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CONTENTS

HAEMATOLOGICAL AND MORPHOLOGICAL EFFECTS OF A B12 AND FOLATE DEFICIENT DIET ON CD1 ALBINO MICE

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Abstract

Dietary B12 and folate deficiency induced visible clinical signs in CD1 albino mice after just 3 weeks of administration. After 5 weeks, the mice were euthanized. Necropsy showed a large array of gross morphological changes in the liver and kidneys of all the animals included in the study. There were also major changes in red blood cell morphology. Complete blood count parameters were normal and there were no morphological changes observed in spleen and bone marrow cytology. Megaloblastic anaemia was not diagnosed.

Keywords: *deficiency, vitamin B12, folate, mice, erythrocyte morphology*

Introduction

Vitamins B12 and B9 are two important factors in nucleic synthesis in the body. Deficiencies in either vitamin can cause megaloblastic anaemia, developing defects in the fetus, and cardiovascular disease due to a compensatory increase of homocysteine [1].

The main causes for cobalamin and folate deficiency are low dietary intake, malabsorption, lactation, and genetic disorders [2]. Mice can be used an animal model for the study of dietary induced megaloblastic anaemia, with findings of ineffective haematopoiesis and pancytopenia. The haematological lesions caused by dietary deficiencies can be reversible, and hyperplasia of the hematopoietic tissue is observed in the spleen rather than the bone marrow [3].

Cobalamin deficiency causes a wide range of disorders such as neurological, gastrointestinal, and haematological. Patients will show changes in bone marrow and peripheral blood cytology and megaloblastic features in epithelial cells lining the gastrointestinal and genital tract. Usually, the first manifestation of B12 deficiency and megaloblastic anaemia is an increase of mean corpuscular volume. Megaloblastic changes in bone marrow cytology are most visible in basophilic and polychromatophilic erythroblast progenitors. Peripheral blood morphology is usually not dramatically modified but aspects such as neutrophil hypersegmentation, poikilocytosis, and anisocytosis may

be observed. Clinical manifestations of cobalamin and folate deficiencies are indistinguishable [4].

Material and methods

The experimental design used six female CD1 albino mice, which were given a cobalamin and folate deficient diet for a period of 5 weeks. Animals were given a week for acclimatization before starting the experiment. The diet was purchased from the National Institute of Medical-Military Research and Development 'Cantacuzino'. The aim of this study was to verify if the administration of a vitamin-deficient diet is sufficient in causing megaloblastic anaemia without the use of absorption inhibiting medication.

Animal health and wellbeing was closely monitored during the experimental period. After 5 weeks, the mice were anesthetized, and blood was collected through cardiocentesis as a terminal procedure.

After euthanasia and blood collection, a full necropsical examination was realized and samples were collected. Haematological examination included complete blood counts, blood smears, bone marrow cytological examination, and spleen cytological examination.

Results and discussion

Some behavioural changes were noted during the administration of the cobalamin and folate deficient diet after only 3 weeks. All the mice tended to be less active and there was a decrease in food consumption. Food and water intake continued to decrease until the end of the experiment. After 5 weeks, one of the mice was found dead and the rest were euthanized for examination.

Necropsy results

Necropsical examination showed the same array of lesions in all the mice. The main findings consisted of a distended stomach, catarrhal gastritis with thickened mucous membrane, edema, and hyperaemia. The stomach was filled with a large amount of gas and liquid content with a light-yellow colouring (Figure 1A and 1B).

The liver was also modified, with a friable consistency and discoloration. There were no visible haemorrhage spots or masses (Figure 1C). The kidneys had a pale brown colour, and many indentations on their surface that looked like fibrotic cortical scarring (Figure 1D).

Complete blood count

Data obtained from the complete blood count was compared to a control group (n = 5) of mice of the same sex, age, and line as the experimental group

and a statistical interpretation war realized using a two-tailed T-test. Values were also compared with an interval range for female CD-1 albino mice. The full set of parameters can be found in table 1.

Complete blood count values showed a slight increase in red blood cell numbers, with a value of 10.96 ± 1.35 M/µL (P = 0.055), slightly over the upper limit of the reference interval. There was also a low mean corpuscular volume (MCV), with a value of 44.92 ± 5.07 fL (P = 0.009), and low mean corpuscular haemoglobin (MCH), with a mean value of 13.82 ± 0.66 pg (P = 0.004). Both MCV and MCH were well below the reference interval values.

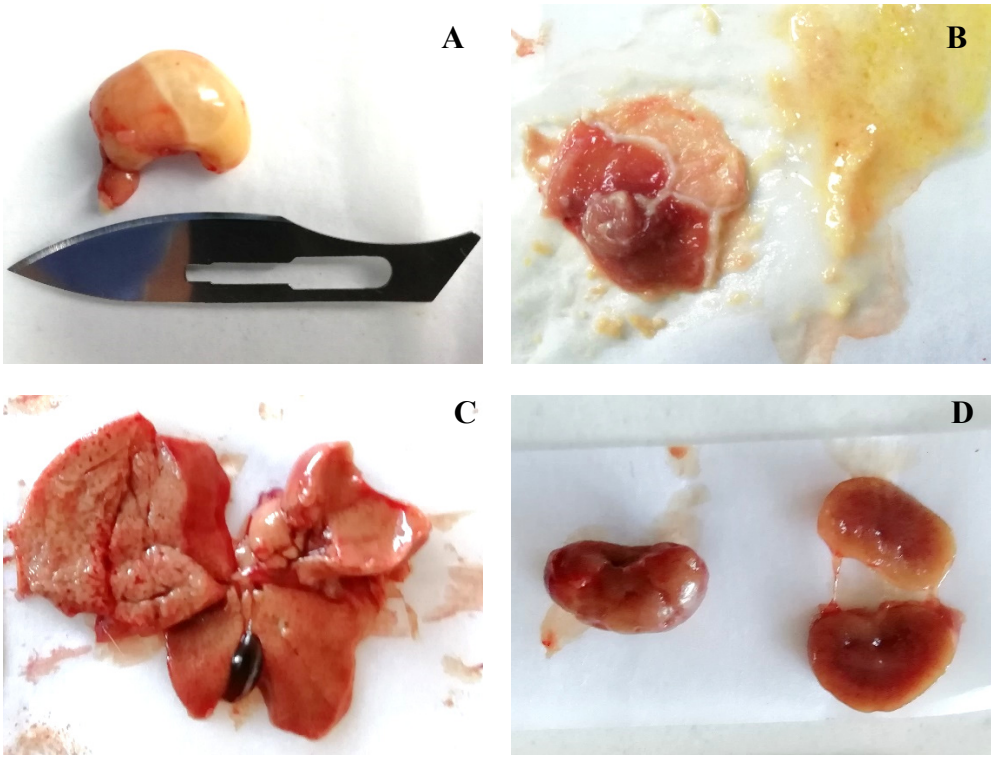

Fig. 1. *Gross lesions found during necropsy after 5 weeks of administration of a cobalamin and folate deficient diet. A – distended stomach and a scalpel blade for size comparison; B – a view of stomach lining and stomach content; C – liver with discoloration; D – kidneys showing cortical fibrotic scarring and a pale brown colour.*

Mice also showed an increased red blood cell distribution width, with a value of 21.06 ± 3.75% (P = 0.003). Reticulocyte absolute count was increased, with a value of 602.74 ± 244.17 K/µL (P = 0.086). White blood cells were elevated with a value of 7.08 ± 3.40 K/µL (P = 0.045), as well as

the neutrophile absolute number, that had a value of 3.83 ± 1.95 K/µL (P = 0.038).

The leukogram also showed elevated values for the absolute counts of monocytes (P = 0.561), eosinophils (P = 0.348) and basophils (P = 0.370). Although these three parameters were over the superior limit of the reference interval, blood smears were checked, leukocyte percentages were calculated and basophilia, eosinophilia, and monocytosis were excluded from diagnosis.

The complete blood count did not point towards anaemia, but there were significant changes in the red blood cell distribution width and a slight increase in the reticulocyte count. Both parameters point towards a change in red blood cell morphology. The leukogram indicated a mild leukocytosis with neutrophilia, consistent with the signs of inflammation seen in the necropsy examination.

Table 1. Complete blood count for CD1 mice after 5 weeks of B12 and folate deficient diet administration

PARAMETERS	UM	CD1 mice (n=5)	Reference value
Red blood cells	M/µL	10.96±1.35 ↑	7.9 – 10.12
Hemoglobin	g/dL	14.94±2.08	13.2 – 16.4
Packed cell volume	%	48±4.07	43.2 – 56.3
Mean corpuscular volume	fL	44.92±5.07** ↓	48.8 – 58.9
Mean corpuscular hemoglobin	pg	13.82±0.66** ↓	15 – 16.7
Mean corpuscular hemoglobin concentration	g/dL	31.46±1.6	27.9 – 33.2
Red blood cell distribution width	%	21.06±3.75** ↑	11.7 – 14.8
Reticulocytes	K/µL	602.74±244.17 ↑	150 – 447
Reticulocytes	%	5.6±2.27	-
Platelets	K/µL	1699.6±330.18 ↑	630 – 1559
Mean platelet volume	fL	6.66±0.98	-
White blood cells	K/µL	7.08±3.40* ↑	0.25 – 5.18
Neutrophiles	K/µL	3.83±1.95* ↑	0.02 – 1.12
Lymphocytes	K/µL	2.74±1.24	0.23 – 4.51
Monocytes	K/µL	0.18±0.05 ↑	0 – 0.09
Eosinophils	K/µL	0.14±0.02 ↑	0 – 0.01
Basophils	K/µL	0.08±0.12 ↑	0 – 0.06

Reference values from Weiss and Wardrop, 2010 [5]
n=number of mice
Data represents a mean value ± standard deviation
*P≤0,05
**P≤0,01

Blood smear evaluation

Red blood cell morphology was significantly altered, with poikilocytosis, and anisocytosis in all 5 mice, along other finds such as hypersegmented neutrophils found in 2 individuals. Polychromasia and other signs of a

regenerative response (such as Howell-Jolly bodies or metarubricytes) were within species parameters.

Red blood cells were modified in shape and volume, with specific finds such as echinocytes, codocytes, elliptocytes, and spherocytes. Morphological aspects are shown in Figure 2.

Elliptocytes are usually associated with bone marrow abnormalities and hepatic disorders, but in this case, the crenated elliptocytes might be attributed to glomerulo-nephritis [6]. Echinocytes might also be a consequence of renal lesions. Advanced types of echinocytes can also lose their spicules and resemble spherocytes.

Red blood cells morphological changes were indicative of renal and hepatic lesions and were not associated with anaemia or a regenerative response. Aside from the two cases with hypersegmented neutrophils, there were no signs that could point towards a megaloblastic anaemia.

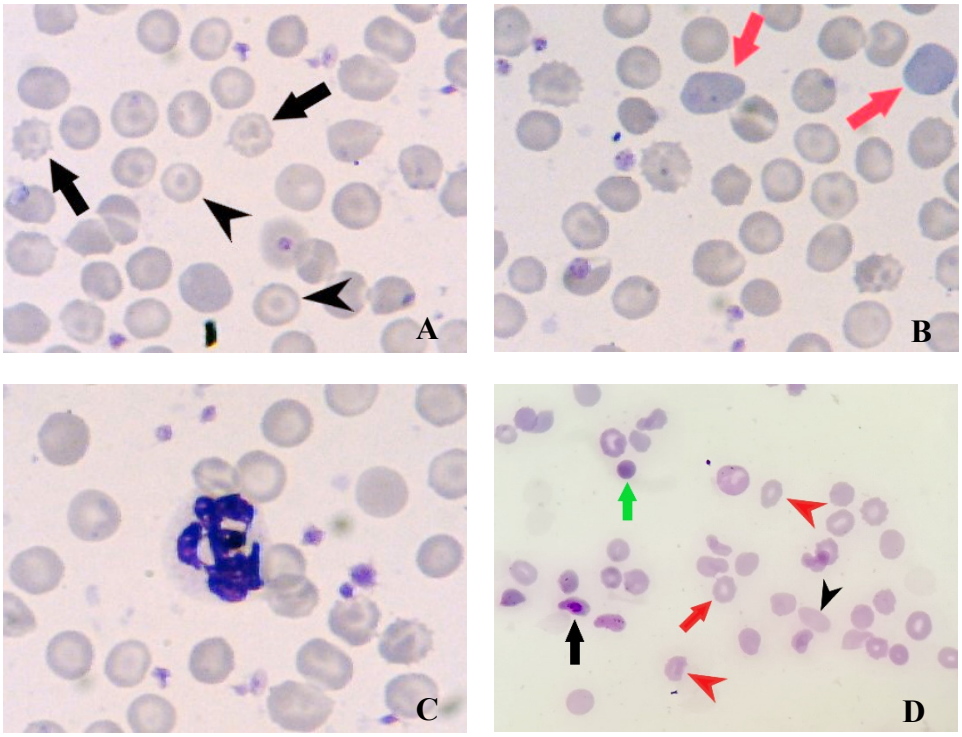

Fig. 2. *Morphological aspects of mouse peripheral blood after 5 weeks of B12 and folate deficient diet (100x, MGG); A – Poikilocytosis with echinocytes (black arrows) and codocytes (black arrowheads); B – reticulocytes (red arrows) and poikilocytosis with echinocytes and spherocytes; C – hypersegmented neutrophil; D – severe poikilocytosis with elliptocytes (black arrowhead), crenated elliptocytes (red arrowheads), echinocytes (red arrow), spherocytes (green arrow) and a metarubricite (black arrow) with modified shape.*

Bone marrow and spleen cytology

Bone marrow examination did not reveal a modified cell distribution or morphology (Figure 3A). The bone marrow as abundant in cells, with a predominance of the myeloid line. There were no changes in cell shape, colour, chromatin, or nucleus – cytoplasm ratios. Elytroid precursors were normal in appearance and the myeloid-elytroid ratio was within interval reference values (3.1:1).

Spleen morphology revealed a predominance of nucleated cells, the majority of which were identified as lymphocytes. The hematopoietic tissue within the spleen also had a physiologic appearance and a normal number of erythroblast precursors (Figure 3B).

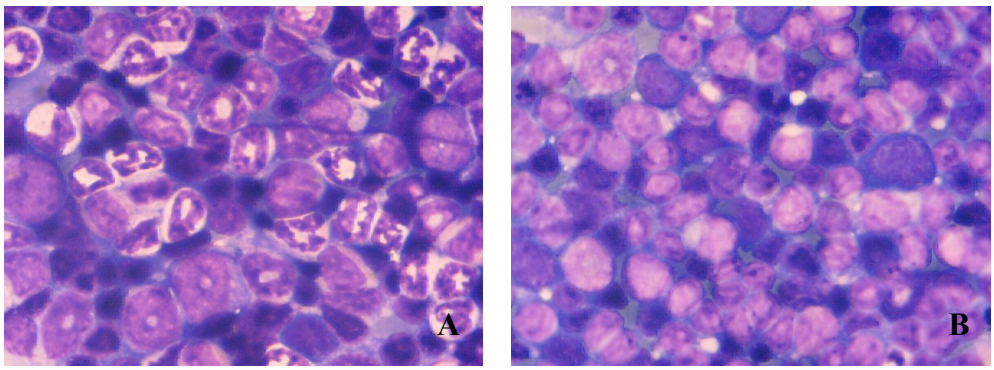

Fig. 3. *Cytological aspects of the bone marrow and spleen in mice after the administration of a B12 and folate deficient diet for 5 weeks; A – bone marrow with a large concentration of elytroid precursors; B – Spleen with normal cytology and elytroid precursors with an orderly distribution.*

Conclusions

The administration of the cobalamin and folate diet did have significant effects on the overall health of the mice. Pathological aspects did not point towards megaloblastic anaemia, but rather others systemic changes related to the digestive and excretory systems.

The lesions were indicative of gastritis, hepatitis, and nephritis, that might have caused the changes found in peripheral blood morphology. Haematological examination also revealed signs of inflammation, revealed by the increased number of neutrophils and the subsequent leukocytosis.

Although this experiment was meant to test if the sole administration of a vitamin deficient diet may be able to cause megaloblastic anaemia, further investigations are required to reach a clear conclusion.

Acknowledgements

During this study, all safety norms regarding the environment, care, manipulation, and euthanasia of experimental animals, were respected as dictated by European Parliament and Council directive 2010/63/UE and following Romanian Parliament laws 43 and 11 from 2014. Housing conditions were followed according to recommendations from the 86/609/CCE Directive and research in the field of laboratory animal welfare (www.animalethics.org.au). Euthanasia was realized following instructions from the Veterinary Medical College guidebook and respecting national laws.

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DYNAMICS OF THE IMMUNE RESPONSE AGAINST NEWCASTLE DISEASE IN CHICKEN FARM WITH ADENOVIRAL INFECTIONS

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Abstract

In order to evaluate the dynamics of the immune response against avian pseudopestle in broiler flocks in which hepatitis with chickens evolves, an experimental model was designed using 2 batches of broiler chickens, one negative for adenoviral infection (hall 1) L1 and another from the hall 11 (L2) in which antibodies against this disease were detected. In this group were found: the presence of specific antibodies at both 1 day and 9 days; at 38 days 40% of the total number of samples were positive and 60% were negative with titers of 1/8 inhibit hemagglutinating units, body weight was 300 g lower and mortality rate 4.4%.

These aspects are due to the fact that in birds with adenoviral infections the dynamics of the immune response is deficient due to liver failure and the impossibility of plasma protein production.

Keywords: *hepatitis including chickens, antibodies, body weight, mortality rate.*

Introduction

Avian pseudo-plague is of great economic importance, due to the economic losses it has produced and can cause to poultry, due to the high morbidity and mortality in combination with other viral diseases. These losses include, in addition to mortality rate, which can reach over 90% of the population and the decrease in egg production and in the case of less virulent strains - the cost of immunoprophylactic measures and losses due to the imposition of restrictive measures imposed by the control plan, as well as those resulting from disruption of the growth program and exploitation (4,5).

It is well established that the disease is a bigger problem and is more difficult to control in countries where poultry farming predominates than in countries where breeding is practiced mainly, or almost exclusively, in intensive systems, in poultry complexes. The explanation is that, in specialized farms, anti pseudopest toxic immune prophylaxis can be systematically applied throughout the life of a herd, in traditional breeding,

full and systematic vaccination of herds scattered in people's yards is a difficult task to achieve (2,3),

Newcastle disease is currently known on all continents but is much more common in Asia, Africa and South America, especially in areas where rigorous immune prophylaxis programs have not been implemented. Due to the permanent and systematic vaccinations applied for more than half a century, most European countries are currently free of this disease, but the threat posed by this disease to poultry remains very serious and current, due to the ease. with which it can be transmitted (6,9).

Materials and methods

In order to highlight the adenoviral infection in the investigated flocks and to determine the immune status of chickens for meat after vaccination against avian pseudopest, an experimental model was designed based on data collection from the farm and systematic determinations of serum antibodies against these diseases.

For this purpose, two halls from the Frumușani farm were chosen, one hall (Hall 1) populated with chickens from the supplier Gallus Hungary and one hall (Hall 11) originating from S.C. BANVIT FOODS SRL, each shelter having an average number of 32,000 heads.

The presence of adenoviral antibodies in the study herds, the level of anti-pseudopestis antibodies, and the mortality rate from the analysis bulletins, and from the existing zootechnical records were analyzed at the farm level.

Blood samples were collected from the auxiliary vein, in syringes without anticoagulant. After collection, the samples were identified and transported to the laboratory in isothermal crates where they were processed immediately, according to methods for the determination of serum antibodies.

A total of blood samples were collected from broiler chickens, respectively 100 per shelter to determine the level of anti-adenoviral antibodies at 1 day and at the age of 9 days by the Elisa technique and those against Newcastle disease at the age of 22 and 38 days of life by the haemagglutination inhibition test (7,8).

Results and discussions

The analysis of the data presented in table 1 shows that the broilers from hall 1 origin Gallus Kft Hungary do not show antibodies against hepatitis including broiler chickens neither at the age of 1 day nor at the age of 9 days.

Table 1. Titration values of anti-adenoviral antibodies in broilers 1

No.	Chicken age	Nr. samples	Seric antibodies titer value				
			0	1052	1137	1235	4881
1	1 day	100	100	0	0	0	0
2	9 days	100	100	0	0	0	0

From the data presented in table 2 it is observed that the broilers from hall 11 from SC BANVIT FOODS SRL present antibodies against hepatitis, including the broilers at the age of 1 day and at the age of 9 days.

The presence of antibodies at such a young age of 1 day denotes a vertical transmission of the disease from the womb to the one-day-old chick.

Table 2. Titration values of anti-adenoviral antibodies in broilers 11

No.	Chicken age	Nr. samples	Seric antibodies titer value				
			0	1052	1137	1235	4881
1	1 day	100	0	10	20	20	50
2	9 days	100	0	10	10	10	70

From the data presented in table 3, we can observe the following:

At the age of the first pseudopest vaccination (9 days), the titers showed values between ¼ and 1/32.

At the age of 22 days, 90% of the samples had protective values of 1/16 and 1/32 and 10% had a low level of post-vaccination antibodies: (1/8).

After 16 days of vaccination at 38 days, all samples indicated a high level of post-vaccine antibodies 1/16, 1/32, 1/64 and 1/128 IU.

Table 3. The values of anti-pseudopest antibody titers in broilers in Hall 1

No.	Chicken age	Nr. samples	Seric antibodies titer value								
			0	1/4	1/8	1/16	1/32	1/64	1/128	1/256	1/512
1	9 days	100	0	5	10	20	65	0	0	0	0
2	22 days	100	0	0	10	20	70	0	0	0	0
3	38 days	100	0	0	0	20	40	20	20	0	0

From the data presented in table 4, we can observe the following:

At the date of the first vaccination of chickens from SC BANVIT FOODS SRL, 30% of them had negative titers and 70% titers between 1/16 and 1/32

At the age of the second vaccination 22 days 10% of the samples had protective values of 1/16 and 90% showed a low level of post-vaccination

antibodies: 20% 1/4, 70% (1/8) fact explained by the presence of hepatitis with inclusions caused by adenoviral infection.

At 38 days of age, 40% of the total number of samples was positive and 60% were negative with titers of 1/8 inhibit-hemagglutinating units, which indicates a very weak protection against the action of Newcastle disease virus.

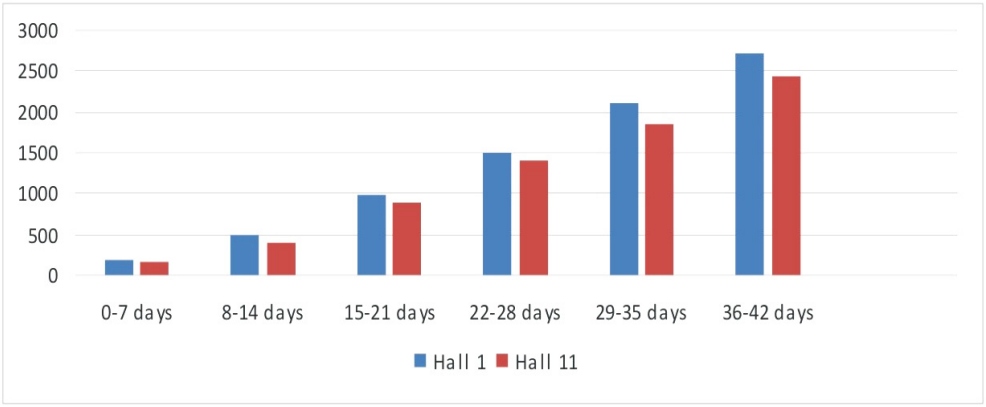
Table 4. The values of anti-pseudopest antibody titers in broilers in Hall 1

No.	Chicken age	Nr. samples	Seric antibodies titer value								
			0	1/4	1/8	1/16	1/32	1/64	1/128	1/256	1/512
1	9 days	100	0	0	30	60	10	0	0	0	0
2	22 days	100	0	20	70	10	0	0	0	0	0
3	38 days	100	0	0	60	40	0	0	0	0	0

From the analysis of the data presented in table 5 and graph no. 1 it is found that there were significant differences between the body weight of healthy broilers and those infected with adenovirus type A.

Table 5. Body weight dynamics in poultry farm in halls 1 and 11

No.	age	Hall 1 (g)	Hall 11 (g)	Observations
1	0-day	40	41	No significant differences in weight were found between batches; body weights being close.
2	7 days	188	175	In the first week, significant weight differences were found between the infected and the uninfected group, respectively, up to 20 g less.
3	14 days	487	410	At the survey scale from week 2, significant weight differences were found between the studied groups. The difference in weight between the 2 lots was 77 g
4	21 days	980	890	At week 3 the differences continued to increase to between 90 g.
5	28 days	1510	1400	At the age of 28 days the difference was 110 g.
6	35 days	2105	1840	At the control scale performed at the age of 35 days, the recorded weight loss was 265 g.
7	42 days	2720	2420	At 42 days when the herds were slaughtered, a difference of 300 g was found in the group with adenoviral infection (hall 11) compared to the healthy group (hall 1).

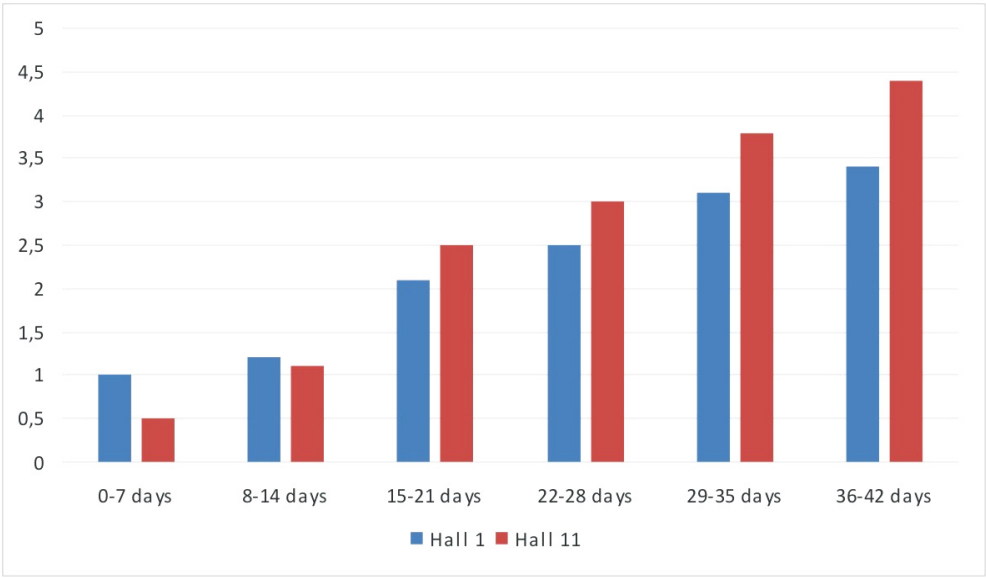


Graph 1. *Body weight dynamics in the study herds*

From the results presented in table 6 and graph no. 2, there are significant changes in the values of the mortality percentage for the batches of chickens in hall 11 compared to those in hall 1.

Table 6. The evolution of the cumulative mortality percentage for the chickens in the halls 1 and 11

No.	week	Hall 1 (%)	Hall 11 (%)	Observations
1	0-7 days	1	0,5	Between the 2 lots, there are significant differences in the mortality rate, especially in the halls that came after a long transport (halls 1).
2	8-14 days	1,2	1,1	No significant differences in cumulative mortality were found between the groups of broiler chickens studied
3	15-21 days	2,1	2,5	Significant differences in the cumulative mortality rate (0.4%) were found in Hall 11
4	22-28 days	2,5	3	In hall 1, no significant differences in the mortality rate were found. In hall 11, on the other hand, the percentage was almost 1.5% higher.
5	29-35 days	3,1	3,8	At hall 11 there is an upward trend in the mortality rate due to complications caused by adenoviral infection.
G	36-42 days	3,4	4,4	At the end of the production cycle, a higher mortality rate is observed for chickens originating from SC BANVIT FOODS SRL.4.4% compared to 3.4% for those from Gallus Kft Hungary.



Graph 2. *The evolution of the mortality percentage in the investigated broiler flocks*

Conclusions

– At 1 day and 9 days of age, chickens originating from Gallus Kft Hungary did not show specific antibodies against hepatitis including chickens.

– Chickens from SC BANVIT FOODS SRL presented at the age of 1 day and 9 days a large number of positive samples, which indicates a vertical transmission of adenoviral infection from the breeding queen to the one-day-old chick.

– At the age of 22 days after 13 days from vaccination in hall 1, there were no positive samples for adenoviral infection, the values of post-vaccination titers were good: 90%.

– At hall 11, where antibodies against hepatitis were included, including chickens at the age of 22 days, 90% of the samples had unproductive values between ¼ and 1/8 and 10% were protective.

– At the age of 38 days, the results showed significant differences between batches, the percentage of positive samples being 100% in the number of uninfected halls.

– In the infected groups coming from hall 11 at the age of 38 days, the percentage of protective samples was small 30%, the rest of the samples being negative.

– The very high percentage of negative samples was due to liver failure induced by the hepatitis virus with inclusions and its inability to secrete albumin and globulin as antibodies.

– The average weekly weight at all control scales showed significant variations between the 2 lots, fact explained by the presence of liver failure in broilers from S.C BANVIT FOODS SRL in hall 11.

– The evolution of the mortality rate had an ascending slope in the infected herd, this being 4.4% in hall 11 at the age of 42 days.

– In the uninfected groups, the mortality rate was within the normal limits of 3.4%.

– In flocks of birds with adenoviral infections the dynamics of the immune response, and body weight is deficient due to liver failure and the inability to produce plasma proteins and structural proteins.

– Also, adenoviral infection induces a higher mortality rate due to the lack of appropriate immunization for major diseases of birds but also due to the intervention of conditionally pathogenic bacterial flora.

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STUDY ON THE INCIDENCE OF SOME VIRAL DISEASES IN A DOG SHELTER

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Abstract

Canine diseases, especially viral diseases, are a highly important area of veterinary pathology, as the dog becomes an increasingly significant presence in the urban environment, on the one hand due to the desire of owners to have a companion, and on the other, the phenomenon of abandonment is very present unfortunately.

Because of this, the species is subject to a highly large number of diseases, among which an important place is occupied by viral diseases that affect the population under one year of age. Among them, canine parvovirus and popular Carre's disease (jigodia) are considered the most dangerous with a global spread and a diverse incidence from tropical to polar areas. These viral agents cause great problems for breeders, but also for dog owners and last but not least for shelters, where the number of animals is increasing.

This paper aims to highlight the prevalence of these viral diseases, the way they act on the animal body and on individuals in the community.

We aimed to identify individuals who had a representative clinical picture, but also those who did not show symptoms and to identify correlations between various factors such as age, sex, race, season.

The shelter where the research was carried out is located on the outskirts of Boldeşti Scăieni located north of Ploieşti in the contact area of the Curvature Subcarpathians with the Romanian Plain.

Keywords: ??

Materials and methods

For the study of the incidence of viral diseases (parvovirus and Carre's disease – distemper) among the dogs in the shelter were observed a number of 20 subjects in the age category between 2 and 8 months, males. The identification data of the individuals that are the object of the study are presented in tables 1 and 2.

Fig. 1. *Outdoor paddock speakers*

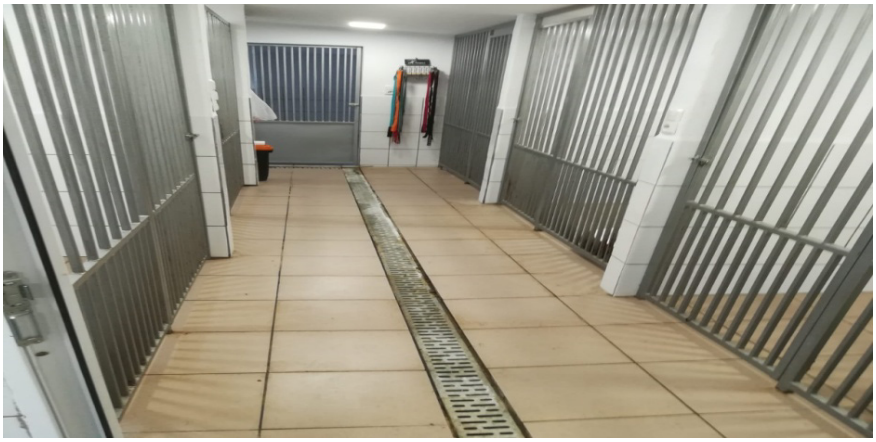

Fig. 2. *Indoor speakers (overview)*

The animals that were the subject of the study were observed for 60 days and come from the animals brought to the shelter a few days at most one week before the start of the investigation.

Results and discussion

Table 1. Patients examined

Nr. No.	Race	Age	Dewormed Vaccine	Sex	Weight
1.	Metis Amstaf	2,5 months	not	M	6 kg
2.	Metis Rottweiler	3,5 months	not	M	12 kg
3.	Dog metis Shepherd G	2,5 months	not	M	4 kg
4.	Common dog	3 months	not	M	3 kg
5.	Common dog	4 months	not	M	3,5 kg
6.	Metis Doberman	3 months	not	M	5 kg
7.	Common dog	6 months	not	M	12 kg
8.	Dog metis Shepherd M	4 months	not	M	10,5 kg
9.	Dog metis Shepherd M	3,5 months	not	M	9,5 kg
10.	Metis Pittbul M	2,5 months	not	M	3,6 kg
11.	Metis Malinois	3 months	not	M	4,5 kg
12.	Dog metis Shepherd M	5 months	not	M	6 kg
13.	Common dog	2,5 months	not	M	3.5 kg
14.	Metis Coker	5.5 months	not	M	4,5 kg
15.	Dog metis Shepherd M	5.5 months	not	M	10 kg
16.	Common dog	5 months	not	M	6 kg
17.	Dog metis Shepherd M	6 months	not	M	11 kg
18.	*Metis Husky*	7 months	not	M	10 kg
19.	*Metis Labrador*	5,5 months	not	M	10 kg
20.	Common dog	8 months	not	M	15 kg

After selecting individuals and distributing them in boxes, such as those shown in the previous images, they were carefully monitored so that when the first symptoms of the disease appear they are isolated and after clinical examination, rapid testing and sometimes after performing a haematological and biochemical examination so that a diagnosis can be made. Subsequently, the treatment corresponding to the discovered viral disease (parvovirus or distemper) was chosen.

A case out of a total of 20 will be described.

Case no.1

Species – canids
Race – metis amstaff
Sex – M
Color – white + brown spots
Weight – 6 kg
Age – 2.5 months.

Anamnesis: it was observed that he is apathetic, has no appetite, vomits and the faeces are soft with a fetid smell.

Clinical examination – general consultation – yellowish white foamy vomit, apathy, anorexia, diarrheal faeces with streaks of blood that started during the night. Temperature – 38.8ºC. Presents abdominal pain on palpation, mild hypothermia, dehydration.

Apparent mucous membranes – pale pink.

Paraclinical investigations:

1. Blood samples were collected for haematological analysis of purple container on anticoagulant EDTA - ethylenediaminetetraacetic acid, (tab. 2) Results:

Table 2. Hemoleukogram - case 1

Measured parameter	Result obtained	Reference range
Leukocyte (WBC)	3,76 m/mm^3	(6.0 – 17.0) m/mm^3
Lymphocytes (Lym)	41,7%	(10.0 – 30.0) %
Monocyte (Mon)	7.8%	(2.0 – 10.0) %
Neutrophils (Snow)	49.1%	(50.0 – 80,0) %
Erythrocytes (RBC)	5.70 M/mm^3	(5.5 – 8.) M/mm^3
Hemoglobin (Hb)	14.9 g/dl	10.0 – 18,0) g/dl
Hematocrit (Hct)	37.1%	(35.0 – 55.0) %
Vol. Eritr. Med. (MCV)	65.2 fl	(58.0 – 73.0) fl
Hb. Eritrea. Med (MCH)	26.1 pg	(19.5 – 24.5) pg
Conc. Er. Med. Hb. (MCHC)	40.1 g/dl	(28.0 – 40.0) g/dl

2. Rapid test Elisa CPV Ag **positive,** dg**. Parvovirus,** (fig. 3).

Fig. 3. *Parvovirus test positive*

Treatment: Lingerate Ringer 20 ml / kg / hour i.v. 3 hours / day - 4 days
Glucose 5% 10 ml / kg / hour 3 hours / day
Cefort 0.3 ml / kg 2 times / day 5 days
Drotaverine 0.6 ml s.c. 2 times / day 4 days
Vitamin K 1.0 ml s.c. 2 times / day 7 days
Canglob P 0.4 ml / kg 4 days

From the 3rd day he started eating dietary food, a sign that his condition improved, after the 5th day he did not vomit, he fed himself - **healed**.

Conclusions

1. The clinical signs found in all patients with parvovirus were sometimes repeated incoercible vomiting, anorexia, abdominal pain, dehydration and bloody diarrhea. In advanced cases, patients also had hypothermia, tachypnea, hypovolemic shock.

2. Clinical signs, in distemper, stuttering were sero-muco-purulent oculo-nasal catarrh, photophobia, apathy, anorexia, dry cough, then prolonged wet cough, vomiting, diarrhea, fetid-smelling soft stools and streaks of blood. Sometimes in older dogs hyperkeratosis in the plantar pads. The nerve shape is manifested by muscle tremors.

3. The establishment of treatment from the first signs of illness increases the chances of cure very much, as evidenced by the cases that received immediate treatment reacted well recovering in proportion of 100%, compared to those who began treatment after 3 days, they also gave the number of deaths from parvovirus or distemper.

4. Animals older than 5 months responded better to treatment, having a higher resistance, and a cure rate of 100%.

5. It has been shown to be of great importance to reduce intestinal peristalsis, prevent bacterial infections and stop gastrointestinal bleeding.

6. The administration of immunoglobulin to patients with parvovirus and distemper, brings an extra chance in the fight against the disease, all patients who received treatment with Canglob P or D at a dose of 0.4 ml / kg were declared cured.

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THE USAGE OF DESLORELIN IN DOGS

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Abstract

Castration, as a recommendation for aggressive dogs or with various other types of behavioural deviations, may not always be a fair recommendation. Sometimes, these males are very valuable from a genetic point of view, and surgical castration, being irreversible, should not be the first option.

The use of Deslorelin (implant) has entered the practice in recent years and statistics can already be made regarding both the effectiveness of the product and the possible side effects. The application method is very simple, similar to the implantation of a microchip, and the duration of action of the active substance is at least 6 months.

Keywords: *Deslorelin, castration, aggressiveness in dogs.*

Introduction

Deslorelin, used at a concentration of 4.7 mg/implant, is a synthetic analogue of GnRH (gonadotropin releasing hormone) that induces temporary infertility of about 6 months in healthy males who have reached sexual maturity.

Sometimes suppression of sexual function is temporarily desired to deduce whether a certain type of unwanted behaviour is sexual in nature, so surgical castration will be the next step that guarantees a change in that behaviour. Other times a temporary sexual inactivity of the patient is desired when left long period in a boarding house, or you want a break in making the irreversible decision of orhidectomy.

Materials and methods

The number of males which participated in the study is 16. They were between 9 months and 6 years old. Of these, eight were small (2 Yorkshire Terriers, 4 Bichons and 2 Shih-Tzu), seven between 8 and 16 kg (2 Dachshunds, 3 Cockers and 2 Beagles) and one German male.

Prior to Deslorelin implantation, testosterone titer was determined, plasma concentrations being 0.4 μg / ml at all participants of the study.

Sampling must comply with certain conditions: to be done exclusively in tubes with activating clot and to be centrifuged immediately after sampling (venous blood, approximately 4 ml / sample, so that at least 1 ml of serum remains after centrifugation).

After implantation, samples were taken (to follow the return to the previous testosterone titer) at 12 months and 18 months. The study took place between January 2020 and September 2021.

Results and discussions

Surgical castration can sometimes result in the tempering of aggressive behaviour or, conversely, its accentuation.

The request for an alternative solution was sought by the owners when it could not be said with certainty whether the aetiology is sexual or not; by reversible inhibition of the androgenic cause, it was followed whether or not the aggressive behaviour was changed.

In 12 of the dogs participating in the study, after a period of 3-4 months after the Deslorelin implant, there was a decrease in aggressive or dominant behaviour, which lasted up to 8 months (in 5 patients) or 10 months (in 7 patients) after implantation. The rest of the patients (4 participants in the study) did not show a decrease of unwanted behaviour.

From this point of view, the conclusion was that a specialist in canine training should be consulted, and patients with sociopathic disorders (intra or interspecies) should be excluded from the application of the implant, when the main purpose is to fix the behavioural disorder.

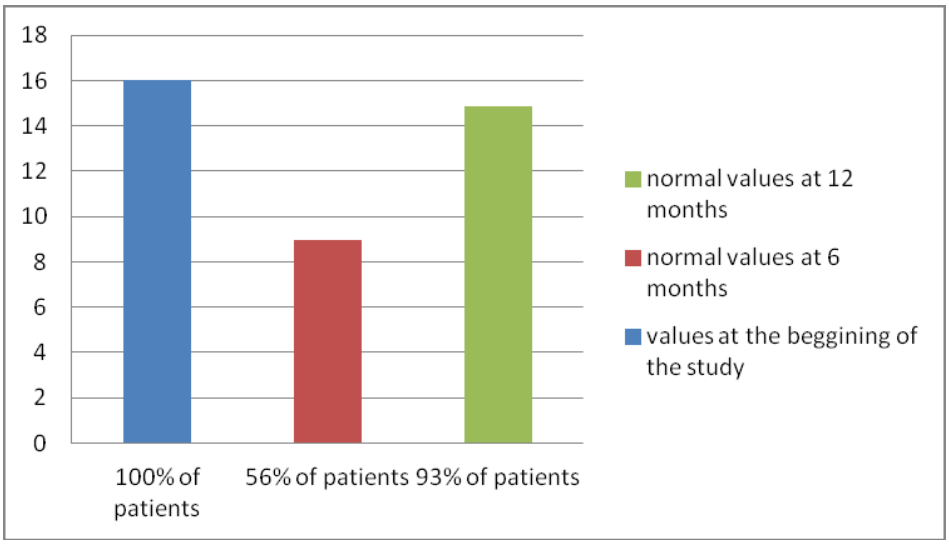
Spermograms were performed on all patients that had the implant and were observed clinically during the 18 months of the study. The conclusions were, in all cases, as follows:

- the size of the testicles was decreased, starting with the 3rd week (in 6 patients) or the 4th week (in 10 patients), without other macroscopic zonal changes or without inducing pruritus or other type of discomfort; hypogonadism was reversible in all cases between 9 and 12 months after implantation;
- libido decreased significantly in all patients, only one (a Yorkshire male) showing a desire to mate, without realizing the actual mounting;
- the spermogram performed before and after the Deslorelin implant showed relevant changes as it follows:
  - in terms of ejaculate volume, all showed a decrease of approximately 30-35% in the specific volume, reported to the size of the animal;

- in terms of sperm count, a decrease of about 60-70% compared to the minimum norm of the species (100-300 million sperm / ml) was also observed.

In one of the patients (Brac 6 years and 1 month), the lowest sperm count was observed, but it was considered that after about 5 years, the sperm quality of any patient may decrease, this study participant having lower values, at the limit of the species, of the number of sperm / ml and before the application of the implant.

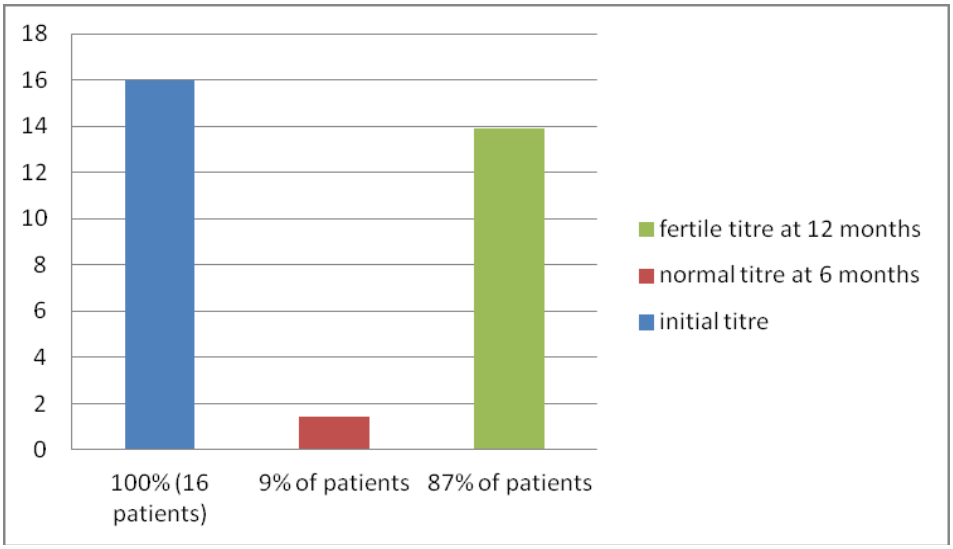
In all patients, spermogram parameters returned to normal at 12 months after implantation (in spermograms performed at 6 months, only 9 of the 16 patients had values comparable to those at the beginning of the study).



Graph 1. *The return of spermogram's parameters back to normal values*

With regard to sperm motility, the useful one is considered to be represented by energetic forward movements; at the beginning of the study, all spermograms showed this type of movement as the majority.

At 6 months, all samples presented a significant decrease in motility, in over 50-60% of sperm, and at 12 months, in 14 of 16 patients, a return to baseline parameters.



Graph 2. *The variation of testosterone's titre at 6 and 12 months after the beginning of the study*

Testosterone titration was performed before the start of the study at 6 and 12 months and it was found that at 6 months only 3 of 16 patients had values of 80% of the initial titer (approximately 0.4 mg / ml) specific to the species; at 12 months already 14 of the 16 patients had a titer similar to the initial titer, the other 2 having about 80% of the initial titer (the two patients were the smallest in size, it is possible that inhibition of testosterone secretion for a longer period 12 months, be influenced by this factor).

Conclusions

1. Deslorelin is an excellent variant of totally reversible inhibition of testosterone production in sexually mature males when surgical castration is not desired.

2. In cases where the aim is to reduce aggression, Deslorelin may not be effective because the disorders are often behavioural in nature, not sexual.

3. Changes in sperm count, libido and testosterone titer are reversible, but it is sometimes necessary more than 12 months after the implant is applied.

4. The macroscopic aspect of the volume decrease of the testicles was encountered in all cases, its lack must involve a new dosage of testosterone to check if the implant is active.

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ASPECTS OF BLOOD COUNT AND BLOOD BIOCHEMISTRY IN CATS WITH LEUKAEMIA

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Abstract

In cats, leukaemia was first described as a morbid entity by Syedamgrotzky in 1871 and later studied by researchers Cotchin, 1957, Smith, 1967, Hoiyworth, 1960 and others. According to Parodi, 1974, of all mammal species, the cat is most commonly affected by leukaemia (3).

Initially, only isolated, sporadic cases were described, later the appearance of the disease was also reported in outbreaks, so we can say that in cats the disease can also register enzootic form.

Leukosis is caused by the feline leukaemia virus FeLV, a Retrovirus that causes anaemia, multiple lymphomas, depression of the immune system. The virus affects cats worldwide, with the prevalence of infection in Europe being low (<1%), but may exceed 20% in some countries / regions. Due to diagnostic tests and vaccines, the prevalence of FeLV infection has decreased considerably over the last 25 years (1,2).

Feline leukaemia virus (FeLV) causes an infectious-contagious disease capable of producing various neoplastic diseases, immunosuppression and a wide variety of associated diseases and paraneoplastic syndromes such as: anaemia, cachexia, hepato-renal, respiratory and reproductive function.

Being one of the most common infectious diseases of cats, the risk factors for infection are represented by sex, age, access to the open air.

It is very useful to know the retroviral status of all cats by testing during life.

Cats infected with FeLV or IVF can live for many years, although these viruses significantly change their health. Proper management can give infected cats a longer and healthier life. No euthanasia decision should be made based on whether or not the fish is infected, no rapid test is 100% specific, all positive rapid tests requiring confirmation by complementary examinations.

Keywords: *leukaemia, retrovirus*

Materials and methods

Materials:

- Animals/cats with uncharacteristic or characteristic symptoms;
- Laboratory equipment: Blood biochemistry analyzer, haematology analyzer;
- Laboratory materials consumables: tubes, pipettes, diagnostic tests, etc.;
- Digital thermometer;

- Ultrasound;
- Medicinal products used in specific therapy: rehydrators, vitamins, antibiotics, immunomodulators;
- Blood for homologous transfusions.

Methods:

- Semiological methods: inspection, palpation, percussion, listening, thermometry;
- Anamnesis;
- Clinical examination on organs, devices, systems;
- Study of observation sheets.

Results

Patient 1. Patient data: Species: Felidae; Race: European; Age: 5 years; Gender: Male; Hormonal status: sterilized; Weight: 5.4 kg.

History: Unappetizing for 3 days, is apathetic, vomits often, liquid faeces, does not drink water, stays hidden, was dewormed externally 2 months ago.

Clinical examination: weight 5.4 kg, RT 39.9 C, stomatitis, flea infestation, reactive submandibular lymph nodes, unchanged cardio-pulmonary auscultation, anaemic apparent mucous membranes, 15% dehydration, slight discomfort at abdominal palpation, not detected organomegaly or intra-abdominal formations.

Recommendations: abdominal ultrasound, blood biochemistry analysis, haematology, IVF / FeLV testing.

Differential diagnosis: Pancreatitis, Renal impairment, IVF, FeLV.

Symptomatic treatment: Fluid therapy, Cerenia i.v, Controloc i.v, Buscopan i.v, Noroclav s.c., Tolfedol

Recommendations: Return to control.

On the 2nd day, blood was collected for biochemistry, hemoleukogram and Fiv / FeLV testing; RT 39.4 C.

Table 1. Patient 1. Biochemistry

Parameters	Results	Reference interval
ALB	1.7 g/dl LOW	2.1-3.3
TP	12.2 g/dl HIGH	7.3-9.3
GLOB	3 g/dl LOW	4.1-5.7
A/G	0.9	0.50-0.80
TB	0.2 mg/dl	0.0-0.3
GGT	3 U/L	0-5
ALT	170 U/L HIGH	0-112
ALP	119 U/L HIGH	0-115

AMY	**1660 U/L**	**0-1600**
CRE	**1.2 mg/dl**	**1.1-2.0**
BUN	**17 mg/dl**	**14-26**
GLU	**201 mg /dl HIGH**	**65-146**
TC	**80 mg /dl HIGH**	**70.00-150.00**
CA	**7 mg/dl LOW**	**8.50-10.00**
PHOS	**5 mg/dl**	**3.70-5.60**
BUN/CREA	**180 mg/dl**	**27.000-182.000**

Table 2. Patient 1. Blood count

Parameters	Results	Reference interval
WBC (*10/ul)	**32.9 HIGH**	**5.5-19**
Undiff. Blasts (*10/ul)	**19.411**	**-**
Neutrophils (*10/ul)	**8.883**	**2.5-12.5**
Lymfocytes (*10/ul)	**3,948**	**1.5-7.0**
Monocytes (*10/ul)	**0,658**	**0-8.5**
Platelets (*10/ul)	**1,6 LOW**	**3-7**
RBC (*10/ul)	**2.5 LOW**	**5-10**
HB (g/dl)	**5.8 LOW**	**10-15**
PCV (%)	**14 LOW**	**30-45**
MCV	**56.8 HIGH**	**39-55**
MCHC (g/dl)	**33.3**	**30-36**

Treatment: Fluid therapy, Cerenia i.v, Controloc i.v, Noroclav s.c., Whole blood transfusion, Vancomycin 80 mg i.v. at 6 p.m.

Recommendations: Repeat blood count at least 5 days post-transfusion, immunostimulation.

Monitoring general condition, water and food appetite, urination, defecation.

Evolution: On the third day of treatment the general condition improved and he started eating alone.

Prognosis: favourable.

Patient 2. Patient data: Species: Felidae; Race: European; Age: 12 years; Gender: Male; Hormonal status: Sterilized; Weight: 4.0 kg.

History: unappetizing, vomiting, apathetic, has been diagnosed in the past with respiratory mycoplasmosis and has been treated with Veraflox, Medrol and has also had a group A whole blood transfusion, 50 ml.

Clinical examination: Intensely anaemic mucosa, TR 39.5 C, gingivitis, dehydration approximately 10%, reactive submandibular lymphocytes, supple

abdomen, painful, free of ocular and nasal secretions at the time of examination, no organomegaly or intra-abdominal formations are detected.

Recommendations: IVF / FeLV testing, biochemistry and haematology analysis, RT-PCR, abdominal ultrasound.

Differential diagnosis: IVF, FeLV, renal failure.

Symptomatic treatment: Fluid therapy, Cerenia, Controloc, Arnetin.

On the 2nd day, blood was collected for biochemistry and haematology tests.

Table 3. Patient 2. Biochemistry

Parameters	Results	Reference interval
ALB	1.1 g/dl LOW	2.1-3.3
TP	15.3 g/dl HIGH	7.3-9.3
GLOB	2g/dl LOW	4.1-5.7
A/G	1.3	0.50-0.80
TB	0.2 mg/dl	0.0-0.3
GGT	5 U/L	0-5
ALT	216U/L HIGH	0-112
ALP	187 U/L HIGH	0-115
AMY	1300 U/L	0-1600
CRE	1.3 mg/dl	1.1-2.0
BUN	17 mg/dl	14-26
GLU	178 mg /dl HIGH	65-146
TC	80 mg /dl HIGH	70.00-150.00
CA	9 mg/dl LOW	8.50-10.00
PHOS	5 mg/dl	3.70-5.60
BUN/CREA	180 mg/dl	27.000-182.000

Table 4. Patient 2. Anaemia screening

Iron	58,1 umol/l	HIGH	8-31

Table 5. Patient 2. Blood count

Parameters	Results		Reference interval
RBC	3.97 T/l	LOW	5-10
Hematocrit	0.27 l/l	LOW	0.30-0.44
HB	76 g/l	LOW	90-150
Leucocytes	7.1 g/l		6-11
Neutrophils	44	LOW	60-78
Lymfocytes	48	HIGH	15-38
Monocytes	6	LOW	0-4
Eosinophils	2		0-6
Basophils	0		0-1
Platelets	271 G/l		180-550

Rapid test Ag IVF / FeLV = IVF negative; **FeLV = POSITIVE**

RT-PCR FeLV test = POSITIVE

Diagnosis: feline leukaemia

Paraclinical investigations: Considering the patient's history, it was recommended to evaluate the type of anaemia – evaluation of peripheral blood cell morphology (regenerative / aregenerative, microcytic / normocytic / macrocytic).

Result: Anaemia is non-regenerative, the change can be associated with the following pathologies:

- Reduction of erythropoiesis following a chronic inflammatory process (infectious-bacterial / viral / fungal)
- Spinal cord aplasia or hypoplasia
- Hypoplasia / selective aplasia
- Inefficient erythropoiesis: nutritional (Fe / Cu / CO / B9 / B12 deficiency
- FeLV-induced erythroid neoplasia

It is recommended to evaluate the possible causes with the testing of the indicated markers; If the tests are negative, a bone marrow biopsy is recommended.

Positive Ag FeLV is usually associated with haemolytic anaemia, which explains circulating iron levels and hypochromia. Lymphocytosis is observed, the more the cell morphology is indicated to detect possible changes in the lymphocyte.

Carriers are prone to leukaemia, mainly mediastinal lymphoma, immunosuppression.

Evolution: not monitored.

Management aspects in feline leukaemia

Maintenance treatment, including fluid therapy, if necessary, along with good medical care. Secondary infections should be treated immediately. Feline omega-interferon may reduce clinical symptoms and prolong the survival of sick cats. AZT (azidotimidine or zidovudine) may also be used, but with the risk of side effects. Cats infected with VLFe should be kept indoors and monitored regularly every 6 months.

Drugs that contain high-dose corticosteroids or other immunosuppressive drugs or that inhibit haematogenous marrow production should be avoided.

VLFe does not survive long outside the host and can be easily destroyed by disinfectants, heat and the dry environment. However, the virus can survive in faeces; may remain active in humid conditions at room temperature, for example in infected syringe needles or in refrigerated blood for transfusions.

Conclusions

• Diagnostic behaviour in leukaemia involves tests that generate high costs for owners such as blood biochemistry tests, haematology tests, rapid Ag or Ac tests, RT-PCR test, ultrasounds, etc.

• For financial reasons, most often, cats are presented late in veterinary offices, the treatments applied being often ineffective and the prognosis reserved.

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