,1	0,1	2	0	0,2	0,2
1x group (n=4)	Mean	2,5	2,5	114	114	4,1	4,1	0,8	0,8	148	149	1,3	1,2
	SD	0,1	0,1	1	2	0,2	0,2	0,1	0,1	1	1	0,2	0,1
5x group (n=8)	Mean	2,5	2,4	114	113	4,1	4,2	0,7	0,7	147	148	1,2	1,3
	SD	0,1	0,1	1	2	0,2	0,2	0,0	0,0	1	1	0,2	0,1
Ref. values		2,1 - 2,9		106 - 120		3,9 - 5,8		0,7 - 1,1		142 - 153		0,9 - 1,7	

Supp. Table 6: Urine pH (mean and SD)

Group		Urine pH	
		D-14	D28
Control (n=4)	Mean	5.91	5.62
	SD	0.73	0.28
1x group (n=3)	Mean	5.74	5.66
	SD	0.33	0.34
5x group (n=6)	Mean	5.82	5.55
	SD	0.26	0.41
Ref. values		5,3 – 7,0	



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Histological Origin and Anaplastic Behavior of Neoplasms Diagnosed in Canines in Guatemala (2015-2019)

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Abstract- The present study sought to statistically relate the histological origin and anaplastic behavior of neoplasms diagnosed in the Pathology Unit of the Faculty of Veterinary Medicine and Zootechnics of the University of San Carlos of Guatemala in the years 2015 to 2019, as well as associate race, age, sex and affected body system. A total of 1,162 canine samples were diagnosed, and a subsample of 289 cases was determined by systematic stratified random sampling of proportional allocation of each year. Regarding histological origin, 44% were epithelial neoplasms, 29% connective and derivatives, 12% other types, 4% endothelial, and 1% nervous. Concerning the anaplastic behavior, 51% of neoplasms were benign and 49% malignant. Likewise, it was determined that the neoplasms affected mostly females, pure breeds, canines of 7-10 years, and affected the integumentary system and soft tissues.

Keywords: *histological, anaplastic, neoplasms, canines.*

GJMR-G Classification: DDC Code: 611.018 LCC Code: QM551



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Histological Origin and Anaplastic Behavior of Neoplasms Diagnosed in Canines in Guatemala (2015-2019)

Andrea Daniela Lemus-Centes ^α, Jazzel Silvia Zea-Muñoz ^σ & Mariano Jarquín-Delgado ^ρ

Abstract- The present study sought to statistically relate the histological origin and anaplastic behavior of neoplasms diagnosed in the Pathology Unit of the Faculty of Veterinary Medicine and Zootechnics of the University of San Carlos of Guatemala in the years 2015 to 2019, as well as associate race, age, sex and affected body system. A total of 1,162 canine samples were diagnosed, and a subsample of 289 cases was determined by systematic stratified random sampling of proportional allocation of each year. Regarding histological origin, 44% were epithelial neoplasms, 29% connective and derivatives, 12% other types, 4% endothelial, and 1% nervous. Concerning the anaplastic behavior, 51% of neoplasms were benign and 49% malignant. Likewise, it was determined that the neoplasms affected mostly females, pure breeds, canines of 7-10 years, and affected the integumentary system and soft tissues. According to the value of the Cramér index ($V = 0.525$), it is concluded that there is a moderate level of association between the histological origin of a neoplasm and anaplastic behavior, with which it is inferred that the different histological origins have greater or lesser probability of developing certain tumor behavior.

Keywords: *histological, anaplastic, neoplasms, canines.*

1. INTRODUCTION

In recent decades, special interest in oncology has been observed in veterinary medicine. The presentation of neoplasms in wild animals is not common, due to the shorter life expectancy, which is why knowledge of oncology in non-domestic animals is scarce. Regarding domestic animals, the dog is of particular interest, since this species is more frequently affected by tumors, due to the fact that neoplasms are among the most common causes of death in dogs ^{5,9}.

Previous studies point out the importance of comparative medical research in this species that share the environment intimately with humans, since it often presents similar risk factors for the development of spontaneous neoplasms that have similar biological behavior and equivalent histological characteristics to the neoplasms seen in the human being. In many aspects, neoplasms in canines can be compared with the biological differences associated with neoplasms

in humans, in addition to their close relationship with them concerning housing, environment and diet ^{1,3,18,20}.

On the other hand, authors indicate that the presence of tumors in canines can be explained by improvements in their health care, better nutrition, immunizations, parasite control, use of antibiotics, etc., which make companion animals live longer, and therefore they are exposed to carcinogens for a longer time and exceed an age in which the cells reach the stage of tumor promotion and progression. For this reason, neoplasms are diagnosed with great efficiency and treated more frequently compared to previous years ^{9,18}.

There is abundant clinical information on diagnosis and therapies for the distinct types of neoplasms, and some studies on risk factors. In an ideal situation, all neoplasms occurring in each population should be microscopically confirmed, either by histopathology or cytology, to identify and enumerate all individuals in the population at risk. Veterinary oncology is a young and growing specialty, and it does not have the resources for studies and treatments that human cancer has. In many cases, the patient is euthanized as soon as there is a presumptive diagnosis of cancer, although it is known that many patients with an early and correct diagnosis can be treated successfully. Likewise, there is limited information on the frequency, prevalence, and incidence of the distinct types of cancer in the canine population in many countries of the world, and Guatemala is no exception ^{4,6,19}.

Different authors assert that the general benefit of these epidemiological studies is not only aimed at better understanding the epidemiology of canine tumors, but also at detecting and understanding future environmental hazards in the development of these diseases when comparing the situation of dogs with humans. Most epidemiological studies of neoplasms in canines in recent years have used canine populations derived from reference university veterinary hospitals ^{15,20,22}.

In Guatemala, the last study conducted on neoplasms diagnosed through biopsies and necropsies was from 2012 to 2014. Despite the interest in the subject, no new epidemiological research has updated it, so the behavior of the disease has likely changed over the years due to the concern of the owners, and

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because of detection methods and greater diagnostic efficiency in veterinary practice¹⁰. The present study seeks to contribute to the knowledge of canine neoplasms in the country, through the analysis of data from canine samples sent in the years 2015 to 2019 to the Pathology Unit at the Faculty of Veterinary Medicine and Zootechnics of the University of San Carlos of Guatemala, a reference center for veterinary histopathological diagnosis in the country; and its objective is to determine and statistically relate the histological origin and anaplastic behavior of diagnosed neoplasms, as well as to associate with the patient's race, age, sex and affected body system.

II. RESOURCES AND TECHNIQUES

A longitudinal study was conducted using canine biopsy and necropsy samples that turned out to be neoplasms sent to the veterinary histopathology and necropsy service provided by the Veterinary Pathology Unit at the Faculty of Veterinary Medicine and Zootechnics (FMVZ) of the University of San Carlos of Guatemala (USAC) in the period from 2015 to 2019. The diagnoses were made by experienced veterinary pathologists who performed a histopathological evaluation of samples stained with standard hematoxylin-eosin.

During that period, 1,162 patients were diagnosed with neoplasms. According to the total population, a probabilistic sample was calculated with the following equation when the size of the population is known:

$$n = \frac{N}{1 + \frac{d^2(N-1)}{z^2 s^2}} \quad n = \frac{1162}{1 + \frac{(0.05)^2(1161)}{(1.96)^2(0.25)}} \quad n = 289$$

n = size of the sample that you want to know

N = known population size

d = deviation or error that is willing to tolerate, expressed in proportion. In this case 5% or 0.05.

z = typical score corresponding to the confidence level; normally, z 95%, or 1.96, is used.

s^2 = population variance in the variable to be measured. In this case we have that $s^2 = pq = (0.50)(0.50) = 0.25$

The calculated size of the sample (289) was subjected to a stratified random sampling of proportional allocation. Subsequently, the subsample corresponding to each year was subjected to a systematic random sampling with which the specimens that made up each specific sample of each year were identified. The investigation focused on the neoplasm in each canine, and if multiple neoplasms were found in one animal, they were all addressed separately. However, biologically identical neoplasms located in the same animal at different anatomical sites were considered a single neoplasm.

For data collection purposes, a form or instrument was used for each canine, in which the data corresponding to the histological origin of the neoplasm (connective tissue and derivatives, endothelial, hematopoietic or lymphoreticular, epithelial, nervous, and others) and the anaplastic behavior were recorded (benign or malignant) according to the records of the pathology unit.

Additionally, general data on sex (female or male), race (pure or mongrel), age (<1 year, 1-6 years, 7-10 years, or >10 years), as well as the patient's affected body system was recorded (integumentary system and soft tissues, gastrointestinal, respiratory, urogenital, hemolymphatic, endocrine, nervous, cardiovascular, mammary gland, musculoskeletal or multicentric). Given the symmetric relationship between the variables to be observed and measured, and the nominal or frequency data they provide, the type of research is correlational, with which Cramér's V coefficient was used to indicate the association index between said study variables or categorical fields.

This work was approved by the Postgraduate Bioethics Committee of the University of San Carlos of Guatemala on October 19, 2021 (Ref. EPVirtual. 190.2021).

III. RESULTS

A total of 1,162 samples of neoplasms from canine biopsies and autopsies were diagnosed during the years 2015-2019 in the FMVZ-USAC Pathology Unit, of which a subsample of 289 cases of neoplasm was determined through a stratified random sampling systematic proportional allocation of each year.

Concerning the histological origin, 128 (44%) were epithelial-type neoplasms, 84 (29%) connective and derivatives, 35 (12%) other types of neoplasms, being mostly mixed type, 12 (4%) endothelial, and 2 (1%) of the nervous type (Table 1). About anaplastic behavior, 146 (51%) neoplasms were benign, and 43 (49%) were malignant (Table 2).

Females presented a higher frequency of neoplasms, with a distribution of 172 (60%) females, compared to 117 (40%) males. Regarding the histological origin, both in males and females, there was a higher frequency of epithelial-type neoplasms (44 and 45% respectively), followed by connective tissue and derivatives (27 and 30%), others (11 and 13%), hematopoietic or lymphoreticular (12 and 8%), endothelial (5 and 3%) and nervous tissue (1%). In relation to anaplastic behavior,

Table 1: Number of canines according to histological origin of neoplasms

Histological origin	N°	%
Connective and derivatives	84	29
Endothelial	12	4
Hematopoietic or lymphoreticular	28	10
Epithelial	128	44
Nervous	2	1
Others	35	12
Total	289	100

Table 2: Number of canines according to anaplastic behavior of neoplasms.

Benign		Malignant		Total	
N°	%	N°	%	N°	%
146	51	143	49	289	100

53 (36%) male canines and 93 (64%) females presented benign neoplasms, while 62 (43%) males and 81 (57%) females presented malignant neoplasms.

Purebred canines presented a higher frequency of neoplasms, being 225 (78%) cases, compared to 64 (22%) of mongrel canines. Regarding histological origin, 105 (47%) purebred dogs presented epithelial-type neoplasms, 63 (28%) connective and derivative types, 27 (12%) other types of neoplasms, and 19 (8%) hematopoietic or lymphoreticular type. In contrast, 23 (36%) mongrel canines presented epithelial-type neoplasms, 21 (33%) connective and derivative types, 9 (14%) hematopoietic and lymphoreticular types, and 8 (12%) other types of neoplasms. Concerning anaplastic behavior, 110 (75%) purebred canines and 36 (25%) mongrel canines presented benign neoplasms, while 115 (80%) purebred canines and 28 (20%) mongrel canines had malignant neoplasms. Commonly found purebreds were Poodle (12.9%), Schnauzer (11.1%), Boxer (8.4%), English Cocker Spaniel (7.6%), Labrador Retriever (7.1%), Siberian Husky (5.8%), Golden Retriever (5.3 %), Dachshund (3.6%), Basset Hound (3.1%) and Chihuahua (3.1%).

Concerning the age, canines between 7-10 years old presented the highest frequency, with 132 (46%) canines, followed by 78 (27%) dogs between 1-6 years, 74 (26%) canines over ten years, and 5 (1%) under one year old. Regarding the histological origin of neoplasms, canines under one year presented a higher frequency of neoplasms of epithelial origin (60%), followed by the endothelial type (20%) and other types (20%). Canines between 1-6 years old showed a higher frequency of neoplasms of connective origin and

derivatives (40%), followed by epithelial neoplasms (31%), hematopoietic or lymphoreticular (11%), and others (10%). Canines between 7-10 years old showed a more significant number of cases of epithelial origin (42%), followed by neoplasms of connective origin and derivatives (29%), hematopoietic or lymphoreticular (13%), and others (11%). Very similar were the canines over ten years, where there was a higher percentage of neoplasms of epithelial type (61%), connective and derivatives (20%), other types of histological origin (15%), and hematopoietic or lymphoreticular (3%). Concerning the anaplastic behavior, benign neoplasms were shown in 70 (48%) canines between 7-10 years old, 42 (29%) canines between 1-6 years old, 30 (20%) canines over ten years old, and in 4 (3%) canines less than one year old while malignant neoplasms were present in 62 (43%) canines between 7-10 years, followed by 44 (31%) canines older than ten years, 36 (25%) canines between 1-6 years, and 1 (1%) canines less than one year old.

Regarding the affected body systems, there was a greater number of cases in the integumentary system and soft tissues (33%), followed by the mammary gland (18%), gastrointestinal system (13%), urogenital (12%), musculoskeletal (9 %), multicentric (7%), respiratory (3%), hemolymphatic (3%), cardiovascular (1%), and nervous (1%). (Figure 1).

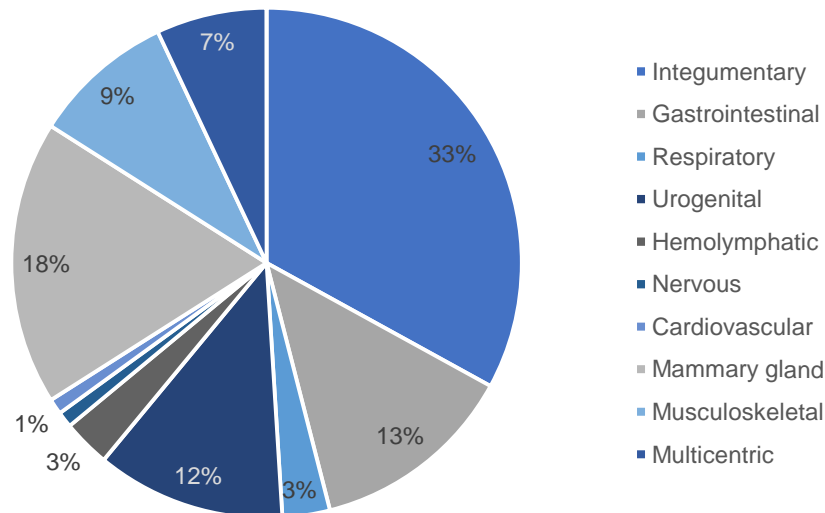


Figure 1: Distribution of canines according to the affected body system.

Concerning the anaplastic behavior according to the affected body systems, the benign neoplasms occurred in the integumentary system and soft tissues, mammary gland, urogenital, musculoskeletal, and gastrointestinal systems; while malignant neoplasms were shown in a higher percentage in the integumentary system and soft tissues, mammary gland, gastrointestinal system, multicentric and urogenital system.

Finally, Cramér's V coefficient was used to indicate the association index between the study variables, where the value was $V=0.525$, indicating a moderate level of association between the histological origin and the anaplastic behavior of the neoplasms in the observed canine sample (Table 3).

Table 3: Number of canines according to the histological origin and anaplastic behavior of neoplasms

Histological origin	Benign		Malignant		Total	
	N°	%	N°	%	N°	%
Connective and derivatives	68	47	16	11	84	29
Endothelial	10	7	2	1	12	4
Hematopoietic or lymphoreticular	2	1	26	18	28	10
Epithelial	41	28	87	61	128	44
Nervous	2	1	0	0	2	1
Others	23	16	12	9	35	12
Total	146	100	143	100	289	100

IV. DISCUSSION

The characterization of a population at risk is essential to understand the epidemiology of a disease. In recent decades there has been a growing interest in comparative oncology, due to the progressive understanding of environmental biological monitoring. In domestic animals such as dogs, there is a high manifestation of tumors, probably because they are

allowed to reach old age, as well as their close relationship with humans regarding the environment and food. The analysis of neoplasms in a population based on their histological origin and anaplastic behavior, in addition to their association with characteristics of the patient's data, can contribute to accurate diagnoses, and early and more appropriate treatments in daily practice^{9,17,19}.

The results of this study reveal interesting aspects of the current epidemiology of neoplasms in canines in Guatemala. Compared to studies previously conducted from 2012 to 2014, where 183 biopsies and 113 autopsies with neoplasm cases were analyzed, the number of cases increased considerably in the years examined in this study, with a total of 1,162 cases as a whole¹⁰.

According to the histological origin, the most common type of tumor was epithelial, followed by connective and derivatives, similar to what was found by Aco (2020) in Peru and Cevallos (2013) in Ecuador; and different from De la Cruz et al. (2017) in Mexico, Mayorga and Ruiz (2016) in Colombia, and Tordova (2016) in Bulgaria, who found a higher frequency of mesenchymal neoplasms (51.5-55%), followed by those of epithelial origin (41.3-45%). Epithelial tumors are usually the most common type in companion animals, primarily affecting the integument and soft tissues, followed by mammary neoplasms. Various authors with equivalent results regarding the affected body system consider that it is because the skin is the tissue most exposed to a wide variety of physical and chemical aggressions, and to various environmental factors that can trigger the development of neoplasms. Likewise, they are easier to recognize through clinical examination as it is the most visible organ, easily explored and accessible to obtain samples, unlike those that affect internal organs that require specific imaging studies, such as X-rays, ultrasound, computed tomography (CT) and magnetic resonance imaging (MRI)^{8,14,16,22}. In second place are mammary gland tumors, which are influenced by sex, being less prevalent in males, and their development in females is due to hormonal factors and increases the probability of suffering from it if they are sterilized, starting from 0.05% if spayed before the first heat, up to 26% if spayed after the second heat, and if spayed later in life, the risk of developing mammary tumors is the same as for an intact female^{8,21}. Other authors (Elgue et al, 2012; Tordova, 2006) differ with this, since they indicate that mammary tumors are the most frequent in canines, and skin neoplasms are in second place.

Regarding anaplastic behavior, there was a similar result for benign and malignant neoplasms, with benign neoplasms being slightly more frequent, mainly those of the connective type and derivatives, like Mayorga and Ruiz (2016) who demonstrated that benign neoplasms and of mesenchymal type were the most frequent. In most studies, benign neoplasms are usually twice as frequent as malignant ones. The close numerical proximity between benign and malignant neoplasms in this study could be due to the fact that benign neoplasms are generally slow-growing, do not represent a danger to the patient, are often missed or detected incidentally during clinical examination, and that owners are reluctant to allow surgical resection

and/or histopathological examination, causing the condition to migrate towards malignancy. A very different result was evidenced by Aco (2020) in Peru, and De la Cruz et al. (2017) in Mexico, where most malignant neoplasms were presented^{1,4,11,13}.

Concerning sex, females presented a higher frequency of neoplasms than males, like results seen by many authors, who consider that gender may be a factor that influences the development of some neoplasms due to the presence of hormones that can stimulate the abnormal cell division and turn into tumors. For this reason, it is considered that hormonal overstimulation of some organs increases the probability of the appearance of neoplasms, as well as working together with other carcinogens that could induce a mutation and neoplastic transformation of tissues^{4,8,14,23}.

Regarding the breed, it was shown that the pure breeds presented a higher frequency of neoplasms, of a malignant nature. Authors who agree with these results have determined that there is a racial predisposition toward the malignant growth of neoplasms, and the reason for this natural predisposition is unknown; it is believed that in specific individuals there is inactivation of tumor suppressor genes or activation of inherited oncogenes, or mutant versions of normal genes, which provide morphological characteristics of the breed together with its other genetic traits^{1,8,11,23}. When comparing dog breeds, neoplasms were found more frequently in poodles, schnauzers and boxers, similar to the results found by Juarez (2017), with the difference that they showed a major presence of neoplasms in canines of the schnauzer breed and in second place the poodles. The results could be influenced by the fact that they are the most popular breeds in Guatemala. However, it is essential to consider that owners tend to exaggerate or idealize the breed of their pets, so the quality of the breed data that depends primarily on owner reports should be questioned¹⁹. Different results were found by Elgue et al (2012) in Uruguay, and De la Cruz et al. (2017) in Mexico, which determined that mongrel canines had a higher presentation of neoplasms than purebreds.

Concerning the age of presentation, it was more frequent in canines between 7-10 years, like Fajardo et al. (2013), Torres et al. (2015), and Aco (2020). This is as a consequence that the tissues have been exposed for extended periods to risk factors, as well as decreased cell repair as the animals age and a reduced immune response that leads to greater vulnerability to activity of carcinogens. The fact that malignant tumors mainly occurred in the older age groups (7-10 years and >10 years), suggests that age could be a significant risk factor, since exposure to endogenous and exogenous risk factors, together with a weakened immune system, can trigger the development of malignant tumors^{8,23}.

According to the Cramér index value, it is concluded that there is a moderate level of association

between the histological origin of a neoplasm and anaplastic behavior. De la Cruz et al. (2017) in Mexico carried out a similar study where they evaluated the existence of a significant statistical association between the five most frequent tumors in the country, and the sex, race, and age of the patients in the development of neoplasms, to which they determined that there was no difference significant after evaluating the behavior of the neoplasm with race and sex, but with the age of the patients. Furthermore, Elgue et al. (2012) concluded through their study in Uruguay that sex, age, and race were factors associated with cancer. When analyzing this information, it is inferred that not only age is an important factor in the development of neoplasms and that senior age increases the risk of developing malignant neoplasms, as well as race and sex, but also the different histological origins of neoplasms are more or less likely to develop malignant tumor behavior, for example, according to the present study, neoplasms of epithelial and hematopoietic/ lymphoreticular origin are more likely to be malignant, while those of connective origin and derivatives are more likely to be benign^{4,7}.

V. CONCLUSION

Studies of neoplasms from the records of a reference veterinary diagnostic center allow a deep understanding of the epidemiology of the disease and point out the differences that may occur from one country to another, which can support a better diagnosis and treatment in daily practice. This study focused on determining and statistically relating the histological origin and anaplastic behavior of neoplasms diagnosed between 2015 and 2019, as well as with the sex, race, age, and affected body system of the patient, revealing interesting aspects about the current epidemiology of neoplasms in canines in Guatemala. With the results obtained, it was found that epithelial-type neoplasms, and of a benign nature, were more frequently presented. In addition, an important level of association between the histological origin and the anaplastic behavior of these neoplasms was evidenced, which represents an important epidemiological factor. Likewise, it was determined that neoplasms in the country mainly affect females in terms of gender, purebred canines, the age group between 7 and 10 years old, and that the disease affects to a greater extent the integumentary system and soft tissues. From all this, it is concluded that it is essential to expand this type of research to understand the behavior of the disease in domestic animals in the country, and to make a continuous effort to evaluate the risk factors and compare them with the risk factors in humans.

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SUPPLEMENTARY MATERIAL

Table 1: Sample size of each year

Year	PT	%	N
2015	106	9	26
2016	215	19	55
2017	269	23	67
2018	290	25	72
2019	282	24	69
Total	1,162	100	289

Table 2: Number of canines according to sex and histological origin of neoplasms

Histological origin	Male		Female		Total	
	N°	%	N°	%	N°	%
Connective and derivatives	32	27	52	30	84	29
Endothelial	6	5	6	3	12	4
Hematopoietic or lymphoreticular	14	12	14	8	28	10
Epithelial	51	44	77	45	128	44
Nervous	1	1	1	1	2	1
Others	13	11	22	13	35	12
Total	117	100	172	100	289	100

Table 3: Number of canines according to sex and anaplastic behavior of neoplasms

Sex	Benign		Malignant		Total	
	N°	%	N°	%	N°	%
Male	53	36	62	43	115	40
Female	93	64	81	57	174	60
Total	146	100	143	100	289	100

Table 4: Number of canines according to breed and histological origin of neoplasms

Histological origin	Purebred		Mongrel		Total	
	N°	%	N°	%	N°	%
Connective and derivatives	63	28	21	33	84	29
Endothelial	9	4	3	5	12	4
Hematopoyetic or lymphoreticular	19	8	9	14	28	10
Epithelial	105	47	23	36	128	44
Nervous	2	1	0	0	2	1
Others	27	12	8	12	35	12
Total	225	100	64	100	289	100

Table 5: Number of canines according to breed and anaplastic behavior of neoplasms

Breed	Benign		Malignant		Total	
	N°	%	N°	%	N°	%
Purebred	110	75	115	80	225	78
Mongrel	36	25	28	20	64	22
Total	146	100	143	100	289	100

Table 6: Number of canines according to age and histological origin of neoplasms

Histological origin	<1 year		1-6 years		7-10 years		>10 years		Total	
	N°	%	N°	%	N°	%	N°	%	N°	%
Connective and derivatives	0	0	31	40	38	29	15	20	84	29
Endothelial	1	20	6	8	5	4	0	0	12	4
Hematopoyetic or lymphoreticular	0	0	9	11	17	13	2	3	28	10
Epithelial	3	60	24	31	56	42	45	61	128	44
Nervous	0	0	0	0	1	1	1	1	2	1
Others	1	20	8	10	15	11	11	15	35	12
Total	5	100	78	100	132	100	74	100	289	100

Table 7: Number of canines according to age and anaplastic behavior of neoplasms

Age	Benign		Malignant		Total	
	N°	%	N°	%	N°	%
<1 year	4	3	1	1	5	2
1-6 years	42	29	36	25	78	27
7-10 years	70	48	62	43	132	46
> 10 years	30	20	44	31	74	25
Total	146	100	143	100	289	100

Table 8: Number of canines according to the affected body system and anaplastic behavior of neoplasms

Affected body system	Benign		Malignant		Total	
	N°	%	N°	%	N°	%
Integumentary and soft tissues	56	38	40	28	96	33
Gastrointestinal	16	11	21	15	37	13
Respiratory	4	3	4	3	8	3
Urogenital	23	16	12	8	35	12
Hemolymphatic	0	0	8	5	8	3
Endocrine	0	0	0	0	0	0
Nervous	2	1	0	0	2	1
Cardiovascular	2	1	1	1	3	1
Mammary gland	26	18	27	19	53	18
Musculoskeletal	17	12	9	6	26	9
Multicentric	0	0	21	15	21	7
Total	146	100	143	100	289	100

Table 9: Contingency table for statistical analysis of Cramér's V association index

Histological origin	Anaplastic behavior		Total
	Benign	Malignant	
Connective and derivatives	68 (42.43)	16 (41.56)	84
Endothelial	10 (6.06)	2 (5.93)	12
Hematopoietic or lymphoreticular	2 (14.14)	26 (13.85)	28
Epithelial	41 (64.66)	87 (63.33)	128
Nervous	2 (1.01)	0 (0.98)	2
Others	23 (17.68)	12 (17.31)	35
Total	146	143	289

Table 10: Distribution of cases of neoplasms according to purebred canines

Breed	No.	%
Poodle	29	12.9
Schnauzer	25	11.1
Boxer	19	8.4
English Cocker Spaniel	17	7.6
Labrador Retriever	16	7.1
Husky	13	5.8
Golden Retriever	12	5.3
Dachshund	8	3.6
Chihuahua	7	3.1
Basset Hound	7	3.1
Pitbull	6	2.7
Shih Tzu	6	2.7
German shepherd	6	2.7
Beagle	5	2.2
Guatemalan dogo	4	1.8
Rottweiler	3	1.3
French bulldog	3	1.3
Sharpei	3	1.3
Bulldog	2	0.9
Fila brasileiro	2	0.9
Doberman	2	0.9
American Bully	2	0.9
Chowchow	2	0.9
Dalmatian	2	0.9
Great dane	2	0.9
Others	22	9.7
Total	225	100

HISTOLOGICAL ORIGIN AND ANAPLASTIC BEHAVIOR OF NEOPLASMS DIAGNOSED IN CANINES IN GUATEMALA (2015-2019)

I. General Data

1. Patient's ID: _____

2. Year
2015 ☐ 2016 ☐ 2017 ☐ 2018 ☐ 2019 ☐

3. Breed
Mongrel ☐ Purebred ☐

4. Age
<1 year ☐ 1-6 years ☐ 7-10 years ☐ >10 years ☐

5. Sex
Male ☐ Female ☐

6. Affected body system

- Integumentary and soft tissues
- Gastrointestinal
- Respiratory
- Urogenital
- Hemolymphatic
- Endocrine
- Nervous
- Cardiovascular
- Mammary gland
- Musculoskeletal
- Multicentric

I. Histological origin

- Connective and derivatives
- Endothelial
- Hematopoietic or lymphoreticular
- Epithelial
- Nervous
- Others

II. Anaplastic behavior

Benign ☐ Malignant ☐

Figure 1: Instrument used in data collection

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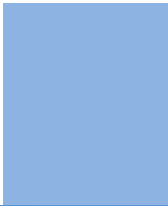
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Acknowledgments

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The following is the official style and template developed for publication of a research paper. Authors are not required to follow this style during the submission of the paper. It is just for reference purposes.



Manuscript Style Instruction (Optional)

- Microsoft Word Document Setting Instructions.
- Font type of all text should be Swis721 Lt BT.
- Page size: 8.27" x 11", left margin: 0.65, right margin: 0.65, bottom margin: 0.75.
- Paper title should be in one column of font size 24.
- Author name in font size of 11 in one column.
- Abstract: font size 9 with the word "Abstract" in bold italics.
- Main text: font size 10 with two justified columns.
- Two columns with equal column width of 3.38 and spacing of 0.2.
- First character must be three lines drop-capped.
- The paragraph before spacing of 1 pt and after of 0 pt.
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- Large images must be in one column.
- The names of first main headings (Heading 1) must be in Roman font, capital letters, and font size of 10.
- The names of second main headings (Heading 2) must not include numbers and must be in italics with a font size of 10.

Structure and Format of Manuscript

The recommended size of an original research paper is under 15,000 words and review papers under 7,000 words. Research articles should be less than 10,000 words. Research papers are usually longer than review papers. Review papers are reports of significant research (typically less than 7,000 words, including tables, figures, and references)

A research paper must include:

- a) A title which should be relevant to the theme of the paper.
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- c) Up to 10 keywords that precisely identify the paper's subject, purpose, and focus.
- d) An introduction, giving fundamental background objectives.
- e) Resources and techniques with sufficient complete experimental details (wherever possible by reference) to permit repetition, sources of information must be given, and numerical methods must be specified by reference.
- f) Results which should be presented concisely by well-designed tables and figures.
- g) Suitable statistical data should also be given.
- h) All data must have been gathered with attention to numerical detail in the planning stage.

Design has been recognized to be essential to experiments for a considerable time, and the editor has decided that any paper that appears not to have adequate numerical treatments of the data will be returned unrefereed.

- i) Discussion should cover implications and consequences and not just recapitulate the results; conclusions should also be summarized.
- j) There should be brief acknowledgments.
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A major lynchpin of research work for the writing of research papers is the keyword search, which one will employ to find both library and internet resources. Up to eleven keywords or very brief phrases have to be given to help data retrieval, mining, and indexing.

One must be persistent and creative in using keywords. An effective keyword search requires a strategy: planning of a list of possible keywords and phrases to try.

Choice of the main keywords is the first tool of writing a research paper. Research paper writing is an art. Keyword search should be as strategic as possible.

One should start brainstorming lists of potential keywords before even beginning searching. Think about the most important concepts related to research work. Ask, "What words would a source have to include to be truly valuable in a research paper?" Then consider synonyms for the important words.

It may take the discovery of only one important paper to steer in the right keyword direction because, in most databases, the keywords under which a research paper is abstracted are listed with the paper.

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Numerical methods used should be transparent and, where appropriate, supported by references.

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Authors must list all the abbreviations used in the paper at the end of the paper or in a separate table before using them.

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Authors are advised to submit any mathematical equation using either MathJax, KaTeX, or LaTeX, or in a very high-quality image.

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Tables: Tables should be cautiously designed, uncrowned, and include only essential data. Each must have an Arabic number, e.g., Table 4, a self-explanatory caption, and be on a separate sheet. Authors must submit tables in an editable format and not as images. References to these tables (if any) must be mentioned accurately.



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3. Ask your guides: If you are having any difficulty with your research, then do not hesitate to share your difficulty with your guide (if you have one). They will surely help you out and resolve your doubts. If you can't clarify what exactly you require for your work, then ask your supervisor to help you with an alternative. He or she might also provide you with a list of essential readings.

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12. Know what you know: Always try to know what you know by making objectives, otherwise you will be confused and unable to achieve your target.

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Verbs have to be in agreement with their subjects. In a research paper, do not start sentences with conjunctions or finish them with prepositions. When writing formally, it is advisable to never split an infinitive because someone will (wrongly) complain. Avoid clichés like a disease. Always shun irritating alliteration. Use language which is simple and straightforward. Put together a neat summary.

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23. Upon conclusion: Once you have concluded your research, the next most important step is to present your findings. Presentation is extremely important as it is the definite medium through which your research is going to be in print for the rest of the crowd. Care should be taken to categorize your thoughts well and present them in a logical and neat manner. A good quality research paper format is essential because it serves to highlight your research paper and bring to light all necessary aspects of your research.

INFORMAL GUIDELINES OF RESEARCH PAPER WRITING

Key points to remember:

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- Write your paper in the form which is presented in the guidelines using the template.
- Please note the criteria peer reviewers will use for grading the final paper.

Final points:

One purpose of organizing a research paper is to let people interpret your efforts selectively. The journal requires the following sections, submitted in the order listed, with each section starting on a new page:

The introduction: This will be compiled from reference matter and reflect the design processes or outline of basis that directed you to make a study. As you carry out the process of study, the method and process section will be constructed like that. The results segment will show related statistics in nearly sequential order and direct reviewers to similar intellectual paths throughout the data that you gathered to carry out your study.

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- Submitting a manuscript with pages out of sequence.
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- Keep paying attention to the topic of the paper.
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- Present your points in sound order.
- Use present tense to report well-accepted matters.
- Use past tense to describe specific results.
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Reason for writing the article—theory, overall issue, purpose.

- Fundamental goal.
- To-the-point depiction of the research.
- Consequences, including definite statistics—if the consequences are quantitative in nature, account for this; results of any numerical analysis should be reported. Significant conclusions or questions that emerge from the research.

Approach:

- Single section and succinct.
- An outline of the job done is always written in past tense.
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- Exact spelling, clarity of sentences and phrases, and appropriate reporting of quantities (proper units, important statistics) are just as significant in an abstract as they are anywhere else.

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The introduction should "introduce" the manuscript. The reviewer should be presented with sufficient background information to be capable of comprehending and calculating the purpose of your study without having to refer to other works. The basis for the study should be offered. Give the most important references, but avoid making a comprehensive appraisal of the topic. Describe the problem visibly. If the problem is not acknowledged in a logical, reasonable way, the reviewer will give no attention to your results. Speak in common terms about techniques used to explain the problem, if needed, but do not present any particulars about the protocols here.



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- Explain the value (significance) of the study.
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- Present a justification. State your particular theory(-ies) or aim(s), and describe the logic that led you to choose them.
- Briefly explain the study's tentative purpose and how it meets the declared objectives.

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Use past tense except for when referring to recognized facts. After all, the manuscript will be submitted after the entire job is done. Sort out your thoughts; manufacture one key point for every section. If you make the four points listed above, you will need at least four paragraphs. Present surrounding information only when it is necessary to support a situation. The reviewer does not desire to read everything you know about a topic. Shape the theory specifically—do not take a broad view.

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Materials may be reported in part of a section or else they may be recognized along with your measures.

Methods:

- Report the method and not the particulars of each process that engaged the same methodology.
- Describe the method entirely.
- To be succinct, present methods under headings dedicated to specific dealings or groups of measures.
- Simplify—detail how procedures were completed, not how they were performed on a particular day.
- If well-known procedures were used, account for the procedure by name, possibly with a reference, and that's all.

Approach:

It is embarrassing to use vigorous voice when documenting methods without using first person, which would focus the reviewer's interest on the researcher rather than the job. As a result, when writing up the methods, most authors use third person passive voice.

Use standard style in this and every other part of the paper—avoid familiar lists, and use full sentences.

What to keep away from:

- Resources and methods are not a set of information.
- Skip all descriptive information and surroundings—save it for the argument.
- Leave out information that is immaterial to a third party.



Results:

The principle of a results segment is to present and demonstrate your conclusion. Create this part as entirely objective details of the outcome, and save all understanding for the discussion.

The page length of this segment is set by the sum and types of data to be reported. Use statistics and tables, if suitable, to present consequences most efficiently.

You must clearly differentiate material which would usually be incorporated in a study editorial from any unprocessed data or additional appendix matter that would not be available. In fact, such matters should not be submitted at all except if requested by the instructor.

Content:

- Sum up your conclusions in text and demonstrate them, if suitable, with figures and tables.
- In the manuscript, explain each of your consequences, and point the reader to remarks that are most appropriate.
- Present a background, such as by describing the question that was addressed by creation of an exacting study.
- Explain results of control experiments and give remarks that are not accessible in a prescribed figure or table, if appropriate.
- Examine your data, then prepare the analyzed (transformed) data in the form of a figure (graph), table, or manuscript.

What to stay away from:

- Do not discuss or infer your outcome, report surrounding information, or try to explain anything.
- Do not include raw data or intermediate calculations in a research manuscript.
- Do not present similar data more than once.
- A manuscript should complement any figures or tables, not duplicate information.
- Never confuse figures with tables—there is a difference.

Approach:

As always, use past tense when you submit your results, and put the whole thing in a reasonable order.

Put figures and tables, appropriately numbered, in order at the end of the report.

If you desire, you may place your figures and tables properly within the text of your results section.

Figures and tables:

If you put figures and tables at the end of some details, make certain that they are visibly distinguished from any attached appendix materials, such as raw facts. Whatever the position, each table must be titled, numbered one after the other, and include a heading. All figures and tables must be divided from the text.

Discussion:

The discussion is expected to be the trickiest segment to write. A lot of papers submitted to the journal are discarded based on problems with the discussion. There is no rule for how long an argument should be.

Position your understanding of the outcome visibly to lead the reviewer through your conclusions, and then finish the paper with a summing up of the implications of the study. The purpose here is to offer an understanding of your results and support all of your conclusions, using facts from your research and generally accepted information, if suitable. The implication of results should be fully described.

Infer your data in the conversation in suitable depth. This means that when you clarify an observable fact, you must explain mechanisms that may account for the observation. If your results vary from your prospect, make clear why that may have happened. If your results agree, then explain the theory that the proof supported. It is never suitable to just state that the data approved the prospect, and let it drop at that. Make a decision as to whether each premise is supported or discarded or if you cannot make a conclusion with assurance. Do not just dismiss a study or part of a study as "uncertain."



Research papers are not acknowledged if the work is imperfect. Draw what conclusions you can based upon the results that you have, and take care of the study as a finished work.

- You may propose future guidelines, such as how an experiment might be personalized to accomplish a new idea.
- Give details of all of your remarks as much as possible, focusing on mechanisms.
- Make a decision as to whether the tentative design sufficiently addressed the theory and whether or not it was correctly restricted. Try to present substitute explanations if they are sensible alternatives.
- One piece of research will not counter an overall question, so maintain the large picture in mind. Where do you go next? The best studies unlock new avenues of study. What questions remain?
- Recommendations for detailed papers will offer supplementary suggestions.

Approach:

When you refer to information, differentiate data generated by your own studies from other available information. Present work done by specific persons (including you) in past tense.

Describe generally acknowledged facts and main beliefs in present tense.

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<i>Introduction</i>	Containing all background details with clear goal and appropriate details, flow specification, no grammar and spelling mistake, well organized sentence and paragraph, reference cited	Unclear and confusing data, appropriate format, grammar and spelling errors with unorganized matter	Out of place depth and content, hazy format
<i>Methods and Procedures</i>	Clear and to the point with well arranged paragraph, precision and accuracy of facts and figures, well organized subheads	Difficult to comprehend with embarrassed text, too much explanation but completed	Incorrect and unorganized structure with hazy meaning
<i>Result</i>	Well organized, Clear and specific, Correct units with precision, correct data, well structuring of paragraph, no grammar and spelling mistake	Complete and embarrassed text, difficult to comprehend	Irregular format with wrong facts and figures
<i>Discussion</i>	Well organized, meaningful specification, sound conclusion, logical and concise explanation, highly structured paragraph reference cited	Wordy, unclear conclusion, spurious	Conclusion is not cited, unorganized, difficult to comprehend
<i>References</i>	Complete and correct format, well organized	Beside the point, Incomplete	Wrong format and structuring



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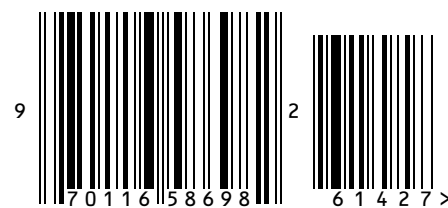
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