Annals of *Spiru Haret* University

Veterinary Medicine

Year XVI, nr. 16, 2015

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ISSN: 1454-8283
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CONTENTS

EPIDEMIOLOGICAL RESEARCHES ON THE POISONING OF DOGS USING A RETROSPECTIVE STUDY

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Abstract

The pathology of toxic etiology in most cases is not explicitly approached in veterinary practice, as regards diagnosis, generally being achieved based on clinical symptoms, and rarely following certain toxicological examinations. For these reasons symptomatology in intoxications, especially in pets, is framed in syndromes with varied etiology, without stating a toxic etiologic agent and in this case, without establishing with certainty the incidence of intoxications at dogs. To establish the incidence and dynamics seasonal of intoxications in dogs was used retrospective longitudinal epidemiological investigation which followed the dynamics of intoxications to the dog during one year period, based on periodicals information detached from history and based on clinical manifestations correlated with laboratory tests. Seasonal dynamics of poisoning incidence in dogs was determined by statistical processing of data using Pearson linear correlation coefficient (r) by interpreting the coefficient of determination (r^2) by multiple linear regression. After processing the data drawn from history, clinical examination and laboratory tests the most common poisonings were with rodenticides (32.24%), followed by those with insecticides (14.42%).

Keywords: *dog, diagnostic, incidence, intoxication, presumptive*

Introduction

Pathology with toxic etiology isn't often regarded in veterinary practice, the diagnosis being usually based on clinical symptoms and rarely on toxicological exams. This is why usually the toxic cases, especially for pets, are considered syndromes with diverse etiology without naming the exact toxic and, in this case, without expressing the concrete incidence intoxications in dogs and cats.

In veterinary medicine toxicology is based on the importance of the dose-response principle, with a graduated and predictable response to the increase exposure to the toxic (6).

Animals are constantly exposed to a wide variety of foreign chemicals, many of which are potentially toxic and some of which result in the clinical poisonings. Pesticides are applied on or around animals for the control of insects and rodents. These chemicals may be placed in areas without regard for accessibility to household pets and domestic livestock. According to same epidemiological study, dogs were the most commonly poisoned species, particularly younger animals. The majority of cases in companion animals resulted from exposure to insecticides, although rodenticides (especially anticoagulants and strychnine) posed a significant risk (1). Pets' intoxications may have a complex etiology, the dog being exposed to accidental contamination with different pesticides (insecticides, rodenticides etc.), antifreeze, decontamination products, etc., as well as a result of a medical error, and here we talk about iatropaties due the disregard of the dosage, concentration, species, age, etc., or to the administration of human medicine. For example even as little as two tablets of an acetaminophen pain reliever can cause severe organ damage in a medium-sized dog. Because animals do not have the natural enzymes necessary for detoxifying and eliminating drugs, medications like ibuprofen and acetaminophen are a major cause of drug poisoning in dogs (10). Dogs tolerate aspirin better than cats; however, prolonged use can lead to development of gastric ulcers. Regular aspirin at dosages of 25 mg/kg, acute ingestion of 450–500 mg/kg can cause GI disturbances, hyperthermia, panting, seizures or coma. Also ibuprofen has been recommended in dogs at 5 mg/kg. However, prolonged use at this dosage may cause gastric ulcers and perforations. GI irritation or ulceration, GI hemorrhage and renal damage are the most commonly reported toxic effects of ibuprofen ingestion in dogs (12).

It is also well known that for the dog certain foods destined for humans are quite toxic, foods such as chocolate, avocado, grapes, etc. Chocolate is derived from the roasted seeds of *Theobroma cacao*. The primary toxic principles in chocolate are the methylxanthines theobromine (3,7-dimethylxanthine) and caffeine (1,3,7-trimethylxanthine). The LD_{50} of both caffeine and theobromine is reportedly 100–200 mg/kg, but severe signs and deaths may occur at much lower dosages and individual sensitivity to methylxanthines varies. In general, mild signs (vomiting, diarrhea, polydipsia) may be seen in dogs ingesting 20 mg/kg, cardiotoxic effects may be seen at 40–50 mg/kg, and seizures may occur at dosages ≥ 60 mg/kg (11).

Dogs metabolized theobromine much more slowly than humans, the biological half-life of theobromine is 17.5 hours; in severe cases, clinical symptoms of theobromine poisoning can persist for 72 hours (3).

Toxic plants represent a different etiology seeing as intoxications can occur after ingesting *Aloe vera; Amaryllis* -lily*; Convallaria mayalis* – lily-of-the-valley; *Philodendrons; Chrysanthemums; Cyclamen persicum; Nerium oleandrum* etc. (4).

The particularities regarding species and especially, race, may as well be leading to intoxications with certain substances for which the dog is susceptible. This is the case for the insecticides which contain ivermectin. The following breeds are most likely to be affected: Old English Sheepdog; English Sheepdog; Shetland Sheepdog (Sheltie); Australian Shepherd; German Shepherd; Long-haired Whippet; Silken Windhound; Skye Terrier; Collie. It is also seen in mixed-breed dogs. Ivermectin prevents or kills parasites by causing neurological damage to the parasite, resulting in paralysis and death for the parasite. But dogs genetically sensitive to the medication have an anomaly that allows the ivermectin to pass the dog's blood-brain barrier and into its central nervous system, which can be lethal for the animal. Symptoms for the dog may be acute or mild. Acute signs will become apparent within 4 to 12 hours of the drug's administration (9).

Material and methods

In order to set the share of intoxications in dogs the monitoring of casuistry was carried for a period of one year in a veterinary practice on 500 cases with diverse etiology. Monitoring the casuistry meant:

- selecting the casuistry with toxic etiology based on the clinical and laboratory diagnosis;
- creating the database needed to assess the incidence of dog intoxications;
- estimating the incidence and seasonal dynamics of dog intoxications encountered during the monitoring period.

Creating the primary database was made using *the retrospective longitudinal epidemiologic investigation* in order to set the incidence and the seasonal dynamics of dog intoxications. The primary database was built based on the cases with diverse etiology in the observation charts for one year. This helped processing the incidence in a mathematical way, representing the number of new diseases which appear in a population in a certain time frame (day/month/year etc.).

Data processing was done by collating the information based on the value of biochemical and serological parameters obtained after performing additional tests and on the information gathered from clinical examination.

The complementary examinations carried out in order to establish the toxicological diagnosis were done by performing biochemical and hematological tests on Vet-Test and LaserCyte apparatuses which belong to the IDEX line. These machines work on plasma/serum as well as ETDA blood.

The clinical diagnosis of poisoning took into account both general symptoms and characteristic signs of toxic syndromes. Clinical examination used general signs adressed by a full semiological exam (palpation, thermometry etc.).

The *seasonal dynamics* of the incidence of toxic events for dogs and cats was established through the statistical processing of data by using the Pearson linear correlation coefficient (r), by interpreting the coefficient of determination (r^2) by multiple linear regressions. This is the method also known as "regression", "linear regression", "multiple regressions" or "the smallest squares" when there's a model built. The purpose of multiple regression (term used by Pearson in 1908) is to highlight the relationship between a dependent variable (explained, endogen) and a multitude of independent variables (factorials, exogenous, predictors). By using the multiple regression it is often tried to obtain the answer to questions such as "which is the best prediction for...?", "who is the best predictor for...?"

The statistical processing of data was done by using the Quatro pro Excel Statistic program with a graphic and/or diagrammatic play.

The data obtained as a result of the epidemic-toxicological survey gave further knowledge regarding the general aspects of possible causes which led to presumptive diagnosis of intoxication.

Results and discussions

Out of the 500 cases with diverse pathology recorded during a year, the smallest share belonged to intoxications in dogs and cats (39 cases, meaning 7.8% of the cases) whereas 92.2% of cases were surgical, infectious or parasitical affections (Fig. 1):

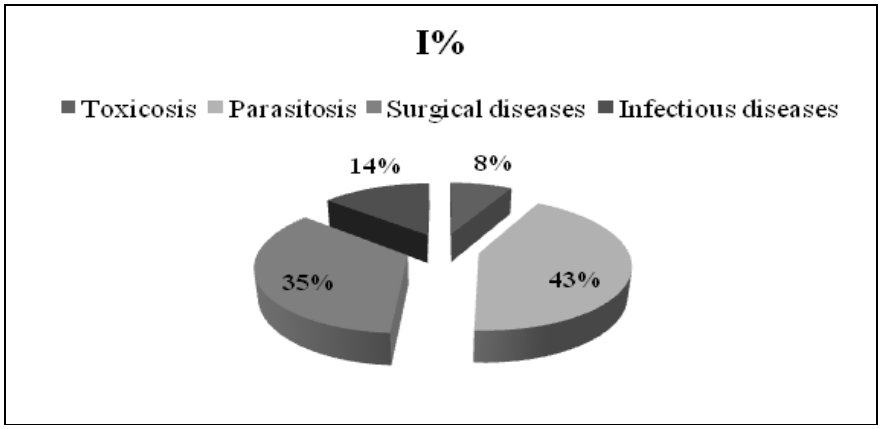

Fig. 1. *Share poisoning cases during the study period*

Out of the 39 cases of intoxications for 41% of them the diagnosis was set based on clinical symptoms and hematological and serum tests; for the rest of 59% of the cases the diagnosis was set based only on the clinical signs and the history the owner told. It is important to mention the fact that during this particular study case, due to economic factors, no case was diagnosed through a toxicological exam. The fact that in present there are less than 5% toxins with an antidote or an efficient antagonist is well known, as well as the fact that veterinary toxicological laboratories can detect about 200 or 300 toxic substances due to the price lists, the time needed in order to obtain the samples, the run tests, the sample volume and the large number of existing potential toxic substances (6).

Out of the total 39 animals which were intoxicated, 28 of them (71.8%) happened to dogs.

In creating the database necessary to estimate the incidence and seasonal dynamics of poisonings in dogs variables were taken into account in order to efficiently process the statistics. Establishing the *presumptive* diagnosis took into consideration the history of the patient as well as clinical and laboratory data.

The dynamics regarding the incidence of poisoning in dogs for the study period was quite varied, as follows (Table 1):

Table 1

The dynamics regarding the incidence of poisoning in dogs

Etiological agent	Ascaron	Insecticide	Medication (antiinflammatory)	Rat poisons	Renal failure (ammonia)	Antifreeze	Detergent	Unknown agent
I%	3,57%	14,428%	3,57%	32,14 %	7,14%	7,14%	3,57%	28,5%

During the study period the most often intoxications encountered in dogs were the ones based on rat poisons (32.24%) followed by insecticides (14.42%), ammonia (7.14%), antifreeze (7.14%), ascaron (5.57%) and washing powder (3.57%) while an important number of cases remained with an unclear toxic agent (28.5%) (Fig. 2):

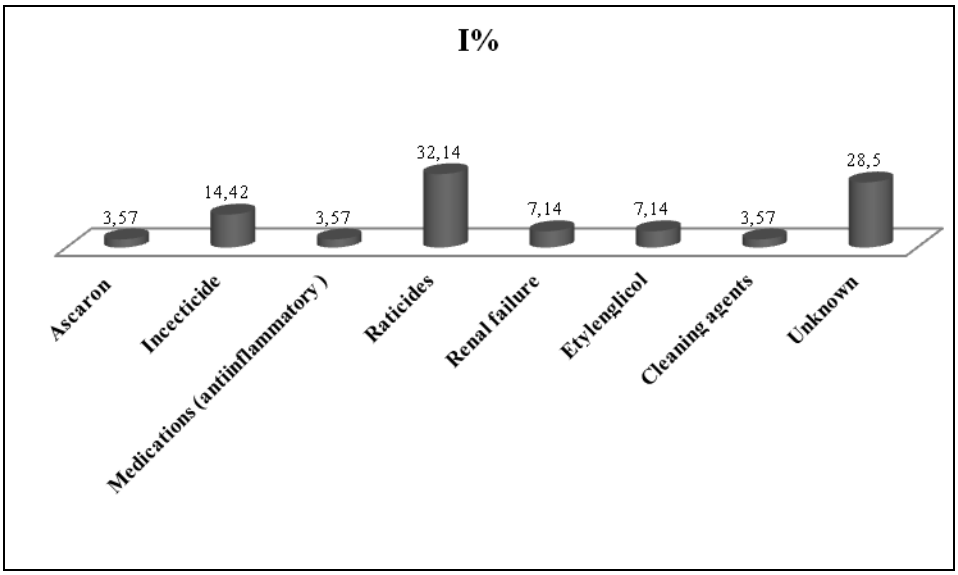

Fig. 2. *The incidence of poisoning in dogs*

Dogs get intoxicated with rat poisons more often than other substances because such disinfestation are regularly twice a year and substances necessary to eliminate rodents are easily accessible to quadrupeds (5).

Insecticide poisoning follows-up close due to the frequency with which these disinfections are made throughout the year. The number of organophosphoric, organochlorine or piretrine intoxications are lower than the rest because, unlike the scheme of getting rid of rats which takes place twice a year, the disinfections take place only once a year either in spring or

in summer when the weather starts to warm up and thus the incidence is lower due to the shorter exposure to the toxic agent.

According to the statistic data from litterature, in 2012, the *ASPCA Animal Poison Control Center (APCC)* in Urbana, Illinois, handled more than 180,000 cases about pets exposed to possibly poisonous substances. Topping the toxins list for the fifth year: prescription human medications; insecticides; over-the-counter human medications; veterinary medications; household products; people food; chocolate; plants; rodenticides; lawn and garden products (8).

A retrospective study was conducted on the exposure of dogs and cats to drugs, reported to the Poison Control Centre of Milan (Centro Antiveleni di Milano (CAV) between January 2006 and December 2012. Calls related to drugs for human use and veterinary drugs accounted for 23.7 per cent of total inquiries (1415) received by CAV and mostly involved dogs (70 per cent of enquiries). The most common class of drugs for human use proved to be CNS drugs (26.8 per cent), followed by NSAIDs (19.6 per cent) and cardiovascular and endocrine drugs (12.9 per cent each) (2).

Seasonal dynamics and incidence trend line of dog intoxications show that the most new cases of disease were recorded in august, september and march (4 cases). During the epidemiological monitoring period it appeared that in January there were no cases of dog intoxications (Fig. 3).

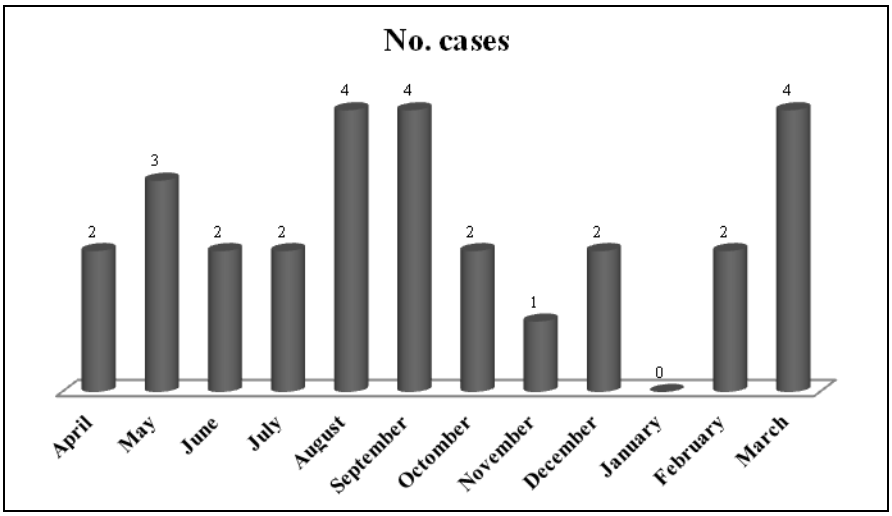

Fig. 3. *The percentage of cases of poisoning in dogs*

Seasonal dynamics of the incidence of intoxications in dogs during the study period presents a lightly descendent trend line for winter months thus making significant weak correlations between the frequency with wich new cases appear and the season (r^2 = 0,015). The highest frequency was recorded in spring-summer (Fig. 4).

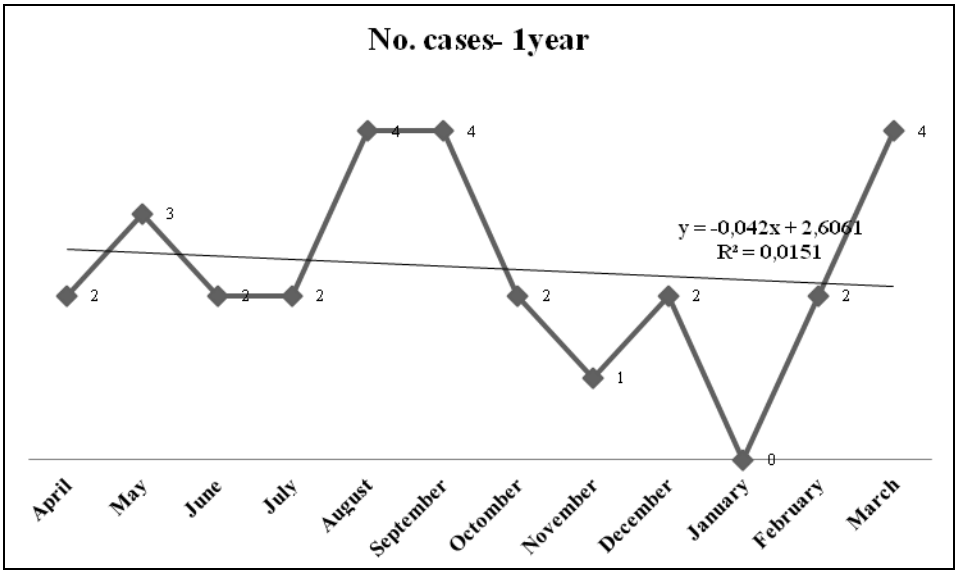

Fig. 4. *Seasonal dynamics and trend line of poisoning incidence in dogs*

The months with the lowest frequency of intoxications were march, august and september. After taking all data into consideration it can be assumed that during spring and summer the risk of a dog to get intoxicated is greater than in autumn or winter and this is due to the intoxications with rat poison (32.14%) and insecticides (14.42%) out of the total number of intoxications. Thus this situation could be blamed on the rise in temperature and on the apparition of insects, microorganisms and vermin which lead to the use of toxic substances. Once the temperature starts rising so does the frequency of bugs (fleas, ticks, mosquitos etc.). In order to fight this effect usually people result to certain toxic insecticides. The transition to cooler seasons led to other types of intoxications. Antifreeze attracts curious animals the same way rat poison does it, and its sweet taste determines the animals to consume it in excess once they tasted it (7).

Conclusions

In order to determine the incidence as well as the seasonal dynamics of poisonings in dogs for a year we used *the retrospective longitudinal epidemiological investigation.*

Out of the grand total of 500 cases with diverse pathology for 39 cases (71.8%) the presumtive diagnosis of intoxication was made.

Out of the 39 intoxicated animals 28 cases (71.8%) made the casuistry for dogs whereas the share of cat intoxications was as low as 11 cases (28.2%).

The most common intoxications in dogs were those with **rat poison** (32.24%), followed by those with **insecticides** (14.42%), **ammonia** (7.14%), **antifreeze** (7.14%), **ascaron** (5.57%) and **washing powder** (3.57).

A significant percent represented the intoxications due to unknown agents (28.5%) which imposes the need of toxicological tests in order to put a clear diagnosis.

Seasonal distribution of the new intoxications cases shows that the most *new cases of disease* were registered in august, september and march (4 cases), while in january there were no reports of intoxications in dogs.

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THE HUMAN – ANIMAL RELATIONSHIP IN PET WELFARE ASSESSMENT

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Abstract

Due to the increase in pet numbers, particularly in dogs, human – animal relationship study has become an extremely important topic for both animal and its owner. Owners expectations of their dogs are closer to human behaviour than to animals'. Cohabitation with a dog is not easy especially when the owner does not have minimum knowledge of canine behaviour and psychology. The purpose of this study has been to assess the manner in which human - animal relationship may be used in establishing pet welfare levels. The study has been carried out by interviewing dog owners or companions (n: 86) in veterinary practices and specially designed dog areas in parks. The people interviewed answered a questionnaire on the topic of their relationship with their pet. Data obtained showed that 60% of the animals live together with their owners in an apartment, 30% of the dogs live in a house but have access to a yard and 10% of the animals spend their entire time in the yard. Animal socializing behaviour was strongly influenced by the time they spent indoors versus outdoors. All dogs that have spent more than 6 hours daily outdoors socialized faster with both other animals and humans, while others needed a longer period of time to approach strangers. Thus human – animal relationship may be an important indicator in pet welfare assessment.

Keywords: *behaviour, dog, relationship, welfare*

Introduction

Due to the increase in number of pets in the recent years, especially in dogs in both our country as well as in the European Union, research in the human – dog relationship has become an important topic for both the animal and its owner. Expectations of owners from their dogs seem to be closer to human like behaviour than canine. Cohabitation with a dog is not simple especially when the owner has little or no knowledge of the specific canine psychology. Life in a close relationship with humans has all the while compelled pets to adjust their behaviour to the owner requirements and

those of their new environment [3, 4, 5]. This aspect has forced dogs to continuously adapt and develop certain abilities that they would not normally have. Csanyi (2005) demonstrates that these animals can mimic humans, can feel emotions, cooperate and obey their commands.

The purpose of this research was to uncover as much as possible of the manner in which owners treat their pet dogs in an attempt to establish the causes that may lead to a faulty relationship between them.

Materials and methods

The research has been carried out over the course of a year in two veterinary practices and three public parks that featured specially designed dog areas. The research consisted in interviewing dog owners whom each received a questionnaire to fill out. The questionnaire was anonymous and was placed in the waiting room of the veterinary practices, while in the parks it was handed out to the dog owners or their companions. 14% of the people that were handed out the questionnaires did not wish to answer or filled them out incompletely.

Only the questionnaires with complete answers were included in the study (n: 86). Out of this number 32 questionnaires were filled out in the two veterinary practices and the rest in the dog areas of the parks. 91% of the interviewees were pet owners, the rest were companions.

The 16 questions framed by the questionnaire focused on the owner – pet relationship and the manner in which this influences animal behaviour towards the owner as well as to other animals all the while establishing a first level of the relationship that proved to be a useful animal welfare indicator.

Results and discussions

Data obtained showed that across the 86 questionnaires included in the study, 60% of the animals live their entire life in an apartment together with their owners, 30% of the dogs live in a house and have access to a yard and only 10% of the interviewees have stated that their animals spend their time outdoor/in the yard, freely (figure 1).

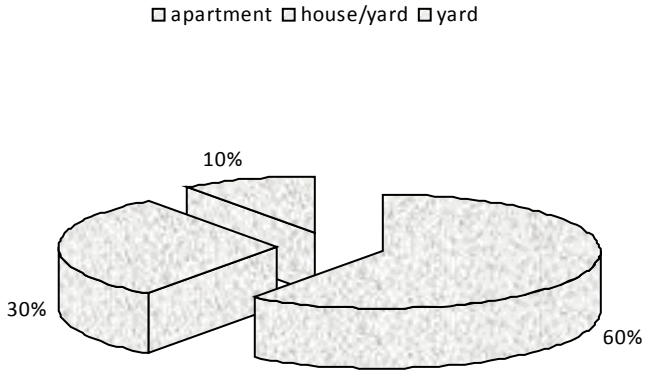

Figure 1. *Percentage (%) of the place dogs live their lives*

Out of the dogs that live in a house but are permitted access to the yard 50% spend between 2 and 6 hrs/day outdoor and the rest indoor, 49% spend between 6 and 12 hrs/day in the yard and only 1% will spend more than 12 hrs/day outdoor (figure 2).

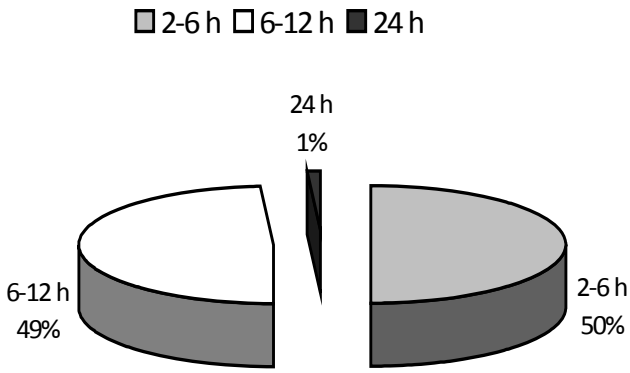

Figure 2. *Time (%) spent by dogs with yard access*

Unlike the former, dogs living with their owners in an apartment (figure 3) will spend more than 20 hrs/day indoor, which means they will spend up to 3 – 4 hrs/day outdoor (in the park, on a leash on the street).

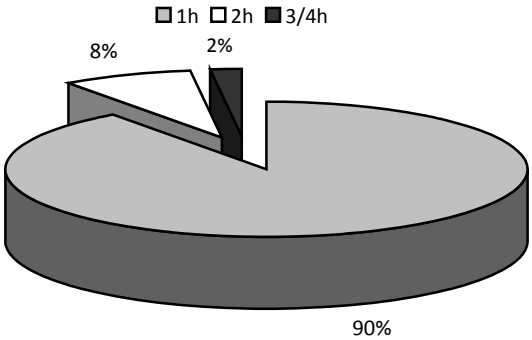

Figure 3. *Time (%) spent outdoor by dogs living in an apartment*

Differences among dogs spending more time outdoor were significant compared to those that do not exceed 3 hrs/day outdoor (90% of dogs living in an apartment).

When answering the question about dogs attitude towards family members – in various positions – results have indicated that 15% will behave aggressively (out of the group of dogs living in an apartment) and only 1% of the animals living in a house/yard will display the same behaviour (figure 4).

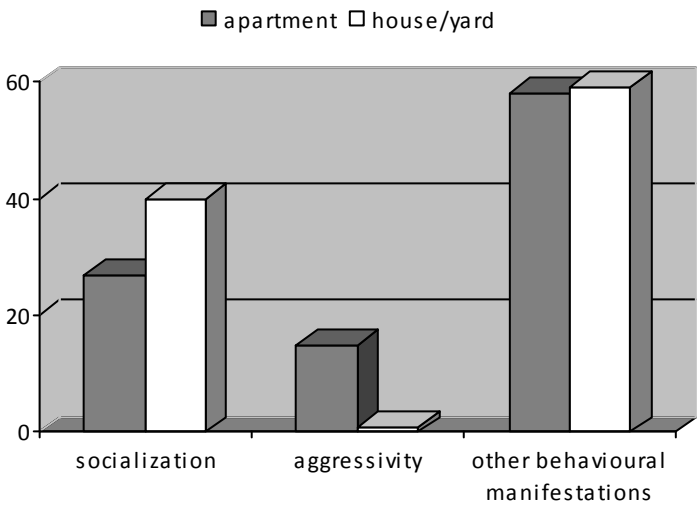

Figure 4. *Socializing and aggressive behaviour (%) in dogs investigated during the research*

This aggressive behaviour witnessed by the owner was difficult to interpret. In the case of owners we have interacted directly with, requiring more details with their answers, we discovered that this aggressiveness was not abnormal behaviour, just a manner in which the animal was trying to attract the owner's attention. In the case of owners interviewed in the veterinary practices however it was difficult to assess the animal's aggressiveness levels based solely on the statements received. We can solidly affirm that animals living in an apartment are less sociable with strangers and even towards other dogs in certain cases due to their limited interaction offered by the owner. It is also clear that these animals are 57% more territorial about their owners compared to those which will spend more time outdoors. The latter will socialize more both with strangers and other dogs due to their longer time spent outside their house/yard.

Duffy et al. (2008) have stated that canine aggressiveness poses serious public health and animal welfare issues. There are breed differences as far as aggressiveness is concerned which have been established based on statistical reports on aggressive behaviour.

It is difficult to assess whether the number of people interviewed was sufficient or not to draw relevant conclusions. Although aware of the fact that the questionnaires were anonymous, a very large number of owners interviewed have not answered accurately and truthfully, potentially influenced by the a legislative bill regarding the tax paid by owners of non-neutered dogs (this has transpired following individual discussions with owners).

Many of the owners whose dogs lived in apartments realised how little time they spent with their pet or how little they knew them only upon filling out the questionnaire and therefore tried to alter their answers. Others were surprised at how long a period the animal would be confined to the apartment each day (20 hrs), realising that there was in fact very limited time dedicated to outdoor exercising, socialising with other animals and humans. A part of the interviewees have realised during this conversation that they do not know much about their pet's behaviour, its reactions, and tried to adjust their answers avoiding an honest approach.

Conclusions

Although our research was based on the owners' account of their relationship to their pet – dogs, both in their living quarters and outdoors, not all owners were willing to participate.

The majority of the owners living in an apartment were astonished to realise, following filling out the questionnaire, that they interact very little with their pet.

Animal socialising behaviour is profoundly influenced by the time spent inside the house compared to how much it will stay outdoors. All dogs that have spent more than 6 hrs/day outdoors have socialised faster with other animals and people, while the others needed a longer period of time to approach strangers. Fear was displayed in a larger number of animals that have spent 1 – 2 hours/day outside.

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IDIOPATHIC FACIAL DERMATITIS OF PERSIAN CATS: CASE REPORT

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Abstract

A 3 year-old, neutered female Persian cat was referred to us with an 10 month history of facial dermatitis. This vaccinated cat lived in a house with 4 other cats. Other than the chronic skin disease, it had no history of medical problems. The skin condition first appeared around the age of 20 months, causing increasingly frequent facial lesions which eventually became chronic, with intermittent flare-ups. Daily local treatment was required, without which the cat would scratch intensively, further exacerbating the lesions. Corticosteroids appeared to have positive but transient effects. Recent FeLV/FIV tests had been negative. A blood test comprising routine haematology and biochemistry had shown no abnormalities, other than eosinophilia. All the animals in the household received flea treatment and the environment was treated twice a year. General examination was satisfactory. The owner indicated that during flare-ups, the cat's behaviour changed and she tended to isolate herself. She also pointed out that at these times, the cat often developed severe conjunctivitis, but no respiratory signs. The skin disease was localised to the face only, affecting the eyelids, facial and nasal folds, lips and chin. There was marked conjunctivitis. The lesions were highly inflammatory with marked erythema. A large number of black follicular casts, adherent to the hairs, were noted in all these areas.

Keywords: *cats, dermatitis, facial*

Introduction

The skin disease was localised to the face only, affecting the eyelids, facial and nasal folds, lips and chin. There was marked conjunctivitis. The lesions were highly inflammatory with marked erythema. A large number of black follicular casts, adherent to the hairs, were noted in all these areas. Presented with this highly inflammatory facial skin disease in a young cat, we considered the following differential diagnosis: allergic dermatitis (age of onset, pruritus, response to corticosteroids), dermatitis associated with herpesvirus infection (facial dermatitis and conjunctivitis), idiopathic facial dermatitis of Persian cats or IFDP (distribution, clinical appearance of

adherent black debris, age, breed), or less probably atypical dermatophytosis or calicivirus infection. The clinical appearance of the blackish adherent material could also have suggested feline acne, but the latter only affects the chin and lips or occasionally the nasal commissure, and does not have such a widespread facial distribution. In all cases, complicating factors such as pyoderma, *Malassezia* dermatitis and/or secondary demodicosis had to be investigated. Impression smears: significant amounts of eosinophils, neutrophils, and both phagocytised and extracellular cocci. No *Malassezia.*

- Negative skin scrapings
- Negative Wood's lamp examination
- Samples collected for fungal culture

To treat the secondary infection, we prescribed an antibacterial treatment only, consisting of marbofloxacin at approximately 2 mg/kg/d, and local treatments were continued until biopsies were carried out. Further examinations:
(local treatments were stopped 3 days before)

- Skin biopsies and conjunctival samples were collected using a cytology brush
- *Chlamydia* testing was also carried out on the conjunctival samples only): negative
- Biopsies for histology:

The biopsies showed marked epidermal hyperplasia (acanthosis) with moderate spongiosis and an inflamed dermis with a large number of eosinophils, neutrophils and mastocytes. The sebaceous glands appeared to be enlarged. A few apoptotic keratinocytes were identified within the epidermis. These histological findings were compatible with, but not specific to, IFDP.

- Fungal culture: negative

Diagnosis

The distribution and aspect of the lesions, together with the test results, were suggestive of idiopathic facial dermatitis of Persian cats. While this diagnosis was very likely, it was not definite. The cat was therefore started on an exclusion diet.

Treatment and disease progression:

The regular flea treatment already instituted in the household was maintained.

A first exclusion diet was carried out for 6 weeks, using commercial hypoallergenic cat food, but the owner was warned that a second diet containing different proteins may be required depending on the results of the first trial (given the diversity of cats' diets, it is usually impossible for an owner who prefers to use ready-made foods to succeed in feeding the cat only novel proteins at the first attempt). Two strict dietary trials were carried out, without success.

During this time, and having taken into account its interference with the exclusion diet, a treatment consisting of corticosteroids, using low dose dexamethasone at 0.05 mg/kg/d, and doxycycline at 10 mg/kg/d was prescribed for the first 3 weeks of the dietary trial, in order to reduce the severe inflammation and soothe the cat. Daily treatment was still meticulously carried out by the owner (removal of debris with a flea comb and disinfection using a diluted chlorhexidine solution). For the full length of treatment, the cat was in good condition, but 2 days after treatment cessation, the inflammation recurred.

A trial using only doxycycline (for its antibacterial and immunomodulatory effects) was carried out for 3 weeks, without success.

Following the exclusion diets, considering that only corticosteroids had an effect, but a transient one, we decided to try off-license cyclosporine treatment. Positive results have been obtained by some authors. We prescribed Atopica 25 mg at a dose of one capsule per day, equivalent to approximately 5 mg/kg/d. During the first month of treatment, the cat improved rapidly (in approximately 10 days), and its condition even became very satisfactory (revisit 1 month later), but once the dose was reduced to alternate day therapy, the skin disease rapidly returned and secondary infection set in.

As the owner did not wish to pursue daily treatment due to costs and administration problems, we suggested a trial with omega interferon for its modulatory effect. The suggested protocol, never previously attempted to the best of our knowledge, consisted of 2 subcutaneous injections of 5 MUI per week for 3 weeks. A very marked improvement was observed after the 3rd injection and the cat presented very few debris on its chin after the 6^{th} injection, in the absence of local treatment. However, the lesions reappeared after 10 days and gradually became increasingly severe.

Discussion:

Idiopathic facial dermatitis of Persian cats (IFDP), also known as "dirty face syndrome" or idiopathic facial seborrhoea of Persian cats, is a recently described condition which seems to affect only Persians and Himalayans. This rare facial skin disease, presumed to have a hereditary origin, takes hold progressively in young Persians, without any gender

predisposition, and presents with highly suggestive lesions. The chin, lips, periorbital area and facial folds are most commonly affected. Findings consist of dark debris, adherent to the hairs, which accumulate as the disease progresses, associated with erythema and exudation. Pruritus is not always a feature initially, but it gradually increases and may become very severe with chronic disease and secondary infections, which are common (pyoderma and *Malassezia* dermatitis). In some cases, otitis externa and/or submandibular lymph node enlargement are found. In the present case, as well as significant blepharitis, marked conjunctivitis was noted and two episodes of corneal ulceration occurred.

The causes of this keratinisation disorder remain unknown.

Diagnosis is primarily clinical, but the presence of this black adherent debris with underlying erythema is not specific. These signs are also observed in feline acne or *Malassezia* dermatitis secondary to allergic dermatitis or severe systemic. Age of onset is not a sufficient criterion as it also corresponds to the age of onset of allergic dermatitis. Dermatitis associated with herpesvirus infection, or even calicivirus infection must be ruled out (these usually cause non-symmetrical lesions, with crusting but no adherent black material). Demodecosis has an extremely variable presentation and must be ruled out. Secondary bacterial infections, often considered rare, should be investigated. Acanthosis is present and often severe, associated with moderate spongiosis. A small number of apoptotic keratinocytes are found throughout the epidermis. Basal cell vacuolation is occasionally seen, but there is no real interface dermatitis (as is found in other types of immune-mediated skin disease), particularly as the inflammatory dermal infiltrate is mixed (neutrophils, macrophages, eosinophils, lymphocytes and mastocytes). In allergic skin disease, spongiosis is also found but there is no apoptosis. The main differential diagnosis is therefore allergic skin disease, particularly when there is secondary *Malassezia* dermatitis, with the diagnostic challenges that this represents. Regular local treatment using flea combs and various topical antiseptic, antibacterial or antifungal agents is very helpful, although it requires considerable commitment. Some systemic treatments help certain cats, but the response is unpredictable. Corticosteroids reduce inflammatory flare-ups but their effects are short-lasting and side-effects may be seen in Persians treated regularly from a young age.

Cyclosporine can have positive effects at a dose of 5 mg/kg/d in some Persians, but it requires daily administration. In the present case, we tried it for one month with positive results, but soon after the dose was reduced to alternate day therapy, the condition worsened again.

We also attempted omega interferon treatment for its modulatory effects.

The tested protocol consisted of 2 subcutanoeous injections of 5 MUI per week for 3 weeks. There was clear improvement after the 3rd injection and the cat presented only very minor amounts of debris on its chin after the 6th injection, in the absence of local treatment. However, the lesions reappeared after 10 days and progressively worsened. It would be worth repeating a trial of this protocol, never previously attempted to the best of our knowledge, using a weekly maintenance injection at a lower dose to limit costs (the drawbacks of an injectable treatment would, however, remain).

The other aspect of treatment which should not be overlooked is that of secondary bacterial and fungal infections, which should be treated appropriately.

Idiopathic facial dermatitis of Persian cats is therefore a condition that carries a guarded prognosis and that clinicians need to be aware of, to ensure owners are informed of its chronicity and the fact that treatment is complex, demanding, has a variable success rate and may be costly. All these factors may lead to early euthanasia in some affected cats.

CLINICAL AND LABORATORY EVALUATIONS IN CRYPTOSORIDIOSIS IN CALVES

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Abstract

This study took place from March to May 2013 and was conducted in the Farm 1 of Research and Development Institute for Cattle Baloteşti, Ilfov County. The aim was to establish guide values about incidence of cryptosporidiosis using speed tests (Rainbow Calf Scour 4) and parasitological exams: Ziehl – Nielsen staining, Anderson flotation method, zinc sulphate flotation associate with clinical signs.

From 10 calves, on coproparasitological exams using Ziehl – Nielsen staining from feces, was tested positive 2 calves by Cryptosporidium *oocyst identification. On coproparasitological exams using zinc sulphate flotation was identificate* Eimeria *unsporulate oocyst and by speed tests (Rainbow Calf Scour 4) was detected one infection with coronavirus. Clinical signs was: diaorhea with yellow-green feces, tenesmus, cramps, loss of appetite, fever.*

Keywords*: cryptosporidiosis, calves, Ziehl – Nielsen staining*

Introduction

Cryptosporidiosis, neonatal opportunistic sporozooza extends across the globe either under clinically inapparent or sporadic forms or epidemic episodes in both human and animals. Following epidemiological investigations, cryptosporidiosis have been identified in sick animals and in the clinically healthy ones from Scandinavia to Australia, the US and Europe to Japan, which shows the worldwide evolving character (Dărăbuş, Gh., 1996, 2001). The various morphological, biological and genetic features differentiating species of *Cryptosporidium* from others is a major problem for practicing veterinarians and physicians in human medicine directly involved in the control of this morbid entity, in understanding the way in which the *Cryptosporidium* infection is transmitted. Also, a lot of studies reveal that, by means of routine clinical or microscopic examination, the *Cryptosporidium* species involved in the infection cannot be exactly

defined (Dărăbuş, Gh., 1997). A study conducted in Romania upon 2802 cattle indicated that the most responsive age to *Cryptosporidiosis* is between 8-14 days. Significant differences were observed ($p < 0.001$) between infection at this age and other categories taken into account (Dărăbuş Gh., 2001). The incidence of infection decreases with age, so positive calves were not detected between the ages of six and twelve months. This decrease in infection extensity can be explained partly on account of age resistance and partly by the immunity gained from repeated contact with the parasite. The presence of a relatively high extensity (8.6%) of infection in cattle over the age of 3 years is probably due to the fact that this category was made up of newly calved cows exclusively (Akam, D and colab., 2001).

Materials and methods

The study took place from March 10 to May 4, 2013 and was conducted within Farm 1 at Research and Development Institute for Cattle in Baloteşti village, county of Ilfov, on a total of 42 cattle aged 0-6 months. 10 calves were part of the study (6 males and 4 females), aged between 5 days to 4 weeks, 3 being raised in maternity and 7 in berth breeding system.

Clinical examination

Serological by immunochromatography lateral flow – Quick Test Rainbow Calf Scour 4

Coproparasitological exams

Quick Test Rainbow Calf Scour 4

This test is composed of five devices each of them containing four strips [red-rotavirus, yellow-coronavirus, blue-e Coli F5 (K99) and green-*Cryptosporidium parvum*] and five tubes with reagent and faeces sampling device. (Peeters, J., Villarcota, 1995)

PROCEDURE

1 – Take feces directly from the anus [if the feces are liquid will take a spatula full (**Fig. 1**)].

2 – Homogenize feces and reagent in the tube and after homogenizing slightly flick the tube against a hard surface so that the liquid is collected at the bottom (**Fig. 2**).

3 – Remove the device from drying tube strips and the reagent tube is inserted therein (**Fig. 3**).

4 – Screw the cap with strips until you hear two clicks, which indicates piercing the septa of the reagent tube; then leave the device on a flat surface and wait for 10 minutes. The liquid in the tube will migrate to reactive strips (**Fig. 4).**

5 – After 10 minutes read results (**Fig. 5, 6**).

Fig. 1. *Sampling faece*

Fig. 2. *Reagent homogenization*

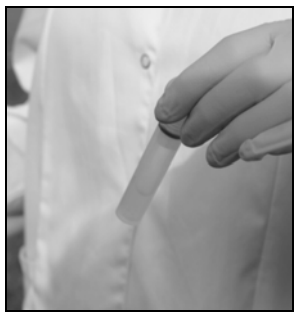

Fig. 3. *Strip contact*

Fig. 4. *Strip contact II*

Fig. 5. *Reading results*

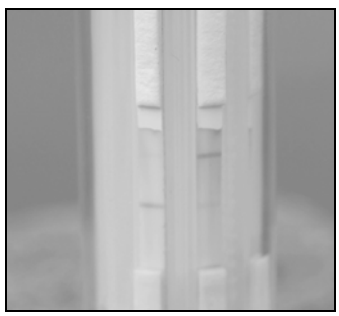

Fig. 6. *Interpretation of results*

Ziehl Neelsen method

Feces smears are fixed with methanol or ethanol for 5 minutes. After removal of the alcohol and air drying, staining with Fuxin fennel (Fuxin Ziehl) for one hour. Stained preparations will be washed in tap water and then differentiated into a 2% solution of sulfuric acid for 20 seconds, stirring the slides continuously. After washing in tap water, the slides will be stained for 5 minutes with 5% solution of malachite green. Rinse again in water, dried in air and examine x40 or x100 objective with immersion.

Cryptosporidiosis appear as spherical or ovoid formation of 4-6 microns, in vivid red color on a green bluish background. Their cytoplasm is granular, with a center often clearer and containing 0-6 sporozoites. By this technique, other non-acid-fast elements from feces are stained by passing through sulfuric acid and then colored green due to

counterstaining. Cells, bacteria and yeasts thus appear green, easily differentiating from Cryptosporidiosis. However, sometimes parasitic elements that do not stain or appear pink may occur, having the same shape and size with bright coloured oocysts. Other coccidia are also stained in vivid red colour, but they are larger. Moreover, there are also traces that appear to be round where oocysts have been placed (Kehl KS, 1995).

Flotation methods

Sheather's sucrose method

Faeces are homogenized with 2 ml formalin, filtered through cheesecloth and put it in a conical centrifuge. Sheather solution is added (500 g of sucrose were dissolved in 320 ml of distilled water containing 6.5 g phenol melted in water bath) until the centrifuge cup is full. Centrifugation is performed at 500 revolutions/min., for 15 minutes. It is a fast, sensitive method and it is recommended for screening.

Anderson's suchrose method

1-5 g feces are mixed by 10-15 ml of water. Mix well and filtered through six layers of cheesecloth. The filtrate is centrifuged 10 min at 500 rev/min. The deposit obtained is stirred with 10 ml of saturated sucrose solution after the formulations given below. Centrifuge again at 500 rev/min., for 10 minutes. The liquid from the surface of the centrifuged tube will be taken with a platinum loop, 4-7 micrometers in diameter and examined between microscope slides with × 40 and × 100 objectives immersion. Sucrose solution density is between 1.27 and 1.29. You can use two types of solutions of sucrose Sheather: – 1500 G sucrose + 320 ml distilled water + 6.5 g of phenol.

Cryptosporidiosis oocysts appear as round microorganisms with a diameter of 5-6 micrometres fine-grained, with a prominent black spot (residual body). Intrinsic color ranging from pink to blue gray in light microscopy. Reading the slides should be made so as not to take more than one hour from execution as it destroys oocysts. Sucrose flotation, before staining methods, has the advantage that by concentrating parasitic elements makes it possible diagnosis of cryptosporidiosis, even in cases of poor infection. Cryptosporidiosis is recognized based on size, shape, colour and the presence of internal structures. Due to its complexity this method lends itself especially to confirm the diagnosis in doubtful evidence. It is not recommended for routine diagnosis. The effectiveness of the method has proven to be 100% in the diagnosis of cryptosporidiosis in fecal samples obtained from calves.

Results and disscusions

Of the total of 10 calves in the study, a number of 2 heads (20%) were identified with Cryptosporidiosis, by coprological examination. The 10 samples collected were examined by two sucrose flotation techniques (Sheather and Anderson) and by staining Ziehl-Neelsen method. In a single sample *Cryptosporidium* oocysts were revealed by all the three methods, in the other one the method Sheather being negative. (Table 1)

Table 1

Coproparasitological examination methods used

Methode	P_1	P_2	P_3	P_4	P_5	P_6	P_7	P_8	P_9	P_{10}
Feces smear Ziehl-Neelsen stain	negativ	pozitiv	negativ	negativ	pozitiv	negativ	negativ	negativ	negativ	negativ
Sheather flotation	negativ	negativ	negativ	negativ	pozitiv	negativ	negativ	negativ	negativ	negativ
Anderson flotation	negativ	pozitiv	negativ	negativ	pozitiv	negativ	negativ	negativ	negativ	negativ

In Ziehl-Neelsen staining oocysts appear red, with various intensities, with the property of being acid-resistant which means they retain Fuxin Ziehl and after contact with the acid-alcohol solution they appear evident as compared to the blue or green background (Figure 7). Their size is 4-6 micrometres, egg-shaped, in which there constantly can be distinguished sporozoites, six in number arranged semicircular. In the samples examined their presence was not significant (Figure 8)

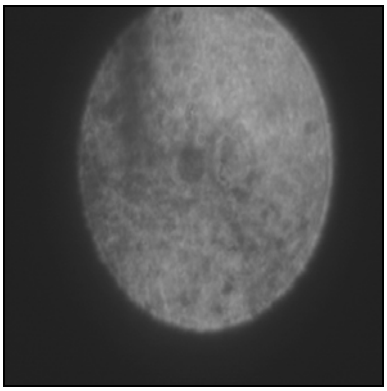

Fig. 7. *Cryptosporidium* oocyst (Ziehl-Neelsen staine)

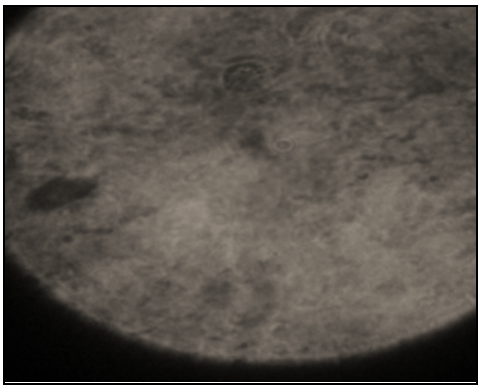

Fig. 8. *Cryptosporidium* oocyst (Sheather method)

Serological quick test “Rainbow Calf Scour 4” positively identified one case, a calf aged 7 days, in maternity, that also presented positive reaction to Coronavirus infection. *Cryptosporidium* oocysts were detected by all three coproparasitological methods.

The usual flotation tests and McMaster method also identified *Eimeria* infections but only in individuals negative for *Cryptosporidium* tests.

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RESEARCH ON SOME ASPECTS OF PATHOLOGY IN PIGEON FANCIERS

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Abstract

The research was conducted over a 2 years based on cases reported in the Veterinary Clinic of the Faculty of Veterinary Medicine Spiru Haret *of Bucharest. Pigeons investigated came from two private farms, one of Bucharest, the other from com. Butimanu, Lucianca. The first farm has about 60 ends and the second ends 35 pigeons for recreation. Both breeders of pigeons nesting structured shelters (boxes) and aviaries [1]. In order to confirm the diagnoses were made: epizootic investigation, clinical, pathological examinations (lesion-necropsy), bacteriological examination, mycological examination, parasitological examination. The pigeons were diagnosed following entities morbid salmonellosis, colibacillosis, mycoplasmosis, ornithosis – psittacosis, pseudomonoza, aspergillosis, paramyxo, canker and cocci.*

Keywords: *pigeons, investigations, morbid entities*

Introduction

The research in this paper started from the practical need to know and also to present new issues regarding this species of birds. It is known that in our country and beyond, pigeon fanciers are particularly passionate about what they do, investing resources often psychological, spiritual and significant material and not insignificant.

The paper presents research on morbid entities that evolve in this species and specific treatment and methods of control and some issues in the biology, physiology and breeding of pigeons.

Thanks to research conducted was possible to draw up a guide of actions and veterinary operations that run of pigeons and a therapeutic handbook bearing the Romanian medicinal products used in the therapy of this species.

Material and methods

They have used a number of methods and materials that:

Epizootic investigation. It performed whenever birds were brought to consultation with various physiological disorders or even morbid conditions. Fetched carefully all data provided by breeders, it is a fact that pigeon owners are very attentive and caring with their birds.

Clinical examination. Clinical examination was performed by the methods of semiological pigeons – inspection, palpation, percussion etc. Sampling pathological material. They collected various materials pathological, bacteriological methodology [5]: conjunctival secretion, pharyngeal exudate, faeces, feathers, crusting, other productions of the skin (serous collections, purulent), eggs (before hatching).

Laboratory confirmation of infections

In order to confirm the diagnosis and bacteriological examinations were performed [5, 6, 7], mycological and parasitological.

Materials used for laboratory examinations: sterile hood, UV lamp, microscope, 0-4ºC temperature refrigerator thermostat to 37ºC, 27ºC thermostat, culture media for bacteria and fungi, surface disinfectants for various substrates and glass (NaOH 3% chloramine etc.), glass Petri dishes or PVC diam. 10 cm, glassware (pipettes, tubing, beaker, flask etc, glass slides and slides PVC) solutions clarified (NaCl 10%), battery coloring Gram solution dye methylene blue 1% other material (filter paper, cedar oleum).

Results and discussions

Morbid entities who were diagnosed in birds in two farms in the study were as follows:

• salmonellosis (clinical, lesion, epizootic investigation and bacteriological);

• colibacillosis (clinical, lesion, epizootic investigation and bacteriological);

• mycoplasmosis (clinical, lesional and epizootic investigation);

• ornithosis – psittacosis (clinical, lesional and epizootic investigation);

• pseudomonoza (clinical, lesional and epizootic investigation);

• aspergillosis (epizootic investigation and mycological egg before hatching);

• paramyxo (clinical, lesional and epizootic investigation);

• trichomonosis (clinical, lesional and parasitological);

• coccidiosis (clinical, lesional and parasitological).

Fig. 1. *Stiff neck and opistotonus in paramyxo (original Badic)*

Following the results obtained, we developed concretely following immunization scheme and treatments in flocks of pigeons:

Day 1-2-3-4 life – fighting canker

Day 5-6 of life – vitamin therapy

7-8-9-10 day life – fighting coccidiosis

Day 11 of life – vitamin therapy

Day 12-13-14-15 life – fighting salmonellosis, colibacillosis, mycoplasmosis, staphylococcus

Day 16-17 life – vitamin therapy and restoring intestinal flora

Day 18 living– internal and external Suppression

Day 19-20-21-22 life – preparing the body before vaccination

Day 21 of life – vaccination against pigeons paramyxo

In April (annual) – vaccination against of pigeon pox

Conclusions

• permanent supervision of the health of pigeons by clinical and laboratory examinations lesion, according to the schemes and programs recommended;

• avoiding the introduction into flocks of pigeons with unknown health status. After their acquisition must be maintained in prophylactic quarantine.

• swift isolation and treatment of pigeons diagnosed with infections and / or infestations to limit the dissemination of morbid entities in those herds or flocks in the neighborhood.

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FOOD ALLERGENS – RISK MANAGEMENT FACTORS FOR FOOD INDUSTRY

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Abstract

The research wants to investigate consumers feedback regarding food allergens based on an exploratory research. December 13, 2014 was the limit for implementing the provisions of Regulation (EU) no. 1.169/2011 regarding mandatory data for food labeling for consumers with the obligation to mention potentially dangerous ingredients for allergic, clearly mentioned – both on product labels, as well as restaurant menus. Researchers have been interested lately on the subject of food allergies, especially about the prevalence of allergies in certain populations, proteins as food allergens, cross-reactivities and effects of food processing on the allergenicity of the food or ingredients, laboratory methods for detecting food allergy or food allergy or adverse reactions to sensitive individuals. About 75% of allergic reactions among children are caused by egg, peanut, milk, fish or nuts. About adults 50% of allergies are caused by fruits (apples, pears, cherries, strawberries, raspberries or almonds), vegetables (celery, carrots, herbs) or different varieties of nuts and peanuts. Other diseases are related to immune-mediated response of food allergens (for example, celiac disease hope or reactions to sulphites in food) or other reactions known as food intolerances, according EFSA. Studies and the general public information provide sufficient data for consumer awareness and for risk management decisions regarding allergen labelling.

Keywords: *food allergens, risk management, labelling*

Introductions

According to Buttriss (2002), food allergy patients may suffer a various of symptoms after consuming problematic foods with a range from uncomfortable (itching, rashes) to a fatal potential (anaphylactic shock).

Food allergy is defined as an adverse immunological (hypersensitivity) response to food and as such it is not a single disease, nor is it caused by one pathophysiologic disturbance (Bruijnzeel-Koomen et al., 1995).

Food allergic disorders can be broadly divided into those that are mediated by IgE antibodies and those that are not. Disorders with acute

onset of symptoms after ingestion are usually mediated by IgE, which arms tissue mast cells and blood basophils, to result in a state termed sensitisation.

Upon re-exposure, the causal food proteins bind to the IgE molecules specific for them and trigger the release of mediators, such as histamine, that cause symptoms. Another group of food hypersensitivity disorders are subacute or chronic, and are mediated mainly by T cells (Sampson & Anderson, 2000).

According to Sicherer (2002) IgE – dependent food allergic reaction may affect one or more target organs like: the skin (urticaria, angio-oedema), respiratory tract (rhinitis, asthma), gastrointestinal tract (pain, emesis, diarrhoea), and cardiovascular system (anaphylactic shock) or an immediate systemic reaction (anaphylaxis), term applied to severe, potentially fatal allergic reactions.

Usually subacute and chronic food allergic disorders typically affect the gastrointestinal tract or skin, with a propensity to affect infants and children.

Some gastrointestinal disorders mainly affect infants and are usually associated with hypersensitivity to cow's milk or soya, but the most serious form of gastrointestinal food allergy in infants is food protein-induced enterocolitis syndrome which has a symptom complex of profuse vomiting and diarrhoea, and potentially a sepsis-like clinical picture with dehydration, acidaemia, methaemoglobinaemia, raised blood neutrophil counts, and shock.

Food allergy and atopic diseases are reported on growing worldwide and Europe according research and survey studies from a range of food intolerance to allergies. The main foods responsible for children are: eggs and milk with atopic dermatitis like a frequent manifestation. For adults: seafood, fruits, and vegetables were the most common causes, with angioedema or urticaria, or both, like the most frequent symptoms.

Materials and methods

Methodology used is an exploratory one based on previous literature review analysis. In literature are presented different studies regarding main allergies, factors, key symptoms, causes, tests available in the market, prevention, food allergies or clinical manifestations for pets, etc., from scientific point of view. Other studies present consumers approach: labelling practices and preferences, life experiences from point of view of allergic consumers, coping with the disease from the human side – on line support group.

Results and disscutions

Research findings are raised from literature review analysis.

Resuming epidemiology, the symptoms may vary from dermatological reactions, oral and gastrointestinal to anaphylaxis.

Table 1 presents the main symptoms and features, divided by immunological reactions, age groups and courses.

Table 1

Features of some food-allergic disorders categorized by pathophysiology
(Source: Sicherer, 2002)

	Age-group	**Key symptoms / Signs / Features**	**Course**
Ig-E dependent , acute onset			
Urticaria / Angio-oedema	Any	Wheal and flare, oedema. Ingestion or by direct skin contact. Food commonly causes acute (20%) but rarely chronic (2%) urticaria	Variable by food
Immediate gastrointestinal reaction	Any	Acute onset nausea, emesis, pain. Diarrhoea may follow	Variable by food
Oral allergy syndrome (pollen-related)	Children/ Adult	Pruritus, mild oedema confined to oral cavity	Wax/Wane
Rhinitis	Any	Congestion/Rhinorrhoea after ingestion or inhalation. Rarely an isolated symptom	Variable by food
Asthma	Any	Bronchospasm after ingestion or inhalation. Isolated chronic asthma is usually not due to food allergy	Variable by food
Anaphylaxis	Any	Multiple organ system reaction can include cardiovascular collapse. Most frequent cause for outpatient anaphylaxis	Variable by food
Food-associated, exercise-induced anaphylaxis	Any	Food triggers anaphylaxis only if ingestion is followed temporally by exercise. Causes: wheat and celery most described.	Unclear
IgE–associated/cell-mediated, delayed-onset/chronic			
Atopic dermatitis	Infant/ Child	Chronic/Relapsing pruritic dermatitis, xerosis, atopic history. 35% of children with moderate-severe disease have food allergy, but rarely a trigger for adults. Common causes: cow's milk, egg, soya and wheat	Resolves: early childhood
Eosinophilic gastroente-ropathies	Any	Symptoms vary with respect to site(s)/degree of eosinophilic inflammation. Oesophageal: dysphagia, pain. Generalised: ascites, weight loss, oedema, obstruction. Causes: multiple foods	Variable
Cell-mediated, delayed-onset/chronic			
Dietary protein enterocolitis	Infants	Chronic exposure: emesis, diarrhoea, poor growth, lethargy re-exposure after restriction: emesis, diarrhoea, hypotension (15%) 2 h after ingestion. Common causes: cow's milk, soya, grains	Resolves: 1-3 years

Dietary protein proctitis	Infants	Mucousy, bloody stools. Common cause: cow's milk via breastfeeding	Resolves: 1 year
Dietary protein enteropathy	Infant/ Child	Malabsorption, oedema, emesis, poor growth. Common cause: cow's milk	Resolves: 1-2 years
Coeliac disease	Any	Malabsorption, diarrhoea, HLA-DQ2-associated. Cause: gluten	Permanent
Dermatitis herpetiformis	Any	Papulovesicular skin lesions, acral distribution. Cause: gluten	Permanent
Associated with precipitating milk-specific IgG			
Pulmonary haemosi-derosis	Infant	Anaemia, pulmonary infiltrates, poor growth. Cause: cow's milk	Unclear

General diagnosis consists in a differential diagnosis regarding some other non-immunological reactions to foods (Panel 1), diagnostic test for IgE, elimination diets and oral food challenges.

Main tests used for allergy are skin prick test allowing the food specific IgE to interact with the surface of the skin mast cells. Other test used is radioallergosorbent test (RAST) where the allergen for study is bounded to a solid matrix and exposed to the patient's serum.

By elimination diets is requested an amelioration of symptoms through simply presumptive evidence of causality. The oral food challenge is done by feeding gradually increasing amounts of the suspected food under observation by a physician over hours or days.

Treatment consists in medications, diet and prevention. Antihistamines are sometimes the only medication needed to reduce itching and rash. However, for patients with more severe symptoms of anaphylaxis, or with respiratory or cardiovascular symptoms, additional treatment is needed like cardiopulmonary resuscitation, administration of epinephrine, antihistamines, corticosteroids, oxygen, intravenous fluids, inhaled bronchodilators, and medications to support blood pressure.

Prevention and dietary management represents the core of the treatment. In most countries, shortcomings on the parts of manufacturers and in labeling make it very difficult to identify allergens in commercial food products. Cross contamination and errors in packaged food shops and restaurants are an additional obstacle. Regulation (EU) no. 1.169/2011 regarding labeling and allergens represents a real support for food – allergic consumers.

For pets the only way to determine whether a dog or cat is food allergic or intolerant is by eliminating the inciting food ingredient from their diet, intradermal skin testing and serum testing aren't accurate diagnostic (Kane, 2012). The elimination diet, with a new protein, must be

implemented for 2-4 weeks up to 10-12 weeks. The goal is to choose a diet that contains only proteins that the pet has never been fed before, and therefore never sensitized to. Owners should be cautioned that in addition to utilizing a novel protein and limited ingredients, preparing a diet that provides complete and balanced-nutrition is necessary.

Panel 1. Examples of non-immunological adverse reactions to food and masqueraders of food allergy
Intolerance (lactase deficiency)
Infection (bacterial, viral, parasitic)
Pharmacological effects (caffeine, histamine, tyramine)
Anatomical (pyloric stenosis)
Digestive (gallbladder disease, pancreatic insufficiency)
Metabolic disorders (galactosaemia)
Toxins (bacterial contamination, scombroid fish, poisoning)
Non-food allergy (reactions to pollen, mould, dust mite dander)
Neurological (gustatory rhinitis from spicy foods, facial, flush from tart foods)
Factitious (Munchausen's syndrome/Munchausen's syndrome by proxy)
Psychological (panic disorder)

Food allergy has potential to detrimentally affect the quality of life experienced by food allergic sufferers (de Blok et al., 2007). Food allergy is a chronic disease that can only be managed through avoidance of problematic proteins in the diet. Inappropriate communication about food allergens can cause stress and insecurity, which may have a negative impact on quality of life (Voordow et al., 2009). Based on the same authors, for some consumers is more expensive to buy non allergic foods, based on cultural approaches. These food risk management systems entail protocols that manufacturers incorporate to cope with allergens in their production processes. These protocols help manufacturers to avoid unnecessary cross-contamination and adequately label the allergens present in their products. Labels should have all ingredients written out in comprehensible national language. Furthermore, the ingredient list and allergens information should have a standardized format that facilitates the decision making process.

Conclusions

Improved strategy for new treatments and prevention are under investigations with hopes for better diagnostic tests and a real treatment.

Prevention with elimination diet is for the moment only treatment. Labelling is a very important part.

The quality of life of food allergic consumers may be improved by consumer protection installed by the new Regulation (EU) no. 1.169/2011.

Traceability is the first step in developing adequate labelling, especially concerning food allergens. Traceability of all ingredients throughout the whole production process is of utmost importance.

Labelling policies and practice should be harmonized, if possible, preferably at international level.

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THE TOPOGRAPHICAL LOCATION OF THE LYMPHATIC DUCT FROM THE THORAX APERTURE IN PIGS AND SHEEP

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Abstract

The purpose of the studies is to contribute with some clarifications to the topographical location of the vascular, lymphatic and nervous formations from the thorax aperture in pigs and sheep. The literature data is little relevant because they depict other formations from the anterior mediastinum, without making a correlation between them. The study was conducted on 20 pig corpses from production farms, with digestive, not respiratory disorders in general, so as not to affect the studied area, and on sheep corpses used by students for dissection. The vascular formations were injected with a mixture prepared in the laboratory of anatomy. The paper shows pictures from several dissections, determining as accurately as possible the topographical location of the anatomical formations, and it has a strong applicative character for human medicine, since the closest species to man as experimental morphological model is the pig.

Keywords: *mediastinum, lymphatic duct, cranial vena cava, caudal cervical ganglion*

Introduction

The fundamental research on the topography of the vascular nervous formations from the aperture of the thorax cavity in animals is approached by many researchers, but the data are presented separately, either for the vascular formations, or for the nervous formations, or for the lymphatic formations (1, 2, 4). These data are a real support to interpret the physiological phenomena and to clarify several aspects regarding the way of approaching the formations during surgery on the anterior mediastinum. The morphology of the species resembles that of the man, which recommends it as an experimental model, provided the European legislation of the experimental animals is observed (4, 5)

Material and methods

The studies were conducted in the laboratory of anatomy of the Faculty of Veterinary Medicine, on 20 pig corpses from a production farm. Before dissecting, the aorta and the veins were injected with a mixture of substances prepared in the laboratory of anatomy. The nervous formations were treated with a solution of acetic acid 10%. The lymph formations were injected with methylene blue. The lymphatic anatomy of 5 pigs was studied and classified and a new technique for lymphatic cannulation was developed. The cannulation success rate was 55%.

Results and discussions

Formation anatomical approach is at chest level as having first milestone coast. It protects the right apical pleural recessive and dissect contained septal formations precardiac mediastinal. In relation to the first rib to show the skull mediastinal lymphonodes who are willing and medial to this axilar lymphonode of the first rib that is located in relation to the edge of the skull. Vegetative plexus is located between cervicotoracic formations located superficial venous and arterial located medial formations (fig. 1).

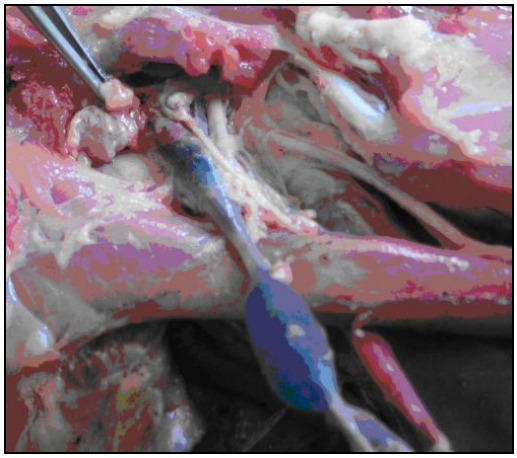

Fig. 1. *Mediastinal aperture approach*

The right caudal cervical ganglion, joined in 15 animals with the thoracic paravertebral ganglion 1 and 2, forms a pericarional aglomeration located on the median face of the first rib in the dorsal side of the anterior mediastinum, being placed dorsally in relation to the long neck muscle, laterally in relation to the vertebral artery, ventrally in relation to the right

subclavicular artery and on the right of the bicarotic trunk. In all studied cases we have identified the middle cervical ganglion which is attached to the caudal cervical ganglion through the subclavicular loop (fig. 2).

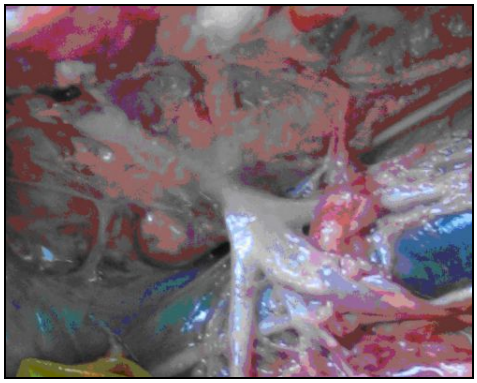

Fig. 2. *Ggl. cervicotoracic*

The right lymph duct passes at a distance of 2 cm ventrally from the cervical-thoracic plexus formed around the cervical-thoracic ganglia, running thereafter sideways vento-cranially, descending from the right side of the aorta towards the cranial vena cava into which it pours. Before pouring in the cranial vena cava, the duct displays a branching which, after passing the aorta-pulmonary ligament, joins again the main duct (fig. 3).

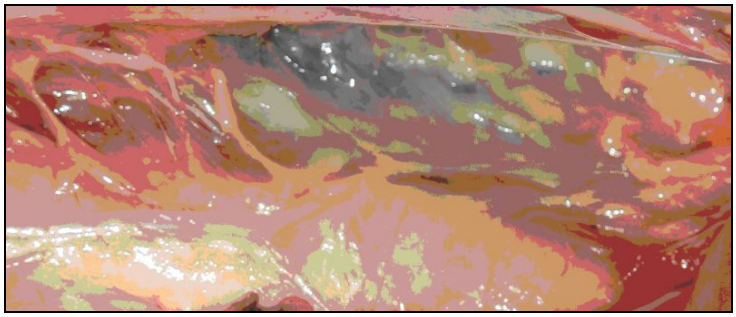

Fig. 3. *Lymphatic duct*

Cardiac lymph is the most direct medium for analyzing metabolical changes in the myocardial cell. Currently, sheep are the animals used for investigation of myocardial lymphatic function. However, questions arise when comparing and interpreting the human system to the experimental model, since the sheep coronary anatomy is different from human anatomy and pulmonary lymph contamination is found in up to 81% of the cases. Swine, having similar coronary anatomy to humans, are a proven model for

cardiovascular research. The purpose of this study was to investigate the cardiac lymphatic anatomy of the swine and to develop a reliable cannulation technique to collect the lymph (fig.4).

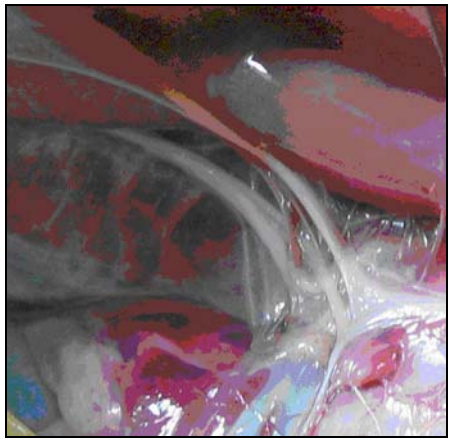

Fig. 3. *Lymphatic duct cannulated*

Conclusion: We conclude that porcine myocardial lymphatics can be successfully cannulated for the investigation of myocardial lymphatic function.

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RESEARCHES REGARDING THE PRESENCE OF SOME SALMONELLA STRAINS ON BIRDS FLOCKS FROM A RESTRAINED AREA IN SOUTH

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Abstract

The Salmonella genus is the biggest in the ***Enterobacteriaceae*** *family, including over 2400 serotypes. Due to the pathogenicity that members of this type possess to humans and the fact that they frequently contaminate various foods,* ***Salmonella*** *genus presents a particular interest for food microbiology, especially those of animal origin. The analysis method for the detection and the confirmation of the germs of* ***Salmonella*** *genus is made accordind to SR EN ISO 6579/2003/AC/2006, using the horizontal method. For detection are used the following steps: isolation and identification, the selection of the colonies for confirmation, the biochimic confirmation, the serological confirmation, the expresion of the results and the perform of the antibiograme. In the present study were subject to interpretation the studies obtained after the monitoring of fecal samples from poultry, fecals which came from economic agents located in a restrained area in south. There were found a number of species of the* ***Salmonella*** *genus including* ***S. enteritidis****,* ***S. infantis*** *and* ***S. Senftenberg****.*

Keywords: *antibiograme, genus, serotypes*

Introduction

National reports continue to point *Salmonella* sp. the most important etiological agent of food poisoning. Establishing a system of investigation and reporting of foodborne disease outbreaks and bacteriological advances in recent years have allowed a more accurate, global dimensioning of zoonotic salmonella incidence.

Members of the Food and Agriculture Organization of the United Ntions (FAO) and the World Health Organization (WHO) expressed concern regarding the safety of food both at national and international level due to increasing incidence of foodborne disease outbreaks caused by microorganisms in food (2).

Data collection is performed for zoonoses under Directive 99/2003, which assigns the European Food Safety Authority (EFSA) task of examining data collected from Member States and the publication of an annual summary report of the Community (3).

This paper followed the evolution of finding *Salmonella* spp germs in samples taken from the units of production (farms, hatcheries) flocks of birds from a restricted zone in the south.

Materials and methods

Analysis method for *Salmonella* detection and confirmation is done according SR EN ISO 6579/2003/CA/2006. Horizontal method for detecting *Salmonella* germs (4). The required quantity of the sample is collected aseptically, so as to provide a dilution of 1/9, after the inoculation of buffered peptone water (for example 25 g of the sample was inoculated in 225 ml).

Apart (or 10 g of sample in 90 ml. apart), depending on the specifications of the Reg. (EC) 2.073/2005 (1). The seeding are abundant with Pasteur pipette, the common culture medium [nutrient broth, nutritional agar, liquid enrichment mediums (selenite cystine broth)] and preenrichment mediums (buffed peptone water).

Aspiration of product seeding is done under sterile conditions (in the seeding box, with flame and with Accu-jet pipette controller, for cadaver organs, long bones, intraplacentar liquid in case of stillbirths.

For seeding other specimen types (shoe covers for the manufacture area, bird droppings, meconium – the product of defecation in incubators, chicken eggs in incubators at different stages, down from hatcheries) use Erlenmeyer flasks (5 for each sample). Place the flask on the balance, bring to zero, and then harvest with sterile instruments 25 grams of sample that is introduced into the Erlenmeyer flask until we make 25 grams. It hands the ball off balance and add 225 mL buffed peptone water.

To be specific we used the following steps: isolation, identification and selection of colonies for confirmation, biochemical confirmation, serological confirmation, expression of results and perform sensitivity testing.

Testing was conducted over the year of 2014.

Testing was done on the following types of probes:

– Pads (protective shoe covers used in production facilities);

– Poultry manure samples from areas of growth;

– Meconium (manure samples inside incubators).

Results and discussions

They have analyzed a number of 778 samples of swabs (protective shoe covers used in production units), 25 samples of bird droppings in areas of growth, and 33 samples of meconium (manure samples inside incubators) (table 1).

Table 1

Types of analysed samples

Analysed sample type	**Total of analysed samples**	**Pozitive samples**	**Procentua results (%)**
Pads (protective shoe covers used in production facilities)	778	60	7,84%
Poultry manure samples from areas of growth	25	9	36.00%
Meconium (manure samples from inside incubators)	33	5	15,15%

From Table 1 it is noticed that were analyzed 836 samples of which were identified 74 positive samples, representing 8.85% percentage. After the working protocol were detected *Salmonella* germs in serotypes following: *S. Bredeney*: 3 strains, *S. enteritidis*: 1 strain, S. *infantis*: 56 strains; *S. Livingstone:* 6 strains, *S. Seftenberg*: 3 strains, *S. corvalis*: 4 strains, *S. albany*: 1 strains. From the results it was noted that identifying the types of evidence serotypes was as follows (Table 2).

Table 2

The distribution of* Salmonella *serotypes sp. on types of evidence

	Shoe covers	Bird droppings	Meconium
S. Bredeney	2	-	1
S. enteritidis	-	1	-
S. infantis	51	4	1
S. Livingstone	6	-	-
S. Seftenberg	2	-	1
S. corvalis	-	4	-
S. Albany	1	-	-

Out of the 7 kinds of strains, prevalent is *Salmonella infantis* and is to be found in different amounts in all samples (Fig. 2).

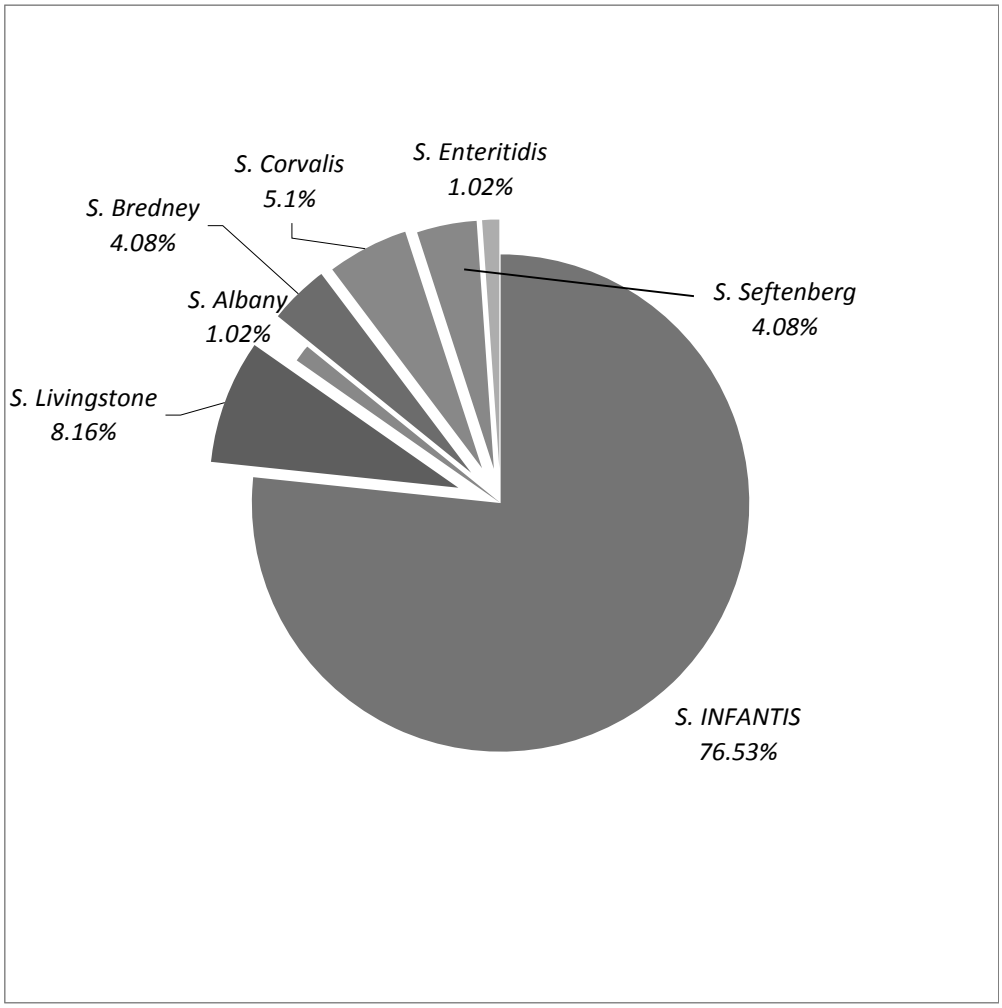

Figure 2. *Procentual distribution serotypes of* ***Salmonella*** *spp.*

For the identified serotypes antibiograms were performed to determine the resistance / sensitivity to various antibiotics (Table 3) and for applying medication to the infected flock.

Table 3

The evolution of antimicrobial sensitivity of tested straines

		S. infantis %			*S. Seften-berg* %		*S. Albany* %		*S. Bred-ney* %		*S. corvalis* %		*S. Living-stone* %		*S. enteridis* %	
		S	R	I	S	R	S	R	S	R	S	R	S	R	S	R
1.	Ampiciline	93,4	5,26	1,3	100	-	100	-	100	-	100	-	37,5	62,5	100	-
2.	Cefotaxine	100	-	-	100	-	100	-	100	-	100	-	100	-	100	-
3.	Ceftazidime	100	-	-	100	-	100	-	100	-	100	-	100	-	100	-
4.	Ciproflox-acine	94,7	3,94	1,3	100	-	100	-	100	-	100	-	100	-	100	-
5.	Gentamicin	-	100	-	-	100	-	100	-	100	-	100	-	100	-	100
6.	Kanamycin	-	100	-	-	100	-	100	-	100	-	100	-	100	-	100
7.	Nalidixic acid	-	100	-	100	--	100	-	100	-	100	-	50	50	100	-
8.	Streptomycin	-	100	-	-	100	-	100	-	100	-	100	-	100	-	100
9.	Sulfonamide	100	-	-	100	-	100	-	100	-	100	-	100	-	100	-
10	Tetracyclines	100	-	-	100	-	100	-	100	-	100	-	100	-	100	-
11	Trimethoprim	100	-	-	100	-	100	-	100	-	100	-	100	-	100	-

As shown in the table it is seen that sensitivity evolution is different for every serotype.

Thus, it can be seen that the sensitivity of *S. infantis* show 100% in the case of certain antibiotics: cefotaxime, ceftazidime, sulfonamides, tetracyclines, and trimethoprim, while developed resistance to other antibiotics ciprofloxacine, gentamicin, kanamycin, streptomycin, nalidixic acid.

For *S. Seftenberg*, *S. Bredeney*, *S. corvalis*, *S. Albany*, *S. enteritidis* isolates are susceptible to most antibiotics: ampiciline, ceftazidime, cefotaxime, ciprofloxacin, sulfonamides, tetracycline, trimethoprim, developing resistance only to: kanamycin, gentamicin, streptomycin.

For *Salmonella Livingstone* sensitivity was observed on: cefotaxime, ceftazidime, ciprofloxacin, sulfonamides, tetracyclines, trimethoprim, while developing resistance to: gentamycin, kanamycin, and spectinomycin and sensitivity to nalidixic acid and have been developed resistance difference in the amount of 50% / 50% depending on the sample to which has been identified. Noteworthy is that moderate sensitivity (I) developed only *S. infantis* at: ampiciline, ciprofloxacine.

Conclusion

1. This paper appreciates the detection of germs of *Salmonella* spp. In samples taken from the units of production (farms, hatcheries) flocks of birds from a restricted zone in the south.

2. During the test were analyzed enrolling 836 samples of which were identified enrolling 74 positive samples, representing 8.85% percentage.

3. Whatever the sample type strain and sensitivity of 100% was recorded in: cephalosporins, tetracycline, trimethoprim, sulfonamides.

4. Ampiciline, ciprofloxacine, cloramphenicol, nalidixic acid sensitivity in the group varies according to several causes (sample's nature, sampling site, the identified serotype).

5. Following the isolated strains antibiograms, we can say that the sensitivity of germs has changed or because repeated treatments and disinfectants used before populating with flocks or because of the changes in the evolution of the studied germs).

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INFLUENCE OF CALCIUM, PHOSPHORUS DISORDERS UPON PRODUCTION PARAMETERS OF EGG HEN

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Abstract

For a group of 3000 hens, 36 weeks old, from a share diagnosed with calcium/phosphorus metabolism disorders, examined for 60 days, comparatively with a control batch, the followings have been found: the decrease of total egg production by 12.5 %, the decrease of laying percentage by 10,2%, the decrease of average body weight of the birds by 350 g, the increase of the number of eggs with soft or deformed shell by 3,81%. For the affected group, flaws of size, form and weight of the eggs were also observed. The affected hens have shown deviations of the chest bone, spontaneous fractures of some bones, difficult walking.

Keywords: *calcium, phosforus, production, hens.*

Introduction

The paper presenting the main metabolic nutritional disorder discovered in a layers hen farm. Metabolic disorders that occur are often caused by deficiencies in several components and are accompanied by a clinical picture. The main problems would affect egg production and the emergence of reproductive disorders.

In many cases these disorders are ignored and is not correlation between clinical and nutrient deficiencies, return flocks would suffer. The most important is that these problems will be detected in time to avoid the decrease in production.

Material and methods

The research was conducted on a group of 3000 hens aged 36 weeks, a hall in which calcium metabolism disorders diagnosed, phosphorus (group A). They were studied and systematized some data on key production parameters in group A compared with the control group (B).

Laboratory work consisted of tests to determine calcium and phosphorus in feed, serum biochemical tests: serum calcium, fosforemie, alkaline phosphatase. The analyzes were carried out using conventional techniques.

Results and discussions

The analysis of 6 samples of feed made from CN 21/7 reveal deficiency of calcium and phosphorus in particular. Calcium concentrations ranged from 0.38%-0.67% (below the normal 0.9%). Determination of phosphorus 0.42% recorded values between -0.58% (below the threshold of 0.7%).

Deficiency in vitamin D has helped trigger state failure in calcium and phosphorus in particular but also to change the ratio Ca/P confirmed by biochemical tests (serum calcium was 14.2 ± 3.1 mg / dl and 6 fosforemia 15 ± l, 84 mg/dl compared to the reference values for calcium 25 ± 5 mg/dl and phosphorus was 7 ± 1 mg / dl).

The main symptoms observed in chickens from the affected batch were deflections of the hull stem tibia and femur fractures spontaneous and travel weight. These signs were recorded at 12% of the flock of studied (group A). The quality of eggs from hens in group affected was an important marker for diagnosis: egg shell was thin, fragile and the eggs were small, with abnormal calcium deposits, with cracks and irregularities of the skin.

The results summarized in Table 1 shows a decrease in the number of eggs in group deficiency with 20,820 units (13.87%) compared to the control group, which is 5.40 eggs/hen the 60 days of the experiment; laying percentage was lower by 10.2 and average body weight was deficient in laying 350 g lower than that of the control group.

Table 1

The evolution herds batch production of eggs and body weight

Lot	Period (day)	Medium effective hens	Total eggs	Eggs/ hen	Percentage of laying	Body weight (g)
A Exp	60	3000	129 240	43.8	71.8	2700
B Control	60	3050	150 060	49.2	82	3050
Differences in the experimental group deficient Ca-P)			20 820	-5,40	-10.2	-350

Table 2

Eggs quality from hens with calcium-phosphorus metabolism disorders

Lot	Medium effective hens	Prod. total eggs	From which				
			Whole-shelled eggs		Soft-shelled eggs or cracked		The average weight of the egg
			Nr.	%	Nr.	%	
A Exp	3000	129 240	124316	96.19	4924	3.81	49,2
B Control	3050	150 060	148 514	98,97	1546	1.03	57,5
Differences in the experimental group		20 820	24 198	-2,78	+ 6470	+2.78	-8.3

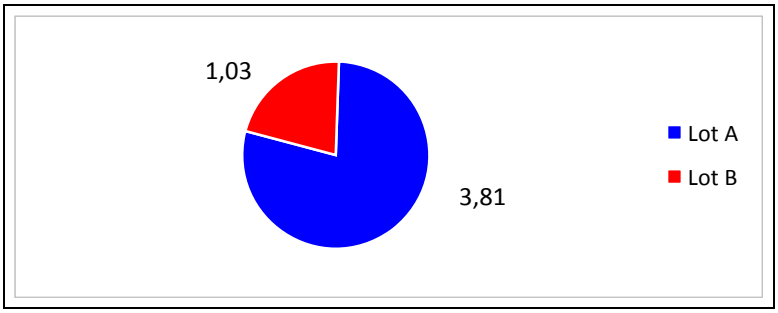

Fig. 1. *Procentual eggs broken*

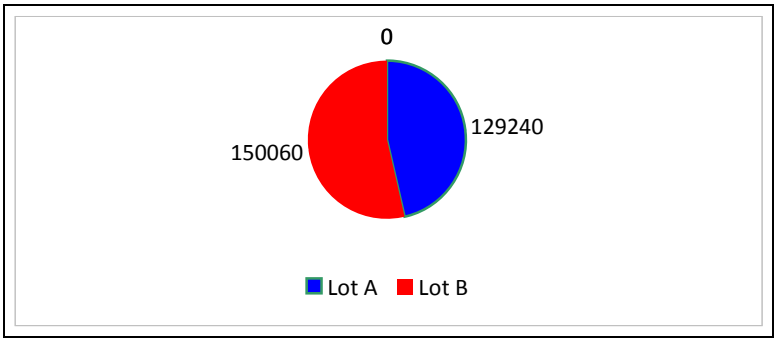

Fig. 2. *Total egg production*

Conclusions

1. In 3000 a group of hens aged 36 weeks, which were diagnosed calcium-phosphorus metabolism disorders were found: total egg production decreased by 13.87%, reducing the percentage of lay 10.2% decrease average body weight of 350 g birds, increasing the number of soft-shelled eggs or deformed by 2.78%.

2. Hens with Ca-P metabolism disorders showed deflections of the hull Stern, spontaneous fractures of bones, weight displacement.

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SUPRAVENTRICULAR EXTRASYSTOLES

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Abstract

Our study aims to identify arrhythmias due to ectopic rhythm disorders, respectively supraventricular extrasystoles products in the atrial area (atrial extrasystole) and atrioventricular node or his bundle (nodal extrasystole). Were clinically examined a total of 20 dogs that showed irregular heartbeat, shortness of breath, discomfort in times of environmental stress. For atrial extrasystole, following electrocardiographic changes were found: premature P wave, deformation T wave who preceding premature cycles, narrow QRS (<0.05 sec.), the disagreement between expiratory phase of the respiratory cycle and slow heart rate cycles. In the nodal extrasystole cases, P wave included in QRS or retrograde P waves were identified. In dogs with supraventricular extrasystoles, echocardiographic parameters showed a healthy heart. Myocardial electrogenesis disorders are due to neurovegetative or possible electrolyte disturbances.

Keywords*: supraventricular extrasystoles, electrocardiography, dog*

Introduction

Arrhythmias may be due either to bioelectric impulse abnormal formation of the embryonic heart tissue or anomalies in its leadership to working myocardium (Irisawa et al., 1995). Alteration of bioelectric impulse generation in the sinus node due to increased sympathetic nervous tone or due to decreased cardiac output in valvulopathy or miocardiopathy initiates the onset of supraventricular ectopic outbreaks. Ectopic discharges occur outside of sinoatrial node, atrioventricular or His bundle areas.

The purpose of this study was to identify supraventricular extrasystoles emerging animal on the background of neurovegetative or some electrolyte disturbances (hypo- or hyperkalemia, hypo- or hypermagnesemia, hypo- or hypercalcemia), but having a healthy heart.

Material and methods

The study was conducted on a total of 20 dogs that showed: irregular heartbeat (with premature beats), shortness of breath, no blood pressure changes. These dogs were investigated with electrocardiograph and ultrasound.

Electrocardiography were performed with a portable ECG device, using the standard bipolar methode and working parameters were: speed 25 mm/sec. and amplitude 10 mm/mV. Were identified and interpreted changes in heart rate, morphology, duration and amplitude waves, electrocardiographic intervals and segments specific routes. Echocardiographic indicators were: shortening fraction, interventricular septum, general morphological aspects of cardiac dimensions, integrity and functionality of valvular and subvalvular endocardium.

Results and discussions

Genesis supraventricular arrhythmias is either triggered by an abnormal automaticity, either by reentry circuits (Martin M., 2007).

Abnormal automaticity occurs by spontaneous discharge, of cardiac cells in the vicinity of the sinoatrial or atrioventricular node, as a result of conditions which induce the decrease of their membrane potential (for example ischemia, stroke, cardiomyopathy or some electrolyte disturbances). Reentry is the reactivation of myocardial fibers by the same wave front repeatedly during the same cardiac cycle; circular motion is due to the difference between conduction velocities of waves excited.

Heart rhythm disturbances are defined by reference electrocardiogram changes identified at regular sinus rhythm characteristic values of reference (Collet and Bobinnec, 2001). Clinical criteria for the diagnosis of extrasystoles were: changes in the frequency precordial shock, heart sounds and pulse, discrepancy between expiratory phase of the respiratory cycle and slow heart rate cycles.

The electrocardiographic evaluation we identified supraventricular extrasystoles only nine of the 20 dogs. The remaining 11 dogs had ventricular extrasystoles.

On ECG routes, supraventricular extrasystoles are generally irregular appearance either isolated or in volleys. Rarely their appearance is regular, called supraventricular aloritmie or bigeminiy (Collet and Bobinnec, 2001).

Supraventricular extrasystoles feature is the appearance of P-QRS-T sequences premature followed post-extrasystolic pause. Premature P waves have a different morphology compared with sinus P waves. Narrow QRS

and T wave deformation preceding premature beats or preceding post-extrasystolic pause (fig. 1, 2 and 3).

In fig. 1 is observed atrial extrasystoles, bigeminy, complete flattening of the T wave or negative T wave. It may be associated with hypokalemia.

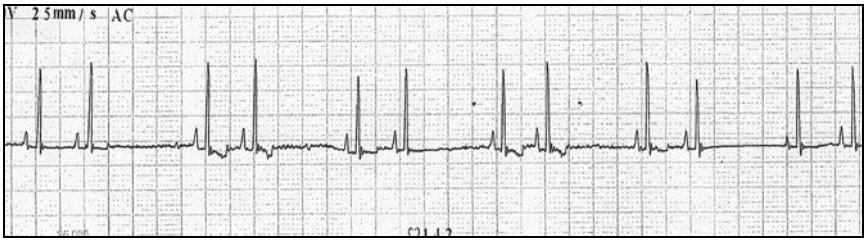

Fig. 1. *Atrial extrasystoles, bigeminy*

Fig. 2 shows atrial extrasystoles, grouped in bursts. Atrial extrasystolic P waves are different in duration and amplitude compared to the sinusal P waves. T waves are negative and high.

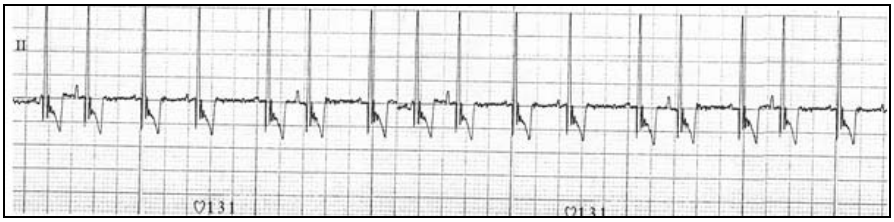

Fig. 2. *Atrial extrasystoles, grouped*

Nodal or junctional extrasystoles are premature and ectopic contractions, which originates in the atrioventricular junction. Assume premature discharge atrioventricular node or His bundle before the sinoatrial node to start a normal cycle.

Fig. 3 shows that the P wave is not visible or is located after the QRS, becoming negative, or is included in the QRS complex. Nodal extrasystoles as well as the atrial extrasystoles were looking narrow QRS complexes.

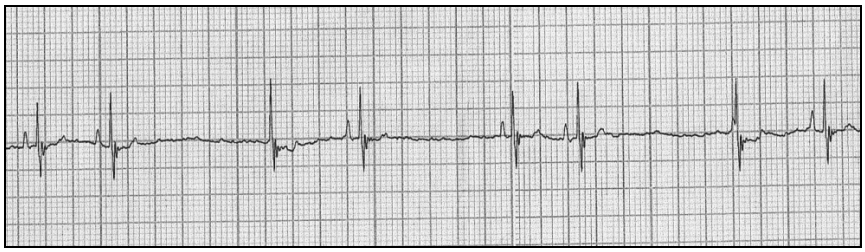

Fig. 3. *Nodal extrasystoles*

Establishing the origin of extrasystoles differentiated respectively atrial or nodal nature is less important and sometimes even difficult. However, the subsequent application of the treatment, it is essential to identify the type of arrhythmia, namely, supraventricular (this includes: atrial and nodal extrasystoles), than the ventricular.

Supraventricular extrasystoles may occur quite frequently in healthy animals, in the absence of changes in the cardiac pathophysiology (Ettinger and Feldman, 2002). But it can also be caused by pathological changes of the heart. Thus, Vollmar et al. (2003) and Dukes-McEwan et al. (2003) mention that atrial extrasystole occurs frequently in heart failure due to mitral insufficiency or dilated cardiomyopathy (in such cases, atrial extrasystole may occur before the development of atrial fibrillation).

Echocardiographic study of ventricular cavity dimensions (diameter and volume) and interventricular septum thickness and ventricular free wall lead to the calculation of specific indicators. The values of these indicators echocardiographic (shortening fraction, ejection fraction, sphericity index) were within normal limits, functionality own heart.

Conclusions

1. Long-term supraventricular extrasystoles may cause hemodynamic deterioration, even in a normal heart.

2. Although some cases of supraventricular remain asymptomatic (which are only detected by electrocardiographic investigations), it is necessary to follow the arrhythmias by regular ECG controls.

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THE COMPARATIVE THERAPEUTIC METHODS USED IN STICKER VENEREAL TUMORS

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Abstract

The study was conducted during 2012 – 2014 in Clinics of Spiru Haret *FMV in the Department of Reproduction. In this study was integrated a total number of 21 cases (11 females and 10 males). The management envisaged both surgery and chemotherapy, the pharmacologically active substances used were: vincristine, cyclophosphamide and doxorubicin. The study recorded the response of patients after the surgery method and after the chemotherapy, both immediate and delayed post-therapeutic intervention; substances have been used alone or in combinations of two, the intervals of administration have respected the general therapeutic protocols or have been adapted to the patient. In some cases surgical management was anticipated with chemotherapy intervention, in others the chemotherapy was after. The positive response of patients requires further research and the extension of therapeutic protocols.*

Keywords: *Sticker's sarcoma, cyclophosphamide, vincristine, doxorubicin*

Introduction

The study was carried out in *Spiru Haret* FMV Veterinary Clinic, Bucharest, Department of Reproduction, Obstetrics and Gynecology, a number of 21 cases of venereal tumor (Sarcoma Sticker). There were examined a number of 11 female dogs and a total of 10 male dogs, breeds and between 2012-2014.

Clinical examination of the patient was done thoroughly, even if Sticker tumor diagnosis did not involve development difficulties. In general, the holders were presented to the clinic complaining bloody vaginal secretions or penis affected. Thanks to a careful clinical examination it could be eliminated the suspicion of other morbid entities of the genitourinary system, both females and males.

Materials and methods

In cases where the overall condition was impaired, a lesser or greater extent, the primary drug treatments rebalanced the animals. The electrolyte solutions were administered, glucose, liver and general tonic, amino, etc. Known therapeutic conduct consists of:

- surgical ablation of tumors;
- chemotherapy:

– Vincristine 0.5 to 0.75 mg/m² body surface area administered strictly, repeat at 7 days to involution and tumor necrosis;

– Cyclophosphamide 8-10 mg/kg/7 days per os or 1-2 mg/kg/7;

– Dexorubicin 30 mg/m^2 body surface area administered to 21 days and all strictly.

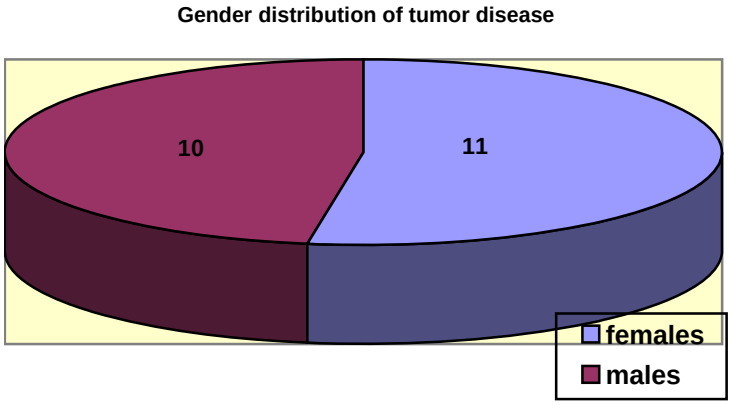
In order to deal surgically, it was considered the extent of tumor process, ease or difficulty of intervention approach, etc. The solution was to curative chemotherapy intravenous use only.

Vincristine is in the form of lyophilized powder, 0.001 g of vincristine sulfate and 0.01 g lactose. The mechanism of action of vincristine, in vivo, vincristine sulfate to secure the tubulin, preventing its polymerization for the formation of the mitotic spindle, thus blocking the metaphase. The half-life in animals hepato-renal function normal is 10.5 to 37.5 hours. The product is eliminated in the bile, so the faeces as metabolites and only 10% by the kidney. The side effects of vincristine sulfate are multiple, but they showed relatively weak. This was possible due to low doses administered, a large time administration (7 days) and, not least, due to reduced number of administrations (1-3 administrations).

Doxorubicin (Adriamycin) is in the form freeze-dried injection preparation (10 mg, 20 mg, 50 mg, 100 mg and 150 mg) and solution of 2 mg/ml, also for injection (5 ml, 10 ml, 20 ml, 25 ml and 100 ml).

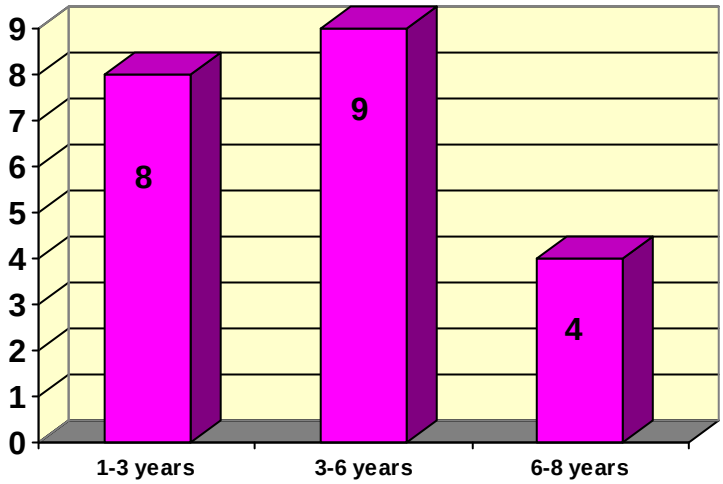
Results and discussions

From the data obtained by analyzing the 21 cases Sticker tumor was found a balance in terms of gender distribution of tumor disease (11 females and 10 males).



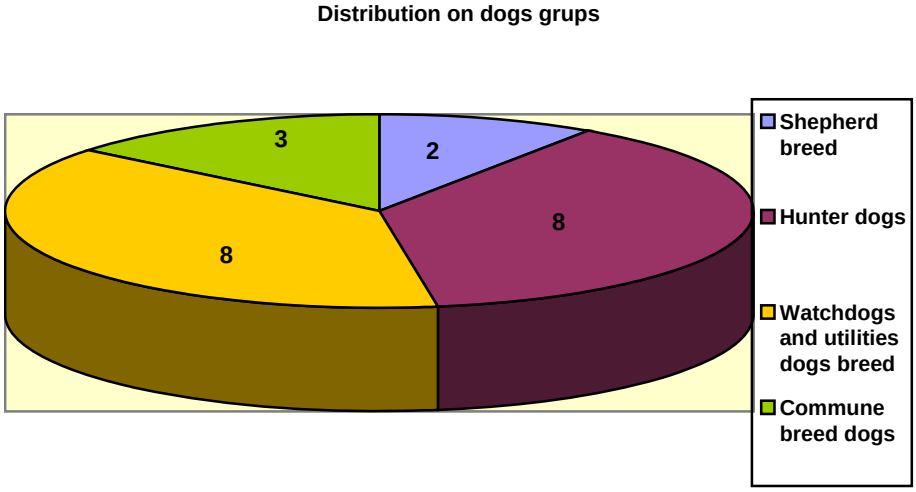
Age animals studied was between 1.6 and 8 years as follows:

- 1-3 years – 8 clinical cases;
- 3-6 years – 9 clinical cases;
- 6-8 years – 4 clinical cases.



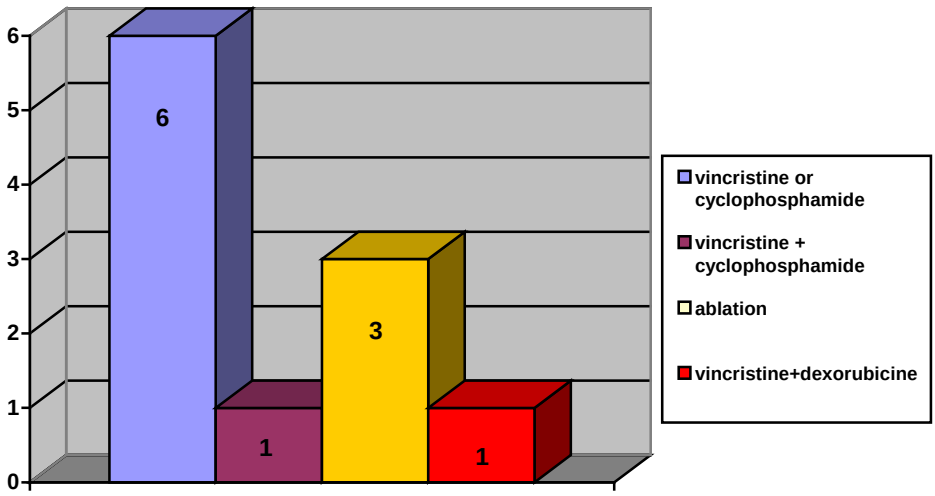
Distribution groups of dogs breed was following:

- Shepherd breed: 2 clinical cases;
- Hunter dogs: 8 clinical cases;
- Watchdogs and utilities dogs breed: 8 clinical cases;
- Commune breed dogs: 3 clinical cases.

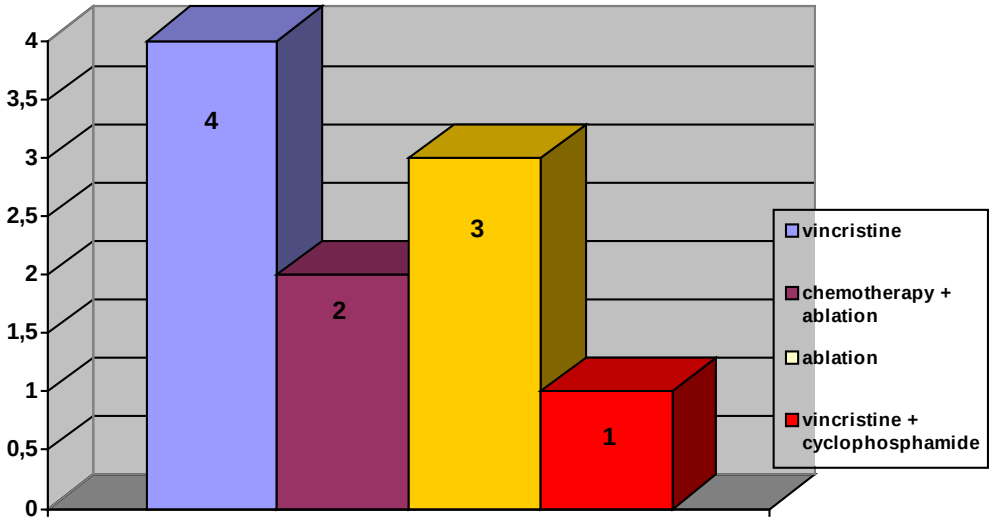


Clinical examination is welcome in determining the etiologic diagnosis of the Sticker tumor. Due to the location (in male dogs the tumor affects the penis and foreskin, in female dogs, it affects the vulva) typical rarely it can make confusion. Always it will establish a correlation between tumor development and existence of a history infecting sexual act, tumor disease transmission being made exclusively by coitus. Additional tests have proved to be useful only in order to assess the organic functionality. These exams were welcomed when administering chemotherapy medication, but also for assessing the type and degree of anestezologic if there was a surgical decision.

Chemotherapy treatments were applied in 13 cases, eight for female dogs and 5 male dogs. 6 female dogs cases received one chemotherapy (cyclophosphamide, vincristine), and in one case there were used together (vincristine and cyclophosphamide). In 3 cases of dogs there was used vincristine, and in 2 cases it was necessary combination of cyclophosphamide and vincristine + vincristine + dexorubicine. In 3 cases of middle female dogs there was used therapeutic surgical ablation of tumors. In 3 cases of dogs there were used surgical therapeutic procedures, with a view for ablation of tumors. In two cases surgical ablation was preceded by chemotherapy.



Female dogs



Males dogs

Conclusions

1. The study of this work was done in 2012-2015 at Veterinary Clinic of *Spiru Haret* FMV, within the Department of Reproduction, Obstetrics, Gynecology and Andrology, on a number of 21 cases (11 female dogs and 10 male dogs). Patient age ranged between 1.6 and 8 years old, period of sexual activity.

2. There were only two of the known therapeutic means: chemotherapy and surgical tumor removal. The method used was chemotherapy – 13 cases. Surgical treatment means were used in 6 cases, and joint (chemotherapy and surgery) in 2 cases.

3. In all cases progress showed a very good therapeutic job, even if therapeutic procedures mean, in some cases, mixed (+ chemotherapy + ablation + chemotherapy or chemotherapy).

4. It is imperative that sexual intercourse takes place after a thorough clinical examination and observation and even intake specialist veterinarian or physician. The use of dogs for breeding, after all clinical signs, if they had the disease tumor Sticker, should be made only after full recovery.

5. Reproductive control and reducing the stray dogs, vectors of transmission of this morbid tumors, should be made with greater rigor.

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STUDIES ON PREPARATION AND PROPERTIES OF SOME POLYMER – SERINE PROTEASE INHIBITOR CONJUGATES

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Abstract

*We studied the conjugation of two types of plant serine protease inhibitors (serpins) with two biodegradable polymers. The serine protease inhibitors were extracted from soybean (STI) and watermelon (**Citrullus vulgaris**) seeds, respectively (CVTI) and were purified by ion exchange and affinity chromatography. The polymers used as conjugation supports were chitosan and poly-3-hydroxybutyrate (PHB)-chitosan blends. The PHB was obtained by microbial biosynthesis and a PHB-chitosan film was prepared by the emulsion blending technique. Two conjugation methods were used: glutaraldehyde mediated conjugation and carbodiimide coupling. The obtained biopreparations were studied with respect to serine protease inhibition capacity and polypeptide release time in different media (physiological saline and buffer solution HCl-KCl 0,015 M, pH 2). The maximum of serine protease initial inhibition capacity (63.4%) was retained through conjugation by covalent binding on chitosan, in case of CVTI and through conjugation by cross-linking with glutaraldehyde on PHB-chitosan blend film, in case of STI. The polypeptide release times were the same for both CVTI and STI at neutral pH (6 hours), meanwhile the acidic pH = 2.0 delayed the STI release time up to 12 hours and accelerated the CVTI release time at 2 hours.*

Keywords: *protease inhibitors, conjugation, poly-(3-hydroxybutyrate), chitosan*

Introduction

The recently achieved progresses in understanding the extra and intracellular interactions at molecular level lead to the validation of a great number of proteins as therapeutic targets. The serine-protease inhibitors

(serpins) play a special role in elucidating the involvement of these enzymes in degradation processes at cellular level [1]. The recent biological researches regarding these inhibitors are revealing a remarkable interest for some of the serine proteases as therapeutic targets and for their inhibitors as potential therapeutic agents in treating of diseases with a continuous increase of frequency, incidence and prevalence indexes [2].

Protease inhibitors are abundant proteins in the storage organs and seeds of plants. The known plant protease inhibitors (PPI) belong to at least nine families: Kunitz (STI) [3], Bowman-Birk (BBI) [4], squash [5], cereal [6], rape seed [7], arrowhead [8], potato I and II [9], and barley [10]. Some of them seem to be restricted to only one botanical family, for example, inhibitors of the squash family (*Cucurbitaceae*). Representatives of others families, for example the potato inhibitors I, were isolated from *Solanaceae* [9], *Gramineae, Leguminosae* [11], *Amaranthaceae* [12], and *Cucurbitaceae* [13]. Among these protease inhibitors, having different specificity towards various proteolytic enzymes [14], plant protease inhibitors of the Bowman-Birk family, a major PPI family from vegetable seeds, have emerged as highly promising cancer chemo preventive agents, being capable of preventing or suppressing carcinogenic processes in a wide variety of *in vitro* and *in vivo* animal model systems.

Using natural, biodegradable polymers (i.e. chitosan or PHB) to obtain bioactive compounds formulations or delivery systems holds considerable promise for improving the safety, effectiveness and cost efficiency of numerous medicines [15, 16]. Such polymeric materials can be used to encapsulate protein drugs (e.g., enzyme or enzyme inhibitor), with the purpose of a controlled release of the protein drug as well as to protect the non-released protein from degradation [17]. Conjugation is known as an useful method to stabilize the biochemical activity of proteins, e.g. enzymatic or inhibitory [18], thereby increasing the availability of the bioactive proteins for longer reactions.

In our experiments we used serine protease inhibitors of plant origin in order to examine their conjugation with chitosan or poly-(3-hydroxybutyrate) – chitosan blend and the behavior of such a bioactive system in different medium conditions.

Materials and methods

2.1. **Isolation and purification of serine protease inhibitors**

The extraction of plant serine protease inhibitors from soybean as well as watermelon seeds was conducted according to the following protocol:

a) defatting of seeds by stirring them, as grounded powders, for 2 hours at room temperature with 5 volumes (v/w) of acetone;

b) protein extraction form the dry defatted powder in an optimal volume of 0.1 M acetate buffer, pH 5.0, with stirring at 4°C, for 1 h;

c) centrifugation of the resulted suspension at 4000 rpm for 30 min, discarding the pellets and separation of the supernatant rich in proteins;

d) fractionated precipitation with ethanol in 2 stages: d1) treatment of the supernatant with an equal volume of ethanol for 1 h at room temperature and removing the precipitate by filtration; d2) the clear supernatant is treated with 4 volumes of ethanol at low temperature (-20°C) and maintained in the refrigerator at 4°C for 24 hours;

e) centrifugation at 4000 rpm of the suspension d2, drying under vacuum of the crude protein precipitate harvested, dissolving it in distilled water and clarifying by filtration;

f) diluting the protein aqueous solution with an equal volume of 0.1 M acetate buffer, pH 5.5, and protein adsorbtion on a CM-Sephadex C25 column, preequilibrated with 0.05 M acetate buffer, pH 5.5;

g) elution of proteins with a linear gradient of NaCl (0.0-0.6 M) in the same buffer, at a flow rate of 60 $mlxh^{-1}$;

h) in the case of watermelon seed extract, the protein solution containing serine protease inhibitor is brought to pH 7.5 and applied to a column packed with trypsin immobilized in chitosan gel and equilibrated with 0.05 m Tris-HCl buffer, pH 7.5; the adsorbed inhibitor is eluted with 0.1M glycine HCl buffer pH 2.8.

2.2. **Preparation of poly-(3-hydroxybutyrate)**

Poly-(3-hydroxybutyrate), PHB, was obtained by fermentation with a *Cupriavidus necator* strain, in batch culture, on glucose and salts, under ammonium limitation conditions. The microorganism was cultivated in a liquid medium in Erlenmeyer flasks, submitted to orbital agitation of 220 rpm at 30°C, for 48 hour. The ratio of PHB accumulated was quite high, up to 89% of the cell dry weight. The recovery of intracellular PHB, as a polymeric membrane, was performed using NaClO digestion, solvent (chloroform) extraction and finally, chloroform evaporation.

2.3. **Preparation of polymer – serine protease inhibitor conjugates**

Conjugation of serpins with the above mentioned polymers was performed according to three experimental variants:

I. glutaraldehyde mediated conjugation of serine protease inhibitors with chitosan (CH);

II. carbodiimide mediated covalent binding of serine protease inhibitors to CH;

III. glutaraldehyde mediated conjugation of serine protease inhibitors with CH-PHB blend.

In the first variant, a CH gel was obtained as follows: 3 g of powder of chitosan was dissolved in a mixture of acetic acid, sodium acetate, water (1:1:24), pH 5.4, on water bath, at 50ºC under continuous agitation. Then, 0.3 g serpin immersed into 20 ml HCl 0.001M were added, under continuous stirring. The homogeneous mixture obtained was treated with 15 ml glutaric aldehyde 50% dissolved in 150 ml bidistilled water, for cross-linking, and maintained for 30 minutes, at a temperature of 4-5ºC; then 1.5 g sodium boronhydride in 50 ml bidistilled water were added in small portions and under continuous agitation, at a low temperature. The granules formed were separated by means of vacuum filtration on filter paper, using a Buchner funnel, and alternately washed with: bidistilled water, 0.5M phosphate buffer, pH 7, 0.5M phosphate buffer pH 5.6 and again with bidistilled water.

In the second variant, a chitosan gel was obtained by dissolving 3 g chitosan in 100 ml solution of acetic acid 1% (v/v). The solution obtained was injected with a seringe of 27G, in a coagulation bath (250 ml), containing sodium hydroxide solution 1N in ethanol 26% (v/v), under agitation, to form spherical gel particles. After a 3 hours static regime for coagulation concluding, the spherical particles formed as such were separated by filtration and then washed in distilled water at a neutral pH. Further on, these were treated with a 0.75% (g/v) solution of carbodiimide in a 0.05M phosphate buffer pH 6, at 25ºC, maintaining them in contact for a10 minutes activation at room temperature. After activation, the particles were abundantly washed in distilled water, filtered, and then suspended in 30 ml serpin (CVTI or STI) solutions in 0.05M phosphate buffer pH 6. The conjugation time was of 60 minutes and then the particles were separated by filtration and washed with distilled water to eliminate the non-immobilized serpin.

In the third variant, 1% chitosan solution in 1.5 M acetic acid-acetate buffer pH 5.4 was prepared at 50^0C, under continuous stirring. Separately, the PHB membrane was dissolved in chloroform, at a concentration of 1% (g/v). The two components were mixed at a 1:1 ratio, at room temperature, then 10 ml of serpin solution, pH 6 and glutaric aldehyde for a final concentration of 1,66% were added, under continuous stirring. The resulting mixture was maintained with stirring at room temperature during 1 hour and then it was placed in a Petri dish for 24 hours, without stirring. The gel thus

obtained was successively washed with distilled water, 5% metabisulphite solution and again distilled water.

Immobilized serpin activities were indirectly measured, by a spectrophotometric method, based on the decrease of the rate of trypsin catalyzed BAPNA hydrolysis. Kinetic characteristics of CVTI or STI release in physiological saline and in buffer solution HCl-KCl 0,015 M, pH = 2, during 12 hours, were recorded.

2.4. **Measurement of protease inhibition**

Trypsin inhibition: The ability of the various trypsin inhibitors (CVTI and STI) to prevent trypsin hydrolysis of BAPNA is measured spectrophotometrically (405 nm, ε = 9.96 $cm^2\mu mol^{-1}$) at 25^0C, with a UV-Vis spectrophotometer by time course measurement of ΔAbs. One trypsin unit hydrolyzes 1.0 µmole of N-a-benzoyl-DL-arginine p-nitroanilide (BAPNA) per minute at pH 7.8 and 25°C and one Trypsin Inhibitor Unit (TIU) will decrease the activity of 2 trypsin units by 50%.

Protein assay: Protein concentration was determined by Lowry method.

Results and dissusions

3.1. **Isolation and purification of the serine protease inhibitor from seeds of *Citrullus vulgaris***

The purification procedure described resulted in the isolation and purification of the homogenous serine protease inhibitor from seeds of *Citrullus vulgaris*. The protease inhibitor was isolated from the ethanol-precipitated proteins by CM-Sephadex C-25 chromatography, followed by affinity chromatography on immobilized trypsin (table 1).

In the first isolation-purification stage, the protease inhibitor was eluted as a symmetrical peak by ion exchange chromatography (figure 1).

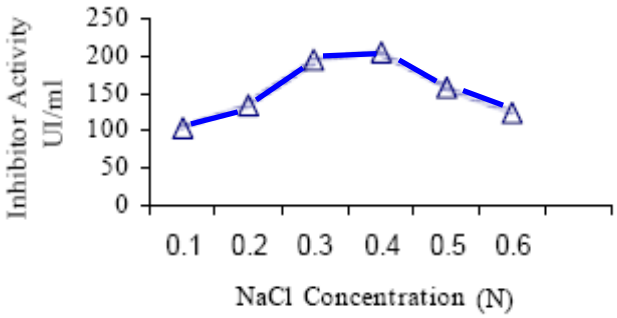

Figure 1. *Desorption of CVTI from CM-Sephadex C-25 column, eluent NaCl 0.0-0.6 N, flow 1 ml/min*$^{-1}$

The mixture of protein fractions no. 2 – 5 was subjected to affinity chromatography purification by applying it onto the affinity column, under slight basic conditions, and elution of the inhibitor at a pH below 3.0. Using this method we purified trypsin inhibitor from watermelon seeds.

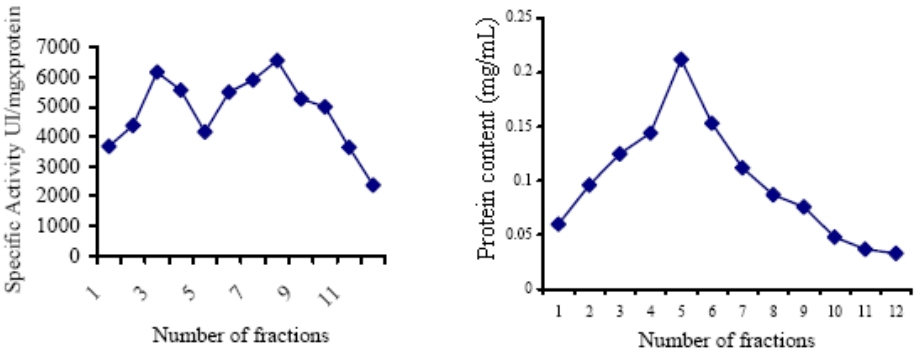

Figure 2. *Graph of desorption of protease inhibitor from the column with trypsin immobilized on chitosan*

Also, we experimented the inhibitor adsorption capacity on immobilized trypsin and we determined optimal desorption yield of the protease inhibitor (table 1 and figure 2).

Table 1

***Biochemical characteristic of the extract and of the purified protein from seeds of* Citrullus vulgaris**

Sample	Protein conc. (mg/ml)	Inhibitor conc. (UI)	Inhibitor specific conc. (UI/mg protein)
Protein extract (precipitated with ETOH)	182	98,716	542
Protein solution (ionic exchange chromatography)	73.9	123,334	1,667
Purified protease inhibitor (affinity chromatography)	16.61	151,512	9,122

3.2. **Obtaining of serpin-polymer conjugates**

The conditions applied for serpin conjugation with biodegradable polymers, CH or CH-PHB, as well as the conjugation efficiency, are presented in table 2.

Table 2

CVTI or STI – polymer conjugates

Sample no.	Type of serpin - polymer conjugate	Initial polymer amount (g)	Initial inhibitor conc. (UI/g)	Amount of resulted conjugate (g)	Serpine – polymer inhibition capacity	
					(UI/g)	% of the initial inhibitory level
1	CVTI / Chitosan (var.I)	10	625	6.74	253	40.5
2	CVTI / Chitosan (var.II)	10	625	5.95	396	63.4
3	STI / Chitosan (var.II)	10	118.2	9.45	31.09	26.3
4	STI / Chitosan-PHB (var.III)	10	118.2	7.9	51,4	43.5

The results obtained show that the conjugation efficiencies are relatively low, except sample 2. Sample 3 had low mechanical resistance and very low conjugation efficiency.

3.3. **Serpin release from conjugate preparations**

Sample 2 and 4 of polymer – serpin conjugates were tested for CVTI or STI release characteristics in physiological saline and in buffer solution HCl-KCl 0.015 M, pH 2, during 12 hours (figures 3 and 4).

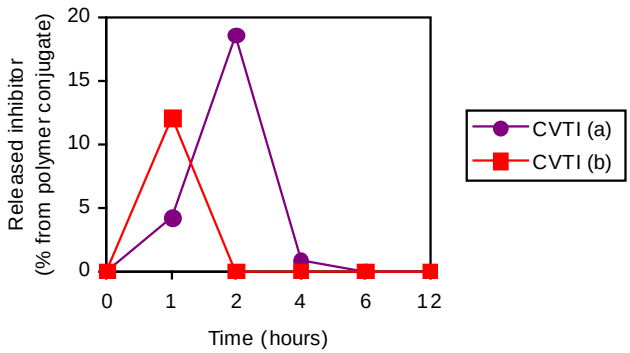

Figure 3. *CVTI release from polymeric conjugate (sample 2):*
***(a)** in 0.9% solution of NaCl and **(b)** in buffer solution HCl-KCl 0,015 M, pH 2*

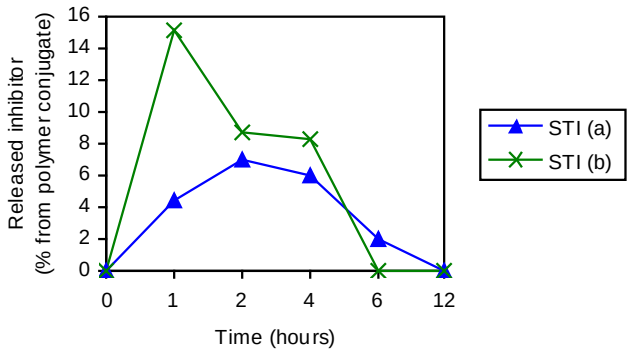

Figure 4. *STI release from polymeric conjugate (sample 4):*
***(a)** in 0.9% solution of NaCl and **(b)** in buffer solution HCl-KCl 0,015 M, pH 2*

Conclusions

The carbodiimide mediated conjugation of CVTI with chitosan led to a recovery of 63.4% of serine protease inhibiton initial capacity of the polypeptide, being more efficient than the glutaraldehyde mediated conjugation, which displayed only 40.5% retained inhibition capacity. STI glutaraldehyde mediated conjugation with chitosan-PHB blend displayed 43.5% of the initial inhibition capacity of the polypeptide, being more efficient than STI – chitosan conjugation by carbodiimide mediated coupling.

As regards the serpin release from the best conjugates obtained, this was accomplished in 6 hours at neutral pH for both CVTI and STI, meanwhile, at acidic pH, the CVTI release time was of 2 hours and the STI release time was of 12 hours.

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ECONOMIC MANAGEMENT AND APPROACH CONTROL OF ORGANIZATION IN BIOCHEMISTRY AND BIOPHYSICS TO ENSURE THE SUSTAINABILITY OF PUBLIC HEALTH IN ROMANIA

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Abstract

The article shows that from among the models known in the scholarly view that mathematical modeling (using symbols, letters, numbers, indices etc.) and the graphic expression modeling (using diagrams, drawings, figures, arrows, continuous lines, dotted lines, circles, rectangles, etc.) are best suited to represent sustainability sanitary condition considered "a situation" in the environment. It was also envisaged to imagine a new model, original, own the first time in literature, as a personal scientific contribution, original, leading to obtain a conception (conceptualization on addressing economic and managerial control (of Biochemistry and Biophysics organization for health sustainability in Romania).

Keywords: *mathematical model, health sustainability, graphic design, biophysics, biochemistry*

Introduction

Health Sustainability is not a physical object, material, tangible that can be defined by specific dimensions of the international system of units. Health is a state sustainability considered "a situation" into the overall quality of the environment.

Measuring health sustainability is part of how to measure intellectual capital, intangible assets.

Therefore, to evaluate, assess and measure the sustainability of health resorting to highlight the processes are part of those measures "knowledge" about a certain concept as sustainability of health.

In the face of scientific investigations concluded that the most appropriate method for highlighting health is shaping sustainability.

Therefore, it was determined that the only way scientific, effective and concrete in this case is represented by a model health sustainability.

In ordinary reality a model is imitating a state, a situation reflects that status by analogy, that situation in terms of quantity (size, content) and qualitative (property, meanings, functions).

Material and methods

1. **Choose a model**

Among the models known in scholarly view that the mathematical modeling (using symbols, letters, numbers, indices, etc.) and the graphic expression modeling (using diagrams, drawings, figures, arrows, continuous lines, dotted lines, circles, rectangles, etc.) are best suited to represent sustainability sanitary condition considered "a situation" in the environment.

It was also envisaged to imagine a new model, its the first time in the literature as a scientific contribution to personal, original, leading to obtain a conception (conceptualization) on tackling the economic and managerial control organization Biochemistry and Biophysics for ensuring the sustainability of sanitation in Romania). (Figure 1).

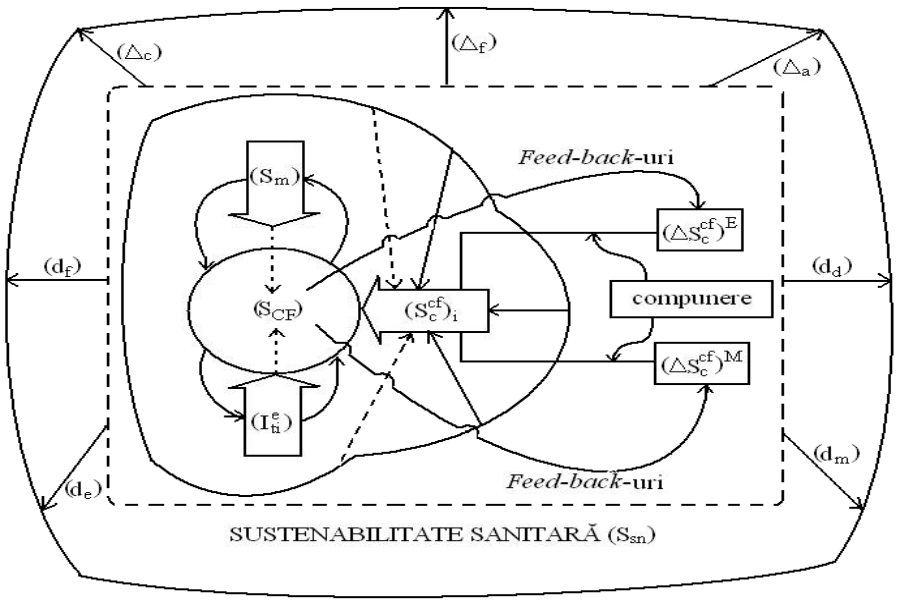

Fig. 1. *Economic concept and managerial approach of biochemical control organization and biophysical health to ensure sustainability in Romania*

In fact, research joined composed, articulate and integrated modeling with two alternatives: mathematical modeling with graphical modeling.

2. **The implications of graphic design**

The first closed contour line, the highest in the whole interior design, includes all other representations and symbols and means the representation of health and sustainability of these accounts has been applied to the symbol (SSN).

In fact, this outline will incorporate the concept of sustainability health, seeking to explain it in terms of quantity and quality by plotting.

Inside the square is formed by drawing the dotted line which, in turn, has representations and symbols inside.

This outline leads us more and more towards health and sustainability is, primarily, feedback, side of the elements found within the square dotted line on the sustainability of health.

In fact, the square is shown with dotted lines Δc.

We note that the square dotted lines are directed by 7 touch-tipped arrows outline the area's main generic health sustainability.

This means that the entire square with dotted lines have feedback, side, retrieved conceptual influences on health sustainability.

These influences are: Δc – general influence on the sustainability of health, Δf – influence the size/functionality on the sustainability of health, Δa – showing sustainability influences health.

Mathematical notation Δ means difference, variation, size is not fixed but expressed influences.

For the same square formed by dotted lines, where there are a number of four arrows that touch the tips outline health and sustainability that are denoted with the letter d, can direct influences.

In fact, it means continuity of health sustainability symbol dm, sustainability means maintaining sanitation, health and sustainable development signifies dd, df is to strengthen the functions of sanitary sustainability.

Further deepening explanatory chart pattern, within the square dotted line has provided the core, essential, which determines, establishes and influences the conception of sustainability and, on this basis, can move to addressing economic and managerial organization biochemical control and biophysics.

The center circle labeled (SCF) means biochemistry and biophysics structures (which can be found in the public health system, health system, hospitals and laboratories etc.).

It states that no variant concept of sustainable health can not be conceived without considering the structures of biochemical and biophysical influence' positive or negative human health and on which, or with their check must be done, in the evaluative Face submit it to the search for economic and managerial organization.

The figure is observed three broad arrows all pointing the nose of the circle representing the biophysical and biochemical structures.

A first arrow broadened, who is from top to bottom, is marked inside with symbol Sm and represents "management structure" (ie, the structures of biochemical and biophysical meet mandatory influences managerial organization, management, establishment, human resources management methods and techniques, etc.).

It is noted that the arrows on biophysical and biochemical structures determine the effect, that is, for a certain type of structure, Biophysics and Biochemistry single arrow oriented widened in the circle which indicates the need for a certain type of management.

Also, simple arrow fired from the circle enlarged structures and showed that a particular type of system can transform the management, modification or adaptation of biochemistry and biophysics structures, so that they can be organized and managed by a real control.

A second arrow is oriented to the enlarged bottom up, with the tip touching the circle biochemical, biophysical structures and is marked inside with I_{ti}^{e} which means "infrastructures applicable to Biochemistry and Biophysics structures" (ie hospitals, laboratories, etc.).

Similar to the situation described in the first arrow widened from top to bottom, we see that the infrastructure can influence I_{ti}^{e} by biophysical and biochemical structures, in turn it can produce influences, eg the system infrastructure requirements.

The arrow representation is noted that the enlarged bottom-up oriented achieved concrete structures of Biochemistry and Biophysics circle.

By comparison it is noted that the enlarged arrow from top to bottom does not touch the concrete circle biochemistry and biophysics structures, since management (systems management) is not active, tangible (material), but conceptual.

A third arrow is oriented widened from right to left and reaches the tip circle of biochemistry and biophysics structures.

It has an internal notation (SCCF) and which means "investigation and evaluation system that control" Biochemistry and Biophysics structures.

Next, it assigns a closed contour line delineating the core of "biophysical and biochemical structures" with three arrows wider influence.

This means that biochemistry and biophysics are targeted structures permanently enlarged by three arrows (management, infrastructure and control/investigation – evaluation) and, in fact, this compulsory core should be subjected to economic and managerial organization.

We note that, in fact, the most important arrow is extended from right to left, control-investigation-evaluation as other arrows are simple continuous or discontinuous lines and influence on the perimeter nucleus where this arrow is extended.

Once outlined this core, we notice that it is the central subject within the square with dotted lines for feedback and influences on the sustainability of health.

Therefore, the most important arrow of the entire model (enlarged arrow flipped left to right) is one that ensures the flow of feedback, reactions and influences, which are variable, denoted by delta (Δ).

The small square top, labeled (ΔSCcf), is "investigating the economic assessment" and the square on the bottom right labeled (ΔSCcf) M is "investigating and evaluating management".

In fact, the two squares (for economic assessment or management) are receiving feedback from biophysical and biochemical central structures on which act throughout the three large arrows widened.

The two types of investigations and evaluations compose and influence the size and quality inside the large square with dotted lines and thus resulting consequences that influence the general contour of health sustainability.

In essence, the original graph shows that the structures of biochemistry and biophysics at the center permanent basis are influenced by management, infrastructure and control system that generate the main influences on qualitative and quantitative evaluation of the sustainability of health.

Below, we reproduce symbolized mathematical model (own creation, original Advanced herein) on the economic outlook managerial approach of biochemistry and biophysics control organization to ensure sustainability of health in Romania:

$$\begin{cases} \left\{\left(\Delta S_c^{cf}\right)^E * \left(\Delta S_c^{cf}\right)^M\right\} \rightarrow \left(S_c^{cf}\right)_i & \text{(i)} \\ \left\{\left(S_m\right) * \left(I_{ti}^e\right)\right\}_{\frac{\max(+)}{feedback-uri}} \rightarrow \left(S_{CF}\right) & \text{(ii)} \\ \left\{\left(S_c^{cf}\right)_i * \left(S_{CF}\right)\right\}_{\frac{(\Delta_c * \Delta_f * \Delta_a)}{(d_f * d_e * d_m * d_d)}} \rightarrow \max\left(S_{sn}\right) & \text{(iii)} \end{cases}$$

So symbolized mathematical model explanations are:

– The mathematical system symbolized discussion of equation (i) is as follows:

• symbol (ΔSCcf) E which is investigating economic assessment, in which the element (ΔSCcf) M represents investigation and assessment management, according to the arrow oriented from left to right in the equation, and together they must determine the size and importance of qualitative and quantitative symbol (SCCF) i.

It specifies that all three symbols are found in the above graphic representation of the model, ie two small squares (upper and lower) and the enlarged arrow oriented from right to left.

The * (asterisk) in mathematics, respectively in equation (i), represents any of the following: addition, subtraction, multiplication, division, integration, derivation, or all together, which means "compounding" and computer scientists, when done the software of the equation, properly established operation/operations concerned.

The interpretation of this equation is the following: the symbols of the two squares composed together, located between accolade, influence the maximum or minimum symbol of arrow widened aimed at controlling, investigating, evaluating, which will be important in organizing economic and managerial control and to finally define the size of the quantitative and qualitative influences on the sustainability of health.

– The mathematical system symbolized discussion of equation (ii) is as follows:

• it marks the first arrow broadened the from top to bottom, that is noted inside the letters Sm and means management structure, consists infrastructures I_{ti}^{e} (hospitals, laboratories) and determines the maximum size of a quantitative and qualitative structures of Biochemistry and Biophysics (SCF).

The equation points that arrow from left to right that have referred above, has to maximize, max (+), and below the arrow, are all conditions expressed by feedback sites belonging to the first two terms of the equation which it is composed.

In other words, the equation shows that the structures of biochemical and biophysical have maximum quantity and quality and are permanently corrected feedback sites and infrastructure management with composition.

– The mathematical system symbolized discussion of equation (iii) is as follows:

• symbol of arrow main broadened oriented from right to left, denoted (SCCF) and that means the system of investigation and evaluation or control compound (SCF), which means places of biochemical and biophysical structures that must determine the sustainability of health at Maximum denoted SSN.

This status/situation must register maximum health sustainability that can be achieved, according to this equation, with the following conditions:

1. above the arrow must be considered together composite Δc influences, which represent general influence on the sustainability of health, Δf – influences the size / functionality on the sustainability of health, Δa – influences that show the sustainability of health;

2. in the arrow must be considered, together, given compounds of the influences which mean the continuity of the sanitary sustainability. Dm symbol – sustainability means maintaining plumbing, sanitary dd sustainability means developing and strengthening the functions df which is the sustainability of health.

So, the model symbolized Matemale original own reported herein and shows that, in fact, the three equations together (i, ii, iii) lead to the expression of essential resolution/representative about the design approach to economic management of Biochemistry and Biophysics control organization to ensure sustainability of health in Romania.

The control system organized by economic and managerial structures that directly influence the biophysical and biochemical structures of management and composition from infrastructure, which is the compound of structure that determines biochemistry and biophysics, in fact, the sustainability of maximum health.

This sentence is expressing relational mathematical conclusions drawn with explanations for the graphical model, namely: essentially, graphic model shows that the structures of biochemistry and biophysics at the core of the topic influenced permanent management, infrastructure and control, generated influences on the main system qualitative and quantitative evaluation of the sustainability of health.

Conclusions and discussions

1. The graph is fully compatible with symbolic mathematical model, that have the same content, same meaning, lead to the same result in enhanced sustainability maximum health, but have different forms of representation, but perfectly complementary, contradictory.

2. This model serves the following processing steps:

– graphical development;

– developing mathematical symbol;

– realization of software;

– achieving widespread IT product that serves economists, managers, developers of programs and strategies.

3. The model is original and is subject to copyright scientific community.

4. There is a possibility that future research to develop this model for the development of applications.

5. The new concept model has the potential to generalize biophysical and biochemical structures that can be replaced with other creative types of structures, areas of economics, knowledge management and specific themes and different subjects.

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FOOD SAFETY – A VETERINARY FORENSICS MEDICINE APPROACH ON INFRACTIONS AND CONTRAVENTIONS IN THIS DOMAIN – STUDY CASE, HORSE MEAT ADULTERATION

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Abstract

Purpose: *The first aim of this paper was to separate and define the concept of food safety from the other concepts defined in the food and feed sectors, as food security, foodstuffs safety and food sovereignity. Food safety may be assessed from different points of view: as matter of food veterinary expertise or control, using health involvements of raw or processed food, from religious point of view, taking into account the existing restrictions within different cultures and people, from economical point of view, both for food with official approved composition and for food submitted to different kinds of adulteration or fraudulent handling, or as a new approach as veterinary forensics medicine, as infractions or contraventions, including several elements from the aforementioned approaches. The last approach is almost entirely new and the second aim of the present paper was to present a study case of horse meat adulteration as beef, by fraudulent label changing of horse meat as beef label.*

Materials and methods: *The drawing up of this paper was based on exploratory checking of specific existing literature regarding food safety, specifically referring to sanitary-veterinary expertise and veterinary official control applied, as well as on assessment and modulation within a descriptive shape of many aspects constituting a case study concerning infractions and/or contraventions correlated with food safety, horse meat adulteration, of less nutritive value, being fraudulent labelled as beef, meat with superior nutritive value and a better price. Procedural order materials, iconographic materials, as well as regulatory legal documents have been used, selected and classified as reference materials for completion of study case.*

Results and discussions: *The proposals drawn up by this paper form into an important tool for veterinary forensics medicine content development and as accurately as possible definition for its competence fields. At the same time, an official classification of methods used to perform infractions or/and contraventions in these domains is proposed, for the first time, up to our knowledge and documentary consulted bibliography, both for well-known-*

traditional fields, as well as within the new domains of food safety, identified in this context. Also, it is used for the first time, the matrix (module) for veterinary forensics medicine expertise, already presented in a previous paper, shape expertise that should be confused with sanitary veterinary and food safety inquiries or expertise, for a complete definition of a veterinary forensics medicine study case.

Limitations: *The drawing up of this paper was based on exploratory checking of specific existing literature regarding food safety, specifically referring to sanitary-veterinary expertise and veterinary official control applied, as well as on assessment and modulation within a descriptive shape of many aspects constituting, by paper consolidation and adaption, a typically study case for frauds produced in food chain.*

Conclusions*: The present work was a good opportunity to test the module proposed, with the view to perform veterinary forensics medicine expertise within the fields of food safety, as a practical example for veterinarians asked, with official ordinance, by bodies of prosecution or by bodies of criminal investigations, to participate within the team performing veterinary forensics expertise, when there is a suspicion of producing some specific infractions in the field.*

Keywords: *food safety, fraud, infraction, contravention, penal, module*

Introduction

As far back as the old civilizations – Greek, Egyptian, Roman, Phoenician, Assyrian, Persian, as well as from old Chinese culture and mosaic cult, elementary food safety rules and principles have been set up and put in place, consisting in provisions for raw food sources control, the manner of their preservation within their shelf life, in order to ensure their quality to be fit for human consumption, without any risk and to prevent or punish any adulteration of raw food, food products or ready to eat food. During the Earlier Middle Age, the Medium Middle Age and the Late Middle Age, the problems related to food safety were under the responsibilities of monks and churches, but the consequences was controversy, face to the policy applied, so, a lot of people died during these periods of time, consuming contaminated foods or non-conforming foods by different reasons, including fraudulent acts on raw materials or products/ready to eat food. During the Modern and Contemporary Era a new approach on food safety arose and situation has been changed, being better than previous periods. A new institutional national (National Sanitary Veterinary and Food Safety Authority/National Authority for Consumer Protection/Ministry of Health/National Organization for Consumer Protection), international framework (World Health Organization/World

Animal Health Organization/Food and Agriculture Organization/World Trade Organization) and at Community level (DG SANCO/DG AGRI) were set up to regulate the food safety aspects and to supervise and control whole food chains. The other institutions having responsibilities to assess and to punish each fraudulent act on food safety are also involved at national or international level. Specific framework legislation on official supervision and controls has been put in place with optimistic results.

Material and methods

Technological and informational development and globalization induce: unprecedented development and diversification of food safety and connected industries, obviously liberalization of food and raw food materials, tendency to self-regulation for food industry and food chain, incomplete or complicated food traceability systems, dissipation of the official supervision of food supply chains and food industry or food processing chains, and all these conducted and facilitated the occurrence and diversification of the infractional or contravention acts on food safety. The drawing up this paper was based on exploratory checking of specific existing literature regarding food safety, specifically referring to sanitary-veterinary expertise and veterinary official control applied, as well as on assessment and modulation within a descriptive shape of many aspects constituting a case study concerning of infractions and/or contraventions correlated with food safety, horse meat adulteration, of less nutritive value, being fraudulent labelled as beef, meat with superior nutritive value and a better price. Procedural order materials, iconographic materials, as well as regulatory legal documents have been used, selected and classified as reference materials for completion the study case. The present work groups specific information, as to follow the classical pattern of a criminal investigation, as follows: initiation of proceedings before the bodies of prosecution or by bodies of criminal investigations, collecting data or information to constitute the probatory, supplementary criminal investigations to formulate the indictment, registration the reactions of national or international institutions having responsibilities in the food safety domain, of political representatives from involved states, of religious cultures, of representatives for food chain or from food industry, against probatory and indictment in case, the substitution of infractions identification, clarifying the causes favouring or facilitating for these kinds of infractions, legal sentence pronounced and preventive measures.

Results and discussions

1. Initiation of proceedings before the bodies of prosecution or by bodies of criminal investigations starts on 15.01.2013, when the Food Safety Irish Services (FSIS) made public the fact that, following strategical examinations in food sector, using PCR techniques, with the aim to determine the species in the meat preparations or products (hamburgers), randomly performed for several food finite products prepared from beef, revealed that these products contained horse meat instead the beef, in different proportions, and supplementary investigations were foreseen. Official notifications were done by Food Safety Irish Services to Irish bodies of prosecution and Irish bodies of criminal investigations, to Community or international bodies of prosecution (EUROPOL/INTERPOL), to all veterinary and food safety authorities from Member States, through Rapid Alert System for Food and Feed and to international institutions or organizations in the specific field.

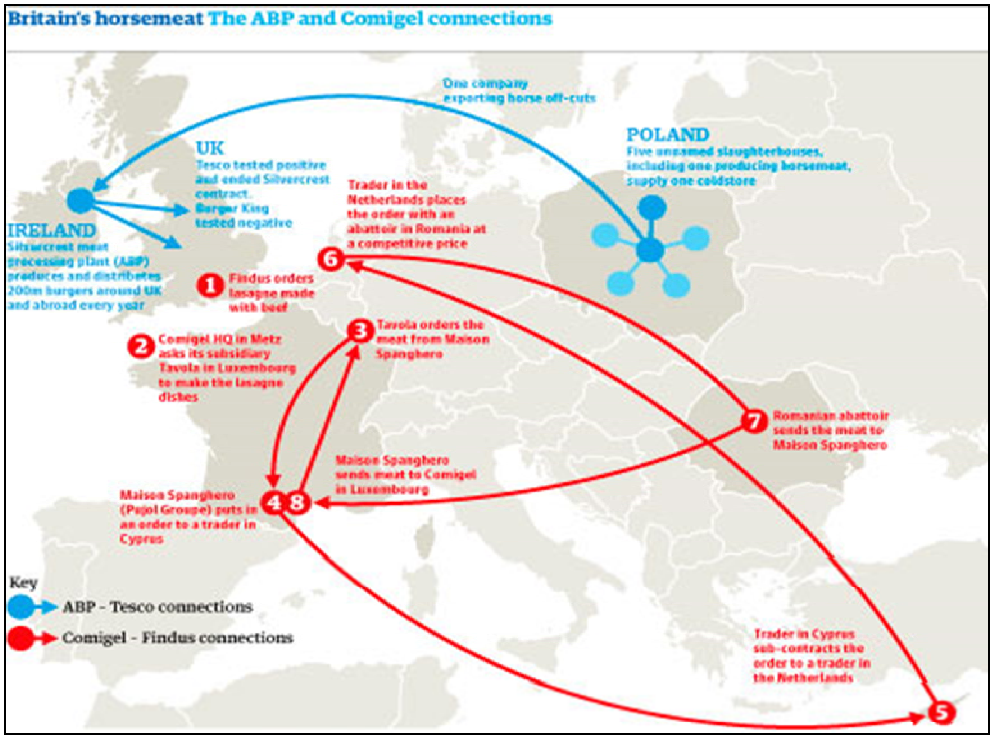
2. Criminal traceability investigations for raw materials, with the aim to constitute the probatory, were done by authorities having responsibilities in food safety and preventing food frauds, but also by operators from food supply chain, from 21 Member States (without Czech Republic, Slovakia, Slovenia, Denmark, Germany and Croatia), as well as from Russia and Azerbaijan.

3. Formulation of the indictment was based on: criminal investigations performed in the involved countries, documentary investigations and recordings realized at those 21 big directly or indirectly involved companies in the scandal, from food chain domain, on PCR tests performed for species detection, by the big transnational companies from food supply chain, with the aim to establish their products conformity, on those asked by national authorities for food safety or asked by Community or international bodies of prosecution and bodies of criminal investigations or by Commission to national authorities responsible for food safety.

4. Recordings and use within the framework of criminal inquiry of concerned reactions of populations, mass media with quotidian breaking news, companies from food supply chain involved in this adulteration, operators from food processing units, involved in distribution and trade process with false horse meat products, animal protection organizations, consumer protection organizations and religious culture representatives (Jewish, Buddhists, Muslims), official position of national authorities in the field or of bodies of prosecution and bodies of criminal investigations.

5. Indictment formulation-detection of the sources for horse meat used for adulteration. Criminal investigations with the aim to detect horse meat sources and their traceability, revealed six mean sources: Poland – fraudulent intra-Community trade with horse meat and horse meat mingled with beef; food companies from France, Netherlands and Cyprus, labelling the horse meat originated from Romania, correctly labelled, as beef; horse meat from horse races located in France, illegally slaughtered for food purposes and supplied to food distribution chain; horse meat from horses originated from Brazil, Mexico and probably United States.

6. Indictment formulation – infraction involvements. The infraction of criminal by substituting resort fraud related to food safety have had multiple consequences: commercial consequences inducing cancelation contracts for meat suppliers, withdrawal from the market of involved type products, losing delivering markets, destruction or wasting a huge amount of food products, loses through interdiction to made some traditional products, expenditure with meat product testing and more important the loss of consumer confidence, as well as the loss in supermarket system confidence; involvements in public health sector related with some medicine traditionally used for race horses treatment, but dangerous for people (fenilbutazone); religious involvements, taking into account specific restrictions applied by different religions (Judaic, Muslims, Buddhism).



7. Establishing the verdict, the guilty legal person and private individual and the sentences pronunciation. The mobile crime was previsedas being label changing (the horse meat label with beef label). The indicted/guilty concurrence-companies directly involved in committing the criminal offenses and companies indirectly involved in committing the criminal offenses, by dubious relations with the previous. The sentence applied consists in prison condemnation for different periods of time for direct counterfeiters from Poland, France, Cyprus, and Netherlands, for concurrent person to counterfeiters, slaughterhouses managers from France, Cyprus and Netherlands, horse races managers from France, as well as substantial contraventions for a lot of incriminated persons, closing some slaughterhouses, some food chain suppliers and suspending several horse races in France.

Conclusions

1. There are a lot of methods to make adulteration on food, but the most frequent method is substitution, both referring to components of raw food materials and to species from this raw materials, products or ready-to eat food is delivered.

2. This study case and its worldwide scope and the huge amount of available information on the subject gave us the opportunity to implement the processual pattern for a veterinary forensics expertize, foregoing proposed.

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THE INFLUENCE OF CROSSBREEDING AT ROMANIAN BLACK SPOTTED DAIRY MAINTAINED IN SEMI-INTENSIV SYSTEM

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Abstract

The aim of study was the application of a crossbreeding scheme to 65 Romanian Black Spotted (RBS) dairy, maintained in semi-intensiv system. In L1 group it was used the infusion cross-breeding one-generation with Holstein (RBS x H → F1 females x RBS → F2 females x RBS → F3 females). In L2 group it was used the repeated cross-breeding during three generations with Holstein (RBS x HF→ F1 females x H → F2 females x H → F3 females). The monitored parameters were: age of first calving, length of lactation, quantity of milk, percent of milkfat and quantity of milkfat. The productive performances data were statistically processed using ANOVA. At F3 metis females, the genetic structure was 87.5% Romanian Black Spotted in L1 and 12.5% Holstein in L2. At infusion cross-breeding, in F3 generation the age of first calving and the lactation length were 782 days and 281 days, respectively. The quantity of milk during the normal lactation increased significantly with 40.5% (from 3280 kg to 4580 kg), by maintaining the standard of breed. Percent of milkfat was 4.85%. In repeated cross-breeding, the performance parameters of F3 generation were statistically close to standard of Holstein breed. In order to maintain the characteristics of Romanian Black Spotted breed, starting with the fourth generation, it is recommended the "itself"cross-breeding, between F4 females and F4 males.

Keywords: *cross-breeding, Romanian Black Spotted*

Introduction

The Romanian Black Spotted cattle was formed as the result of a long crossing between the native cattle breed cows with Friesian type bulls imported since 1960 [3]. After the approval (1987), the breed was amended, genetically approaching by Holstein [2].

The breeding program pursues the consolidation of the standard and realization of 6000 kg milk [4].

Material and methods

The aim of study was the application of a crossbreeding scheme to 65 Romanian Black Spotted (RBS) dairy, maintained in semi-intensiv system.

The study was conducted on a private farm from Giurgiu country, in the period 2006 – 2013.

The Romanian Black Spotted cattle was reared both in purebred and crossing with Holstein.

The crossing scheme is shown in Table 1.

Table 1

Crossing scheme

Generation	L1 Infusion cross-breeding	L2 Repeated cross-breeding
P	♀ RBS x ♂ Holstein ↓	♀ RBS x ♂ Holstein ↓
F1	♀ F1 x ♂ RBS ↓	♀ F1 x ♂ Holstein ↓
F2	♀ F2 x ♂ RBS ↓	♀ F2 x ♂ Holstein ↓
F3	♀ F3 x ♂ RBS	♀ F3 x ♂ Holstein

The monitored parameters were: age of first calving, length of lactation, quantity of milk quantity, percent of milkfat and quantity of milkfat.

The data were processed statistically by T-test.

Results and discution

The data concerning the genetic structure are shown in figure 1.

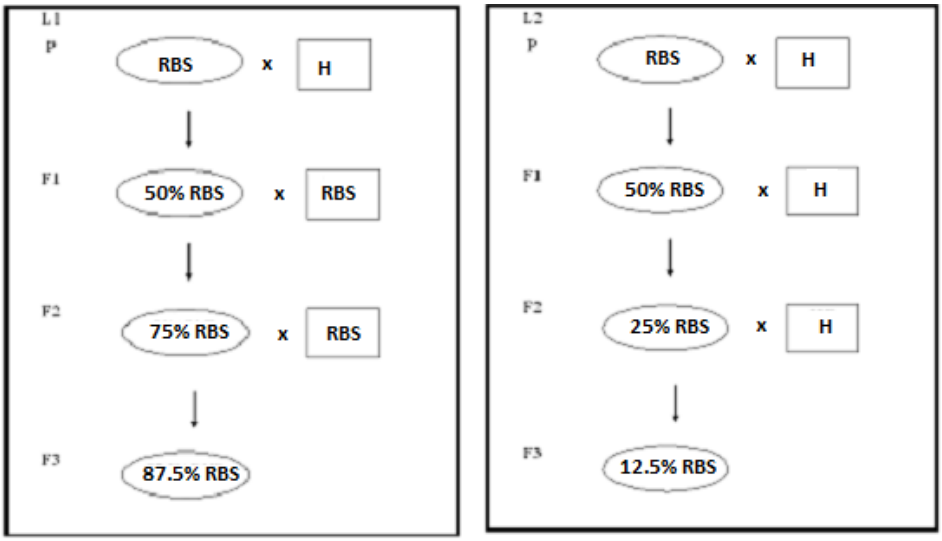


Figure 1. *Genetic structure*

In parental generation, the females of both groups were 100% Romanian Black Spotted (RBS). At group L1, it was applied infusion cross-breeding with Holstein bulls (for one generation), then out-breeding. At group L2 it was applied repeated cross-breeding with Holstein.

In third generation, the genetic structure of females was 87.5% RBS at group L1 and 12.5% RBS at group L2.

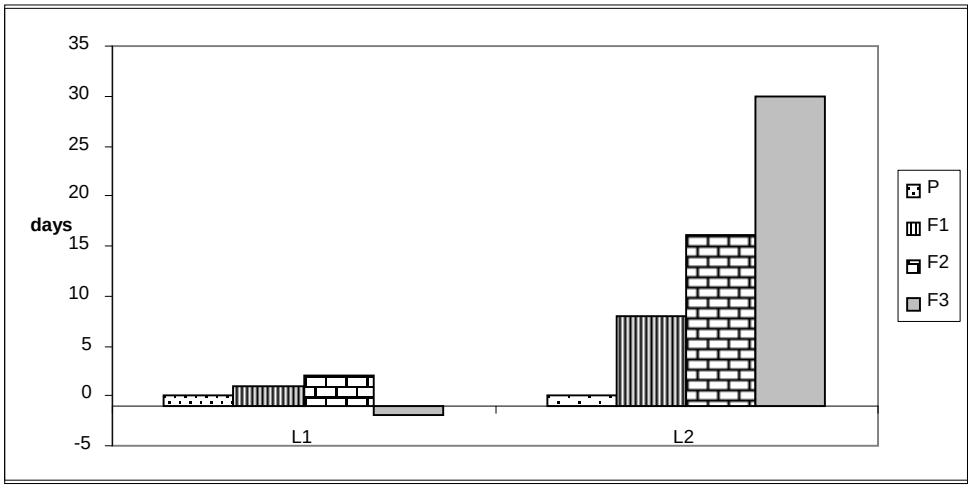
Table 2 shows the data concerning the age of first calving.

Table 2

Age of first calving (days)

Specification	L1	L2
P	784 ± 14.87	781 ± 15.01
F1	791 ± 12.14	789 ± 12.33
F2	786 ± 13.28	797 ± 12.41
F3	782 ± 13.12	811 ± 12.16

The age of first calving is very important, because influencing the interval between generations and it is a selection criteria to cattle. Comparing with parental generation (P), the females F3 recorded an equality statistics to group L1 (graphic 1), while at group L2 it was observed an increase with 30 days ($p \leq 0.05$).



Graphic 1. *Age of first calving*

The results from group L2 can be explained by the strong influence of Holstein breed, given that the age of first calving is 780-840 days at Holstein and 840-870 days at RBS [5].

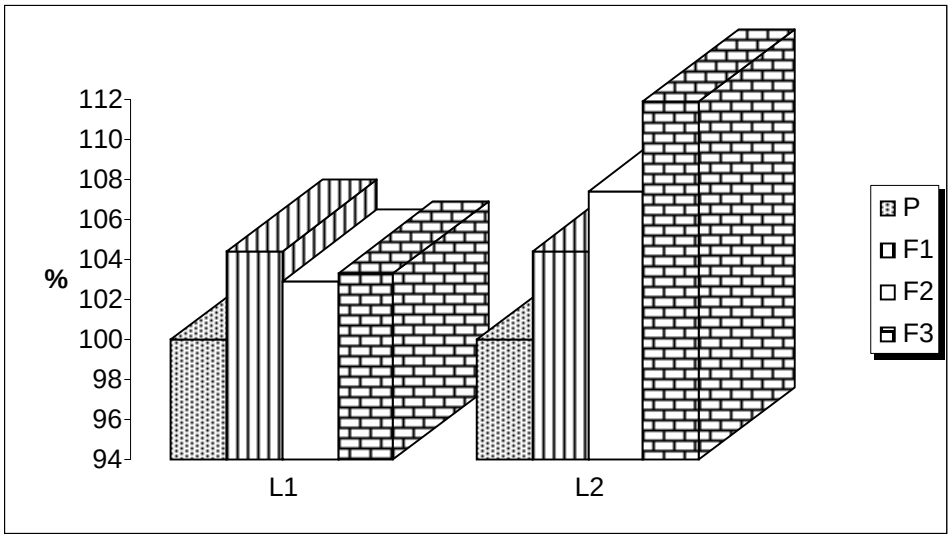
Data concerning the length of lactation are shown in Table 3.

Table 3

Length of lactation (days)

Specification	L1	L2
P	272 ± 26.02	270 ± 25.78
F1	284 ± 23.42	282 ± 23.51
F2	280 ± 20.17	290 ± 18.94
F3	281 ± 19.18	302 ± 16.06

The length of lactation influences both the productive performances and the reproductive activity. Comparing with parental generation (P), this parameter increased with 3.3% at group L1 (NS, $p \geq 0.05$) and with 11.8% at group L2 ($p \leq 0.05$), according to Graphic 2.



Graphic 2. *Length of lactation (%)*

These results can be explained by the different scheme of cross-breeding. In case of the one-generation infusion cross-breeding (L1) the parameter was not significantly influenced (from 272 days to 281 days). In repeated cross-breeding (L2), the length of lactation increased significantly (from 270 days to 302 days), and the population approaching the Holstein standard [1].

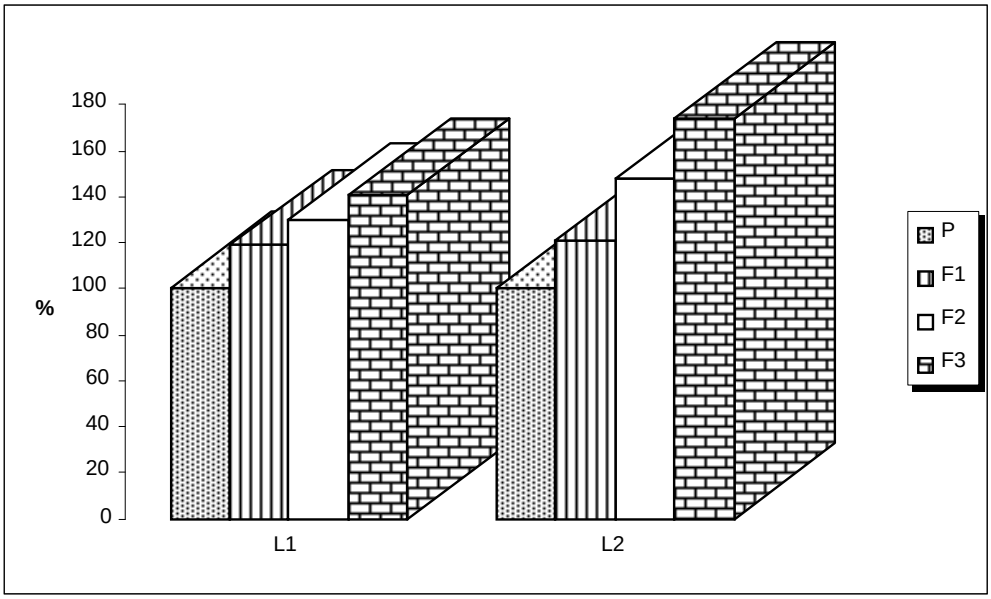
Table 4 shows the quantity of milk during the normal lactation.

Table 4

Quantity of milk (kg)

Specification	L1	L2
P	3260 ± 392.2	3230 ± 388.7
F1	3870 ± 324.1	3900 ± 346.3
F2	4240 ± 401.5	4780 ± 406.3
F3	4580 ± 418.2	5630 ± 435.5

The quantity of milk is a selection criteria and an efficiency parameter for dairy farms. Comparing with parental generation (P), the quantity of milk increased significantly with 40.49% at L1 ($p \leq 0.01$) and with 74.30% at L2 ($p \leq 0{,}001$), according to Graphic 2.



Graphic 3. *Quantity of milk (%)*

The interval between the parental and F3 generations was 6.46 years at L1 and 6.49 years at L2. In this period, the quantity of milk increased with 204.43 kg (L1) and 369.80 kg, respectively.

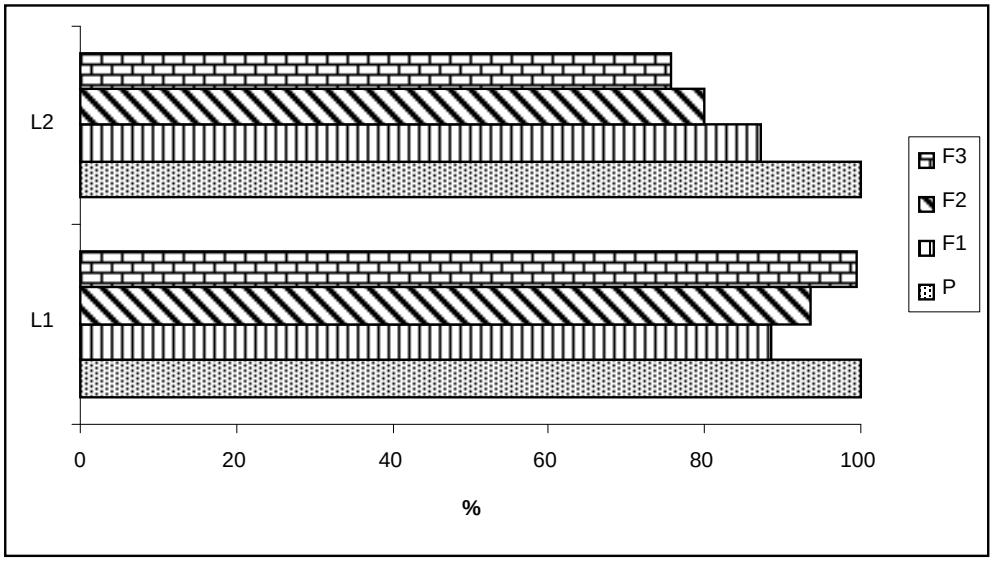
The data concerning the percent of milkfat are shown in Table 5.

Table 5

Percent of milkfat

Specification	L1	L2
P	4.87 ± 0.53	4.91 ± 0.50
F1	4.31 ± 0.44	4.28 ± 0.43
F2	4.56 ± 0.47	3.93 ± 0.42
F3	4.85 ± 0.46	3.72 ± 0.39

Comparing with parental generation (P), the percent of milkfat was statistically equal at L1 and decreased significantly with 24.2% at L2 ($p \leq 0.05$), according to Graphic 4.



Graphic 4. *Percent of milkfat*

Table 6 shows the data concerning the quantity of milkfat during the normal lactation.

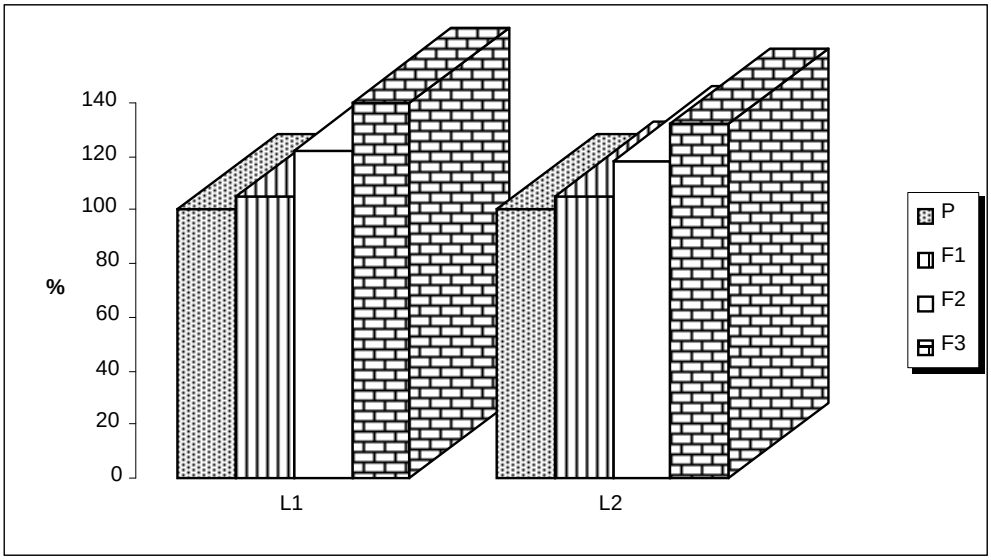
Table 6

Quantity of milkfat (kg)

Specification	L1	L2
P	158.76 ± 22.58	158.59 ± 23.58
F1	166.80 ± 21.25	166.92 ± 22.08
F2	193.34 ± 26.00	187.85 ± 23.22
F3	222.13 ± 29.28	209.43 ± 26.05

The quantity of milkfat is a synthetic parameter, being conditioned by the quantity of milk and the percent of milkfat.

Comparing with parental generation (P), the quantity of milkfat increased significantly with 39.91% at L1 and 32.05% at L2 ($p \leq 0.01$), according to Graphic 5.



Graficul 5. *Cantitatea de grăsime pe lactația normală (%)*

Conclusions

1. The infusion cross-breeding one-generation with Holstein (group L1) improved the productive performances and secured a genetic structure in F3 of 87.5% Romanian Black Spotted.
2. The repeated cross-breeding during three generations with Holstein (group L2) determined a genetic structure of 12.5% Romanian Black Spotted.
3. In group L1, the females F3 had higher productive performances, but within Romanian Black Spotted breed characteristics.
4. In group L2, the females F3 suffered productive modifications approaching Holstein breed.

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RESEARCHES ON MORPHOLOGICAL ASPECTS OF BLOOD IN CHICKENS AGED 1-10 DAYS

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Abstract

Research has sought morphological assessment of blood and percentage of leukocytes in chickens aged 1 to 10 days. Morphology study of figurative elements was performed by performing peripheral blood smears stained with May-Grünwald Giemsa method. Quantitative assessment of figurative elements was performed using white by examining the leukocyte formula of 10 slides/lot. Morphology of figurative elements and their percentage representation recorded at day old chicks from the values indicate differences in adult hens. The 1-day-old chicks are found in the peripheral blood of a significant proportion of erythroblasts policromatofile or acidophilic, higher than that found in the adult hen. The percentage as opposed to adult birds is a large percentage of lymphocytes large, medium and pseudoeozinofile. Unlike adult hens, chickens aged 1-10 days has smaller proportion of lymphocytes and pseudoeozinofile percentage is higher.

Keywords: *chicken 1-10 days, figurative elements, morphology, leukocyte formula*

Introductions

Elements of the birds are different morphologically from those of mammals, but also by cells hematopoiesis. This is because different phylogenetic origin and evolution of the two classes of vertebrates (1).

Birds, primitive reptiles, shall be formed of a Jurasic vertebrate class after the occurrence of mammals and develop independently of mammals and reptiles (8).

Figurative elements in chicken blood morphology was studied since 1801 when Romanowsky has put in place the first method of staining smears cationic and anionic colorants using (eosin and methylene blue) (7).

Knowledge of blood morphology have evolved in the twentieth century with the development of methods for staining and May-Grünwald

Giemsa Wright, methods used today. They were considered by most authors as a modification of the method Romanowsky (Marschall, 1978 cited by Manolescu N. et all) (5).

Blood morphology was studied systematically at birds after 1935 when a series of parasitic diseases of poultry were producing significant losses in industrial grown chicken flocks (malaria, spirochaetosa, microfilariosis). Kracke and Garver (3), Osgood and Ashworth (6) have made important contributions to the establishment and standardization of terminology birds figurative elements by publishing atlases and participation in congresses.

Morphology studies birds figurative elements undertaken after 1940 (Kinred, (2), Lucas AM, (4), Lucas AM, C. Jamroz, (4) and Olson, 1965 Archer, 1971 Leonard 1982 cited by Lucas and Jamroz (4), Pârvu et al. (7), have made important contributions to knowledge of the morphology and physiology in birds figurative elements.

The formula of differential blood count adult at birds is opposite to that of mammals, it is characterized by a significant lymphocytosis (average 60% lymphocytes variation limits between 45-75% depending on species, breed, age, sex), a minority neutrophil count (called heterofile on average 28% with a range of variation between 15-40%), a high percentage of monocytes (average 8% of the variation range 5-10%), and a number of rare basophils to 4 % eosinophils (range 1.5-6% variation) (9).

Materials and methods

The purpose of this study was figurative elements blood morphology between 1-10 days after hatching and dynamic leukocyte posteclozional formula.

They formed batches of 10 chicken breeds aged 1 day light that were brought up in identical conditions of food and accommodation until age 10 days. In the range of 1, 10 days samples were taken from peripheral blood. The smears were stained by May-Grünwald Giemsa method.

Findings of white blood cell counts were performed on a total of 10 samples for each age group by counting more than 100 leukocytes for each smear.

Results and discussions

Morphology of figurative elements in the first weeks of posteclozional life is close to that of adult birds and little different from the eclozional period. Figurative elements in chicken blood present the following morphological characters 1-10 days in the posteclozional period and differs

from that of mammals through significant number of figurative elements met the peripheral blood blast.

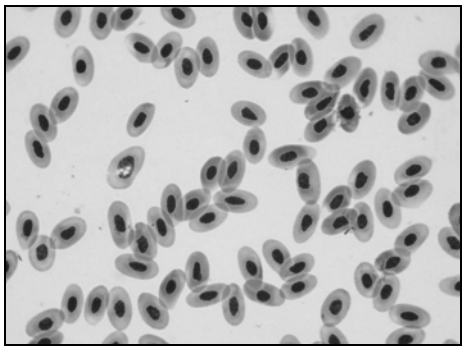

Fig. 1. *Bird blood smear – chicken one day – erythrocytes; May-Grünwald Giemsa staining; Ob. 100x*

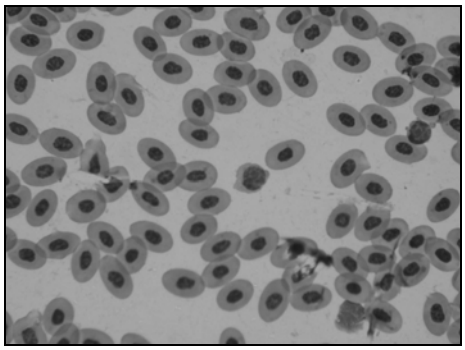

Fig. 2. *Bird blood smear – chicken one day – erythrocyte late policromatofil; May-Grünwald Giemsa staining; Ob. 100x*

Erythrocyte is a nucleated cell, the ovoid shape, without condensed chromatin nucleolus and in bulk form (fig. 1). The cytoplasm stained red acidophilic by May-Grünwald Giemsa method. In the studied period have met frequently immature forms of erythrocytes from blast stages (erythroblasts) policromatofil later stage of erythrocyte (fig. 2).

Trombocyte is a ovoid cell with a size smaller than a red blood cell, the cytoplasm slightly colored, easy acidophile May-Grünwald Giemsa in coloring, with one or more azurophilic granules in the cytoplasm (fig. 3).

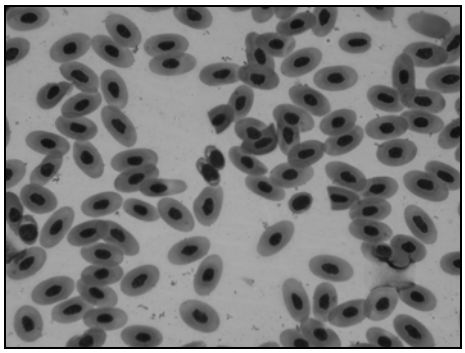

Fig. 3. *Bird blood smear – chicken one day – trombocytes; May-Grünwald Giemsa staining; Ob. 100x*

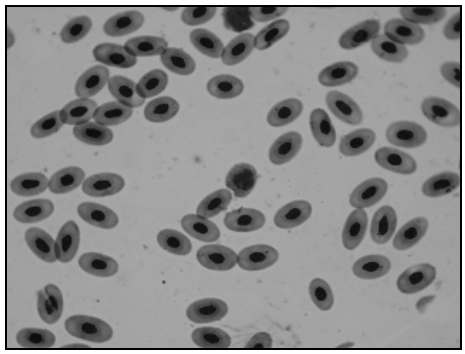

Fig. 4. *Bird blood smear – chicken one day – immature trombocytes; May-Grünwald Giemsa staining; Ob. 100x*

The nucleus is spherical, with condensed chromatin, homogeneous, without nucleolus and can be easily confused with lymphocytes smears. Frequently encountered in smears were immature forms of trombocytes (fig. 4).

Adult lymphocytes variable waist cells are characterized by a nucleo-cytoplasmic ratio for the nucleus, which occupies over 70% of the cytoplasm.

The nucleus is spherical, highly chromatic condensed chromatin, bazicromatic, lacking the nucleolus (fig. 5).

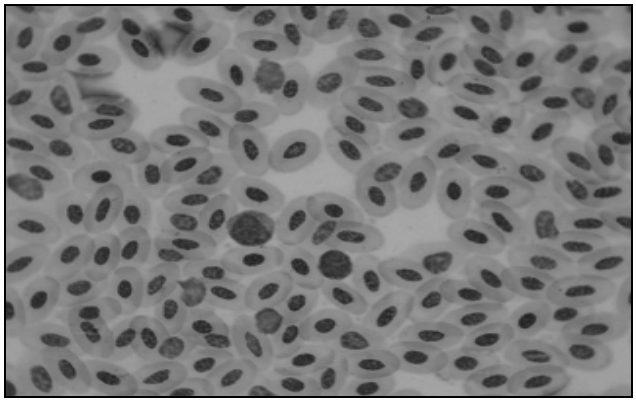

Fig. 5. *Bird blood smear – chicken 10 days – medium and small lymphocytes; May-Grünwald Giemsa staining; Ob. 100x*

At one day old chickens lymphocyte predominant large (fig. 6) in more than 70% of the total lymphocyte (tabel 1).

The proportion of small and medium lympho cytes changes from the age of 10 days by following to predominate small and medium lymphocytes.

Table 1

Dynamics of Gallus domesticus lymphocytes to 1-10 days old

Blood cells	**Age (days)**			
	1		10	
	No.	%	No.	%
Small and medium lymphocytes	83	27,3	266	45,20
Large lymphocytes	221	72,7	322	54,80
Total lymphocytes	304	100	588	100
Total blood cells	1004	30,27	1129	52,08

In peripheral blood posteclozional smears at the age of 1 day, lymphocyte prevails elements of large (over 72%) which confirms Lucas AM and C. Jamroz (4), the elements are elements of large lymphocytic immature, they form after a period of maturation in peripheral blood lymphocytes mature elements.

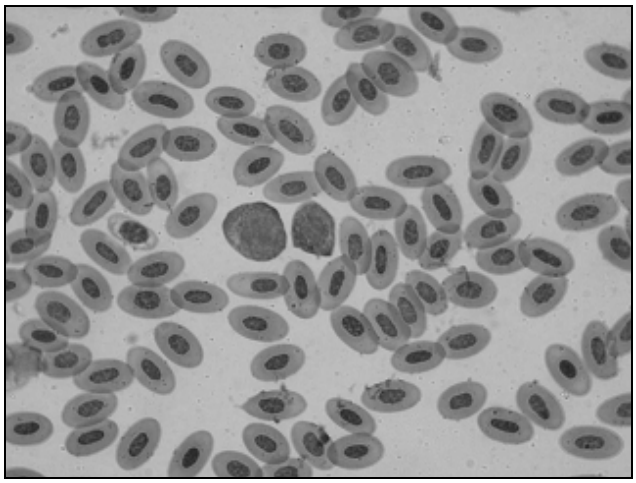

Fig. 6. *Bird blood smear – chicken one day – lymphoblasts; May-Grünwald Giemsa staining; Ob. 100x*

Monocytes are cells with the largest size (above 14 microns) with a nucleus eccentric less bazicromatic that presents edentations having kidney-shaped form (fig. 7).

Monocyte is similar to the cell mammalian, the nucleus occupying 45-50% of the cell. The cytoplasm stained basophil and presents azurophilic granules.

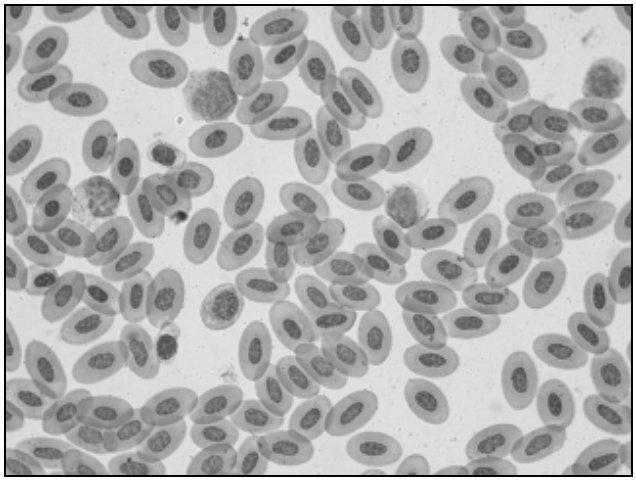

Fig. 7. *Bird blood smear – chicken 10 days – monocyte; May-Grünwald Giemsa staining; Ob. 100x*

Heterophils (*pseudoeosinophils*) are cells with medium size (8-9 microns) spherical with weakly acidophile cytoplasm, nucleus lobate (2 or 3 lobes), having granular cytoplasm aciforme long and narrow or short elongated and large, occupying throughout the cytoplasm, grouped in packets or dispersed (fig. 8).

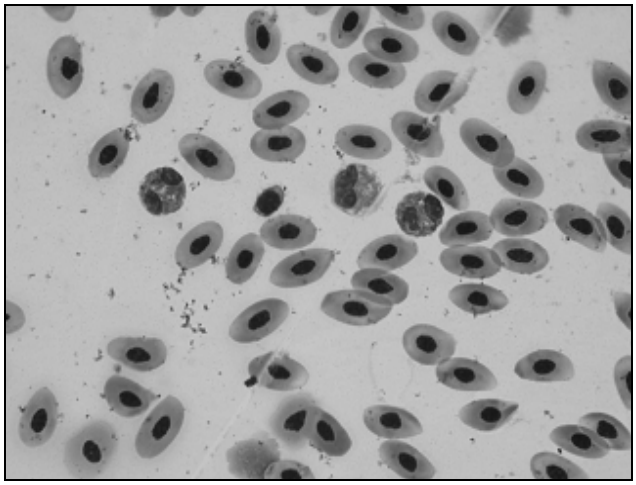

Fig. 8. *Bird blood smear – chicken one day – heterophils and monocytes; May-Grünwald Giemsa staining; Ob. 100x*

In the period 1-10 days, all heterophils presented bilobate nucleus (fig. 8).

The large number of heterophils the first day posteclozional allows advancing the hypothesis that these cells ensure immune defense processes chicken until final form T and B lymphocytes, which grow numerically and reverses the age of 10 days report (table 2). During this period most of the contaminants are such as bacterial pathogens, which could explain the high percentage of heterophils.

During this period (1-10 days) the most common diseases are bacterial nature of chickens (tifopulorosis, colibacillosis) heterophils cells have an important role in defense of the organism against bacterial infections.

Table 2

Dynamics of white blood cell counts in the period 1-10 days posteclozional

Age (days)	The formula differential blood count %					Total
	Lymphocytes	Heterophils	Eosinophils	Basophils	Monocytes	
1	30,27	63,94	2	0,89	2,88	100
10	52,08	32,23	0,7	0,62	7,35	100

Eosinophils are cells heterophils similar to the shape and size, the nucleus is bilobate and cytoplasm covered by spherical formations red colored (fig. 9).

Frequently can be noticed smears and eosinophils with one lobe. The cytoplasm is weak basophil. The granules are uniform in size and evenly distributed in the cytoplasm.

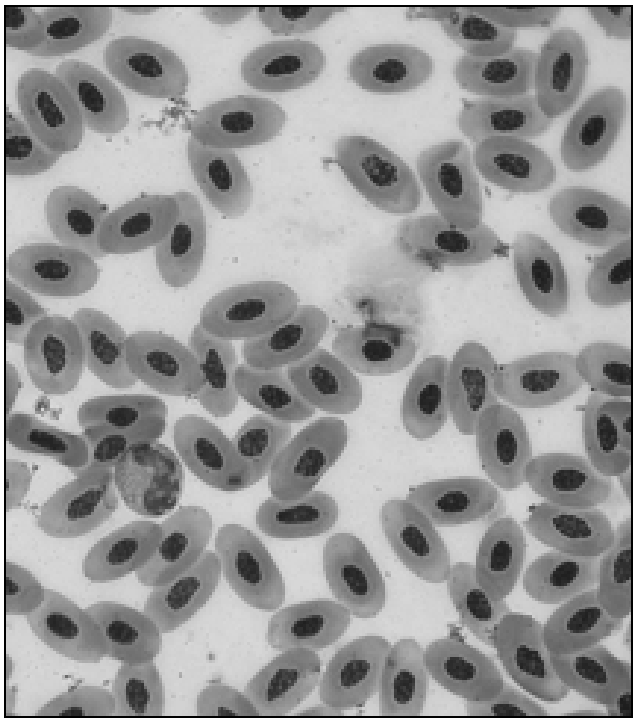

Fig. 9. *Bird blood smear – chicken one day – eosinophil; May-Grünwald Giemsa staining; Ob. 100x*

Basophils are very rarely seen in smears. The size and shape of the cell are identical with heterophils. Frequently basophils present a single nucleus nelobe or lobe, but are present in the cytoplasm waist granules similar to those of eosinophils, only are chromatic basophil (fig. 10).

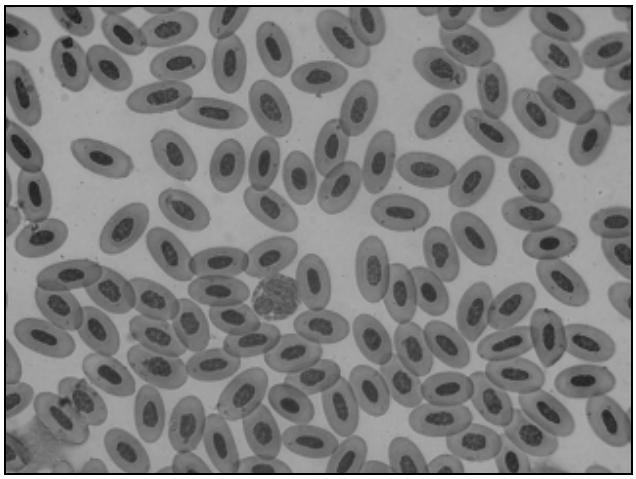

Fig. 10. *Bird blood smear – chicken one day – basophilic; May-Grünwald Giemsa staining; Ob. 100x*

Basophil granules are much smaller than those of the eosinophil. Basophil cytoplasm is very weakly colored or less basophil. The nucleus is chromatically disposed in with chromatin stacks. The nucleus and nucleolus lacks sometimes present two lobes.

Chicken white blood cell counts values recorded in the period 1-10 days are shown in table 3.

Table 3

The dynamics of the* Gallus domesticus *leukocyte formula aged 1-10 days

Leukocytes	**1 day**		**10 day**		**Grown***	
	No.	**%**	**No.**	**%**	**No.**	**% the mean/variance**
Heterophils	642	63,94	443	39,23	-	28 (15-40)
Eosinophils	20	2	8	0,7	-	4 (1,5-6)
Basophils	9	0,89	7	0,62	-	rare
Monocytes	29	2,88	83	7,35	-	8 (5-10)
Lymphocytes	304	30,27	588	52,08	-	60 (45-70)
Total	1004	100	1129	100	-	100

* after Nemi J. - Schalm's Veterinary Hematology

The data presented indicate the age of one day a report lymphocytes/ pseudoeosinophils for heterophils, for 10 days to establish a change in the ratio lymphocytes/ heterophils in favor of lymphocytes when the recorded values are close to those referred to adult hens.

Conclusions

1. The frequency of immature red blood cell (erythrocytes policromatofile early and late immature) at day 1 of age is over 11% and gradually decreases from the age of 10 days.

2. Frequently they were observed in the peripheral blood immature forms of figurative elements of blood (erythroblasts, erythrocytes policromatofile early and late, lymphoblasts, promyelocytes, early and late immature thrombocytes).

3. Chicken blood count is predominant in the early days posteclozional heterophile. Modifying the report heterofile/lymphocyte happens after 10 days of age, the percentage of heterofile decreases from 63.94% to 39.23% at the age of 10 days.

4. Except age of 1 day, lymphocyte blood count is dominant.

5. The first week posteclozional lymphocytosis is determined by lymphocytes (over 72.7%), a percentage that decreases with age.

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ASSIMILATING INFORMATIONS – LEADING ROLE IN THERAPEUTIC. CLINICAL CASE STUDY

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Abstract

The dialogue of life requires first stage of curiosity and observation on the clinical pathology of indirect animal. Elapsed time for the purpose of the collection of information by the owner played the animal must not represent a moment of boredom, because behind this information always finds a conflict what surely underlies the pathophysiology mechanism elucidation of morbid condition. In other news, the elevation dialog offers the possibility of getting to know the affective condition — and the attitude of the owner against the disease.

Keywords: *history, clinical observation, affective, emotional behavior, emotional process.*

Introduction

Very often the affective implication of owner-lack of discernment in the light of rational thinking reduction-enslaved or hidden feelings towards wholeness extrapolated excessively complicate the clinical picture animal through a paddle clinic sometimes nonexistent (grassroots or set-animal versus the lack of a child, parent, friend; pet remembrance of a loved one versus). The role of the physician is to sort out information and clinical affective processes monitoring behavior of owner against emotional pathology of the animal in question.

Materials and methods

This first stage in settling and staying of diagnosis is performed when the doctor discern, categorizes and so-called “psychic conflict” involved and otherwise disrupted the so-called “disturbing” factor here represented the modification of your pet, the absence of a state of which the owner is fine

with chants. The specific importance of affective deficiencies proprietors unlocking, heavily tested in terms of animal disease, presents major significance especially when morbid condition is serious prognosis, rehabilitation or recovery (animal) showing very small chances.

Results and disscusions

Overcoming the so-called “critical threshold” obliges the owner to the awareness of reality around the morbid Act of animal suffering. Taking into account the different mode of involvement of each of us is hard to deal with a standard template to different personalities-entities. This can be achieved by contribution and what doctor has an obligation to explain the meaning of each forecast on the evolution of the disease based on the analysis of symptomatic.

In such situations is that the simplicity to replace the vanity of the veterinarian. Unfounded ambition or desire to do by exposing the impression of a conflict of hitherto unknown not morbid simplifies events but they complicate even further in the mind of the owner precisely through the probable lack of confidence on the fact the incapacity started managing the reality or the acceptance thereof. In other news a morbid condition need not be justified but argued, the physician-owner's perspective-often being “confused” with morbid condition triggers cause, actually in fact inconsistent in most situations with reality.

That is why it is urgently necessary to clear explanation at the outset regarding the conduct of medical instrument-even stages where it lends itself to logical and constructive therapeutic, professional implications and its materials, but also the prognosis and diagnosis. The purpose of the clinical and laboratory evaluations has as their objective the ultimate deciphering the mechanism underlying pathological physiology of disease. The end result should be brought to the knowledge of the owner, or the ways in which it is here is not binding.

Explanation of certain interest owners towards the pursuit of the intermediate stages of condition assessment of disease reflects among other collateral interest, curiosity, and desire for involvement or disbelief, sometimes given to the verification of the accuracy of the therapist's conduct investigation forms. Constructive collaboration, but especially the one based on a vein of distrust in the physician-owner combination (pet) commits to regular or permanent analysis of collaborative activity-along with the effectiveness of therapeutic instrument of communication-ability and expression of the status of the two in order to diminish the agreements based on mutual compromise-the emergence of the harsher realities.

Very often are reported events that originally had a different point of view compared to the one that took place at the time of the commencement of conflict situation. Lack of authority and firmness to the doctor regarding the coordination of the medical act of default owner desire-to return to the previous implications in case of non rewarding/share (proper animal feeding failure, ignoring the prophylaxis, deviation from the advice of a doctor, etc.) explains on the one hand the relationship between imbalance physician-owner (animal), the effects of being pursued through the improvement of the deficit. Reported cases of irreversible evolution toward November, exhaustion and metabolic status-comprehensive degradation of major tissue senility etc.– with “screenplay” obligatory explained by doctor since the beginning of the collaboration with the owner of the animal.

And in such situations, with serious prognosis, irreversible is a great care regarding therapeutic agreement granted the animal through the understanding of the physician-owner (pet). Very often the continuation of conflicts arises on the basis of the therapy in case of morbid conditions stranded, on the one hand the doctor treatment procedures resulting in extremis there often influenced indirectly by “lack of reason”, which involved owner sentimental sometimes forgets that becomes obliging the egoist to maintaining a living just suffering from a lack of discernment.

It is not enough that the veterinarian to expound their opinion towards intensive treatment interruption in critical cases, irreversible and terminal, the final decision being assigned and usually taken by the animal's owner everything under medical coordination proved not justified. Whatever morbid condition, events – whatever their nature – a relationship of dynamic equilibrium, so the reader's attention that the dialogue of life requires psychoanalyzing and indirect clinical observation over the animal pathology. This fact is the basis of stage building plan through which one can reach the diagnosis, thus healing the patient.

THE COMPARATIVE FELINE CONTRACEPTION METHODS

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Abstract

Clinical study was conducted in the Faculty of Veterinary Medicine from the Spiru Haret *University, at the Department of Obstetrics and Reproduction, the 20 feline, aged 5 months to 4 years, 14 females and 6 males, clinically healthy without reproductive function disorders. Our previous clinical observation lasted about two years. Side effects are insignificant if the relevant protocols, especially with the administration in a specific period of sexual cycle. The effect of the substances is fully reversible, with few exceptions, which is very important for specimens genetically valuable. Deslorelin contraceptive methods and Proligestona can successfully replace surgical methods of suppressing estrus.*

Keywords: *contraceptive, methods, desloreline, proligestona*

Introduction

Adjusting feline reproductive function aims, as in other mammals, three floors that are secreted hormones involved in genital area. The hypothalamus, pituitary and at the gonadal. At the level of the hypothalamic GnRH is secreted as, gonadorelin, which causes the release of FSH and LH, and PRH-PIF which controls the secretion of prolactin in pituitary. In cats, because ovulation is induced breeding, the GnRH peack only appears after some repeated matings made in a short time. At both anterior pituitary is secreted FSH and LH induce, along with FSH, ovulation, although the cat may exist and ovulation. After ovulation, LH helps the corpus luteum formation that secrete progesterone.

LH has a role in the secretion of androgens in males or maintenance of secondary sexual characteristics. At gonadal estrogens are secreted by ovarian follicles and follicular cysts and progesterone by the corpus luteal cysts or yellow. As a particular species, cats have only mammary gland

progesterone hormone receptors. In males, the testicles, androgens are secreted under the influence of LH, which is called ICSH in males. They are involved in the sexual instinct and marking territory. It is also known that an important role in reproductive function feline plays melatonin, the hormone that causes the pituitary and suppresses anestrus seasonal reproductive function. Reproductive control methods feline can be both surgical and conservative, this study refers only to the conservative.

Materials and methods

Clinical study was conducted in the Faculty of Veterinary Medicine from the *Spiru Haret* University, at the Department of Obstetrics and Reproduction, the 20 feline, aged 5 months to 4 years, 14 females and 6 males, clinically healthy without reproductive function disorders. Our previous clinical observation lasted about two years.

The goal was the discontinuation reversible reproduction and observation of possible adverse effects of drug substances used. They used Proligestona and Deslorelin. Other progestins, for example medroxyprogesterone acetate, are avoided, since it is known that the severe side effects at the level of the breast and the uterus.

Deslorelin is a synthetic agonist of GnRH which are in the form of an implant with 4,7 mg active substance. If it is implanted before puberty, delaying its emergence with 7-8 months. The adult provides maximum contraceptive effect, if implanted in diestrous progesteronemia the value is high. Implantation is made in the neck, lumbar or post umbilical area, where you can extract more easily in case of severe adverse effects are observed. It avoids implantation of adipose tissue in areas poorly vascularized because it blocks the release of the active substance. Where is constant and low dose administered, Deslorelin suppresses the hypothalamic pituitary gonadal function, therefore cease synthesis and release of FSH and LH.

The male reproductive organs decreases function, spermatogenesis and serum testosterone. The maximum plasma concentrations Deslorelin should be installed in 10 to 30 days, and infertility is installed for about 10-11 months. Also, there was a maintenance of male fertility about 1 to 2 months after the application of the implant. The product has been used 6 males and 6 females.

Proligestona is a retard, antigonadotropic properties, which is given in interestrus or proestrus. Perform booster 3 months after the first administration, then 4 months after the second injection, then 5 months after the third administration, then 5 in 5 months. If administered early estrus, its signs will regress in 3-5 days. After a first inoculation can return to estrus in

30% of cases, only after about 5 months. It was administered to eight of the surveyed females, subcutaneously, in a region less visible, because quite often there is a discoloration of hair at the inoculation site, often accompanied by hair loss.

Results and discussions

For all 20 cases, both Deslorelin and Proligestona exercised their contraceptive effect. The animals were observed for a period of about 2 years after the first administration of contraceptives. Adverse effects were observed which did not affect negatively the overall condition of the animals and not put their lives in danger. They are presented in the table below.

Table 1

The behavior following administration of clinical contraceptive substances

The active substance used	*Gender*	*Cases*	*Side effects observed*
Desloreline	female	1	Swelling at the implant site
		2	Conjunctival reaction to form a capsule that embraced implant
		3	Ovarian cysts sided
		1	Pyometra
	male	3	Decrease in volume of the testicles
		1	Testicular swelling accompanied by pain
Proligestone	female	2	Pyometra – both cases were inoculated in proestrus
		1	Fibroadenomatose in mamary tissue
		3	Alopecia at the injection site
		1	Oestrus suppression final

Each case was clinically healthy and was subject prior to administration of contraceptive substances, clinical and laboratory examinations, blood biochemistry examinations, blood counts, ovarian and testicular ultrasound measurements.

Conclusions

1. Deslorelin contraceptive methods and Proligestona can successfully replace surgical methods of suppressing estrus.
2. Side effects are insignificant if the relevant protocols, especially with the administration in a specific period of sexual cycle.
3. The effect of the substances is fully reversible, with few exceptions, which is very important for specimens genetically valuable.

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MONITORING SOME PHYSIOLOGICAL PARAMETERS DURING ANESTHESIA WITH ISOFLURANE AND KETAMINE TO LABORATORY MICE

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Abstract

Experimental were perfomed inside the unit for laboratory animals testing in the Cantacuzino Institute – Băneasa Station. Studies have been conducted on 15 laboratory animals – NMRI mice, 3 months old, divided into 3 groups of 5 individuals.

Experimental animals were placed on three experimental groups:

a) group 1 that was used inhaled Isoflurane anesthesia;

b) group 2 that was used intraperitoneal anesthesia with Ketamine;

c) group 3 that was used intraperitoneal anesthesia with Ketamine combined with Isoflurane inhalation anesthesia. Research has followed computerized monitoring of physiological parameters with importance in laboratory mice anesthesia (heart rate, respiratory rate and pulse amplitude) using STARR device and related software. Isoflurane is the first choice in mice anesthesia. He was administered in oxygen for precise vaporization in exact percentage (5% for inducing, 1-3% for maintenance). The use of Ketamine into mice produces an accentuated bradypnea. An excellent combination is the Ketamine associated with Isoflurane.

Keywords: *Isoflurane, Ketamine, laboratory mice, some of physiological parameters.*

Introduction

Anesthesia and analgesia are crucial components of the most experimental protocols. All experimental standards require express preventing pain whenever possible and treating pain whenever it is discovered. Exceptions to these principles are allowed in rare cases and only with special permission.

Materials and methods

Experimental were perfomed inside the unit for laboratory animals testing in the Cantacuzino
Institute – Băneasa Station.

1. **Experimental animals**

Studies have been conducted on 15 laboratory animals – NMRI mice, 3 months old, divided into 3 groups of 5 individuals (fig. 1). Experience mice were trained through preoperative depilatory post auricular areas using a depilatory pasta filled with sensors monitoring application.

Throughout the test period an attempt was made to limit to the minimum the influence her environmental factors by providing an appropriate habitat and food balanced on the animals being housed in separate cages.

Fig. 1. *Experimental animals*

2. **Materials used**

Ketamine is a product of synthesis which forms part of the group nonbarbiturates anesthetics (fig. 2). Unlike classical anesthetics induce anesthesia, ketamine, often characterized by shallow sleep with analgesia but since blocks the cortex nervous influx without vital centers of the depression.

Fig. 2. *Ketamine bottle*

Izoflurane is an isomer of the analgesic enflurane lower and no toxicity (fig. 3). It is due to the expansion of hypotensive, but not sensitizes heart to catecholamine. For representations and resuscitation are fast being preferred halotane.

Fig. 3. *Izoflurane bottle*

3. **Apparatus used**

Facilities necessary for carrying out the inhaled anesthesia is device cover (fig. 4) of oxygen source, flow meter, vaporizer, breathing in respiratory inspiration/circuit with unidirectional valves, endotracheal tube connector, for mixing gas balloon and ventilation control, filter with carbon dioxide fixative trample sodata, valve opening/closing of the circuit, the room of anesthesia (fig. 5) and the necessary anesthesia monitoring equipment.

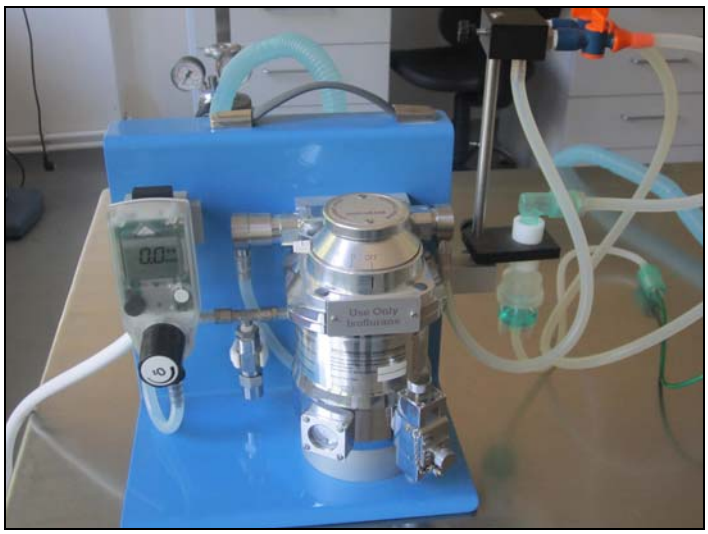


Fig. 4. *Inhaled anesthesia apparatus*

Fig. 5. *Room of anesthesia*

4. **Experimental model**

Experimental animals were placed on three experimental groups:

a) group 1 that was used inhaled Isoflurane anesthesia;

b) group 2 that was used intraperitoneal anesthesia with Ketamine;

c) group 3 that was used intraperitoneal anesthesia with Ketamine combined with Isoflurane inhalation anesthesia.

5. **Methods of results evaluation**

Research has followed computerized monitoring of physiological parameters with importance in laboratory mice anesthesia (heart rate, respiratory rate and pulse amplitude) using STARR device (fig. 6 and 7) and related software (fig. 8).

Fig. 6. *STARR device*

Fig. 7. *STARR device*

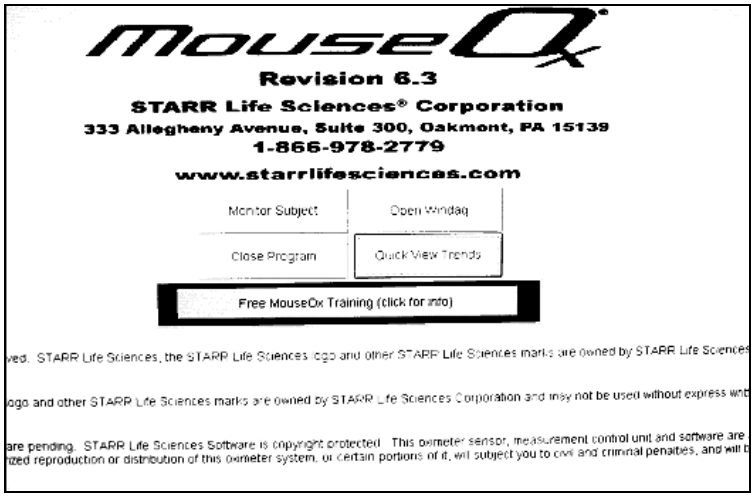


Fig. 8. *Related software*

Results and discussions

Research regarding with inhaled Izoflurane anesthesia

Five mice representing lot 1 have been sleeping by using Izoflurane in a room of anesthesia (fig. 9). For induction it was used the concentration of 5% and for maintenance of anaesthesia concentration was 1-3%.

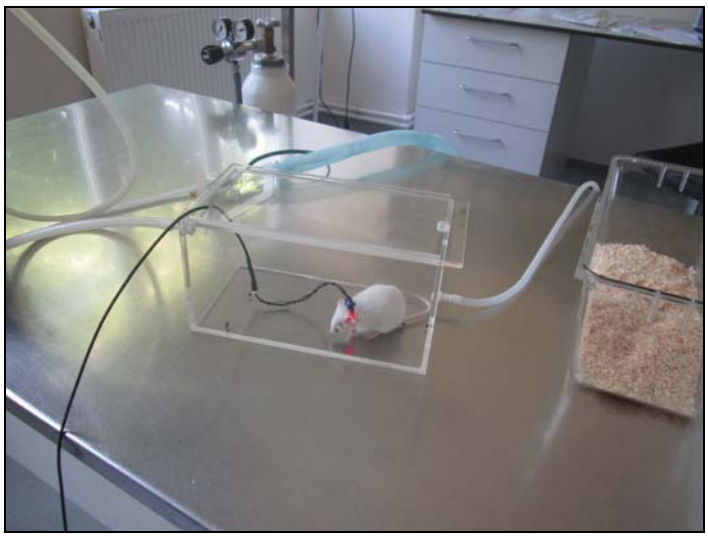


Fig. 9. *Induction of Izoflurane the animals of group 1*

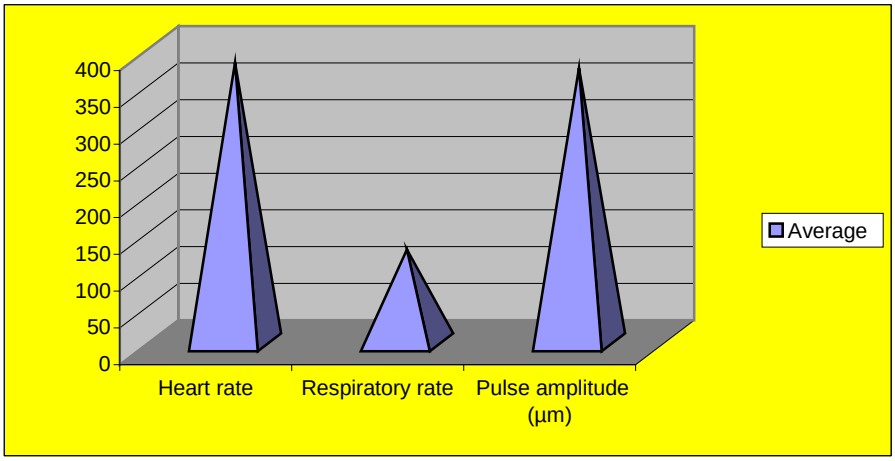
During the experiment were recorded heart rate frequency expressed in beats/min., respiratory rate expressed in respirations/min. and pulse amplitude expressed in micrometers. Pulse amplitude is the difference in diameter of carotid artery during cardiac systole and diastola.

The analysis of the results obtained at intervals of 5, 15 and 30 minutes was:

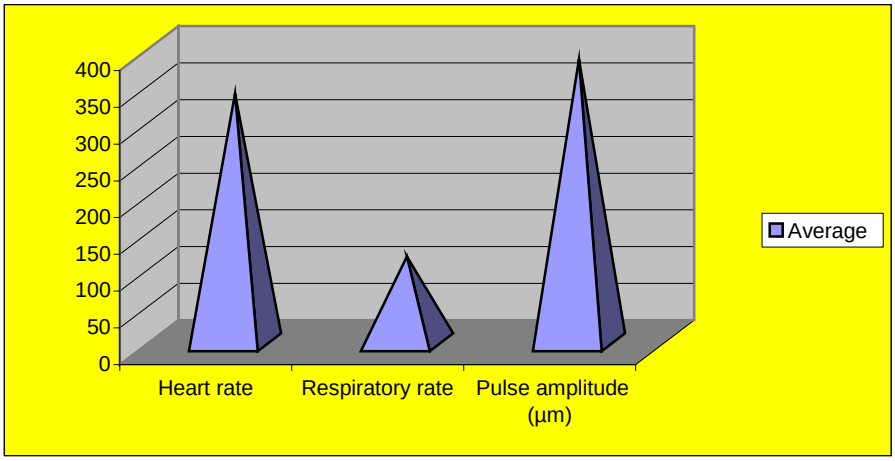
– the heart rate has been stored in physiologic limits throughout the experiment;

– pulse amplitude preserved in physiological limits throughout the experiment;

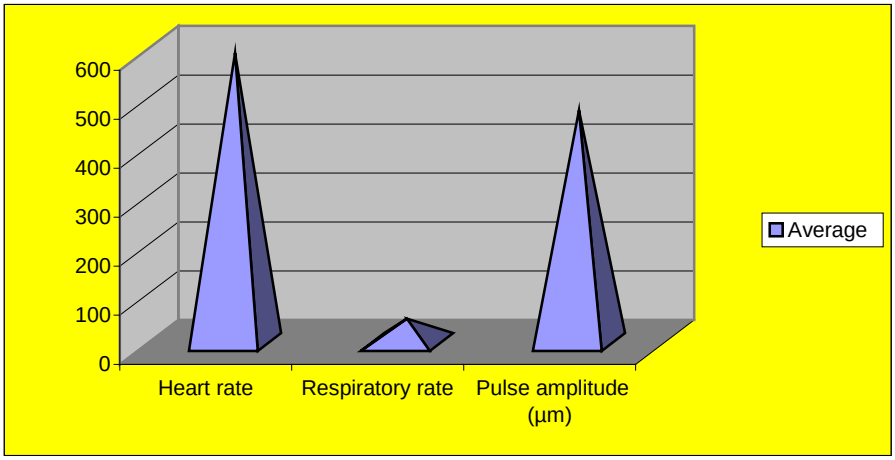
– the respiratory rate was kept in the physiological limits in the first 15 minutes by registering a slight bradipnee in the next 15 minutes corresponding installation sleeping anesthetic (graphics 1-3).



Graphic 1. *The average of heart rate, respiratory rate and pulse amplitude in minute 5 at group 1*



Graphic 2. *The average of heart rate, respiratory rate and pulse amplitude in minute 15 at group 1*



Graphic 3. *The average of heart rate, respiratory rate and pulse amplitude in minute 30 at group 1*

Research regarding intraperitoneal anesthesia with Ketamine

Five mice representing group 2 have been sleeping by injecting a intraperitoneal solution Ketamine 10% at a dosage of 200 mg/kg body weight (fig. 10).

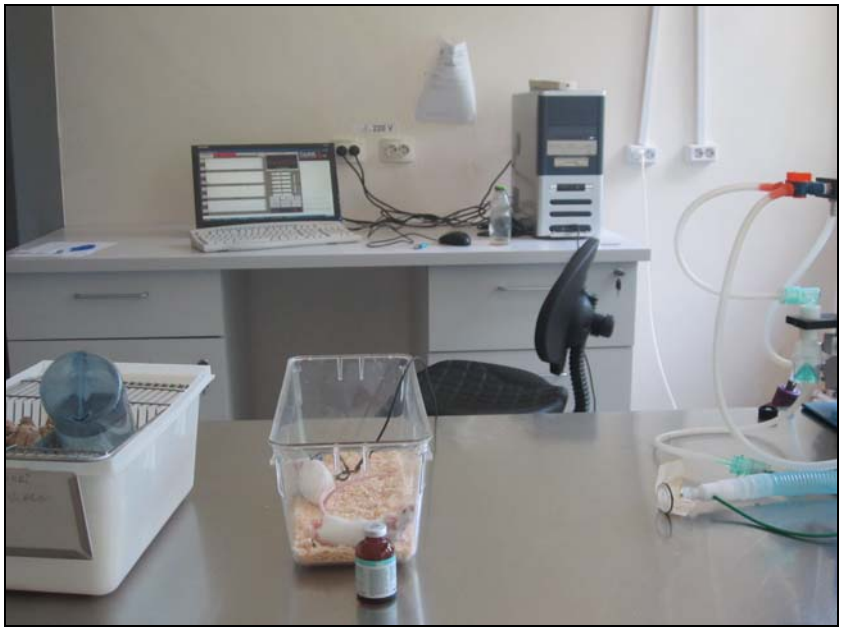


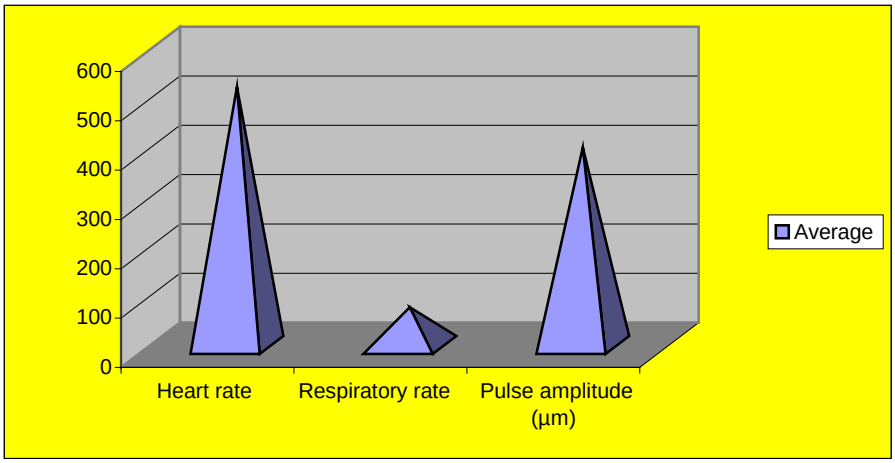
Fig. 10. *Installation of anesthesia in animals from group 2*

The analysis of the results obtained at intervals of 5, 15 and 30 minutes was:

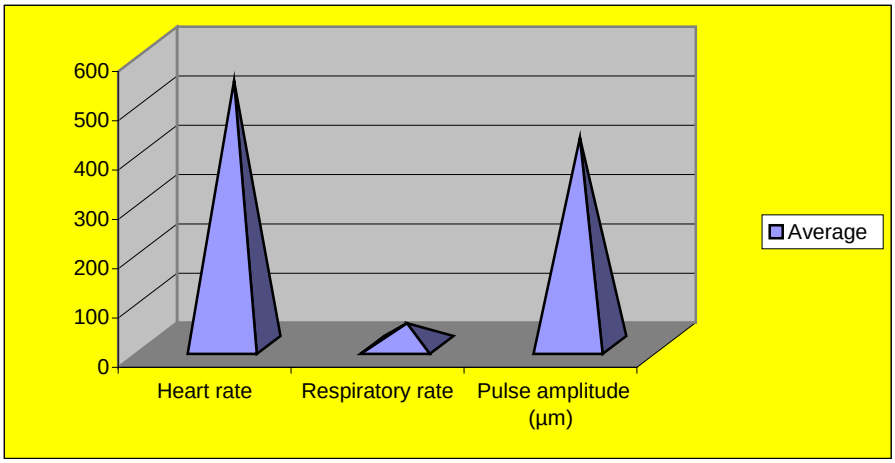
– the heart rate has been stored in physiologic limits throughout the experiment;

– pulse amplitude preserved in physiological limits throughout the experiment;

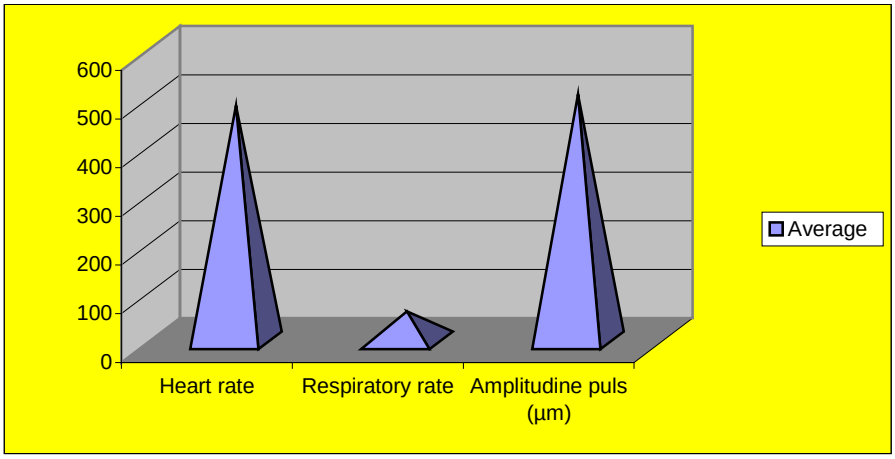
– the respiratory rate has registered a record bradipnee throughout the duration of the experiment (graphics 4-6).



Graphic 4. *The average of heart rate, respiratory rate and pulse amplitude in minute 5 at group 2*



Graphic 5. *The average of heart rate, respiratory rate and pulse amplitude in minute 15 at group 2*



Graphic 6. *The average of heart rate, respiratory rate and pulse amplitude in minute 30 at group 2*

Research regarding anesthesia used intraperitoneal anesthesia with Ketamine combined with Isoflurane inhalation anesthesia.

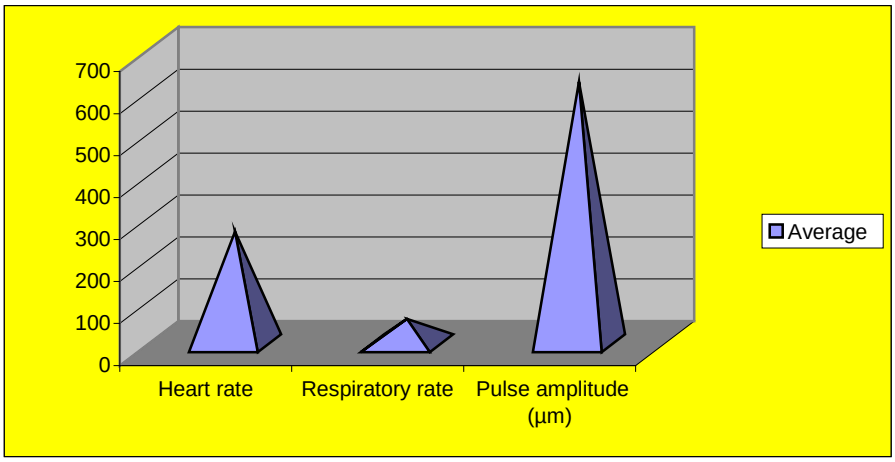
Five mice representing group 3 have been sleeping during step 1 intraperitoneal injection of ketamine 10% solutions at a dosage of 200 mg/kg G.V. completed with Izoflurane in the administration of high concentration of 5% and 3% for induction and maintenance during step 2.

The analysis of the results obtained at intervals of 5, 15 and 30 minutes was:

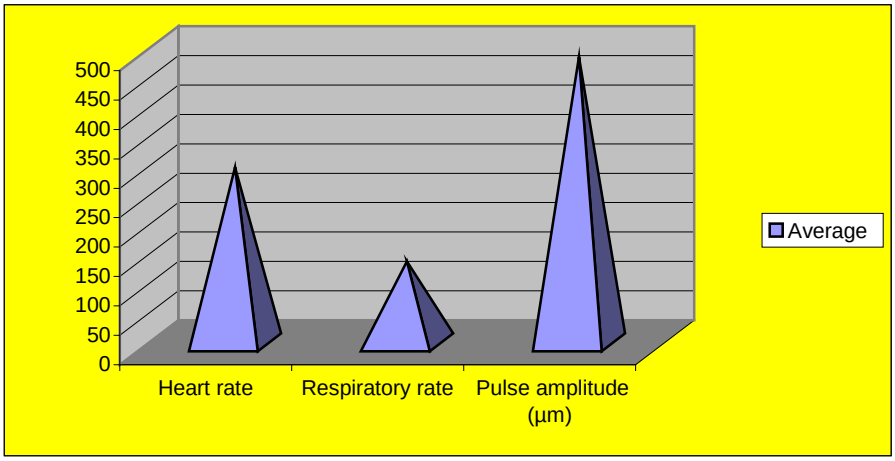
– the heart rate has been stored in physiologic limits throughout the experiment;

– pulse amplitude-preserved in physiological limits throughout the experiment;

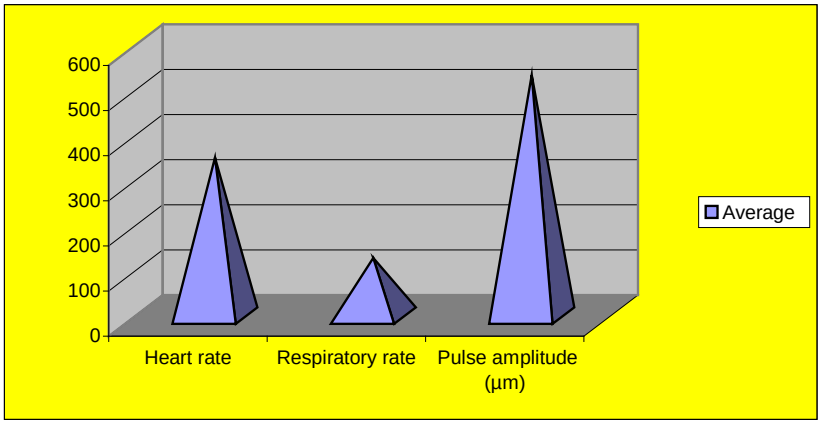
– the respiratory rate has registered a slight bradipnee in the first five minutes, returning to normal values within the next 25 minutes (graphics 7-9).



Graphic 7. *The average of heart rate, respiratory rate and pulse amplitude in minute 5 at group 3*



Graphic 8. *The average of heart rate, respiratory rate and pulse amplitude in minute 15 at group 3*



Graphic 9. *The average of heart rate, respiratory rate and pulse amplitude in minute 30 at group 3*

Conclusions

Research carried out in experimental settings of the Laboratory Animal Research Laboratory of Cantacuzino Institute in Bucharest have led to the following conclusions:

1. All animals used in the experiment were awakened from anesthesia without accidents.
2. Isoflurane is the first choice in mice anesthesia. He was administered in oxygen for precise vaporization in exact percentage (5% for inducing, 1-3% for maintenance).
3. Injectable anesthetics are administered intraperitoneally with usually. Intramusculary administration it should be avoided due to low muscle mass.
4. The use of Ketamine into mice produces an accentuated bradypnea.
5. An excellent combination is the Ketamine associated with Isoflurane.

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Bun de tipar: 26.02.2016; Coli tipar: 8,75
Format: 16/70×100

Editura Fundației *România de Mâine*
Str. Fabricii nr. 46G, București, Sector 6
Tel./Fax: 021/444.20.91; www.spiruharet.ro
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