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**CONTENTS**

# ANATOMIC CONSIDERATIONS ON THE MIDDLE EAR IN DOGS

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***Abstract***

*The middle ear is a bone cavity coated with mucous and full of air. This cavity is implanted inside the temporal bone between the ear drum. Inside the cavity there are four small bones articulated between them in a chain. The lateral wall is membraneous, formed of the tympanum membrane and of a tympanum ring. The median wall is formed of the stone part of the temporal bone and displays three characteristic anatomic formations: promontory, oval window and round window. The promontory looks like an elongated prominence which separates the oval window from the round one. The oval window or the vestibular window is like a slightly ovoid orifice located dorso-medial from the promontory, by which the vestibule communicates with the middle ear. The round window or the cochlear window is a circular orifice located caudo-lateral from the promontory. It is covered by a membrane called the secondary tympanum, which separates the tympanum cavity from the tympanic ramp of the cochlea. In the ventro-lateral side, the tympanum cavity has the tympanum opening. The acoustic bones, the hammer, the anvil, the lenticular bone and the ladder, are articulated between them and form a chain which connects the tympanum and the vestibular window. The bones are driven by two muscles, the tensor muscle and the ladder muscle. The mucus lining the irregularities inside the cavity is in connection with the pharyngeal mucous by means of the auditive tube.*

***Key words:*** *ear, hammer, anvil, ladder*

## *Introduction*

The middle ear forms, together with the external ear and the inner ear, the peripheral receiver of the auditory system and of the vestibular system. The literature has references to the middle ear of the domesticated mammalians, but there are few descriptive data on the middle ear of the dog (2).

The middle ear is an air-filled cavity (tympanic cavity) carved out of the temporal bone, between the tympanic and stony parts, lined with mucous. The cavity holds for articulated ossicles. The *tympanic membrane (eardrum)* is an oval membrane, thin and resistant, separating the external ear from the middle ear; it has a more or less oblique position according to the species. It has a tensed part inserted on a fibro-cartilaginous ring and a flaccid part. The tympanic cavity is oval, with two walls: external and internal, with a concave aspect and a circumference. The side wall is membranous and it consists of the tympanic membrane and the tympanic ring. The middle wall consists of the stony part of the temporal bone and it has three characteristic anatomic parts: the *promontory, oval widow* and *round window*. The *promontory* has an elongated prominence which separates the oval window from the round window. The *oval widow* or vestibular window is like a slightly ovoid orifice, located dorso-medial from the promontory, by which the vestibule communicates with the middle ear. The *round window* or the cochlear window is a circularly orifice, located caudo-lateral from the promontory. It is covered by a membrane, the *secondary tympanum*, which separates the tympanic cavity from the tympanic ramp of the cochlea. On the ventro-oral side, the tympanic cavity has the tympanic opening (1, 2).

The ossicles, hammer (*malleus*), anvil (*incus*), round bone and the stirrup (stapes) form a chain linking the tympanum and the vestibular window. The ossicules are driven by two muscles: the *tensor muscle of the tympanum* and the

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*stirrup (stapes) muscle*. The mucous lining the cavity is continued with the throat/nasopharynx mucous via the Eustachian tube (3, 4, 5).

### *Material and method*

The study was conducted on two dog skulls (9 months and two years) from common, large size dogs. The skulls were processed by maceration and submitted to a treatment of mechanical cleaning with perhydrol. The temporal bone was collected first; the external wall was opened carefully to study the tympanic cavity. The ossicles were collected separately and subsequently described.

### *Results*

The **middle ear** is located inside the tympanic part of the temporal bone, being delimited medially by the stony part of the temporal bone. The tympanum cavity has two walls and a circumference. The external wall is closed by the tympanic membrane (ear drum) which separates the external ear from the middle ear. The *tympanic membrane* has an elliptical form, it is inserted obliquely, ventro-dorsally on the tympanic ring; it is concave towards the exterior and convex towards the interior. The *tympanic ring* is located on the external side of the tympanic cavity and has an oval shape. The internal wall is formed by the bony part of the stony portion of the temporal bone. Several formations can be observed on this wall:

– the *promontory,* with its bony prominence looking like a mammilla, which separates the oval window from the round window and it belongs to the basal prominence of the cochlea;

– the *oval window* is located in front of the promontory. It is a small oval opening through which the tympanic cavity communicates with the vestibule. The base of the stirrup, the footplate, fills the oval window;

– the *round window* is larger than the oval window and is located posterior to the promontory. It has a circular opening corresponding to the tympanic ramp and it is covered by a membrane, the secondary tympanic membrane. The circumference of the tympanic cavity is made of an almost smooth bone, divided in the middle by a thin relief which separates the cavity in two compartments.

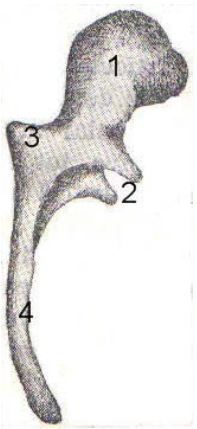


Fig. 1. Picture of the hammer, anvil and lens-shaped bone

Inside the tympanic cavity there are the following ossicles:

– The *hammer (malleus)* is the longest of the ossicles and has two ends, an upper end and a lower end. *The head of the hammer* – the upper end – is rather irregular than round, and it joins the inside of the eardrum. Its caudo-ventral part has a joining area fitting to the anvil (incus). *The neck of the hammer* is quite long. Its external part relates to the upper part of the tympanic membrane (eardrum). *The arm of the hammer* – the lower end – is long and fits perfectly to the tympanic membrane ending in a spatula-shape. The arm of the hammer has two sides, anterior and posterior, and two edges, lateral and medial. In its dorsal part, the hammer has two processes, one short and thick and the other longer and thinner. The short and thick process is located laterally, it has the shape of a conic prominence and emerges from the ventro-lateral side of the hammer – this is the *lateral process*. The long process is located rostraly, close to the tympanic ring – this is the *rostral process* (fig. 2a).

– The *anvil (incus)* is located ventro-medial from the hammer. It has a body and two arms. The *body of the anvil* has two sides: lateral and medial. The anterior part of the body has a joining facet which fits to the joining head of the hammer. The two arms are a horizontal one and a vertical one. The horizontal arm has a triangular shape – this is the *short arm*. The vertical arm ends in an apophysis in the shape of a lens – this is the *long arm* (fig. 2b).

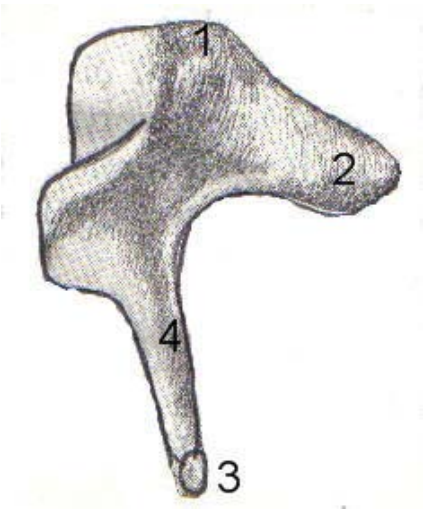


Fig. 2a. The hammer

*1) head; 2) rostral (long) process; 3) lateral (short) process; 4) arm; 5) muscular process.*

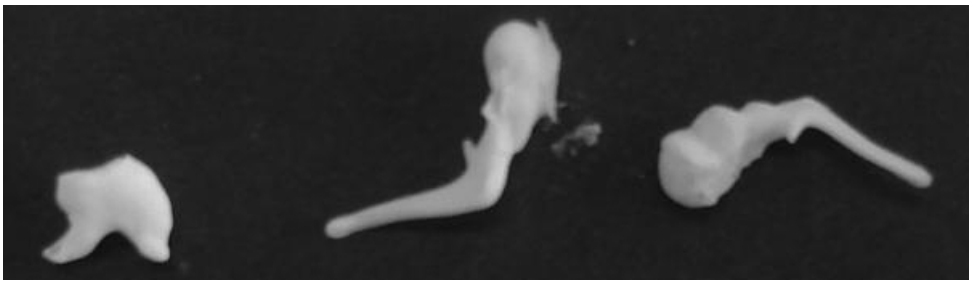

Fig. 2b. The anvil

*1) anvil body; 2) short arm; 3) long arm.*

– The *stirrup (stapes)* has the shape of a saddle stirrup; it has a head, a basis and two arms. The head has, on the lateral end, a small particular facet which fits the lens-shaped apophysis of the anvil. The footplate of the anvil consists of an oval bony plate filling the oval window. The arms of the stirrup, one caudal and one rostral, describe a slight curve (fig. 2c).

The middle ear ossicles are driven by the following muscles:

– *The hammer muscle* is an elongated muscle in dogs. It originates close to the upper end of the pharynx-tympanum tube, goes upwards and ends in a tendon which recurves before the oval window to insert on the extremity of the long hammer apophysis.

– *The stirrup muscle* is a rather long, very thin muscle. It originates in the pyramidal tube where most part of it lies through a tendon inserted on the upper end on the stirrup head.

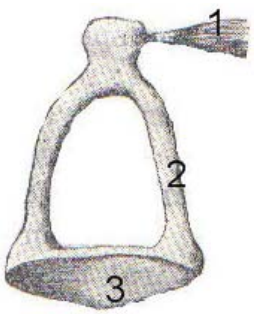


Fig. 2c. The stirrup

*1) footplate; 2) caudal arm; 3) rostral arm; 4) head.*

The middle ear communicates with the pharynx through the pharynx-tympanum tube, which is short – 1-1.5 cm – in dogs and which has a very elongated opening. This opening is rather difficult to reveal because of the mucous membrane covering it.

## *Conclusions*

1. The tympanum cavity is very roomy and it is not lined with mastoid cells.
2. The ear ossicles are rather large and resemble very much the human ossicles.
3. The lens-shaped bone is represented by the lens-shaped process from the long arm of the stirrup.
4. The stirrup muscle is very thin, almost invisibly for the human eye.
5. The pharynx-tympanum tube is short (1-1.5 cm).

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# THE VIRUCID ACTIVITY OF *DECONTAMINOL*** BIOCIDE

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***Abstract***

*The purpose of the paper was to investigate the virucide activity of a disinfecting, product based on quaternary amines mixed with glutaraldehyde. The virucide activity was determined against the viruses of the Newcastle disease (ND), aviary influenza (AI), infectious aviary bursitis (IAB) and the infectious aviary bronchitis (IB). Solutions in phosphate buffered saline (PBS), each of $10^{-4}$ $DIE_{50}$/ml of the mentioned viruses were put into contact for 10, 30 and 60 minutes with DECONTAMINOL 0.1% dilutions in sterile hard water[4]. After the time for contact ended, the mixtures of virus and DECONTAMINOL have been into SPF embryos, with an age of 9 days. Each embryo received $2x10^{-3}$ $DIE_{50}$. The alanto-amniotic fluid was examined for hemaglutinant activities (ND, AI), the embryos for the presence of the specific lesions of IAB, IB. DECONTAMINOL 0.1% dilution had an intense virucide activity (100%) against ND, AI and IAB viruses, which were destroyed after 10 minutes of contact. IAB virus was by the 0.1% DECONTAMINOL solution after 60 minutes of contact.*

***Key words***: *quaternary amines, glutaraldehyde, virucide activity, aviary viruses (ND, AI, IAB, IB)*

## *Introduction*

*Quaternary amines* are ammonium salts in which the organic radicals have been substituted by 4 hydrogen atoms. This substitution allowed important biological and physico-chemical properties, among which we mention the biocide activities, a high electrolytic power and good water solubility. The biocide activity was mentioned in an ample monography, which studied the bactericide, fungicide and bacteriostatic activity of the quaternary amines (14).

Due to these properties, the aminoquaternary are used as antiseptic agents, detergents, disinfecting and sanitation agents, algaecide and emulsifiants (6).

As antiseptic agents they have bacteriostatic and bactericide effect on Gram positive and Gram negative germs and display antifungal activity and fungicide activity on protozoa (13).

The *quaternary amines* have a low toxic activity on the teguments and are not toxic to the environment (6).

*Glutaraldehyde* is a saturated dialdehyde used as disinfectant and chemical sterilizer due to the wide bactericide, fungicide, tuberculocide, virucide and slow sporicide spectrum (5, 8).

*DECONTAMINOL* is a disinfecting solution based on quaternary amines (alkyl dimethylphenol o/p-ammonium chloride) mixed with glutaraldehyde. Due to its bactericide and bacteriostatic, antifungal and algaecide activity, the manufacturer recommends the use of DECONTAMINOL for prophylactic and necessary disinfection of various surfaces (flooring and walls) in animal farms, in the food industry (dairy products factories, slaughterhouses, meat processing units, etc.) and to disinfect the tires of the cars and vans hauling animals.

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* *DECONTAMINOL* is a ROMVAC Co. S.A. disinfecting solution prepared from a mixture of quaternary amines 15 % and glutaraldehyde 5 %.

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[3] SC ROMVAC COMPANY SA.

[4] The hard water contains 300 µl/calcium carbonate.

DECONTAMINOL is used as 1% disinfecting solution after the hydromechanical cleaning of the flooring, walls, working tables, installations, etc.

The working dilution has a low level of aggressiveness on the environment and is not toxic to the persons handling the substances. The mechanism of action of the quaternary amines and glutaraldehyde on the microorganisms is not known.

The literature mentions the high resistance in the environment of the aviary viruses, which cause important economic damages, the viruses of the *Newcastle disease* (ND), *aviary influenza* (AI), *infectious aviary bursitis* (IAB) and the *infectious aviary bronchitis* (IB) (1, 4, 7, 9).

These viruses resist at 56°C for 6 minutes to 6 hours. IAB resists in the environment between 2 months and 2 years, which makes it one of the toughest viruses (2, 9).

The purpose of the paper was to study the virucide activity of *DECONTAMINOL* on ND, AI, IAB and IB viruses.

### *Material and method*

1. **Working solution**, *DECONTAMINOL* diluted in sterile hard water was tested for embryonic toxicity in dilutions of 10%, 1%, 0.1%, and 0.01%. The 0.1% dilution was selected to study the virucide activity; this dilution ensured the survival of all inoculated SPF embryos. The hard water was processed according to SR EN 14675 Standard and it contains 300 μg/l calcium carbonate. After preparation, the hard water was sterilized for 15 minutes at 121°C.

2. SPF[5] embryos age 9 days were inoculated via the intraalantoamniotic route with 0.2 ml of a mixture of equal parts of dilution $10^{-4}$ $DIE_{50}$/ml of the virus with 0,1% *DECONTAMINOL* solution, after a contact of 10, 30 and 60 minutes. Each embryo was inoculated with $1 \times 10^{-3}DIE_{50}$ ND, IB, AI and IAB virus.

3. The used viruses are from the strain collection of ROMVAC COMPANY SA, as follows:

a) *Newcastle disease* virus, La Sota strain, with titre $10^{-9,2}DIE_{50}$/ml;

b) *Infectious aviary bronchitis* virus, ***hot*** P.A. strain, with titre $10^{-7,7}DIE_{50}$/ml;

c) *Aviary influenza* virus, ***Avx*** – 7367 strain, with titre $10^{-9}DIE_{50}$/ml;

d) *Infectious aviary bronchitis*, $\boldsymbol{H_{120}}$ strain, with titre $10^{-6,7}DIE_{50}$/ml.

The virus dilutions have been done in saline phosphate buffer with pH 6.8-7.0, at the temperature of 20°C.

### *Experimental model*

Groups of 10 SPF embryos age 9 days were inoculated with 0.2 ml as follows:

- 3 groups of embryos with a mixture of ND virus and *DECONTAMINOL* 0.1%, after a contact of 10, 30 and 60 minutes;
- 3 groups of embryos with a mixture of IAB virus and *DECONTAMINOL* 0.1%, after a contact of 10, 30 and 60 minutes;
- 3 groups of embryos with a mixture of AI virus and *DECONTAMINOL* 0.1%, after a contact of 10, 30 and 60 minutes;
- 3 groups of embryos with a mixture of IB virus and *DECONTAMINOL* 0.1 %, after a contact of 10, 30 and 60 minutes;
- 3 groups with 0.1 ml of ND virus dilution $10^{-4}DIE_{50}$/ml at intervals of 10, 30, 60 min.;

[5] Specific Pathogen Free.

- 3 groups with 0.1 ml of IAB virus dilution $10^{-4}DIE_{50}$/ml at intervals of 10, 30, 60 min.;
- 3 groups with 0.1 ml of AI virus dilution $10^{-4}DIE_{50}$/ml at intervals of 10, 30, 60 min.;
- 3 groups with 0.1 ml of IB virus dilution $10^{-4}DIE_{50}$/ml at intervals of 10, 30, 60 min.;
- 1 control group not inoculated;
- 1 control group inoculated with 0.2 ml PBS/embryo;
- 1 control group inoculated with 0.2 ml hard water/embryo;

During the contact period between the virus and the *DECONTAMINOL* solution, the tubes with virus were kept in the refrigerator, same as for the virus dilutions.

After inoculation, the embryos were evaluated daily by ovoscopy for 7 days. During this interval, the embryos which died within 24 hours after inoculation were regarded as accidents and removed, while the embryos which died after this interval were examined for the hemaglutinant activity of the alanto-amniotic fluid for viruses ND and AI.

Viruses IB and IAB produce embryo lesions, each dead embryo being examined. At the end of the experiment, the live embryos were slaughtered and examined.

The experiment conducted at the refrigeration temperature of + 4-8°C was repeated, this time the suspensions being kept at +20°C.

The alanto-amniotic fluid collected from the slaughtered embryos after 60 minutes of contact, were used to make two blind passages for each category of virus. The SPF embryos age 9 days were inoculated with 0.2 ml sterile alanto-amniotic fluid and evaluated daily by ovoscopy. At the end of the 7 days, the embryos were slaughtered and the alanto-amniotic fluid was used for the third passage.

## Results and discussions

Tables 1, 2 and 3 show the experimental results.

Table 1

Testing the embryotoxicity of the *DECONTAMINOL* solutions

| Group | DECONTAMINOL solution in hard water | Number of inoculated embryos | Amount of inoculum | Embryo mortality | | | |
|---|---|---|---|---|---|---|---|
| | | | | Dead | Survivors | % | Total dead/ total inoculed |
| 1 | 10% | 10 | 0,2 | 10 | 0 | 100 | 10/10 |
| 2 | 1% | 10 | 0,2 | 9 | 1 | 90 | 9/10 |
| 3 | 0,1% | 10 | 0,2 | 0 | 10 | 0 | 0/10 |
| 4 | 0,01% | 10 | 0,2 | 0 | 10 | 0 | 0/10 |

Table 2

Virucide activity of 0.1% *DECONTAMINOL* solution

| No. | Inoculum | Period of contact | | | Virucide activity, % | | |
|---|---|---|---|---|---|---|---|
| | | 10 minutes Positive*/ Total | 30 minutes Positive*/ Total | 60 minutes Positive*/ Total | 10 minutes | 30 minutes | 60 minutes |
| 1 | ND virus + D | 0/9 | 0/10 | 0/10 | 100 | 100 | 100 |
| 2 | IAB virus + D | 0/9 | 0/10 | 0/9 | 100 | 100 | 100 |
| 3 | AI virus + D | 0/10 | 0/10 | 0/10 | 100 | 100 | 100 |
| 4 | IB virus + D | 8/10 | 2/10 | 0/10 | 20 | 80 | 100 |
| 5 | ND virus | 10/10 | 10/10 | 10/10 | - | - | - |
| 6 | IAB virus | 10/10 | 9/10 | 8/10 | - | - | - |
| 7 | AI virus | 8/10 | 9/10 | 8/10 | - | - | - |
| 8 | IB virus | 9/9 | 10/10 | 8/10 | - | - | - |
| 9 | Control | 0/10 | 0/10 | 0/10 | - | - | - |
| 10 | PBS control | 0/9 | 0/10 | 0/9 | - | - | - |
| 11 | Hard water, control | 0/10 | 0/9 | 0/10 | - | - | - |

***** Positive/Total (with HA activity or with embryo lesions/total inoculated).
D – DECONTAMINOL

Table 3

Results of the blind passages of the alanto-amniotic fluid (a.a.f.) collected from the slaughtered embryos

| Group | a.a.f. inoculum virus | Embryo inoculum | First passage | | | Second passage | | |
|---|---|---|---|---|---|---|---|---|
| | | | Positive* | Survived with no lesions | Total positive/ inoc. | Positive* | Survived with no lesions | Total positive/ inoc. |
| 1 | BN | 20 | 0 | 10 | 0/10 | 0 | 10 | 0/10 |
| 2 | IA | 20 | 0 | 10 | 0/10 | 0 | 10 | 0/10 |
| 3 | BIA | 20 | 0 | 10 | 0/10 | 0 | 10 | 0/10 |
| 4 | BI | 20 | 0 | 10 | 0/10 | 0 | 10 | 0/10 |

Positive* – embryos with specific embryonic lesions and with hemaglutinant activity.

The results from Table 1 show that the 0.1% *DECONTAMINOL* dilution ensured the survival of all inoculated embryos, which is why this dilution was selected for the experiment. The 0.1% dilution supports the completely atoxic activity of the product.

The virucide activity of Decontaminol was manifested after the first 10 minutes of contact against the viruses of the Newcastle disease, IAB and aviary influenza. The quaternary amines and the glutaraldehyde may act on the

neuraminidases and hemaglutinins encountered on the surfaces of the two viruses and cancelled their infectivity and pathogenicity. The hemaglutinine and neuraminidase receptors are very receptive to the lytic action of the quaternary amines. The quaternary amines and glutaraldehyde, particularly, destroy the viral capside, preventing the infection mechanism of ND, IAM and AI viruses on the erythrocytar cells (10, 11).

In the case of IB virus, even though it also has hemaglutinine on the surface of the capside, the biochemical structure of the hemaglutinine is different from that of the other two viruses. The hemaglutinine of IB virus has 51% proteins, 34% lipids, traces of carbohydrates and RNA (12). It is possible that the lipids and, particularly, the proteins within the viral hemaglutinine make it more resistant to the action of the biocide substance.

In the case of the IAB virus, the action of the quaternary amines in combination with the glutaraldehyde was fast and didn't need a long time of contact with the virus to destroy the viral capside, such it was the case with Catiorom. IAB virus is particularly resistant in the environment (2). The literature shows the virucide and slow sporicide activity of glutaraldehyde, but gives no concrete evidence on its action on different types of viruses, and doesn't mention whether this action is accompanied by the destruction of the viral particle. The blind passages we did after collecting the a.a.f. from the slaughtered embryos, which had a 60 minutes contact with the biocide substance, showed the virucide activity of the two substances against the aviary viruses. This supports the stronger virucide activity of Decontaminol biocide compared to Catiorom (3).

## *Conclusions*

1. Decontaminol biocide, prepared from quaternary amines and glutaraldehyde, when used in 0.1% dilution, didn't produce embryo mortality in SPF embryos.

2. The 0.1% dilution of Deconaminol has 100% virucide activity against ND, IAB and AI viruses after 10 minutes of contact.

3. The 0.1% dilution of Deconaminol has 100% virucide activity against IB virus after 60 minutes of contact.

4. The virucide activity of Decontaminol biocide has been confirmed by two blind passages.

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# IMMUNOGLOBULIN G RESPONSE OF STREPTOCOCCUS SUIS BACTERIN – VACCINATED PIGS

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***Abstract***

*Thirty SPF pigs known to be free of Streptococcus suis infection were randomly selected from four litters at 10 to 12 days of age and allocated to 20 vaccinates and 10 controls. Each of the vaccinates was injected intramuscularly with a 1 ml dose of a S. suis bacterin at 10-12 days of age, followed by a 2.0 ml dose at 26 days of age. All of the animals were challenged with S. suis 16 days following the second vaccination. The vaccinates were protected and the controls showed clinical signs and necropsy lesions. Western blot analysis identified the special proteins between 35 and 50 kD that were recognized by all sera from pigs vaccinated twice and also by sera from pigs surviving challenge. These 35-50 kD proteins were not recognized by sera from prevaccinates and negative controls. An indirect ELISA using 35-50 kD proteins for coating plates was standardized for the detection of specific antibodies following vaccination and challenge.*

***Key words*:** *Streptococcus suis, immunoglobulin G*

## *Introduction*

*Streptococcus suis* has become one of the major pig infections in today's modern pig operations using medicated early weaning (MEW), segregated early weaning (SEW) or any other high health technologies to eliminate most of the pathogens from pig herds (9). The reason is that pigs are colonized with *S. suis* during parturition or between 5 and 10 days of age, which is far earlier than the actual weaning day in the above early weaning systems. Colonized piglets will transmit *S. suis* into other nursery pigs and cause outbreak of clinical disease as maternal antibody declines (1). For this reason, to vaccinate pigs with a S. suis bacterin at an appropriate age could be one method of keeping this disease under control. Attempts to control this disease with vaccines have been made by several researchers (2, 4, 6). However, in the absence of a reliable serological method to detect the antibody response, it is difficult to evaluate the immune response and the level of protection induced in vaccinated pigs. The aim of this study was to identify certain bacterial protein fractions that can only be detected by sera from vaccinated pigs which survived virulent challenge, and to develop an ELISA to measure specific IgG levels against these proteins in vaccinated or challenged pigs.

## *Materials and methods*

**Test Bacterin:** Streptococcus Suis Bacterin, MVP Serial LS 101, is a killed bacterin adjuvanted with Emulsigen (an oil-in-water adjuvant).

**Challenge Strain:** *S. suis*, Serotype 2 (MVP Reference Strain 7837).

**Efficacy Test and Serum Collection:** Thirty *S. suis* free SPF pigs from four litters were randomly assigned to a test group (20 pigs) and a control group (10 pigs) when they were 10-12 days old. Each of the 20 pigs from the test group was injected intramuscularly with a 1 ml dose of test bacterin at 10 and 11 days of age, followed by a 2.0 ml dose at 31 and 32 days of age. At the same time as the test group, each of the ten control pigs was injected with similar volumes of a sham vaccine which contained only the growth medium plus adjuvant.

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All 30 pigs were challenged with a 1.0 ml intravenous dose containing 107 CFU of virulent *S. suis,* 16 days after the second injection. Clinical signs were recorded daily.

Serum samples were taken prior to each vaccination and prior to challenge. Serum samples were also taken at 10 days after challenge or just prior to death by euthanasia.

The challenged pigs were temperatured and observed daily for clinical signs of S. suis infection. Observations were made for 7 to 10 days following challenge.

Clinical signs such as dyspnea, lameness (joint swelling), meningitis and depression (anorexia, etc.) were evaluated daily on a 3 point scale as follows:

| Score/Day | Degree of Dysfunction |
|---|---|
| 0 | Normal |
| 1 | Mild |
| 2 | Moderate |
| 3 | Severe |

Necropsy observations and histopathology results were scored in a similar fashion (normal, mild, moderate, severe) on a scale of 0 to 3.

Fever was scored daily as follows:

| Score/Day | Temperature Range |
|---|---|
| 0 | < 103.0oF |
| 1 | 103.0 to 103.9oF |
| 2 | 104.0 to 104.9oF |
| 3 | => 105.0oF |

Death attributed to *S. suis* infection received a score of 15.

**Production of Hyperimmune Pig Serum:** Prior to immunization, a young pig was bled and its serum was tested by slide agglutination to confirm absence of *S. suis* antibodies. The sero-negative pig was immunized with two doses of MVP Streptococcus Suis Bacterin (Serial LS 101, above), followed by weekly intravenous injections of 107 CFU of washed, formalin-inactivated *S. suis* for 3 months. The hyperimmune sera was aliquoted and stored at -20$^{o}$C. Non-reactive sera from 10 SPF pigs were also stored at - 20$^{o}$C for use as a negative control.

**SDS-PAGE and Western blotting:** Ten (10) ml of the formalin-killed *S. suis* whole culture was centrifuged at 1000 G for 10 minutes at room temperature. The culture was lysed by resuspending the pellet in 1 ml of cold (4oC) Lysis Buffer (0.1M Tris pH 8.0, 0.8 mg EDTA, 1 mg lysozyme) for 5 minutes. Approximately 240 µl of the lysed whole cells were mixed with 120 ul of gel loading buffer (DTT: 0.11 gm, 20% SDS: 0.71 ml, 1M Tris pH 6.8: 0.57 ml, glycerol: 1.07 ml, 1% Bromophenol Blue: 4 µl, H2O: qs. 7 ml) and heated to 100oC for 1 minute. The SDS-PAGE (7.5%) gels were prepared and 20 µl of the heated sample was loaded on each well of 2 gels and 10 µl of the molecular weight marker was also loaded on the outside well of each gel. After electrophoresis and electroblotting, blots were probed with pig serum.

**ELISA Procedure (Using Special Fractions of S. suis as Antigen):** A formalin inactivated culture of *S. suis* was concentrated 10 fold and an SDS-PAGE was performed as described above. After electrophoresis, the gel was removed and

placed in a staining box for silver staining. As soon as the bands started to appear, the stain reaction was stopped by adding 5% citrate solution. With a scalpel, the gel band of interest was excised (the area between 35 and 50 kD) and fragmented using a tissue grinder. One (1.0) ml of extraction buffer (0.5% SDS in 125 mM Tris-HCl, pH 6.8) was added and the suspension was incubated overnight at 5°C.

The suspension was centrifuged at 1000 G for 10 minutes and the supernatant was transferred to a fresh centrifuge tube containing 1 ml of cold precipitation buffer (90% acetone, 5% acetic acid, and 5% trimethylamine). The tube was put on ice for 30 minutes and then centrifuged at 5000 G at 5oC for 10 minutes. The supernatant was removed and the pellet was resuspended with an appropriate amount of PBS (pH 7.2) to make an antigen solution containing 0.125 mg protein per ml. Each well of the 96-well microtiter plates (Dynatech Immulon-2) was coated with 50 µl (6.25 ug/well) of the diluted antigen. The plates were air dried overnight at room temperature. Plates were then filled with 50 µl per well of a blocking agent (1% BSA in PBS pH 7.2) and incubated at room temperature for 30 minutes. After washing 3 times with 150 µl per well of PBS-Tween 20, the antigen-coated microplates were incubated at room temperature for 90 minutes with 50 ul of diluted test serum. Hyperimmune pig serum was used as a positive control and serum from non-exposed pigs was used as negative control. Each plate contained positive and negative control samples diluted in the same manner as test serum samples. Serial dilutions of test sera were made from 1:400 to 1:12,800. After another three washes with PBS-Tween 20, 50 µl of alkaline phosphatase conjugated rabbit anti-porcine IgG (Sigma Chemical Co.) diluted 1:3000 was added to each well. Plates were incubated at room temperature for 90 minutes. After three washes with PBS-Tween, 100 µl of alkaline phosphatase substrate solution (Sigma Chemical Co.) was added to each well. The plates were allowed to react at room temperature until the OD 405 of the negative control was between 0.20 and 0.30. Then 50 µl of 5N NaOH was added to stop the color reaction. The plates were read at 405 nm. Results were reported as titers which were defined as the reciprocal of the lowest serum dilution having a ratio (OD test / OD negative control) of 1.5 or larger.

**ELISA Procedure (Using Washed Whole Cells of S. suis as Antigen):** One (1.0) ml of a whole culture containing 107 CFU per ml of *S. suis* was added to 9 ml of sterile PBS and mixed well by vortex. After centrifugation at 500 G for 10 minutes, the pellet was saved and resuspended in 10 ml PBS. More PBS was added to adjust the cell suspension to an OD of 0.10 at 405 nm. A 1:100 dilution of this cell suspension was made in PBS and used to coat each well of a 96-well microtiter plate with 100 µl of cell suspension. The plate was centrifuged at 1000 G for 20 minutes. The supernatant was removed and the plate was allowed to air dry before the ELISA was started. The ELISA procedure was the same as described above.

**ELISA Procedure (Using Heat Extracted Antigens of S. suis as Antigen):** The confluent growth from overnight cultures of *S. suis* serotype 2 was grown on a sheep blood agar plate and was harvested with sterile 0.85% NaCl solution and heated at 121°C for 2 hours. Cells were pelleted by centrifugation and the supernatant was adjusted to 6.25 ug of protein per ml with PBS. Fifty (50) µl of the

diluted antigen extract was used to coat each well of a 96-well plate and air dried. The ELISA procedure was the same as described above.

**Serological study of baby pigs from vaccinated and nonvaccinated sows using ELISA:** A total of 16 baby pigs, 8 from a vaccinated sow and 8 from a nonvaccinated sow, were randomly selected from their siblings for *S. suis* serological study after receiving colostrum by nursing. All of the 16 baby pigs were bled at the age of 2, 5, 8 and 11 days old. The sows were bled at parturition. The antibody titer profiles of each of the 16 pigs and 2 sows were determined by using the ELISA procedure described above.

## *Results*

**Efficacy Test:** The vaccinates were protected from a virulent challenge with 1 x 107 CFU of *S. suis* serotype 2 while the controls showed clinical signs and necropsy lesions. The mean cumulative scores of clinical signs, necropsy lesions, histopathology and mortality was 3.9 for vaccinates and 51.0 for the controls ($P < 0.05$, see Table 1).

**Western blots:** Western blot analysis of the cellular proteins from homologous *S. suis* serotype 2 with pig sera obtained from prevaccinates and vaccinates identified protein bands between 35 and 50 kD which were recognized by all sera from pigs vaccinated twice and also by sera from pigs surviving virulent challenge. These bands were not recognized by sera from pre-vaccinates and negative controls. A band of 59 kD was frequently recognized by most of the pig sera including pre-vaccinates, vaccinates, and survivors after challenge.

**Development of ELISA:** Serial dilutions of pig sera from negative control pigs, *S. suis* bacterin-vaccinated pigs, and *S. suis*-hyperimmune pigs were assayed using 3 different antigens of *S. suis*, serotype 2 as described above. At a serum dilution of 1:800, absorbance values from *S. suis*-vaccinated and *S. suis*-negative pigs were widely separated when 96-well microtiter plates were coated either with 35-50 kD proteins of *S. suis* serotype 2. or with heat extracted antigens of *S. suis* serotype 2. Absorbance values from *S. suis*-vaccinated and *S. suis*-negative pigs were quite similar to each other when plates were coated with washed whole cells of *S. suis* serotype 2.

ELISA procedures using either 35-50 kD proteins of *S. suis* serotype 2 or heatextract antigens of *S. suis* serotype 2 for coating plates were compared in their detections of antibody response in the same group of pigs, before and after immunization, or before and after virulent challenge. Comparison was also made between the vaccinated and nonvaccinated groups of pigs (table 2, table 3). At a serum dilution of 1:800, background readings (absorbance value from S. suis-negative pigs) were higher when heat extract antigens were used as coated antigens, and low when special fractions (35-50 kD proteins) were used as coated antigens. IgG antibody titers increased from 0 (prevaccinate) to 800 (after vaccination) and 3200 (post challenge) when 35-50 kD proteins of *S. suis* serotype 2 were used as antigens.

**Sensitivity and specificity:** Sensitivity of ELISA using 35-50 kD proteins of *S. suis* serotype 2 as coated antigens was established with sera from 64 pigs which were obtained from infected pig herds showing clinical signs of *S. suis* infection and at least one positive culture for *S. suis* serotype 2. Sera from 2 pigs had a S/N (signal to noise) ratio below the 1.5 cutoff value, giving a sensitivity of 97%. Specificity of the ELISA was also determined with sera from another group of 64 pigs, which were obtained from SPF pig herds and conventional pig herds showing no clinical signs and no positive culture for *S. suis* serotype 2. Sera from 6 *S. suis* serotype 2-negative pigs had a S/N ratio above the 1.5 cutoff value, giving a specificity of 91% (table 4).

**Serological study of baby pigs:** Antibody titer of the unvaccinated sow was 0 while the titer of the vaccinated sow was 1600. Piglets from the vaccinated sow had titers of 800 at 2 days, 5 days, and 8 days, and 0 at 11 days and 15 days. Piglets from the unvaccinated sow had titers of 0 at 2 days, 5 days, 8 days, 11 days and 15 days.

Table 1. Scores of clinical signs, necropsy lesions, histopathology and mortality after challenge of both vaccinates and nonvaccinated pigs with virulent *S. suis* serotype 2.

| **Test Animals Vaccinates** | **Clinical Cumulative Signs** | **Necropsy Lesions** | **Histopathology Lesions** | **Score** |
|---|---|---|---|---|
| Pig # 1 | 5 | 1 | 0 | 6 |
| 2 | 2 | 0 | 0 | 2 |
| 3 | 3 | 0 | 0 | 3 |
| 4 | 4 | 0 | 0 | 4 |
| 5 | 0 | 0 | 0 | 0 |
| 6 | 1 | 0 | 1 | 2 |
| 7 | 6 | 0 | 2 | 8 |
| 8 | 4 | 0 | 1 | 5 |
| 9 | 7 | 0 | 2 | 9 |
| 10 | 6 | 0 | 0 | 6 |
| 11 | 0 | 0 | 0 | 0 |
| 12 | 0 | 0 | 0 | 0 |
| 13 | 5 | 0 | 0 | 5 |
| 14 | 2 | 0 | 0 | 2 |
| 15 | 3 | 2 | 0 | 5 |
| 16 | 1 | 0 | 0 | 1 |
| 17 | 0 | 0 | 0 | 0 |
| 18 | 7 | 0 | 0 | 7 |
| 19 | 11 | 0 | 0 | 11 |
| 20 | 2 | 0 | 0 | 2 |
| | | | | Mean cumulative score: 3.9 |

| | | | | |
|---|---|---|---|---|
| Controls: | | | | |
| Pig # 21 | 41 | 3 | 0 | 44 |
| 22 | 64 | 3 | 2 | 69 |
| 23 | 69 | 3 | 2 | 74 |
| 24 | 28 | 0 | 0 | 28 |
| 25 | 33 | 2 | 0 | 35 |
| 26 | 52 | 3 | 0 | 55 |
| 27 | 59 | 3 | 0 | 62 |
| 28 | 51 | 1 | 2 | 54 |
| 29 | 36 | 2 | 0 | 38 |
| 30 | 50 | 1 | 0 | 51 |
| | | | | Mean Cumulative Score: 51 |

Table 2. Absorbance and Antibody Titers Obtained with Pooled Sera from S. Suis Bacterin-Vaccinated and Non-Vaccinated Pigs by ELISA Using a Specific Fraction (35-50kD) of *S. suis* serotype 2 as Antigens

| | **Dilution of Pig Serum** | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1:800 | | 1:1600 | | 1:3200 | | 1:6400 | | 1:12800 | | Antibody Titer |
| Group | OD | S/N | OD | S/N | OD | S/N | OD | S/N | OD | S/N | |
| Vaccinates Pre-vaccination | 0.29 | 1.0 | 0.26 | 0.9 | 0.20 | 1.0 | 0.27 | 1.0 | 0.26 | 0.9 | 0 |
| After 1st Vaccination | 0.49 | 1.8 | 0.33 | 1.2 | 0.32 | 1.1 | 0.29 | 1.0 | 0.26 | 0.9 | 800 |
| After 2nd Vaccination | 0.57 | 2.0 | 0.39 | 1.4 | 0.34 | 1.2 | 0.29 | 1.0 | 0.26 | 0.9 | 800 |
| Post-Challenge | 1.14 | 4.1 | 0.75 | 2.7 | 0.53 | 1.9 | 0.35 | 1.3 | 0.29 | 1.0 | 3200 |
| Controls Pre-challenge | 0.27 | 1.0 | 0.30 | 1.1 | 0.25 | 0.9 | 0.23 | 0.8 | 0.22 | 0.8 | 0 |
| Post-Challenge | 1.42 | 5.1 | 0.80 | 2.9 | 0.53 | 1.9 | 0.48 | 1.7 | 0.40 | 1.4 | 6400 |
| Hyperimmune Pig | 0.96 | 3.4 | 0.54 | 1.9 | 0.44 | 1.6 | 0.35 | 1.3 | 0.31 | 1.1 | 3200 |
| Negative Pig | 0.28 | | 0.27 | | 0.26 | | 0.22 | | 0.27 | | 0 |

Table 3. Absorbance and Antibody Titers Obtained with Pooled Sera from S. Suis Bacterin-Vaccinated and Non-Vaccinated Pigs by ELISA Using Heat-Extract Antigens of *S. suis* serotype 2 as Antigens

| | **Dilution of Pig Serum** | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1:800 | | 1:1600 | | 1:3200 | | 1:6400 | | 1:12800 | | Antibody Titer |
| Group | OD | S/N | OD | S/N | OD | S/N | OD | S/N | OD | S/N | |
| Vaccinates Pre-vaccination | 0.36 | 0.6 | 0.36 | 0.6 | 0.40 | 0.7 | 0.41 | 0.7 | 0.42 | 0.7 | 0 |
| After 1st Vaccination | 0.70 | 1.1 | 0.58 | 0.9 | 0.53 | 0.9 | 0.48 | 0.8 | 0.47 | 0.8 | 0 |
| After 2nd Vaccination | 1.06 | 1.7 | 0.79 | 1.3 | 0.67 | 1.1 | 0.58 | 0.9 | 0.57 | 0.9 | 800 |
| Post-Challenge | 0.94 | 1.5 | 0.79 | 1.3 | 0.65 | 1.0 | 0.55 | 0.9 | 0.54 | 0.9 | 800 |

Continuing table 3

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Controls Pre-challenge | 0.62 | 1.0 | 0.47 | 0.8 | 0.42 | 0.7 | 0.42 | 0.7 | 0.45 | 0.7 | 0 |
| Post-Challenge | 0.99 | 1.6 | 0.78 | 1.3 | 0.63 | 1.0 | 0.55 | 0.9 | 0.55 | 1.9 | 800 |
| Hyperimmune Pig | 1.19 | 1.9 | 0.92 | 1.5 | 0.67 | 1.1 | 0.56 | 0.9 | 0.53 | 0.9 | 1600 |
| Negative Pig | 0.62 | | 0.52 | | 0.43 | | 0.43 | | 0.45 | | 0 |

Table 4. Sensitivity and Specificity of the ELISA using 35-50 kD proteins of *S. suis* serotype 2 as Coated Antigens in Detecting Antibodies to *S. suis* serotype 2 in Pigs.

| ELISA Result | Number of Pigs with Indicated *S. suis* Antibody Test Result | | Total |
|---|---|---|---|
| | Positive | Negative | |
| Positive | 62 | 6 | 68 |
| Negative | 2 | 58 | 60 |
| Total | 64 | 64 | |

Table 5. Antibody Titers Obtained with Pooled Sera from Baby Pigs at 2-15 Days of Age by ELISA Using 35-50 kD Proteins of *S. suis* as Antigens

| Age of Pig (Days) | Titers of Pooled Sera from 8 Baby Pigs Farrowed by Unvaccinated Sow | Titers of Pooled Sera from 8 Baby Pigs Farrowed by Vaccinated Sow |
|---|---|---|
| 2 | 0 | 800 |
| 5 | 0 | 800 |
| 8 | 0 | 800 |
| 11 | 0 | 0 |
| 15 | 0 | 0 |
| Unvaccinated Sow | 0 | NA |
| Vaccinated Sow | NA | 1600 |

## *Discussion*

Previous experiments have shown that IgG and IgM directed against surface components of *S. suis* serotype 2 are important in protection of pigs that had been inoculated with live and killed cultures of *S. suis* serotype 2 (2, 5). It has also been reported that rabbit IgG generated against such cell surface components could passively protect mice against the challenge with reference *S. suis* serotype 2 strain (7, 10). While one report indicated that mice injected with cell proteins of 33, 128 and 136 kD were protected against challenge with the homologous *S. suis* strain (10), another paper indicated rabbit serum against 78 and 94 kD proteins protected mice against challenge (7). However, no experiment has been done to identify such bacterial cell surface components by sera from pigs surviving virulent challenge.

In this experiment, we found that 35-50 kD proteins were recognized by all sera from pigs vaccinated twice with S. Suis Bacterin and also by sera from pigs

surviving virulent challenge. These 35-50 kD proteins were not recognized by sera from prevaccinates and negative controls. These data suggest that these 35-50 kD proteins of *S. suis* serotype 2 may play a role in stimulating protective antibodies in pigs. In a separate experiment, we also found that such 35-50 kD proteins could be identified by sera either from mice immunized with a subunit vaccine containing 35-50 kD proteins or from mice immunized with a *S. suis* serotype 2 bacterin using Western blot analysis and an indirect ELISA (8). This further confirms the usefulness of mice for studying S. suis infection in pigs as described before by other authors (3, 11). This may indicate the feasibility of developing a standardized in-vivo serological test to compare the antibody titers induced by two or more S. suis bacterins. Further experiments using mouse as an animal model for pigs will be needed.

Test results in this experiment indicated that it was difficult to get reliable results with an indirect ELISA using formalinized whole culture of *S. suis* serotype 2 for coating of plates. This is consistent with a previous report (2).

In order to confirm a strong correlation between protection of pigs vaccinated twice with *S. suis* bacterin and the presence of antibody titers of 800 or higher against the specific proteins (35-50 kD) of *S. suis* serotype 2 an indirect ELISA using 35-50 kD proteins for coating of plates was developed. The present study has shown an acceptable sensitivity and specificity of the ELISA, using 35-50 kD proteins of *S. suis* for coating plates. In the present study, a heat extract antigen of a whole cell preparation of *S. Suis* serotype 2 was also used to develop an indirect ELISA. Test results showed that the ELISA using 35-50 kD proteins for coating of plates gave lower background readings than that using heat extract antigens for coating of plates.

Due to the present lack of knowledge about the specific immunogenic components of *S. suis* serotype 2, it is still premature to adopt such an ELISA for routine *S. suis* serology study. However, data from the present study did show that a specific antibody response against *S. suis* could be measured by an indirect ELISA. Further use of such ELISA for determining the protection levels in pigs after immunization will need further studies.

It is known that pigs are colonized with *S. suis* before 15 days of age and that such colonization is largely responsible for *S. suis* disease (9). Therefore, it may be necessary to immunize sows with *S. suis* bacterin to provide maternal protection for young pigs on farms adopting the SEW system. However, there are no reported data about the level of maternal antibody titers in young pigs shortly after birth and before 15 days of age. In this experiment we found the titer of antibodies against 33-50 kD proteins of *S. Suis* serotype 2 was 0 for an unvaccinated sow and its offspring, 1600 for a vaccinated sow when pigs were farrowed, 800 when pigs from a vaccinated sow were 2 days, 5 days and 8 days of age, and 0 when pigs from a vaccinated sow were 11 days and 15 days of age. In order to study the maternal protection of young pigs on farms using a SEW system, further experiments are needed.

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# THE ELECTROCARDIOGRAPHIC PARAMETERS EVALUATION OF THE SPORT HORSE

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***Abstract***

*A group of 24 sport horses was surveyed for the characteristic elements of the electrocardiograms done before and after a period of 5 months of physical exercises specific to the competition trainings. The sportive performance was positively correlated with the morphology of the ECG waves: the biphasic aspect of wave P, the sS-type aspect of the QRS complex, the monophasic aspect of the T wave. After the training, the average amplitude of $P_2$ biphasic wave increased by 14.7%, the average duration of the QRS complex increased by 16%, of the S wave increased by 21.8% and of the monophasic T wave increased by 22.2%.*

***Key words:*** *sport horse, ECG parameters, adaptative capacity*

## *Introduction*

Many authors (Landolsi et al., 1997; Pellecia et al., 2000; Kronfeld, 2001; Piccione et al., 2003; Fernandez, 2004; Lightowler et al., 2005) determined correlations between some morphological modifications of the electrocardiographic parameters with the athletic performance of the sport horses and characterized the bioelectric activity of the heart in relationship to some pathological aspects (electrolyte misbalances, hypoxia, epitasis, syndrome), conditioned by intense, unbalanced trainings. The study aimed to characterise the specific morphological traits of the electrocardiographic parameters in sport horses before the physical training and after 5 months of training.

## *Materials and Methods*

The experiment was conducted on 24 sport horses (12 females and 12 males), clinically healthy, aged between 3.5-8 years, managed by a private equitation club. In January 2007, the horses started the training program for the sportive competitions of the year. Before the start of the training session, the first electrocardiographic recording was performed at rest, while standing, with a portable DELTA 1 PLUS electrocardiograph. We used the technique of the uni-and bipolar derivations of the limbs; the working parameters were the amplitude of 10 mm/mV and the speed of 25 mm/sec. The second electrocardiographic recording was done under the same conditions as before, in June 2007, after 5 months of physical exercises progressive as intensity and duration, corresponding to the age and stage of training of the trained horses. The experimental results were interpreted statistically using the Student test.

## *Results and Discussions*

Before the training, while recording the bioelectrical activity of the heart, we also calculated the average value of the heart beat frequency (40 beats per minute) and of the breathing frequency (18 breathings per minute). Table 1 shows the average values defining the amplitude and duration of the electrocardiographic parameters.

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Table 1

Characteristics of ECG waves and intervals
(uni- and bipolar derivations of the limbs)
in sport horses before the training program

| ECG parameters | Duration (sec) | | Amplitude (mV) | |
|---|---|---|---|---|
| | M | SD | M | SD |
| **monophasic P** | 0.128 | 0.010 | 0.180 | 0.020 |
| **biphasic $P_2$** | 0.130 | 0.012 | 0.176 | 0.018 |
| **P-R** | 0.265 | 0.020 | - | - |
| **QRS** | 0.100 | 0.010 | 1.420 | 0.480 |
| **S** | - | - | 0.780 | 0.082 |
| **R-T** | 0.230 | 0.020 | - | - |
| **monophasic T** | 0.135 | 0.035 | 0.310 | 0.028 |
| **biphasic $T_2$** | 0.210 | 0.040 | 0.505 | 0.051 |

The normal morphology of P wave had two characteristics: the positive monophasic aspect observed in DII and a VF, in 15% of the individuals; the biphasic aspect, with the first deflection ($P_1$), positive, and the second ($P_2$), negative, observed in the other derivations (DI, DIII, aVR, aVL), in 75% of the individuals. The 10% difference, characterized the abnormal P wave, with biphasic aspect, in which $P_1$ wave was negative.

The average duration of the monophasic P wave was 0.128 sec, and of the biphasic $P_2$ wave was 0.130 sec. There were no major statistical differences between the average values of the duration.

The average amplitude of the monophasic P wave was 0.180 mV, and of the biphasic $P_2$ wave was 0.176 mV (while $P_1$ had the amplitude of only 0.080 mV). The difference between the average amplitude of the monophasic P wave and of the biphasic $P_2$ wave was not significant.

The P-R interval represents the duration of propagation of the depolarization from the sinusal node to the atrio-ventricular node. The duration of the P-R interval translates the vagal tone, its instability is related to the respiratory sinusal arrhythmia or to the atrio-ventricular block which doesn't always have a pathological significance (Fernandez, 2004).

The QRS complex corresponds to the ventricular depolarization wave. In derivations DIII and aVF the QRS complex appears as rS type in 68% of the individuals. The average duration of QRS complex duration is 0.100 sec, and of its amplitude is 1.420 mV (of which 0.780 mV is the amplitude of wave S).

T wave is the ventricular repolarization. The predominant morphology of T wave was monophasic positive, observed in DII, DIII and aVR, in 65% of the individuals, then biphasic with the first deflection ($T_1$), negative and the second one $T_2$), positive, observed in aVF in 25% of the individuals. Much seldom, in 10% of the individuals, was observed the negative monophasic aspect of T wave in DI and aVL.

After 5 months of training, the horses were in the competition period for the obstacle race, being at their height of physical condition. The second electrocardiogram

was done 30 minutes after the end of the effort when the average value of the cardiac frequency was 36.5 beats/minute and of the respiration was 16 breathings/ minute. Table 2 shows the average values of the ECG parameters in horses.

Table 2

Caracteristics of ECG waves and intervals
in sports horses after five months of training programme

| **ECG parameters** | **Duration (sec)** | | **Amplitude (mV)** | |
|---|---|---|---|---|
| | **M** | **SD** | **M** | **SD** |
| **monophasic P** | 0.125 | 0.013 | 0.210 | 0.020 |
| **biphasic $P_2$** | 0.128 | 0.014 | 0.202 | 0.020 |
| **P-R** | 0.280 | 0.024 | - | - |
| **QRS** | 0.116 | 0.010 | 1.565 | 0.350 |
| **S** | - | - | 0.950 | 0.088 |
| **R-T** | 0.300 | 0.030 | - | - |
| **monophasic T** | 0.165 | 0.027 | 0.360 | 0.042 |
| **biphasic $T_2$** | 0.214 | 0.036 | 0.514 | 0.055 |

The monophasic aspect of P wave persisted, observed in DII and aVF, in 15% of the individuals; the biphasic aspect with positive $P_1$, observed in the other derivations (DI, DIII, aVR, aVL), increased to 80% of the individuals. The abnormal P wave, with biphasic aspect and negative $P_1$ was observed only in 5% of the trained horses. The presence of the abnormal P wave, with biphasic and negative $P_1$ aspect suggests neurovegetative instability, but according to some authors (Landolsi et al., 1997) it is correlated with the poorer performance of some of the trained horses.

The average duration of the monophasic P wave was 0.125 sec, and of the biphasic $P_2$ wave was 0.128 sec. There were no major statistical differences between the average values of P wave duration between the trained an untrained horses.

The average amplitude of the monophasic P wave was 0.210 mV, and of the biphasic $P_2$ wave was 0.202 mV (while $P_1$ had amplitude of only 0.090 mV). A 16.6% increase of the average values was observed in the amplitude of the monophasic P wave and a 14.7% increase of the average values was observed in the amplitude of the biphasic $P_2$ wave, which correlated positively with the high level of physical performance after the training.

P-R interval varies with the cardiac frequency: it increases when the cardiac frequency decreases. The difference between the value of the P-R interval in the trained and untrained horses was poorly significant statistically. It was observed, however, that the too long duration of the P-R interval (in excess of 0.40 sec) is in a direct relation with a state of hypervagotony and, to a certain extent, with a training unfitted to the stage of the physical training of the horse (Pellecia et al., 2000).

The rS-type morphology of the QRS complex was observed in DIII and aVF in 72% of the horses. The average duration of the QRS complex in horses was 16%

longer after the training than before training. The longer duration of the QRS complex is positively correlated to the mass of the ventricular myocardium. The hypertrophy of the ventricular myocardium which appears subsequently to the sportive training may generate a higher distribution of the Purkinje fibres in the epicardium and therefore, the time of the electric impulse conduction increases. The amplitude of the QRS complex increased by 10% after the training and the amplitude of S wave increased by 21.8%.

R-T interval corresponds to the ventricular electric systole, but also to the atrial repolarization (Dojana, 2000). During the recovery period after the training, the duration of the R-T interval increased by 30%.

After the training most horses (85%) displayed a T wave with monophasic positive aspect, which correlates to the best shape of the functional capacity of the heart in the horses involved in the experiment. The duration of the monophasic T wave increased by 22.2% and the amplitude increased by 16%, which is the increase of the time required for the myocardial repolarization and for the velocity of the electric process.

## *Conclusions*

1. The adaptative modifications of the heart functionality which were observed in the sport horses trained for obstacle jumping have generated changes in the electric activity of the heart, as shown by the specific electrocardiograms.

2. The morphological characteristics of the ECG were the biphasic aspect of P wave, with positive $P_1$ and negative $P_2$, observed in most of the uni- and bipolar derivations of the limbs of 80% of the horses; the rS-type aspect of the QRS complex in derivations DIII and aVF, observed in 72% of the horses; the positive monophasic aspect of T wave observed in derivations DII, DIII and aVR, in 85% of the horses.

3. The increase of P, S and T waves voltage was correlated positively with the sportive performance of the horses.

4. The effect of the training also determined increases in duration of QRS and T waves and on the R-T interval.

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# HAEMATOLOGICAL CHANGES INDUCED BY EFFORT IN SPORT HORSES

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***Abstract***

*The study was conducted on 24 sport horses in best shape as result of the previous intensive training sessions. The variability of the haematological parameters was monitored in relation to the individual physiological particularities of age and sex before and after the routine training sessions. The adult horses displayed after the training exercise a 32.4% increase of the erythrocytes number, a 29.4% increase of the neutrophils number and a 23% increase of the hematocrit; the lymphocytes number decreased by 14.3%. The erythrocytes number increased after the training exercise by 18.5% in males horses and by 16.6% in female horses, the hematocrit increased by 20% in the male horses and by 16% in the female horses, while the N/L ratio increased by 56% in the males and by 26% in the females.*

***Key words*****:** *sport horse, adaptative capacity, haematological indices*

## *Introduction*

The superior athletic performances of the Romanian sport horses, as force, resistance and capacity to jump over obstacles is a corollary of the functional integration of the major systems of the organism involved in the production and release of energy. Horse adaptation to standardized physical effort requires complex physiological modifications of the cardiovascular, respiratory, locomotor apparatuses, accompanied by the intervention of the neuro-hormonal activity.

Many studies (Szarska, 2001; Piccione et al., 2001; Fazio et al., 2002; Krumrych, 2006) have shown that the parameters of the training exercises (intensity, duration and frequency) determine, according to the individual reactivity, changes in the value of the haematological parameters.

The importance of this study resides in the evaluation of the efficiency by type of training exercise in relation to the adaptative potential expressed by haematological parameters.

## *Materials and methods*

The experiment was conducted on 24 sport horses (12 males and 12 females) from a private equitation club. Two groups were formed, one consisting of 14 horses aged 3.5 to 5 years and one group consisting of 10 horses aged 5 to 8 years. These horses underwent daily exercise practices adequate to their age and stage of training. A progressive training session includes three stages. The first stage is warming up: normal walk – 10 minutes; trotting – 10 minutes; gallop alternating with trotting – 10 minutes; jumping over separate obstacles, 80-90 cm high, for 10 minutes. The second stage was the stage of intense effort in which the horses from the first group had to jump over obstacles 100-110 cm high, and the horses from the second group had to jump over obstacles 120-130 cm high along a distance of 600 m within 90 seconds, similar to the conditions of competition. The last stage was relaxation, 10-15 minutes of normal walk; over the following 15 minutes all effort ceased by rest in the stable. Before the training started (at the stable) and

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thereafter for the first 3 minutes after the effort (the stage of relaxation) and after the period of recovery, blood sample were collected from the jugular vein, in syringes on heparin. The blood was assayed on a performing haematological analyser for red blood cells (RBC), haemoglobin (Hb), hematocrit (PCV), white blood cells (WBC), neutrophils count (N) and lymphocytes count (L).

The experiment was conducted during the first decade of July 2007; the training exercises were conducted on a sandy trail, at 22-26°C, 36-48% humidity and 1012-1023 hPa atmospheric pressure.

The experimental results were interpreted with the Student test.

### *Results and discussions*

The clinical parameters recorded before and after the effort, at the stable, were as follows: for group I – the average value of the pulse was 32 heart beats per minute and the respiration rate was 16 breathings per minute; for group II – 31 heart beats per minute and 12 breathings per minute. In the first 3 minutes after effort, group I had 82 heart beats per minute 80 breathings per minute; group II had 78 heart beats per minute 66 breathings per minute. After 30 minutes from ending the effort, group I had 38 heart beats per minute 18 breathings per minute; group II had 35 heart beats per minute 18 breathings per minute. These values characterise the optimal physical condition for competition of the horses involved in the experiment.

Important changes were noticed, for the surveyed haematological parameters, compared to the values recorded before the effort, which were induced by the intensive exercise of the training session.

Within the first 3 minutes after effort (Table 1) an increase was noticed in the value of the surveyed haematological parameters: RBC, Hb, PCV, WBC, N and L; after the end of the effort, the indicators showed a trend to return to the values they had before the training exercises. The post-exercise increase of RBC was 28.8% in group I and 32.4% in group II, which shows a faster and higher mobilization of the erythrocytes stored in the spleen of the adult trained horses adapted to physical effort. The increase of the erythrocytes count was accompanied by the 19.4% increase of the total haemoglobin in group I and 17.9% in group II, the difference between groups not being significant.

A significant increase of PCV value was observed in group II, both during the post-exercise period (23%) and during the recovery period (12.8%). This is explained by the translocation of the blood serum water outside of the vascular system, towards the muscles, due to the intensive, long training exercises (Persson et al., 1996).

The requirement of the organism for oxygen is met by a two-fold increase of the number of capillaries and arteriolar anastomoses, by the release in the peripheral blood of a higher volume of erythrocytes by the contraction of the spleen smooth muscles triggered by the increase of sympathetic vegetative nervous system activity and by the increase of the circulating catecholamines concentration. The higher number of red blood cells accomplishes an important supply of oxygen, mainly through the haemoglobin, from the lungs towards the muscles, to support the oxidative degradation of the glycogen and triglycerides during cell respiration (Kunugiyama et al., 1997).

Due to the increase of the serum corticoadrenal hormone (cortizol mainly) concentration, the process of leokocytosis appeared. The cortizol stimulates the

production of neutrophils in the bone marrow and their release into the peripheral blood (neutrophils), but it suppresses the circulating lymphocytes (lymphopenia), (Shinkai et al., 1996). During the post-exercise period, the N/L ratio increased by 58.2% in group I due to a stronger emotional stress of the younger horses and by 51% in group II.

Table 1

Hematological parameters in the sport horses
from the two groups at different ages

| Haematological indices | Groups | Before effort | | During the first 3 minutes after effort | | At 30 minutes after effort | |
|---|---|---|---|---|---|---|---|
| | | M | SD | M | SD | M | SD |
| **RBC $10^6/mm^3$** | **I** | 9.7 ± 1.15 | | 12.5 ± 1.12 | | 10.2 ± 1.05 | |
| | **II** | 10.8 ± 1.05 | | 14.3 ± 1.14 | | 11.2 ± 1.10 | |
| **Hb g/dl** | **I** | 13.4 ± 1.13 | | 16.0 ± 1.15 | | 14.2 ± 1.16 | |
| | **II** | 15.6 ± 1.48 | | 18.4 ± 1.20 | | 15.8 ± 1.18 | |
| **PCV %** | **I** | 38 ± 3.56 | | 45 ± 4.25 | | 40 ± 3.80 | |
| | **II** | 39 ± 3.65 | | 48 ± 4.62 | | 44 ± 4.24 | |
| **WBC $10^3/mm^3$** | **I** | 8.5 ± 1.01 | | 12.4 ± 1.12 | | 9.7 ± 1.01 | |
| | **II** | 9.7 ± 1.12 | | 13.8 ± 1.14 | | 10.8 ± 1.05 | |
| **N $10^3/mm^3$** | **I** | 4.6 ± 0.36 | | 6.2 ± 0.58 | | 5.7 ± 0.48 | |
| | **II** | 5.1 ± 0.46 | | 6.6 ± 0.60 | | 5.8 ± 0.52 | |
| **L $10^3/mm^3$** | **I** | 2.6 ± 0.20 | | 2.2 ± 0.28 | | 2.3 ± 0.23 | |
| | **II** | 2.8 ± 0.30 | | 2.4 ± 0.32 | | 2.6 ± 0.24 | |

Table 2 shows the differences observed in the dynamics of the values of the surveyed haematological indicators. Sex-related physiological differences were notices at rest, before the effort: RBC value was 13.7% higher in the males than in the females; Hb value was 12% higher in the males than in the females; PCV value was 16.6% higher in the males than in the females.

Table 2

Hematological parameters in the sport horses according to the sex

| Haematological indices | Sex | Before effort | | During the first 3 minutes after effort | | At 30 minutes after effort | |
|---|---|---|---|---|---|---|---|
| | | **M** | **SD** | **M** | **SD** | **M** | **SD** |
| **RBC $10^6/mm^3$** | **M** | 10.8 ± 1.03 | | 12.8 ± 1.13 | | 10.3 ± 1.02 | |
| | **F** | 9.6 ± 1.10 | | 11.2 ± 1.12 | | 9.8 ± 1.01 | |
| **Hb g/dl** | **M** | 14.8 ± 1.15 | | 18.2 ± 1.20 | | 15.8 ± 1.16 | |
| | **F** | 13.4 ± 1.12 | | 16.3 ± 1.14 | | 14.2 ± 1.13 | |
| **PCV %** | **M** | 40 ± 3.85 | | 48 ± 4.64 | | 44 ± 4.20 | |
| | **F** | 38 ± 2.88 | | 44 ± 4.46 | | 40 ± 3.80 | |

Continuing table 2

| | | | | |
|---|---|---|---|---|
| **WBC $10^3/mm^3$** | **M** | 9.1 ± 1.01 | 12.8 ± 1.12 | 10.5 ± 1.04 |
| | **F** | 9.5 ± 1.02 | 13.4 ± 1.13 | 11.2 ± 1.10 |
| **N $10^3/mm^3$** | **M** | 4.8 ± 0.38 | 6.2 ± 0.46 | 5.0 ± 0.42 |
| | **F** | 5.2 ± 0.50 | 5.8 ± 0.58 | 5.4 ± 0.52 |
| **L $10^3/mm^3$** | **M** | 2.2 ± 0.20 | 1.8 ± 0.26 | 2.5 ± 0.22 |
| | **F** | 2.6 ± 0.24 | 2.3 ± 0.28 | 2.8 ± 0.30 |

The increased values of these indicators in the males compared to the females are determined by the presence of the steroid hormones, testosterone and cortizol particularly, which have a higher concentration in the males. The testosterone stimulates the production of erythropoietin and the cortizol reduces the number of circulating lymphocytes and stimulates the production of neutrophils (Marc et al., 2000).

No significant differences were observed during the post-exercise period in the values of the surveyed haematological parameters according to the sex. RBC value increased by 18.5% in males and 16.6% in females; Hb value increased by 23% in males and 21.6% in females; PCV value increased by 20% in males and 16% in females; WBC increased by 40,6% in males and 41% in females; N/L ratio increased by 56% in males and 26% in females.

## *Conclusions*

1. The modifications observed in the values of the surveyed haematological and clinical parameters were generated by the intensity and duration of the exercises conducted during the training, they appeared immediately after the effort and were short and transient.

2. The evaluation of the efficiency by type of training in sport horses must consider the correlation between the individual age and sex and the variability of some haematological indicators.

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# DIAGNOSIS OF DISMINERALOSES IN CATTLE BY DETERMINATION OF SOME MINERALS IN HAIR. METHODS AND REFERENCES VALUES

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***Abstract***

*In order to elaborate the work technology and establish reference values of some minerals in hair, 50 hair samples were drawn from cows in two farms with best production and reproduction parameters and good sanitary veterinary state. The following parameters were determinated in hair: ash, calcium, phosphorus, magnesium, iron, cupper, manganes, zinc. Analyses were performed by colorimetric and complexometric methods. Zinc was determined by spectrophotometry in atomic absorbtion. The following reference values for minerals in cattle hair were established: ash 1000-2600 mg/100g; calcium 100-230 mg/100g; phosphorus 13-19 mg/100g; magnesium 40-85 mg/100g; iron 30-107 ppm; copper 10-18 ppm; manganes 6-40 ppm; zinc 92-120 ppm. The values of mineral in cattle hair were compared and analyzed with those in blood serum (serum mineral prophile).*

***Key words***: *dismineraloses, cattle, hair analyses*

## *Introduction*

Diagnosis of dismineraloses in cattle can be realized by complexes methods (4, 7). An important method is determination of the minerals in hair (1, 2, 3, 5, 6, 8, 9, 10). The hair is able to reflect real content of the minerals transported by the plasma. Anke and al. (1) pointed out the correlation between the content of some minerals in fodder and animal's hair, respectively, they also evidenced higher values of hair's minerals during winter's months; the autors proposed hair test to be used in order to diagnosis mineral deficiency in cattle.

Several factors, among breed, sex, age, colour of the hair, period of drawing of the samples can influence the concentration of the minerals in hair (8, 10). Agreements between magnesium and copper in hair and the concentration of these minerals in the blood serum and cows fodder respectively were established (5). The copper deficiency was established in the cattle by determination of copper concentration in the hair (6). Toxical action of same chemical pollutants, especially heavy metals, could by noticed by hair analyses of the cattle (3, 4, 9).

This paperwork was intended to pointed out main methods for mineral determination in cattle's hair and also established reference values for analyzed minerals.

## *Materials and methods*

Methods for samples drawing, preparation and processing were pointed out using 50 cows from two standard farms with optimum production and reproduction parameters and good sanitary veterinary statement. Using also dry or wet mineralization methods, different laboratory technique were used according to the characteristics of the minerals in hair and the degree of technical endowment of the laboratory.

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Eight parameters were analyzed using the following methods:

- total minerals (ash) – calcinations method;
- calcium – complexometric method;
- phosphorus – colorimetric method with ammonium molibdovanadat;
- magnesium – colorimetric method with titan gelb;
- iron – colorimetric method with ammonium sulphocyanide;
- copper – colorimetric method with sodium diethyldityocarbamat;
- manganese – colorimetric method with ammonium persulphate;
- zinc – spectrophotometry in atomic absorbition.

Some methods were comparated and analyzed using different techniques, colorimetries or spectrophotometry in atomic absorbition respectively.

Results of some mineral determination in the cattles' hair were also compared with those recorded in the blood serum using a semiautomatically biochemical analyzer of some parameters and cows.

## *Results and discussions*

The investigations were performed in two cattle ferms with optimum production and reproduction parameters having a properly beeding, feeding and housing conditions.

The morbidity and mortality recorded in the farms were in technological limits; no infectocontagious diseases were recorded during the investigation period.

Comparative results of the mineral determinations in the cattle's hair also by colorimetric method and by spectrophotometry in atomic absorbition are centralized in table 1.

Table 1

Mineral concentrations in the cattle's hair by colorimetry and spectrophotometry in atomic absorbition ($\overline{X} \pm S$)

| Parameter | UM | N | Colorimetric method | SAA** method | Student test |
|---|---|---|---|---|---|
| Calcium* | mg/100g | 10 | 187.25 ± 56.55 | 148.75 ± 57.97 | p>0.1 (NS) |
| Magnesium | mg/100g | 10 | 13.22 ± 6.70 | 15.13 ± 6.18 | p>0.2 (NS) |
| Iron | ppm | 10 | 72.78 ± 20.31 | 70.10 ± 14.52 | p>0.5 (NS) |
| Copper | ppm | 10 | 16.06 ± 1.87 | 16.39 ± 2.28 | p>0.5 (NS) |
| manganes | ppm | 10 | 7.56 ± 1.22 | 6.37 ± 1.90 | p>0.1 (NS) |

* complexometric method

** spectrophotometry in atomic absorbition

NS unsignificant

According to the table 1, the differences recorded by comparison in the two methods were nonsignificant. This establishement can indicate the accuracy of colorimetric and complexometric methods for determination of some minerals in cattle's hair; these methods are accesibles, economic and efficients for the laboratories with medium tehnical endowement.

In order to establish reference values in the two farms epidemiological, clinical and anamnetical investigations were performed. Hair and blood samples were drown from the cows. These cows were clinically healthy with optimum production and reproduction parameters and good sanitary veterinary statement.

Results recorded at the light mineral parameters, in the cattle's hair are centralized in table 2, as variation intervals (minimum and maximum limit).

Table 2

Reference values of some minerals in the cattle's hair

| Parameter | UM | Limits |
|---|---|---|
| Ash | mg/100g | 1000-2600 |
| Calcium | mg/100g | 100-230 |
| Phosphorus | mg/100g | 13-19 |
| Magnesium | mg/100g | 40-85 |
| Iron | ppm | 30-107 |
| Copper | ppm | 10-18 |
| Manganese | ppm | 6-40 |
| Zinc | ppm | 92-120 |

Supposing that the results of the analyzed samples are joined in the reference values interval that reflects a normal mineral statement. These reference values are generally comparable with those noticed by others authors in the cattle's hair (1, 2, 5, 6, 10).

The results of the biochemical exams accomplished to the blood serum samples drawn from the same animals and in the same period as the hair's drowing were recorded and centralized in table 3.

Table 3

Mineral blood serum concentrations recorded at the cows ($\overline{X} \pm S$)

| Parameter | UM | Recorded values |
|---|---|---|
| Calcium | mg/100g | 8.03 ± 0.76 |
| Phosphorus | mg/100g | 6.64 ± 0.75 |
| Magnesium | mg/100g | 2.33 ± 0.33 |
| Iron | ppm | 178.5 ± 23.20 |
| Copper | ppm | 63.35 ± 9.35 |
| Zinc | ppm | 125.53 ± 58.52 |

Recorded values of the analyzed blood serum samples are joined in the reference values for the cows (7). Decreasing of the recorded values of the hair samples under minimum references limit indicated the evolution of the deficiency statement for the analyzed parameter; increasing of the values over maximum reference limit can be explained as a dismineralose by excess. Certainly the interpretation of the recorded data by comparation with the reference values has to be made prudently; it is necessary to analyse different others complexes data: anamneticals, epidemiologicals, pathophisiologicals, pathomorphologicals, terapeuticals, nutritional (quantitatives and qualitatives) and, of course, the results of the blood serum biochemical determinations.

The metods of analyzing the cattle's hair mineral had proved to be useful for the diagnosis of dismineraloses due to several considetrations: the hair samples are

easy to drawing (noninvasive method); the samples are easy to be stored and transported to the laboratory; the processing is relatively easy and colorimetric, and complexometric indicated methods are accessibles, economic and can be reproduced.

### *Conclusions*

1. The methods for minerals determination in the cattle's hair were pointed out and reference values were established as variation intervals.
2. Main analyzed parameters were: ash, calcium, phosphorus, magnesium, iron, cupper, manganes, zinc.
3. Following references values were established: ash 1000-2600 mg/100g; calcium 100-230 mg/100g; phosphorus 13-19 mg/100g; magnesium 40-85 mg/100g; iron 30-107 ppm; copper 10-18 ppm; manganes 6-40 ppm; zinc 92-120 ppm.
4. The norms of interpretation of the analyzed parameters versus reference values were indicated.
5. The colorimetric and complexometric indicated methods for mineral determination in the cattle's hair were proved to comparable to those recorded by spectrophotometry in atomic absorbition; in addition, these methods are economic, accessible and can be reproduced.

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# CORRELATION BETWEEN THE VALUES OF SOME ERYTHROCYTE AND SIDEREMY PARAMETERS IN COWS DURING LATE PREGNANCY AND IN THEIR CALVES

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***Abstract***

*The investigation monitored some haematological and biochemical parameters that characterise iron metabolism in cows during the last month of pregnancy and in their calves during the first three days of life. Haemoglobin and hematocrit were 11.8% and 8.5%, respectively, lower and sideremy was 34.5% higher in the pregnant cows that were cared for improperly, with low protein diets, compared to the cows that received proper diets. The newborn calves from the cows maintained and fed properly had 5.6% and 7.9% values for haemoglobin and, respectively, hematocrit than the calves that resulted from improperly nourished cows. In both categories of calves sideremy had lower values, close to the lower physiological limit, which requires the preventive supplemental with iron of the newborn calves. At birth calves weight was positive correlated with their haemoglobin value and those of their mothers too.*

***Key words**: haemoglobin, hematocrit, sideremy, pregnant cows, new born calves*

## *Introduction*

The major role iron plays in the organism consists of it transporting and preserving oxigen by means of haemoglobin and myoglobin. Nevertheless, being a mineral addicted enzyme, iron is responsible for the control of catalase and peroxidase, reactive oxigen species, involved in oxigen extraction from oxygenated water, while by means of citocromoxidasa, iron plays an essential role in reducing the intracellular oxygen (4, 6).

Iron deficiency is responsible for the presence of hypocrom microcitar anaemia in almost all the species, except ruminants in which case we deal with normocrom microcitar anaemia. Feriprive anaemia is frequent in young animals and it is the result of the insufficient iron level in maternal milk or milk substitutes used to feed suckling calves, which at birth are characterized by small iron stocks. Iron deficiency can be the secondary effect of an insufficient capitalization of the iron quantity provided (1, 3, 5, 7).

The present study consists of an investigation regarding the relation existing between iron metabolism disturbances in late pregnancy cows and their calves, in the purpose of establishing some prophylactic and early curative anti-iron anaemia measures.

## *Materials and methods*

The research was carried out in two cow-farms (A, B), during the February-May period. The farms presented particularities at the level of their maintenance and food providing (quantity and quality) systems, as well as at the level of their sanitary-veterinary status. A 35 three-four weeks antepartum pregnant cows group was created at the level of each farm, following the criteria of clinical health. The

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animals were randomly chosen. In the first three days of life, the resulted calves were clinically examined and individually weight. Blood samples were collected in syringes with heparin from both categories of animals and the following parametres were determined: haemoglobin (Hb), using cianhaemoglobin, photocolorimetric method; hematocrit (Ht), by means of micro-method; total seric protein (Pt), by biuret method; sideremy (Fe) by o-fenantroline method; the total iron binding capacity (TIBC) was established by means of the same method like the one used in case of seric iron after being treated initially with magnezium carbonat; transferina's saturation coefficient (CS) was determined according to the calculation formula. The mean corpuscular volume (MCV) and the mean corpusculare haemoglobin concentration (MCHC) was determined by means of consecrated formulae (2).

### *Results and discussions*

In comparison with cows in farm B, cows in farm A presented a good sustenance state, balanced and qualitative feeding, and less cases of illness in new born calves. Late pregnant and nursing cows in farm B presented bad sustenance conditions and improperly feeding. The results of the laboratory examinations were recast by arithmetical mean and standard deviation and afterwards statistically interpreted by means of the Student test. Haemoglobin and hematocrit were 13% and 8,8% respectively higher in the cows in farm A than in the cows in farm B (table 1).

Table 1

Values of some haematological and biochemical parameters ($\overline{X}\pm S\overline{X}$) in advanced pregnancy cows

| Specification | Hb g/dl | PCV % | Pt g/ml | Iron µg/dl | TIBC µg/dl | CS % | MCV $\mu^3$ | MCHC g/dl |
|---|---|---|---|---|---|---|---|---|
| A | 9.85 | 33.8 | 8.20 | 110.35 | 318.96 | 35.94 | 40 | 29.80 |
| | 0.90 | 3.10 | 0.80 | 12.10 | 29.80 | 3.40 | 3.75 | 3.20 |
| B | 8.70 | 30.5 | 7.60 | 150.14 | 308.14 | 49.27 | 32 | 28.32 |
| | 0.80 | 3.10 | 0.72 | 16.12 | 26.42 | 5.20 | 2.87 | 2.60 |
| Normal values | 8-11 | 25-35 | 7-8.5 | 100-150 | 250-320 | 30-40 | 40-60 | 27-35 |

The higher value of proteins in lot A was positively correlated with the better protein quota in feeds. The value of sideremy (table 1) was 36% higher in the cows form the lot B, than in the cows form lot A. The values of seric iron in animals belonging to lot B were correlated with the comparatively lower levels of haemoglobin and hematocrit, indicating the presence of deficiencies in iron metabolism mainly as a result of the low protein content of the feeds. The TIBC of animals in lot A was insignificantly higher than in lot B. The saturation value of transferina in the animals form the lot A was 27% lower than in the animals belonging to lot B. Haemoglobin and hematocrit values in calves during their first three days of life were higher in lot A than in lot B (table 2).

Table 2

Values of some haematological and biochemical values ($\overline{X} \pm S\overline{X}$) in new born calves

| | Hb g/dl | PCV % | Pt g/ml | Iron µg/dl | TIBC µg/dl | CS % | Weight (Kg) | MCV $\mu^3$ | MCHC g/dl |
|---|---|---|---|---|---|---|---|---|---|
| A | 9.81 | 37.52 | 6.85 | 102.59 | 336.37 | 31.10 | 33.40 | 52 | 26.31 |
| | 1.10 | 3.84 | 0.68 | 10.90 | 25.32 | 2.90 | 3.74 | 4.35 | 2.50 |
| B | 9.28 | 34.78 | 6.00 | 96.47 | 332.71 | 29.18 | 29.60 | 42 | 26.86 |
| | 1.05 | 3.38 | 0.56 | 9.10 | 22.85 | 3.10 | 3.25 | 3.80 | 2.65 |
| Normal values | 9-10.5 | 35.6-38.5 | 5.8-7.6 | 100-150 | 250-320 | 25-30 | 30-40 | 45-70 | 26-29.7 |

The values of sideremy in calves belonging to lot B was low – an average of 100 g/dl, value considered the normal low limit of reference (5). Consequently, calves in lot B presented an iron deficiency starting at birth, which triggers the necessity of oral or parenteral supplementing their food with this microelement, which is responsable for the genesis process of haemoglobin taking place in normal conditions. The medium body weight of the calves in lot A at birth was 14% higher than in lot B, which implies a positive correlation between it and haemoglobin level.

The strong variability of the haemoglobin and hematocrit values registered in calves belonging to both groups might be the result of collecting the majority of the blood samples 18 hours after the moment of birth, period of time during which some preclinical intestinal problems appeared that might had caused deshydration.

Positive correlations could be observed between the values of haemoglobin in pregnant cows and the weight of the calves at birth (table 3).

Table 3

Values of haemoglobin in last month pregnant cows and the body-weight of the new born calves

| Specification | Lot A | | Lot B | |
|---|---|---|---|---|
| Hb pregnant cows | < 10 g/dl | > 10 g/dl | < 10 g/dl | > 10 g/dl |
| Nr. new born calves | 18 | 17 | 15 | 20 |
| Kg. new born calves | 35.8 ±3.65 | 30.5 ± 3.15 | 31.2 ± 3.29 | 28.0 ± 2.72 |
| Kg. diference | +5.3 | | +3.2 | |
| „t" test | 4.042 | | 2.847 | |
| p value | $p < 0.001$ | | $p < 0.01$ | |

The results pointed out the fact that anaemia is frequently found in pregnant cows, the main cause being the food administrated – low in proteins, minerals and vitamins, but also the presence of the factors of toxicity – vegetal, mineral, micotic – which make difficult or even block iron absorption at the level of the intestines. The prevention of iron deficiency anaemia in new-born calves becomes obligatory

as a consequence starting from the first days of life, but iron administration must be correlated with the body weight and the results of the paraclinical tests.

### *Conclusions*

1. The value of sideremy higher in the group of cows that were given low protein food had as correspondents lower haemoglobin and hematocrit levels, indicating dysfunctions in haemoglobin synthesis iron based metabolism.

2. The study proved the existence of a positive correlation between haemoglobin values and body weight in calves, as well as between the values of haemoglobin and hematocrit values in pregnant cows and their calves weight at birth.

3. Iron administration in the case of new born calves starting from the first days of life represents an imperative measure of preventing iron deficiency anaemia.

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# INFLUENCE OF THE PHYSIOLOGICAL STATE ON THE MINERAL STATUS OF CATTLE HAIR

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***Abstract***

*The mineral status of the cattle hair was assayed in 20 hair samples from pregnant cows and in 20 hair samples from lactating cows from the same farm. Calcium was determined with the complexonometric method; phosphorus was determined with the colorimetric method with ammonium molibdovanadate; magnesium was determined with the colorimetric method with titan yellow. The following values were determined: calcium 177.64± 19.44 mg/100g in pregnant cows and 151.86±13.06 mg/ 100g in lactating cows; phosphorus 15.92±3.84 mg/100g in pregnant cows and 10.70± 4.24 mg/100g in lactating cows; magnesium 47.89±6.32 mg/100g in pregnant cows and 40.21±2.08 mg/100g in lactating cows. The values for the three minerals were significantly lower in the hair samples from lactating cows than in the hair samples from the pregnant cows, which shows that these minerals are used intensely during lactation, sometimes exceeding the homeostatic capacity for retention in the skeleton and, implicitly, in the hair.*

***Key words***: *cattle, hair, physiological state*

## *Introduction*

The high incidence of mineral deficiency in cattle is caused by quantitative and/or qualitative nutritional imbalances. Generally, these pathological states have a subclinical, insidious evolution, and their detection is most times difficult and late.

In the attempt to maintain its mineral homeostasis, the animal organism uses its reserves located in the bones, liver and in other tissues, which it mobilizes. The biochemical parameters of the blood and of other biological fluids reflect the mineral status at the moment of sample processing (1, 4, 7, 8). The evaluation of the mineral status by hair sample analysis, unlike other methods of investigation, offers several advantages in terms of sampling, transportation, conservation, animal protection since it is a non-invasive method (3, 4, 8). The evaluation of mineral status by hair analyzis, as well as the blood metabolic profile tests, must take into consideration several exogenous or endogenous factors which may bear an influence on the analyzed parameters. Among these are the physiological status of the cows, which was proved to influence the status of minerals such as calcium, phosphorus, magnesium, copper (2, 3, 4, 5).

The purpose of the paper was to study the influence of the physiological state of the cows, pregnancy and lactation, on the calcium, phosphorus and magnesium levels in the hair.

## *Material and method*

Two groups of 20 cows each with optimal production and reproduction parameters were formed in a dairy farm. The animals were clinically healthy and they were in different physiological states as follows:

- group 1 – pregnant cows during their last 3 months of pregnancy;

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- group 2 – lactating cows at peak lactation (months 3-4).

Hair samples were collected from the forehead area, amounting to 5-7 g. The hair was washed, degreased in ethylic ether, rinsed several times in distilled water and finally in bidistilled water and thereafter processed by dry mineralization. Calcium was determined with the complexonometric method; phosphorus was determined with the colorimetric method with ammonium molibdovanadate; magnesium was determined with the colorimetric method with titan yellow.

### *Results and discussion*

Table 1 and Chart 1 show the results of the analyses.

Table 1

Trace mineral status in pregnant and lactating cows ($\overline{X} \pm S$)

| Physiological state | Number of samples | Calcium mg/100 g | Phosphors mg/100g | Magnesium mg/100g |
|---|---|---|---|---|
| Pregnant cows | 20 | 177.64±19.44 | 15.92±3.84 | 47.89±6.32 |
| Lactating cows | 20 | 151.86±13.06 | 10.70±4.24 | 40.21±2.08 |
| T test | | p<0.01 | p<0.01 | p<0.01 |

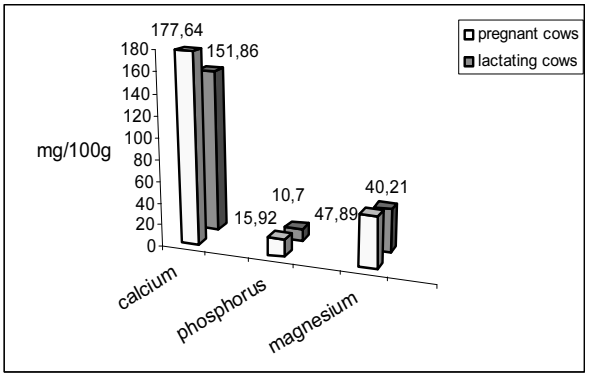


Chart. 1 Average values for calcium, phosphorus and magnesium in the hair samples from pregnant and lactating cows

The physiological limits of variation of calcium range between 100 and 230 mg/100g, of phosphorus between 13 and 19 mg/100g, and of magnesium between 40 and 85 mg/100g. The recorded values show that the physiological state of the animal obviously influences the level of calcium, phosphorus and magnesium in the hair of the dairy cows. The lower values if the surveyed trace minerals in the hair samples from lactating cows compared to the pregnant cows shows that these minerals, calcium particularly, are consumed in large amounts during lactation and may disturb the mineral homeostasis. It is thus known that one litre of cow milk contains about 1.25 g calcium and 0.95 g phosphorus (4), which makes the elimination of trace minerals to exceed their retention in the bones. Under the influence of lactation, particularly in the high yielding cows, the bone may undergo demineralization, phenomenon which can also be determined in the hair tissue (6). The decrease of blood calcium during late pregnancy and particularly during lactation may be regulated hormonally with parathormone, but only up to a given

level, after which a high hipocalcemia may cause clinical signs, paresis especially (2, 5, 6).

The level of hair calcium, phosphorus and magnesium may be an accurate marker for the evaluation of these trace elements' status in the organism.

## *Conclusions*

1. The physiological status of the cows influenced significantly the levels of hair calcium, phosphorus and magnesium.

2. Significantly lower values of the hair calcium, phosphorus and magnesium were observed in the lactating cows compared to the pregnant cows ($p<0.01$).

3. The evaluation of Ca, P and Mg in the hair of dairy cows may be an accurate marker for the evaluation of these trace elements' status in case of marginal deficiency, when the bone may undergo early demineralization.

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# THE BREEDING OF DAIRY SHEEP BY GENETIC MARKERS ASSESTED

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***Abstract***

*The investigation involved 66 Teleorman Black Head Tsigai sheep. The animals were monitored throughout their twelve controls determining the total amount of milk. The genetic markers considered by the investigation were the haemoglobin and transferrin. They were identified two genotypes ($Hb^A/Hb^B$ and $Hb^B/Hb^B$) at the haemoglobin locus and eight genotypes at the transferrin locus. The simultaneous analysis of the two studied markers reveals the superiority of the heterozygous sheep $Hb^A$ $Hb^B/Tf^MTf^E$, which displayed the highest productive performance for the studied character.*

***Key words***: *genetic marker, sheep, haemoblobin, transferrin*

## *Introduction*

The prediction of the breeding value of the farm animals is important for the selection of the most valuable specimens which to yield the next generation and for pair matching. Among the modern methods assessing the productive capacity of the animals and of their breeding value is the use of biochemical markers whose identification allows the determination of the population's genotypes and the correlation with the productive results (3).

## *Material and methods*

The experiment used 66 Teleorman Black head Tsigai sheep. Sheep were monitored during 12 tests, determining the yield of milk by each test (according to the method of Nica), calculating thereafter the total amount of milk by lactation. The genetic markers under study were the haemoglobin and the transferin.

The genotype categories were identified by vertical electrophoresis using polyacrylamide as migration carrier by means of the Meriaux J.C. technique (2) adapted by Mariana Rebedea and the biochemistry collective of the Faculty of Biology (1, 3).

The data were processed statistically by variance analysis.

## *Results and discussion*

For the haemoglobin marker (Table 1), the frequency of the observed genotypes was 0.3 for the heterozygous genotype $HB^AHB^B$ (P) and 0.2 for the homozygous genotype $HB^BHB^B$ (Q). The frequency of gene A (p) was 0.15 and the frequency of gene B (q) is 0.85, according to the Hardy – Weinberg law.

Table 1

Gene frequency at the haemoglobin locus

| Genotype | $HB^AHB^A$ | $HB^AHB^B$ | $HB^BHB^B$ | Total |
|---|---|---|---|---|
| Genotype frequency | 0 | 0.3 | 0.7 | 1 |
| Gene frequency | p (gene A) 0.15; q (gene B) 0.85 | | | |

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For the transferrin marker, the frequency is shown in table 2.

Table 2

Gene frequency at the transferrin locus

| Genotype | $Tf^{B}$ $Tf^{C}$ | $Tf^{B}$ $Tf^{E}$ | $Tf^{B}$ $Tf^{M}$ | $Tf^{C}$ $Tf^{C}$ | $Tf^{C}$ $Tf^{D}$ | $Tf^{C}$ $Tf^{M}$ | $Tf^{M}$ $Tf^{E}$ | $Tf^{M}$ $Tf^{M}$ | Total |
|---|---|---|---|---|---|---|---|---|---|
| Genotype frequency | 0,18 | 0,05 | 0,04 | 0,14 | 0,14 | 0,22 | 0,14 | 0,09 | 1 |
| Gene frequency | p (gene B)= 0,227; q (gene C) = 0,204; r (gene D) = 0,068; s (gene E) = 0,25; t (gene M) = 0,250 | | | | | | | | |

There have been identified eight genotypes. The gene frequencies were: 0.227 for gene B, 0.204 for gene C, 0.068 for gene D, 0.251 for gene E and 0.250 for gene M.

Table 3 shows the results obtained for the milk yield trait at the haemoglobin locus.

Table 3

Average performance achieved at the haemoglobin locus

| Genotype | Milk yield |
|---|---|
| $Hb^{A}Hb^{B}$ | 131.13 ± 16.35 |
| $Hb^{B}Hb^{B}$ | 89.91 ± 7.58 |

The best results for this marker were obtained in the heterozygous animals, in which the milk yield per lactation was 131.12 kg, 45% more than in the homozygous animals for gene B. The differences were significant ($p \leq 0.05$).

The productive results according to the genotype at the transferrin locus are shown in Table 4.

Table 4

Average performance achieved at the transferin locus

| Genotype | Milk yield | Ranking |
|---|---|---|
| $Tf^{M}/Tf^{E}$ | 131,30 ± 4,88 | 1 |
| $Tf^{C}/Tf^{D}$ | 115,65 ± 5,41 | 2 |
| $Tf^{C}/Tf^{M}$ | 112,04 ± 7,03 | 3 |
| $Tf^{C}/Tf^{C}$ | 104,08 ± 6,32 | 4 |
| $Tf^{M}/Tf^{M}$ | 99,02 ± 8,57 | 5 |
| $Tf^{B}/Tf^{E}$ | 92,06 ± 3,66 | 6 |
| $Tf^{B}/Tf^{C}$ | 91,28 ± 4,27 | 7 |
| $Tf^{B}/Tf^{M}$ | 86,88 ± 2,97 | 8 |

The superiority of genotype $Tf^{M}/Tf^{E}$ was observed. In this case, the total milk yield per lactation was 131.30kg, 13.5% more than the genotype on the second position.

Table 5 shows the data obtained by genotype aggregated from the haemoglobin and transferrin loci.

Table 5

Average performance by aggregate genotype

| Genotype | Milk yield | Ranking |
|---|---|---|
| $Hb^{A}Hb^{B}/Tf^{M}Tf^{E}$ | 135.61 ± 6.68 | 1 |
| $Hb^{B}Hb^{B}/Tf^{M}Tf^{E}$ | 129.15 ± 7.33 | 2 |
| $Hb^{B}Hb^{B}/Tf^{C}Tf^{D}$ | 115.65 ± 7.24 | 3 |
| $Hb^{B}Hb^{B}/Tf^{B}Tf^{C}$ | 114.62 ± 7.37 | 4 |
| $Hb^{A}Hb^{B}/Tf^{C}Tf^{M}$ | 112.05 ± 8.06 | 5 |
| $Hb^{B}Hb^{B}/Tf^{C}Tf^{C}$ | 104.08 ±6.87 | 6 |
| $Hb^{A}Hb^{B}/Tf^{M}Tf^{M}$ | 99.02 ± 5.72 | 7 |
| $Hb^{B}Hb^{B}/Tf^{B}Tf^{E}$ | 92.06 ± 3.16 | 8 |
| $Hb^{A}Hb^{B}/Tf^{B}Tf^{M}$ | 86.88 ± 6.23 | 9 |
| $Hb^{A}Hb^{B}/Tf^{B}Tf^{C}$ | 83.50 ± 5.9.0 | 10 |

The analysis of the aggregated genotype showed that genotype $Hb^{A}Hb^{B}/Tf^{M}Tf^{E}$ ranked first, with a total milk yield of 135.61 kg, 5% more than genotype $Hb^{B}Hb^{B}/Tf^{M}Tf^{E}$ ranked secondly. The differences between groups are not significant ($p \leq 0.05$). Taking into consideration the data obtained at the transferrin locus, one may observe that irrespective of the genotype existing at the haemoglobin locus, the $Tf^{M}Tf^{E}$ heterozygous individuals will have better results of the total milk yield.

## *Conclusions*

1. At the *haemoglobin* locus, two types of migration were observed by electrophoresis, corresponding to two genotypes $Hb^{A}/Hb^{B}$ and $Hb^{B}/Hb^{B}$. Gene frequency was: 0.15 for gene $Hb^{A}$ and 0.85 for gene $Hb^{B}$.

2. At the *transferrin* locus, eight types electrophoretic movements were noticed, determined by genotypes $Tf^{B}/Tf^{C}$, $Tf^{B}/Tf^{E}$, $Tf^{B}/Tf^{M}$, $Tf^{C}/Tf^{C}$, $Tf^{C}/Tf^{D}$, $Tf^{C}/Tf^{M}$, $Tf^{M}/Tf^{E}$ şi $Tf^{M}/Tf^{M}$. Gene frequency was: 0,227 for gene $Tf^{B}$, 0,204 for gene $Tf^{C}$, 0,068 for gene $Tf^{D}$, 0,251 for gene $Tf^{E}$ and 0,250 for gene $Tf^{M}$.

3. The simultaneous analysis for haemoglobin and transferin revealed the superiority of $Hb^{A}Hb^{B}/Tf^{M}Tf^{E}$ group in analyzed trait (total amount of milk).

4. Irrespective of the genotype existing at the haemoglobin locus ($Hb^{A}Hb^{B}$ or $Hb^{B}Hb^{B}$), the $Tf^{M}Tf^{E}$ heterozygous individuals will have better results of the total milk yield

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# GROWTH PROCESS IN FEMALES QUAIL CHICKS MATHEMATICAL ASSISTED

**Monica PÂRVU[*], R. BURLACU[**], Ioana Cristina ANDRONIE[*]**

***Abstract***

*In order to work out a mathematical modelling, the growth process on quail chicks was studied. The experiment used 55 one day-old quail chicks raised in cages, assigned to 5 randomized groups (one control and four experimental groups). The experimental period was of 42 days. The chicks received isocaloric diets with three levels of feeding (ad libitum, 90% and 80% of ad libitum) and three protein levels (according to the standard and lower with 5% and 10%). The growth energy and the growth speed were significantly influenced by the level of feeding and by the dietary protein level. The highest daily weigh gain was assessed, by mathematical modelling, at 17.12 g, achieved at 25 days. The weight at maturity (39 days) was assessed, by mathematical modelling, with the highest value being 156 g.*

***Key words**: mathematical modelling, quail*

## *Introduction*

Mathematical modelling is based on the simulation of the processes of broiler growth and development considering the efficiency of nutrient utilization [2]. General equations are developed, which consider the initial weight and the chemical composition of the diet. The literature shows various mathematical models by species and production category, without mentioning the breed or hybrid that was used.

## *Materials and Methods*

The experiment used 55 one day-old females quail chicks raised in the cages, assigned to 5 randomized groups (one control and four experimental groups). The experimental period was of 42 days.

The chicks received isocaloric formulations with different dietary levels of protein and amino acids. The experiment used various conditions of feeding in order to provide a wide range of applicability to the model. We used three levels of feeding (*ad libitum*, for the control group, 90% and 80% of *ad libitum* for the first and second experimental groups) and three protein levels (according to the standard for the control group, lower with 5% at experimental group 3 and 10% at experimental group 4).

Since the main aspects of the growth process, in the case of meat production, are the growth energy, the speed of growth and feed conversion ratio, throughout the experimental period we monitored the weight gain, feed intake and feed conversion ratio.

The environmental conditions were according to the standard of the growth technology.

The forage samples were assayed with the method of Weende and with an adiabatic calorimeter.

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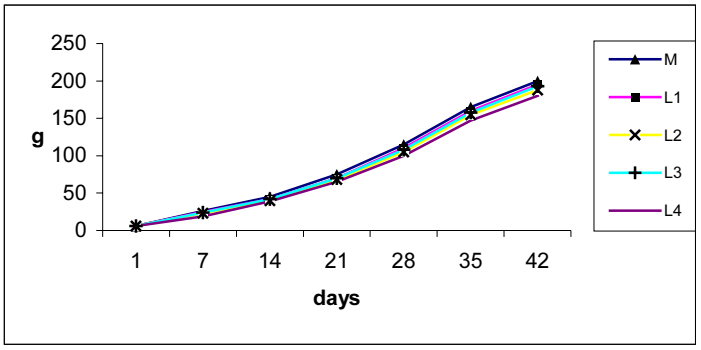
The mathematical modelling of the growth process used Gompertz-type functions.

### *Results and Discussions*

Growth energy (see the chart) was not the same throughout the growth period, being higher during the period 30-40 days. It was observed that related to the weight at hatching (initial weight), body mass at 14 days increased in average by 5.5 times ($p \geq 0,5$). Starting with the age of 28 days, the level of feeding and the level of dietary protein influenced significantly the growth energy ($p \leq 0.05$). At the age of 42 days, body mass had increased 21 times in the control group, 18 times in group 1, 17 times in group 2, 13 times in group 3 and 10 times in group 4.

The conditions of feeding (level of feeding and level of dietary protein) did not bear an influence on the overall curve, which was an "S" for all groups.




Growth energy

Growth speed (average daily gain) was 4.62 g in the control group, 4.15 g in group 1, 3.97 g in group 2, 4.30 g in group 3 and 3.38 g in group 4. The average daily gain was significantly influenced by the level of feeding ($p \leq 0.05$) and distinctly significant ($p \leq 0.01$) by the depression of the dietary protein level. The decrease of dietary protein and essential amino acids (lysine, methionine+cystine) level inhibited the growth and a high feed intake mare [1].

Processing statistically the data obtained from all groups with the Gompertz-type growth functions, we obtained the following equations:

$$\textit{Weight } W\,(g) = 6.5 \times e^{\frac{0.1271}{0.028} \times (1 - e^{-0.028 \times t})}$$

$$\textit{Average daily gain } \Delta W\,(g/day) = \Delta GW/dt = 0.1251 \times W \times e^{-0.028 \times t}, \textit{ where } t = 25 \textit{ days}$$

$$\textit{Highest weight gain } \Delta W(t^x) = 17.12\ g/day$$

$$\textit{Highest weight } W_{max} = 156\ g$$

The highest daily weight gain was assessed at 17.12 g/day, at the age of 25 days. The highest weight at maturity was assessed at 156 g, at the age of 39 days.

## *Conclusions*

1. The variability of the feeding conditions (both as quality and quantity) has to be provided for the development of a mathematical model for the simulation of growth processes in females quail.

2. The growth energy is significantly influenced by the level of feeding and by the dietary protein level.

3. The highest daily weigh gain was assessed, by mathematical modelling, at 17.12 g, achieved in 25 days.

4. The weight at maturity (39 days) was assessed, by mathematical modelling, with the highest value being 156 g.

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# ASSESSMENT OF PHYSIOLOGICAL RESPONSE IN SPORT HORSES TO TRAINING INDUCED STRESS

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**Monica PÂRVU**[1], **Ş. CONSTANTIN**[2], **Ş. ZAMFIRESCU**[3]

***Abstract***

*Research monitored the induced response of some physiological welfare indicators in sport horses during training. We assessed the stress intensity during training by measuring the variations of heart rate, lactic acid, cortisol and creatinine levels. The horses included in the study were grouped in: untrained (A1 n: 15) and trained (A2 n: 22). Physiological responses differed between the two groups, even if the training programme they underwent was the same. The heart rate and cortisol values increased in untrained horses compared to the trained ones but we recorded an increase of their values in the latter when repeating the exercises during the monitored period (98-120 nmol/l). The plasma lactate levels recorded increased values in the case of the first group (10,2 mmol/l), only until the animals got used to that effort, after which they dropped (9,9 mmol/l). Creatinine concentration increased in the case of trained horses (1,5 mg/dl) compared to the untrained ones (1,2 mg/dl). Horse training may be regarded as stressful under certain circumstances thus leading to a depreciation of their welfare depending on the exercises taken, training intensity and animals' physical condition.*

***Key words***: *horses, training, welfare*

## *Introduction*

Horses are labor animals in many developing countries, used mostly for companionship, leisure and sport activities in the majority of the developed countries as well as meat source in many other countries. The interest fields related to horse welfare assessment were transport, veterinary care, faulty owner management and insufficient nutrition (Johnson, 1998).

Horse welfare may be assessed by monitoring the behavioural indicators and measuring the physiological ones (Schwean, K., 1999), and their responses may occur to a wide variety of stressors such as transport, foot illnesses, lack of exercise, or even training, thus affecting the animals' welfare. Foreman and Ferlazzo (1996) state that animal responses to stress are unique and differ from one animal to another in case of the same given situation.

The levels of physiological indicators show us the manner in which horses respond to training and the changes that appear following training, during relaxation period.

## *Materials and method*

The present study monitored 37 horses of different breeds (n: 37) aged 3 to 10 years that were divided into two groups: A1 untrained horses (3-5 years old) and A2 trained horses (5-10 years old). When not in training, horses participant to the research were sheltered in individual boxes with optimal housing conditions for this category. All horses were subjected to the same light (walk), moderate (trot), and intense (canter) training programme. We monitored the indicators for 30 minutes of training/day and week, over 3 months (July, August and September).

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Among the physiological indicators assessed to establish the quality level of their welfare we chose: heart rate, lactic acid, creatine and cortisol levels.

We measured the heart rate by means of a non-invasive method, using a Polar type of cardio-monitor. Its electrodes were placed under the girth on each side of the saddle, in close contact with the animal skin by means of a gel. The transmitter was horizontally placed on the withers and it was also fixed on the harness while the rider wore the recording device on his/her hand. The data recorded throughout the training period were downloaded on a computer by means of an infrared device.

Blood samples were collected by puncture of the jugular 2h prior and after training. The blood was collected in 1.3 ml lithium-heparin vacutainers (Vacutainer System) (LH/1.3). Immediately after sampling, the blood was stored in ice before processing (centrifugal action at 2000 rpm, 15 minutes) according to the working protocol. The plasma samples obtained were stored at $-20^0$C until they were analyzed in the laboratory.

The plasmatic cortisol levels were obtained by radioimunoassey RIA (IDEXX SNAP* READER), the lactic acid and creatinine levels by chemical analysis with commercial reagents in a biochemical analyser (IDEXX Vet Test 8008).

The statistical data analysis included the T student test in order to compare the biochemical and haematological parameters of the two horse groups whereas for the heart rate recorded, data were processed by means of the Polar Equine SW programme installed.

### *Results and discussions*

The recorded heart rate (fig. 1) during training shows a significant increase in untrained horses in the first month of their training as compared to the ones used to training.

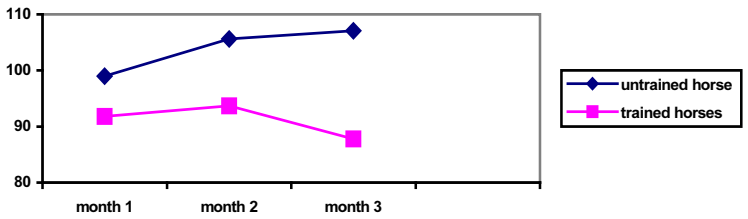


Fig. 1. Heart rate (bpm) recorded during research

This increase is present also in the third month of their training depending on the intensity of their exercise. In trained horses the heart rate decreases in the third month of training, which indicates the fact that the animals are getting used to the exercise. Repetitive training may be considered a chronic stress factor for trained horses.

The heart rate varied with training intensity (fig. 2) within relatively the same increase rate in untrained horses over the entire research period. Hyperpnoea combined with a decrease in the neurovegetative system control over the heart rate during intense training may result into a prevalence of its highly frequent variability (HRV) (Evans et al., 1995).

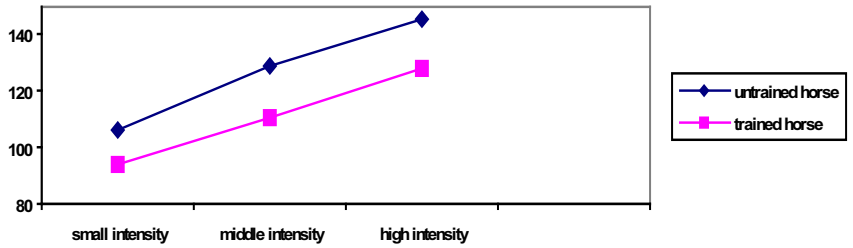


Fig. 2. Heart rate (bpm) recorded depending on the training intensity

Besides other physiological parameters, which respond to horse training, the heart rate modifications are useful in training assessment (Art and Lekeux, 1993).

The plasmatic cortisol levels (fig. 3) measured during training shows a concentration increase in untrained horses as opposed to trained horses; its values are different according to the exercise's intensity. This aspect is due especially to the horses' effort to adapt to training.

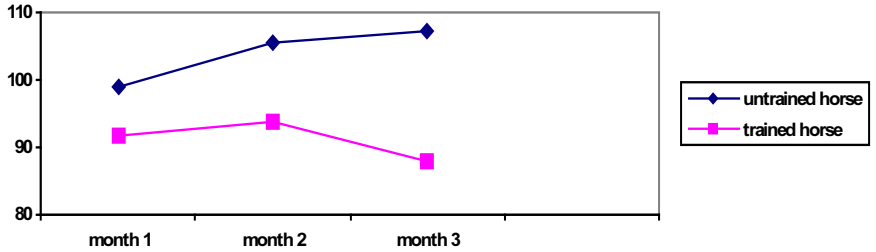


Fig. 3. Plasmatic cortisol level (nmol/l) recorded during research

Plasmatic lactate shows a decrease in trained horses compared to untrained horses (table 1), probably due to exercises thus testifying for a good athletic condition.

Measuring the seric enzymes may assess muscular activity and cellular metabolites resulted during training. The muscular cells produce lactate during anaerobic glycolyse (Sciliano et al., 1995). Its concentration level may be used as horse performance (Evans et al., 1995) as well as the animals' welfare level indicator.

Table 1

Level variations in biochemical indicators monitored during research

| **Biochemical indicators** | **Trained horse** | | | **Untrained horse** | | |
|---|---|---|---|---|---|---|
| | **month 1** | **month 2** | **month 3** | **month 1** | **month 2** | **month 3** |
| Plasmatic lactate mmol/l | 6,8 | 9,6 | 8,3 | 7,8 | 10,2 | 9,9 |
| Plasmatic creatine mg/dl | 1,42 | 1,48 | 1,52 | 1,18 | 122 | 1,39 |

Plasmatic creatine recorded an increase in trained horses (table 1) as opposed to untrained horses, which indicates a normal renal activity of the animals. Exercise intensity leads to an increase in muscular mass and implicitly in plasmatic creatine levels, which is a by-product of creatine decomposition, a nitrogenic compound

used by muscular cells to stock up energy. Seric creatine concentration varies with creatine synthesis and the animal muscular tissue (Stockman, 1995).

In 2005, Martins et al. suggested the hemato-biochemical profile in sport horses as physiological and training status marker.

## *Conclusions*

Heart rate may be an efficient method to monitor horse training, whose variations indicate horses' response to duration, type and intensity of training. It may also be suggested as marker of horse welfare during training.

The plasmatic cortisol records different levels in trained horses as compared to untrained horses as well as depending on exercise intensity.

Plasmatic lactate shows a decrease in trained horses which is indicative of the fact that, correlated to cortisol levels, it does not make for a major stimulus to the animal during training.

Seric creatinine levels are high in trained horses, especially when considering a higher intensity of exercises, as a result of muscular mass growth, which indicates significant horse athletic capacity.

The haematological and biochemical indicators can be used in assessing the welfare quality level of sport horses during training.

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# RESULTS OF INVESTIGATIONS ON THE QUALITY OF FEEDS GIVEN TO DAIRY COWS WITH DIFFERENT STATES OF DISEASE

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***Abstract***

*In a dairy cow farm, during November 2006-March 2007, several morbid states were observed displayed as placenta retention (75-80%), uterus-vaginal secretions and losses by mortality of suckling calves, lower milk yield, etc. Several factors were analyzed to determine the causes of the health problems. Among the analyzed factors, an important role was played by the quality of feeds. The raw ingredients and compound feeds were examined oganoleptically, physico-chemically, mycologically and micotoxicologically in order to determine their involvement in worsening the health state. The results showed significant deviations of the crude protein content, a moderate contamination with toxicogenous fungi and the presence of mycotoxins in excess of the admitted maximal loads. Of the 9 samples examined mycotoxicologically, aflatoxin was determined in 3 samples (compound feed for cows, wheat bran and untoasted sunflower meal) and ochratoxin was determined in 5 samples (compound feed for nursing calves, toasted sunflower meal, compound feed for cows, coarse grinded wheat and corn and untoasted sunflower meal). The presence in feed of aflatoxin and ochratoxin establish the alteration of the health status of the dairy cows.*

***Key words***: *dairy cows, morbid states, feed quality, mycotoxins, organoleptic and laboratory examination*

## *Introduction*

Despite the fact dairy cow farming is no longer required to achieve yields established at the "center", some farms still can not observe the technological principles. There seem to be multiple causes, but the most frequent one being the acute lack of financing. Because of this reason they can not provide a rational, balanced feeding with safe feeds, as fresh as possible or preserved according to proper technological norms (1, 6). The lack of equipment for feed preparation and homogenization and the manual distribution of feeds are other factors contributing to an irrational feeding. It is an established fact that the dairy cows have to be fed according to their milk yield and to their genetic potential (4, 7), which can be done by individually tailoring their daily diets.

In some former communist states which have recently joined the European Union, the only measured taken in the dairy cow farms was to introduce the minimal guaranteed price for the delivered milk that meets EU quality parameters (9). Following this decision of the government, investment was done in modern milking parlours and in installation for feed preparation and automated distribution. It is known that even though the dairy cow diet is correctly formulated, it is not efficient if the feeds are not broke down in the nutrients which they contain so they can be absorbed into the blood stream (12). Digestion is done both with the assistance of the stomach enzymes and with the enzymes produced by the microorgsanisms from the forestomachs. If the feeds are contaminated by mycotoxins for long periods or if their level exceeds the admitted one, serious disturbances occur both in organs (2, 3, 8) and in the synthesis of proteins, therefore of the enzymes working for digestion. Cows' capacity to resist to the different pathogens is impaired with the decrease of specific immunoglobulin counts, which sometimes is significant (11).

Some dairy cows forms from Romania invested in modern milking parlours but didn't purchase automatic installations for forage preparation and distribution. Feed mixes are still done with the shovel and they are still distributed by the sack carried on the back. Under these conditions one can not speak of technological rearing and exploitation.

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In such a farm, which keeps the animals indoor for long periods worsening health state and poor production performance were observed. The calved cows displayed placenta retention (75-80%) and uterus-vagina secretions; the newborn calved got sick and died within 2-8 days from calving (77-100%), while the milk yield was at least 15% lower. The dead suckling calves revealed at necropsy serious haemorrhagic-dystrophic lesions of the myocardium, liver and kidney, cataral haemorrhagic enteritis with mucous denuding, thickened mucous of the urinary bladder with numerous red spots, sometimes hemorrhagic urine, dystrophic skeletal muscles with the looks of boiled muscle. All the information acquired during the visual examination and during the epizootologic inquest suggested us that the feeds might play a very important role in altering the health status.

## *Material and method*

The investigations were conducted on 15 samples of raw ingredients and finished feeds (corn silage and compound feeds). The samples were examined organoleptically, physico-chemically, mycologically and mycotoxicologically. The samples were collected in February and June. Concomitantly, blood samples were collected from 10 cows with different disorders, placenta retention mainly, for biochemical examinations; hemogram was also done for 5 of them.

The biochemical examination determined the total proteins, albumins, globulins, calcium, phosphorus and gammaglutamyltransferaze (GGT). The laboratory examinations were conducted using the usual techniques.

## *Results and discussion*

### The organoleptic examination

The organoleptic examination revealed that 4 of the 9 samples collected in February were improper. The compound feed (CF) for calves smelled rancid, the sunflower meal (toasted or not) contained a large amount of husks, the compound feed for cows was dominated by bran and sunflower husks, the corn silage contained a large amount of earn corn. Upon sampling for laboratory examination it was observed that all operations for compound feed preparation were done with shovels.

### Physical-chemical examination

Table 1 shows the results of the physical and chemical examination being analzsed the moisture, crude protein, ether extractives and crude fiber.

Table 1

Values of the physical and chemical parameters

| No. | Feed | Moisture% | CP% | EE% | CF% |
|---|---|---|---|---|---|
| 1. | Corn silage | 31.94 | 2.90 | - | |
| 2. | CF for suckling calves | 8.80 | **13.65** | 3.01 | 4.88 |
| 3. | Sunflower meal | 9.03 | 24.03 | 1.10 | 25.84 |
| 4. | Corn meal | 11.36 | 8.6 | 5.30 | 4.20 |
| 5. | CF cows | 10.00 | **18.02** | 2.51 | 6.20 |
| 6. | Wheat and barley coarse grinding | 8.69 | 10.75 | 2.36 | 4.20 |
| 7. | Ground peas | 13.07 | 22.20 | 3.58 | 6.08 |
| 8. | Wheat bran | 7.14 | 14.00 | 3.58 | 10.84 |
| 9. | Sunflower meal, not toasted | 7.18 | 35.01 | 2.20 | 20.25 |

Table 1 shows many deviations from the normal values. Thus, moisture ranged between 7.14% (wheat bran) and 31.94% (corn silage). In 7 of the 9 samples, humidity was below the normal values (normal values are 12-14% for the raw ingredients and for the finished feeds, and 40-55% for the silage). The dietary crude protein from the compound feed for cows was 18.02% higher than the normal 13-14%, and of only 13.65% for the calves. The level of ether extractives from the compound feed for cows was 4.88% and 6.20% much lower than the normal values 15-18%. These results show that the deviations observed during the organoleptic and physical-chemical examination may characterise the feeds as improper.

**The mycological examination**

The examinations conducted on the samples collected in February and June revealed the presence of different types of fungi, some of them known as very toxicogenous. The total fungi count (TFC) was evaluated according to the value imposed by the EU and by the Romanian authorities (SR 7954/2001 and Ord 249/2003), which is ≤ 5000/g feed. Table 2 show the results of the mycological examination.

Table 2

Mycological examination

| No. | Specification | Fungi genera | TFC | |
|---|---|---|---|---|
| | | | February | June |
| 1. | Corn silage | Yeasts | **47,000** | NT* |
| 2. | CF for suckling calves | *Aspergillus, Penicillium, Fusarium*, Cladosporidium, Mucor, Rhisopus | **7,000** | 5,000 |
| 3. | Sunflower meal | *Aspergillus,* Cladosporidium, yeasts, Mucor | **6,000** | **16,000** |
| 4. | Corn meal | *Aspergillus, Penicillium*, yeasts, Cladosporidium, Absidia | **6,000** | NT |
| 5. | CF cows | *Penicillium, Fusarium*, Aspergillus, yeasts, Absidia | **24,000** | **16,000** |
| 6. | Wheat and barley coarse grinding | *Aspergillus, Penicillium*, Fusarium, yeasts, Absidia | **10,000** | 5,000 |
| 7. | Ground peas | *Aspergillus*, Cladosporidium, Mucor, Rhisopus | **8,000** | 5,000 |
| 8. | Wheat bran | yeasts | **27,000** | 5,000 |
| 9. | Sunflower meal, not toasted | yeasts (February); Aspergillus, Fusarium, Penicillium, Mucor, Rhisopus (June) | **36,000** | NT |

*NT = Non-tested

During the first TFC examination, all analyzed samples exceeded the maximal count of 5000, but during the examination of June, only 2 of 6 samples exceeded this value. At 6 of 9 samples collected in February and in 7 of 9 samples collected in June, toxicogenous fungi were detected, at least one of the three genera very much involved in the veterinary pathology, respectively *Aspergillus*, *Penicillium* şi *Fusarium*.

**The mycotoxicological examination**

The samples of raw ingredients, compound feeds and corn silage were examined mycotoxicologically. Tables 3 and 4 show the results of the examination.

Table 3 shows that two of the most active mycotoxins have been identified in all samples: **aflatoxin and ochratoxin**. In 3 and 5 feeds, they had values in excess of the admitted limit, i.e. 4 ppb for aflatoxin and 5 ppb for ochratoxin. The compound feed for dairy cows contained both mycotoxins, while the compound feed for suckling calves contained ochratoxin. Both mycotoxins have been identified in the not-toasted sunflower meal and only ochratoxin was identified in the toasted sunflower meal.

Table 3

Mycotoxicological examination – February 2007

| No. | Specification | Aflatoxin ppb | Ochratoxin ppb |
|---|---|---|---|
| 1. | Silage | 0.64 | 2.05 |
| 2. | CF for suckling calves | 3.01 | **5.07** |
| 3. | Sunflower meal | 2.23 | **5.02** |
| 4. | Corn meal | 0.71 | 2.32 |
| 5. | CF cows | **4.64** | **8.76** |
| 6. | Wheat and barley coarse grinding | 3.03 | **5.91** |
| 7. | Ground peas | 0.79 | 3.69 |
| 8. | Wheat bran | **4.09** | 3.21 |
| 9. | Sunflower meal, not toasted | **4.91** | **7.76** |

In June, the samples were collected for the second examination, while the results showed lower values than in winter, with the values exceeding the upper allowed limit rarely. Table 4 shows that the CF for cows and for the nursing calves still has ochratoxin in large amounts, particularly the CF for cows.

Table 4

Mycotoxicological examination – June 2007

| No. | Specification | Aflatoxin, ppb | Ochratoxin, ppb | Citrinin (ppb) |
|---|---|---|---|---|
| 1. | CF dairy cows | 1.56 | **7.34** | 60.42 |
| 2. | CF for suckling calves | 1.63 | **4.50** | 43.79 |
| 3. | Wheat bran | 1.72 | 2.78 | 61.36 |
| 4. | Wheat and barley coarse grinding | 1.24 | 0.66 | 68.95 |
| 5. | Ground peas | 0.95 | 1.19 | 52.52 |
| 6. | Sunflower meal | **4.25** | 2.64 | 177.02 |
| | | LA≤4 ppb | LA≤5 ppb | |

Citrinine was detected in the samples collected in June, a mycotoxin produced like ochratoxin, by *Penicillium fungi*. Citrinine has structural similarities with ochratoxin A, poteting it in its adverse effects on the renal function (5).

The results of this study support the literature data (10) which show that the mixtures of ingredients increase the risk that the feed contains mycotoxins, which produce toxicological synergies, increasing the severity of the mycotoxicoses. Thus, at the first examination, in the compound feed for cows were determined both the aflatoxin and ochratoxin, while at least one mycotoxin was found in the raw materials (wheat bran, sunflower meal and wheat+barley coarse grind).

The literature (2, 3, 10) shows that all three mycotoxins are involved in altering the health status of the dairy cows. The degradation of aflatoxin $B_1$ in the rumen is low, being under 10% for the concentration ranging from 1 to 10 μg/g. **The aflatoxicosis** of cattle produces jaundice, diffuse hemorrhagic syndrome, serious hepatic disorders, ascitis and serous oedema, while in the very young calves, which are very sensitive, it appear the acute diarrhoea syndrome and depression. In the dairy cows, a few days after the intake of feeds contaminated with aflatoxin, the milk yield decreased drastically. **The ochratoxicosis** affects more seriously the kidney compared to the liver, the weight gain decreases very much, the calves display depression, oliguria and dehydration, diarrhoea syndrome and ciphosis. **Zearalenone** is involved particularly in the reproductive disorders producing abortion, placenta retention, prolonged puerperium and anestrum, chronic endometritis followed by infecundity, estral cycle deregulations, hipertony or hipotony of the uterine horns, serous, leucoreic or yellowish discharges at the lower corner of the vulva in impuber females. All these pathological aspects were present in the dairy cows in February, when the investigations started.

**The biochemical examination**

The biochemical investigations on 10 cows which displayed reproductive disorders showed the increase of the total proteins, of the albumin and globulin, supporting the literature data (2) which show that *aflatoxin produces metabolism disorders, especially hiperproteinemia* due to its concentration in the liver, determining the fatty chronic hepatitis, inducing enzymatic misbalances, and affect of the hepatic function. Table 5 and fig. 1 show the increase of the total protein (8 samples), albumin (7 samples) and globulin (all 10 samples).

Table 5

Biochemical examination

| Sample | Total protein (g/dl) | Albumin (g/dl) | Globulin (g/dl) | Calcium (mg/dl) | Phosphor (mg/dl) | GGT (U/l) |
|---|---|---|---|---|---|---|
| 1. | **9.20** | 3.21 | **5.99** | 9.20 | **8.35** | NT |
| 2. | 7.85 | 3.17 | **4.68** | 8.40 | **7.21** | **58** |
| 3. | **9.36** | **4.35** | **5.01** | 9.60 | **8.86** | 12 |
| 4. | **9.35** | **4.36** | **4.99** | NT | **8.76** | NT |
| 5. | **9.20** | **4.14** | **5.06** | 10.4 | **8.51** | **34** |
| 6. | **8.97** | **4.27** | **4.70** | NT | **8.30** | **65** |
| 7. | 8.38 | 3.50 | **4.88** | NT | **8.88** | **33** |
| 8. | **9.71** | **5.23** | **4.48** | NT | **9.12** | NT |
| 9. | **9.49** | **4.18** | **5.31** | NT | **7.15** | NT |
| 10. | **8.96** | **4.41** | **4.55** | 9.6 | **7.79** | **53** |
| Normal value | 7.6 ±0.8 | 3.4±0.5 | 4.2±0.3 | 9-10 | 6.7-5.6 | 14.9±3.6 |

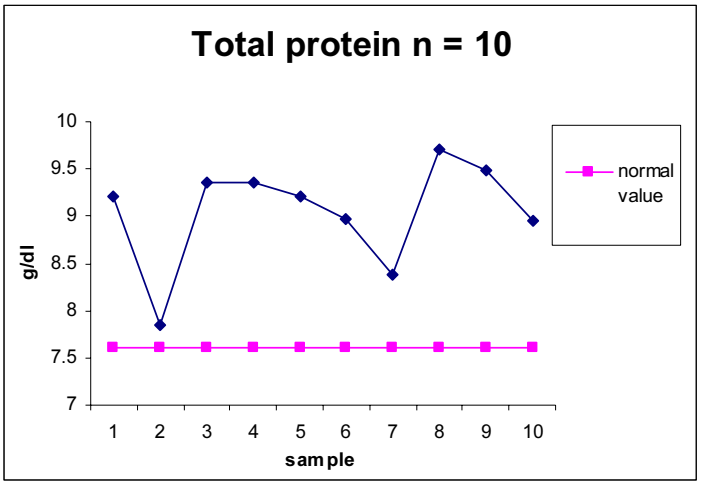


Fig. 1

The **haemogram** generally had results similar in the 5 examined samples (Table 6).

Table 6

Haemogram results

| Analyzed parameters | MU | Sample | | | | | Normal values |
|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 | |
| Leukocyte number | Thousands/mm$^3$ | 8.30 | 7.30 | 9.00 | 15.90 | 4.20 | 8.00±1.5 |
| Red cells number | Million/mm$^3$ | 5.27 | 4.94 | 5.13 | 5.40 | 5.13 | 6.5±1.4 |
| Haemoglobin (Hb) | g/dl | 2.30 | 8.80 | 8.50 | 9.70 | 8.80 | 10.2±1.0 |
| Hematocrit (Ht) | % | 22.90 | 25.00 | 23.50 | 26.80 | 24.30 | 35.0±3.0 |
| VEM | $\mu^3$ | 43.00 | 51.00 | 46.00 | 50.00 | 47.00 | 53.4±2.2 |
| HEM | pg | 15.70 | 17.80 | 16.70 | 17.90 | 17.10 | 16±2.5 |
| CHEM | g/dl | 36.20 | 35.10 | 36.30 | 36.10 | 36.10 | 29.1±2.1 |
| Nr platelets | Thousands /mm$^3$ | 544 | 301 | 124 | 201 | 452 | about 300.000 |

VEM = volume of average erythrocytes
HEM = haemoglobin of average erythrocytes
CHEM = amount of haemoglobin of average erythrocytes

Table 6 shows that the number of red cells and the haemoglobin were close to the normal values. Ht was lower, while CHEM was increased. These data were supported statistically after the hemogram results have been processed (table 7).

Table 7 shows that the number of leukocytes, Ht values, VEM and the number of platelets display very high values of the standard deviation and standard error.

Table 7

Statistical analysis of haemogram results

| Parameters | MU | Average ($\overline{X}$) | Standard deviation | Standard error of the average |
|---|---|---|---|---|
| Leukocyte number | Thousands/mm$^3$ | 8.94 | 4.301 | 1.923 |
| Red cells number | Million/mm$^3$ | 5.174 | 0.172 | 0.077 |
| Haemoglobin (Hb) | g/dl | 8.82 | 0.535 | 0.239 |
| Hematocrit (Ht) | % | 20.5 | 9.913 | 4.433 |
| VEM | $\mu^3$ | 47.4 | 3.209 | 1.435 |
| HEM | pg | 17.04 | 0.898 | 0.401 |
| CHEM | g/dl | 35.96 | 0.487 | 0.218 |
| Nr platelets | Thousands /mm$^3$ | 324.4 | 173.52 | 77.601 |

For CHEM, with almost homogenous values for the 5 samples (a standard deviation of 0.487), the average being 35.96 compared to 29.1±2.1 considered normal value. The number of platelets varied very much, from animal to animal (from 544 thousands /mm$^3$ to 124 thousands /mm$^3$).

## *Conclusions*

The complex examinations of feed samples collected in February and June revealed an improper quality of the feeds both for dairy cows and for calves.

1. The organoleptic examination revealed a rancid smell of the compound feed for calves and a large amount of sunflower husks and wheat bran in the dairy cows compound feed; the corn silage had a high level of earn corn.

2. The physico-chemical examination of the feed revealed a lower than normal moisture, a high dietary crude protein level of the compound feed for dairy cows and a low dietary crude protein level of the compound feed for calves. The ether extractives and the crude fiber were also lower than the normal values.

3. The mycological examination conducted in February showed that all analyzed samples exceeded the higher limit of TFC, while in June only 2 of 6 samples exceeded the higher limit. Most raw feeds and finished feeds had at least one of the three genera of toxicogenous fungi involved in the veterinary pathology: *Aspergillus, Penicillium* and *Fusaruim*.

4. The mycotoxicological examination revealed levels in excess of the aflatoxin in the compound feed for dairy cows, wheat bran and toasted sunflower meal; other five samples, respectively feeds for dairy cows and calves, wheat and barley coarse grinding, toasted and untoasted sunflower meal were positive for ochratoxina Both mycotoxins are involved in altering the health status of the dairy cows and of the very young calves.

5. The dairy cows with reproductive disorders displayed at biochemical examination hyperproteinemia, a characteristic feature of the aflatoxicoses.

6. Haemoglobin showed dramatic alterations of the leukocytes count. Of the hematocrit, VEM and for the thrombocyte count, the standard deviation and the standard error of the mean being very high. The mean and individual value of

CHEM was much higher compared to the normal values. These results show important alterations of the blood parameters following the administration of feeds with mycotoxic load.

*

* *

These conclusions were supported by the response of the animals after measures were taken to alleviate the mycotoxic effect on the pregnant and lactating cows and on the suckling calves during their first two months of life. Thus, the number of diseases and mortalities in calves decreased (with 7-12%) and so did the placenta retention disorders (with 18%), while the milk yield returned to normal.

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# ASSESSMENT OF BLOOD PRESSURE IN HUMAN-DOG INTERRELATION

**V. ANDRONIE*, Ioana Cristina ANDRONIE*, Ş. ZAMFIRESCU****

***Abstract***

*Humans with their own dogs, between whom a companion bond had been established, were used as an experimental group (n=5). The control group was represented by dog loving humans with a friendly, but unfamiliar dog, between whom no bond existed (n=5). The cardiovascular response of human respondents (n=17) were also evaluated during quiet reading. The neurochemical and cortisol analyses fall beyond the purpose of this paper. In individuals with their own dogs there was a significant ($p<0.0052$) percentage of decrease in the MAP, SP, and DP. The MAP of 5 out of 8 individuals decreased by more than 5% during the experiment and remained decreased by more than 5% in 3 individuals, until the end of the experiment. In individuals with unfamiliar dogs there was a non-significant percentage increase in the MAP and DP, and a significant ($p=0.00189$) percentage of decrease in SP. The MAP for 6 out 8 individuals decreased by more than 5% and remained decreased by more than 5% in 3 individuals, until the end of the experiment.*

***Key words***: *dogs, blood pressure, interrelation human-dog*

## *Introduction*

Various studies have shown positive short and long term physiological and psychological benefits to humans when they are in the presence of a dog, or when they interact with a dog. The physiological effects include decreases in blood pressure and pulse rates and they can be due to a moderation of the neuroendocrine cardiovascular stress response. The purpose of this study is to investigate the cardiovascular effects of positive human-dog interrelation for both species. To our knowledge, this is the first time that blood pressure and pulse rate change were evaluated for both species during same positive interrelation.

## *Material and methods*

Humans with their own dogs, with which a companion bond had been established, were used as an experimental group (n=5). The control group was represented by dog loving humans with a friendly, but unfamiliar dog, with which no bond existed (n=5). The respondents (humans and dogs) were placed in a comfortable experimental settlement. Non-invasive blood pressure monitoring was done for both species by means of a Dinamap. An average of 5 measurements were done before interrelation and a venous blood sample was collected for neurochemical and cortisol analysis. A decrease in main arterial pressure (MAP) was used as an indicator of positive interrelation and further blood samples were collected over a period of 25 minutes, i.e. measurements were taken at every 5 to 30 minutes. The cardiovascular response of human respondents (n=17) were also evaluated during quiet reading. The neurochemical and cortisol analyses fall beyond the purpose of this paper.

An analysis of variance was performed on each of the variables PMAP_V, PSP_V, PDP_V (Percentage change in main arterial pressure, systolic pressure and diastolic pressure respectively). A factorial design was used. The factors used to explain the variance in each of the variables were the person (or dog), the activity (interrelation with dog or reading), whether the interrelation happened with a known dog/person or not and the time elapsed since the beginning of the

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experiment. Since the time variable is continuous, an analysis of covariance was done with time as the covariate. A nested design was used to make provisions for the fact that not all persons interracted with a known as well as with an unknown dog. The person/dog was therefore nested within the factors of the known and unknown dog. Last squares estimates of each factor's means of the levels were obtained. The significance of each of these means was tested and the means of the different levels of each factor were compared (using t-tests in both situations). It was, for instance, found that MAP_V is significantly different when interrelating and reading.

### *Results and discussions*

In case of quiet reading blood pressure changes in humans perform accordingly to the data from table 1.

Table 1

Quiet reading: blood pressure changes in humans

| BLOOD PRESSURE | LINEAR RELATIONSHIP | EXPECTED CHANGE PER MINUTE* | EXPECTED CHANGE IN 25 MINUTES* |
|---|---|---|---|
| MAP | Yes (p=0.00177) | 0.269 % | 6.73 % |
| SP | Yes (p=0.0021) | 0.284 % | 7.10 % |
| DP | Yes (p=00057) | 0.373 % | 9.33 % |

MAP = Main arterial pressure, SP = Systolic pressure, DP = Diastolic pressure
*If all other variables (individual and type of activity) remain constant.

The MAP of 11 out of 17 individuals decreased by more than 5 % during 10 minutes of quiet reading and remained decreased until the end of the experiment.

Quiet reading versus positive human-dog interrelation see in bar diagram number 1.

In case of positive human-dog interrelation the changes of blood pressure in humans is presented in table 2.

Table 2

Positive human-dog interrelation: blood pressure changes in humans

| BLOOD PRESSURE | LINEAR RELATIONSHIP | EXPECTED CHANGE PER MINUTE* | EXPECTED CHANGE IN 25 MINUTES* |
|---|---|---|---|
| MAP | No | - | - |
| SP | Yes (p=0.0037) | 0.276 % | 6.90 % |
| DP | Yes (p=0.0370) | 0.278 % | 6.95 % |

*If all other variables (individual and type of activity) remain constant.

In individuals with their own dogs there was a significant ($p<0.0052$) percentage of decrease in the MAP, SP, and DP. The MAP of 5 out of 8 individuals decreased by more than 5 % during the experiment and remained decreased by more than 5 % in 3 individuals, until the end of the experiment.

In individuals with unfamiliar dogs there was a non-significant percentage increase in the MAP and DP, and a significant (p=0.00189) percentage of decrease in SP. The MAP for 6 out 8 individuals decreased by more than 5 % and remained decreased by more than 5 % in 3 individuals, until the end of the experiment.

Individuals with their own dogs versus individuals with unfamiliar dogs can be seen in bar diagram 2.

In case of positive human-dog interrelation the changes of blood pressure in dogs is presented in table 3.

Table 3

Positive human-dog interrelation: blood pressure changes in dogs

| BLOOD PRESSURE | LINEAR RELATIONSHIP | EXPECTED CHANGE PER MINUTE* | EXPECTED CHANGE IN 25 MINUTES* |
|---|---|---|---|
| MAP | No | - | - |
| SP | Yes (p=0.0042) | 0.723 5 | 18.10 % |
| DP | No | - | - |

*If all other variables (individual and type of activity) remain constant.

In case of dogs with owners there is non-significant percentage increase in MAP and SP and a significant percentage increase in DP (p=0.0384). However, the MAP of 6 out 8 dogs decreased by more than 5 % and remained decreased in 1 individual until the end of the experiment. In case of dogs with unfamiliar individuals there is a non-significant increase in all three pressures. However, the MAP of 6 out of 8 dogs decreased by more than 5 % and remained decreased in 3 individuals until the end of the experiment. Dogs with familiar individuals versus dogs with unfamiliar individuals see bar diagram 3.

Quiet reading rendered the best average decrease in all blood pressures, since the humans were relaxed, on their own and not influenced by the stress of handling a dog. Many individuals found it difficult to keep the dog sitting or lying next to him/her.

Less favourable results in humans and dogs during human-dog interrelation can be attributed to:

– stress throughout the experiment due to taking of blood samples, unfamiliarity of circumstances, controlling the dog, etc.;

– stress at the onset of the experiment due to unfamiliarity of the circumstances, which caused an initial increase in blood pressure followed by a decrease;

– a possible greeting response in human when the blood pressure increased at the onset of petting;

– excitement at the onset of the experiment. This effect was better emphasized in familiar dogs, since they probably anticipated playing/working with the owner;

– a secondary increase in the blood pressure at the end of the experiment due to discomfort (high cuff pressure).

## *Conclusions*

1. Blood pressure changes in both humans and dogs during the same positive interrelation were evaluated for the first time.

2. Although the average blood pressure changes were not remarkable and often non-significant.

3. The blood pressure decreased by more than 5% in 62.5% in humans with familiar dogs and by 75% in humans with unfamiliar dogs, dogs with owners and dogs interracting with unfamiliar individuals.

4. This supports the belief that pet ownership *per se* is not necessary for an individual to receive physiological benefits from the presence of a pet.

5. From this experiment it seems that dogs experiment similar beneficial physiological effects as humans.

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# ASPECTS OF PROTOZOA INFECTION IN DOGS AND CATS

**Sofia COMAN*, B. BĂCESCU*, Andreea NEAGOE[*]**

***Abstract***

*Investigations were carried out during February 2006-January 2008 on a total of 153 dogs and 23 cats of different breeds and ages. The animals had diarrhoeic syndrome and were examined by coproscopy with Willis and Mc. Master methods. The incidence of infestation with Isospora sp. in puppies was 49.6%, of which 88.1% was recorded in puppies aged 1-3 months and 11.8% in puppies aged 4-6 months; in cats the frequency of infestation was 30.4% of which 71.4% in kittens aged 1-3 months and 28.5% in kittens aged 4-6 months. The incidence of infestation with Giardia in dogs was 15.6% of which 83.3% in puppies and 16.6% in full grown animals. In the young cats the incidence of infestation with Giardia was 26.08%. The efficacy of Ultrasol in Isospora sp. infestation was 96 %, while the efficacy of Flagyl in giardiosis treatment was 85%.*

***Key words***: *dog, cat, Giardia, Isospora sp.*

## *Introduction*

The diarrhoeic syndrome has quite a high frequency in the pet carnivores, manly in the young pets aged few months. The classical therapy with antibiotics associated with rehydration, general stimulants and a specific diet is not successful in all patients because of the polyfactorial aetiology. The determination of the pathogens involved in the aetiology of enteritis requires specific laboratory tests.

Psapini R. et al. (7) mentioned a 15.7% prevalence of Giardia infestation in kittens up to the age of one. The authors concluded that cats are an important source for environmental pollution with Giardia cysts. Capelli et al. (2) reported a 17.3% prevalence of Giardia infestation in puppies.

Mitchell S.M. et al. (5) proved that the infestation with *Isospora sp*. in puppies and adult dogs with immunodepression can start the diarrhoeic syndrome. The authors conducted experiments with *Isospora canis* of Beagle female puppies aged 6-8 weeks. The prepatent period was of 9-11 days and the patent period of 7-18 days. The diarrhoeic syndrome started 2-3 days before the presence of oocysts in faeces in all infected female puppies. No bacteria or viruses were isolated, the diarrhoea being caused by *Isospora canis*. Heusinger A. (4) conducted a study on the incidence of infestation with *Giardia lamblia* (duodenalis), on its cycle of evolution and on the diagnostic methods in dogs and humans. Diagnosis was done by ELISA on coprology samples and showed 21% infestation in puppies.

Papazahariadou M. et al. (6) investigated the incidence of parasite infestation in dogs on 281 coprologic samples and observed a prevalence of 4.3% for *Giardia sp.* and 3.9% for *Isospora sp.* mainly in the young animals. Diaz et al. (3) observed a 21.9% incidence of Giardia infestation in puppies aged few months and 10.8% in adult dogs. Barr S.C. et al. (1) showed experimentally that the administration of Fenbendazol in dose of 50 mg/kg body mass controls the infestation with Nematoda species, also being efficient in giardiosis control in dogs.

## *Material and methods*

The investigations were conducted during February 2006 - January 2008 on a total of 153 dogs and 23 cats of different breeds and age. The animals displayed weakness, capricious appetite, inappetence, dehydration and persisting diarrhoea even after the antibiotics therapy.

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The parasite infestations with protozoa and the level of infestation were evaluated with the flotation methods of Willis and McMaster for helmints determination.

The infestation with Giardia species was determined by means of the direct examination of the fresh coprologic samples treated with Lugol solution to reveal the cysts or the trophosoits.

The infestations with Isospora were treated by intramuscular injections with Ultrasol (sulfamethoxydiazin) in doses of 20-30 mg/kg body mass for 3 consecutive days.

The infestations with Giardia were treated orally with Flagyl (Tinidazol) in doses of 30-40 mg/kg body mass for 6 days.

### *Results and discussion*

The coproscopic examinations conducted using flotation methods showed the presence of infestations with *Isospora sp., Giardia canis* and *Giardia cati* in the examined puppies and kittens.

The results of the laboratory examinations revealed a high incidence of sporozoa infestation both in the puppies aged a few months (49.6%) and in the kittens (30.4%) (Table 1).

Table 1

Incidence of *Isospora sp.* infestation

| Species | Nr. of animals | Positive identification | Percent | Young animals | | Adults | |
|---|---|---|---|---|---|---|---|
| | | | | Nr. | % | Nr. | % |
| Dogs | 153 | 76 | 49.6 | 76 | 100 | - | - |
| Cats | 23 | 7 | 30.4 | 7 | 100 | - | - |
| total | 176 | 83 | 47.1 | 83 | 100 | - | - |

Tables 1 and 2 show that in dogs, the *Isospora sp.* infestations occurred only in puppies, 88.1% being recorded in puppies aged 1-3 months and 11.8% in puppies aged 4-6 months. In the examined cats the frequency of infestation was 30.4 % of which 71.4 % in kittens aged 1-3 months and 28.5 % in kittens aged 4-6 months.

Table 2

Incidence of *Isospora sp.* infestation by age categories

| Species | Positive identification | Age 1-3 months | | Age 4-6 months | |
|---|---|---|---|---|---|
| | | Nr. | % | Nr. | % |
| Dogs | 76 | 67 | 88.1 | 9 | 11.8 |
| Cats | 7 | 5 | 71.4 | 2 | 28.5 |
| total | 83 | 72 | 86.5 | 11 | 13.2 |

Tables 3 and 4 show the incidence of infestation with *Giardia genus flagellate*. Table 3 shows a 15.6% incidence of *Giardia canis* infestation, of which 83.3 % in puppies and 16.6 % in full grown animals. The coproscopic examination conducted on 23 cats showed Giardia infestation in 6 animals (26.08 %) all of them kittens.

Table 3

Incidence of *Giardia sp.* infestation

| Species | Nr. of animals | Positive identification | Percent | Young animals | | Adults | |
|---|---|---|---|---|---|---|---|
| | | | | Nr. | % | | |
| Dogs | 153 | 24 | 15.6 | 20 | 83.3 | 4 | 16.6 |
| Cats | 23 | 6 | 26.08 | 6 | 100 | - | - |
| total | 176 | 30 | 17.04 | 26 | 86.6 | 4 | 13.3 |

Since 86.6% of the young carnivore pets submitted to coproscopic examination showed infestation with Giardia, the pet owners must be cautioned to apply adequate sanitary measures to prevent contamination with these flagellate species.

Table 4 shows the incidence of *Giardia sp.* infestation by age categories. The table shows that 62.5% of the animals infested with *Giardia canis* were puppies aged 1-3 months, 33.3% were puppies aged 4-7 months and 4.1% were puppies aged 8-10 months. In the kittens, 66.6% of the positive cases were recorded in the age group 1-3 months and 16.6% of the positive cases were recorded in each of the age groups 4-7 months and 8-10 months.

Table 4

Incidence of *Giardia sp.* infestation by age categories

| Species | Positive identification | 1-3 months | | 4–6 months | | 7-10 months | |
|---|---|---|---|---|---|---|---|
| | | Nr. | % | | | Nr. | % |
| Dogs | 24 | 15 | 62.5 | 7 | 33.3 | 1 | 4.1 |
| Cats | 6 | 4 | 66.6 | 1 | 16.6 | 1 | 16.6 |
| total | 30 | 19 | 63.3 | 9 | 30.0 | 2 | 6.6 |

The incidence of protozoa infestation followed by the diarrhoeic syndrome in the carnivore pets was 65.3% in dogs, of which 96.0% in puppies and 4.0% in adult dogs, and 56.5% in cats, of which 92.3% in kittens and 7.69% in adult cats (Table 5).

Table 5

Incidence of protozoa infestation

| Species | Nr. of animals | Positive identification | Percent | Young animals | | Adults | |
|---|---|---|---|---|---|---|---|
| | | | | Nr. | % | | |
| Dogs | 153 | 100 | 65.3 | 96 | 96.0 | 4 | 4.0 |
| Cats | 23 | 13 | 56.5 | 12 | 92.3 | 1 | 7.69 |
| total | 176 | 113 | 64.2 | 108 | 95.5 | 5 | 4.42 |

Table 6 shows the incidence of protozoa infestation by age categories in puppies and kittens. The incidence of protozoa infestation was 65.3%, of which 82.0% in puppies aged 1-3 months, 17.0% in puppies aged 4-7 months and 1.0% in puppies aged 8-10 months. The incidence of protozoa infestation was 56.5% in kittens, of which 69.2% in kittens aged 1-3 months, 23.07% in kittens aged 4-7 months and 7.6% in kittens aged 8-10 months.

Table 6

Incidence of protozoa infestation by age categories

| Species | Positive identification | 1-3 months | | 4-7 months | | 8-10 months | |
|---|---|---|---|---|---|---|---|
| | | Nr. | % | Nr. | % | Nr. | % |
| Dogs | 100 | 82 | 82.0 | 17 | 17.0 | 1 | 1.0 |
| Cats | 13 | 9 | 69.2 | 3 | 23.07 | 1 | 7.6 |
| total | 113 | 91 | 80.5 | 20 | 17.6 | 2 | 1.7 |

The aetiology of the diarrhoeic syndrome in the puppies and kittens also involved parasitic agents, namely sporozoa and flagellate species. 49.6% of the puppies with diarrhoeic syndrome and 30.4% of the kittens with diarrhoeic syndrome were infested with *Isospora canis (Cystoisospora canis)*. Giardia infestations were detected in 15.6% of the puppies with diarrhoeic syndrome and in 26.08% of the kittens with diarrhoeic syndrome. Because the control of the diarrhoeic syndrome in puppies and kittens is done by antibiotics therapy associated to general stimulants, rehydration etc., we recommend laboratory investigations to reveal the parasitic infestations followed by the specific therapy with coccidiostatics and drugs for flagellate infections.

Table 7 shows the products used to control protozoa infections in pet carnivores and their efficacy after 10 days of treatment.

Table 7

Efficacy of protozoa infestation treatment

| Infestation | Product | Dose mg/kg body mass | Efficacy |
|---|---|---|---|
| *Isospora sp* | Ultrasol | 20-30 IM. 3 days | 96% |
| *Giardia sp* | Flagyl | 30-40 per os 6 days | 85% |

Ultrasol (sulfamethoxydiazin) administered in doses of 20-30 mg/kg body mass for 3 days displayed an efficacy of 96% in the *Isospora sp.* in puppies and kittens.

The oral treatment with Flagyl in doses of 30-40 mg/kg body mass for 6 consecutive days displayed an efficacy of 85% on the Giardia species.

## *Conclusions*

1. The incidence of *Isospora sp.* infestations in puppies was 49.6%, of which 88.1% were recorded in puppies aged 1-3 months and 11.8% in puppies aged 4-6 months.

2. The incidence of *Isospora sp.* infestations in kittens was 30.4 %, of which 71.4 % in kittens aged 1-3 months and 28.5 % in kittens aged 4-6 months.

3. The prevalence of *Giardia canis* infestations was 15.6%, of which 83.3% in puppies and 16.6% in adult dogs.

4. The prevalence of Giardia infestation in kittens was 26.08%.

5. The efficacy of Ultrasol in controlling *Isospora sp.* infestations was 96 %.

6. The efficacy of Flagyl in controlling *Giardia sp*. infestations was 85 %.

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# REMARKS ON CEPHALIC CLINICAL MANIFESTATIONS AS A RESULT OF MITRAL INSUFFICIENCY IN DOGS

**A. SALLAY*, Elena Luiza BĂDIC[*]**

***Abstract***

*Over February 2007-March 2008, 34 dogs of various breeds, aged between 10 and 14, were examined and studied. They had been diagnosed with mitral insufficiency. The aim of this paper is to reveal the correlation between the cephalic clinical manifestations (xerostomia, pharyngeal constriction, lingual changes, epiphora, the hyperaemia of the conjunctival mucous membrane and ceruminous hypersecretion with auricular hyperkeratosis) and the mitral insufficiency in dogs. Xerostomia, pharyngeal constriction, lingual changes and epiphora were to be found in all the dogs, the hyperaemia of the conjunctival mucous membrane and the ceruminous hypersecretion with auricular hyperkeratosis were found in five dogs only. The x-ray photographs revealed the heart hypertrophy in all subjects, their heart exceeding the size of three intercostal spaces. Following the laboratory tests no specific bacterial or mycotic bacterial pathogenic flora was revealed. The clinical expression of the cephalic manifestations simultaneously with the heart disease and the radiological image and the negative results of the bacteriological or mycological examinations confirm the interdependence between facial manifestations and heart diseases.*

***Key words*****:** *mitral insufficiency, xerostomia, epiphora, auricular hyperkeratosis, ceruminous hypersecretion, pharyngeal constriction*

## *Introduction*

Theoretical and practical knowledge is useful in getting information about the health or illness of the patient, therefore the diagnosis. And this is so as both the prognosis and the rational treatment plan entirely depend on the diagnosis. The accurate determination of the disease is based on the interpretation of the results of the general clinical examination and of the various organs, implicitly the facial one, the facies offering many clues to the diagnosis.

In most diseases, the facies expresses the condition of the animal due to the connections between the organs and the innervation of the facial region, which makes possible the reflection of numerous sensations at this level, the final result being the face expression [8]. Due to the complexity and variability of the physical and chemical, neuro-vegetative or endocrine reactions, depending on the physiological and/or pathological processes, the facies can express the condition of the animal. The facial expression is rendered either under the form of a physiognomy lacking expression, of an immobile ”mask” or under the form of real contraction (mimics), accompanied by the contraction of the various muscles, blinking movements of the eyeballs, etc. [10]

Veterinary medicine describes the nervous, sad, immobile, mobile, influenza, mitral, wolf-like, hyper-thyroidian and hemiplegic facies as being quite relevant for the diagnosis [11].

The paper wants to go thoroughly into the description of the facial expression accompanying various extra-cephalic diseases, as the information currently available in the Romanian veterinary literature is presented briefly or even telegraphically.

## *Material and methods*

Over February 2007-March 2008, 34 different breeds of dogs, age between 2 and 14, were examined and studied.

With the help of the methods of examination, the cephalic and systemic clinical manifestations were observed, analyzed and monitored. They used the

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main examination procedures (inspection, palpation, percussion, listening, and thermometry) and the complementary ones (radiography).

By using sterile instruments, samples for bacteriological and mycological tests were taken. The samples representing pathological secretions, crusts or hairs were inoculated on culture media (nourishing bullion or horse serum, simple or blood gel, violet lactose agar and Chapmann medium for bacteriological examinations and Czapeck or Sabouraud media for mycological examinations). The media were incubated at 37° C for 24 to 48 hours for bacteriological examinations, for 3 to 5 days at 25° C for mycological examinations. The culture examinations characterized the types of colonies obtained on the culture media. Colour smears were made by means of Gram's Method and methylene blue for fungi.

### *Results and discussions*

Thirty-four dogs were diagnosed with mitral fibrosis, the clinical picture being outlined by the seriousness of the pathological process. Following the anamnetic investigation and the clinical examination, extra-cephalic manifestations materialized in apathy, whimsical appetite, intolerance to effort, night-time cough, pulmonary oedema, vertigo and tachypnea. By indirectly listening, a double endo-cardiac onomatopoeic breath was detected, translated as "fff-dup". With the help of radiograms the cardiac area was established, which was actually enlarged, in 32 subjects, (figs. 1 and 2). Cephalic manifestations consisted of dry mouth, pharyngeal constriction (the sensation of a lump in the throat), soft or dry coated tongue, epiphora, hyperaemia of the conjunctival mucous membrane and ceruminous hypersecretion accompanied by auricular hyperkeratosis [8].

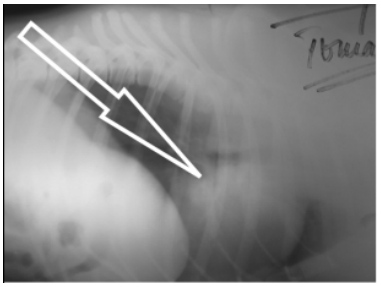

Fig. 1. Cardiac hypertrophy in mitral insufficiency
in dogs (French bulldog, 8 years old)

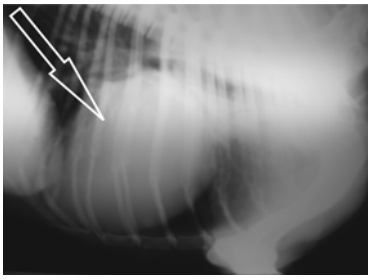

Fig. 2. Cardiac hypertrophy and pulmonary fibrosis
in mitral insufficiency in dogs (Tackle, 10 years old)

The apathy and the whimsical appetite were correlated with the help of xerostomia, pharyngeal constriction and dry or soft tongue in 20 dogs, while the intolerance to effort, tachypnea or the pulmonary oedema were cephalically represented by the coated tongue, the hyperaemia of the conjunctival mucous membrane and epiphora in 14 dogs. In most cases, the dry mouth and the sensation of a lump in the throat (pharyngeal constriction) were reported in the compensated phase of the mitral insufficiency [9]. These manifestations would account for the cephalic mechanisms and vegetative neuro-reflexes accompanying the mitral insufficiency [2, 3]. The tongue and peri-ophthalmic congestion accompanied the decompensated phase (increase in the time necessary for emptying the left ventricle and the maximum pressure) of the heart disease and were consecutive to the pressure and the lung venous stasis [5, 10].

The ceruminous hypersecretion accompanied by the auricular hyperkeratosis was reported in four dogs diagnosed with decompensated mitral insufficiency. Before the emergence of the heart insufficiency, according to anamnetic data, the subjects did not show pathology with auricular expression (otitis, allergic reaction, food poisoning dermatosis, dermatitis under the form of eczema, nutritional-metabolic or endocrine disorders). The results of the bacteriological and mycological tests did not reveal auricular pathogenic microbial flora. As a rule the development of the pathogenic microbial flora at an auricular level (staphylococcus, streptococcus, and pseudomonas) is favoured and maintained by the alkaline pH of the ceruminous secretion [6]. In the cases under consideration, the pH of the ceruminous secretion was acid (5.0-6.8), the possibility of the development of the pathogenic microbial flora being quite low. The explanation of maintaining the acid pH at an auricular level would be due, on the one hand, to the presence of the cerumen and, on the other hand, to the lower speed of the venous blood circulation and the hypoxia generated valvular insufficiency at this level [1]. The auricular hyperkeratosis was more evident in dogs that showed stasis and pulmonary oedema, a phase in which the metabolic acidosis is much more intense. The thickening of the auricular horn-like layer could be due to the ceruminous hypersecretion.

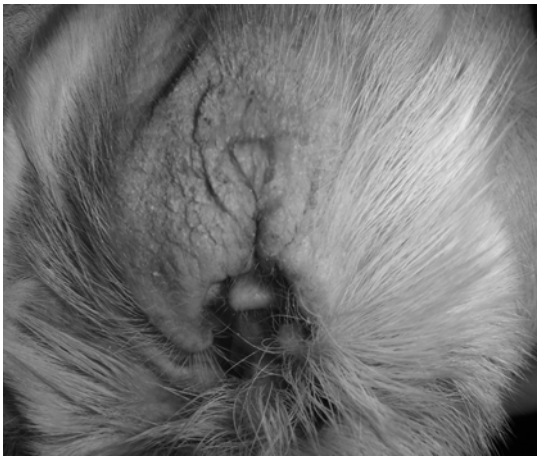

Fig. 3. Ceruminous hypersecretion accompaniedby auricular hyperkeratosis in mitral insufficiency in dogs (mongrel, 18 kg, 12 years old)

The clinical cephalic evolving dynamics was accounted for by blood disorders and pulmonary complications (stasis and pulmonary oedema) [11]. In this respect, we think that the cephalic manifestations were expressed depending on the cardiovascular adapting capacity and the blood redistribution (opening of the arteriovenous shunting to reduce resistance or peripheral blood storage in organs, which limits the mechanical working condition of the heart).

## *Conclusions*

1. The cephalic manifestations in the mitral insufficiency are represented by xerostomia, soft or dry coated tongue, epiphora, hyperaemia of the conjunctival mucous membrane, ceruminous hypersecretion accompanied by the hyperkeratosis of the auricular conchea and retromandibular ganglionic hypertrophy.

2. Xerostomia was reported in the compensated phase of the mitral insufficiency. The blood tongue and peri-ophthalmic accumulation accompanied the decompensated phase of the heart disease.

3. The auricular hyperkeratosis is evident in dogs suffering from pulmonary oedema.

4. The retromandibular ganglionic hypertrophy accompanies cough, dyspnoea or the faulty lymphatic draining. Regional adenopathy is more evident during crises of night-time cough.

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# FINDINGS IN CEPHALIC CLINICAL MANIFESTATIONS CAUSED BY SECONDARY ORGANOPATHIES RESULTING FROM CANINE BABESIOSIS

**A. SALLAY*, B. BĂCESCU*, N. BERCARU[*], R. CONDRUȚ[**]**

***Abstract***

*This paper aimed at describing the cephalic clinical manifestations brought about by the secondary organopathies resulting from babesiosis in 42 dogs that were examined and studied in the Medical Pathology Clinic of the Faculty of Veterinary Medicine, Spiru Haret University and the Tiovet Centre for Diagnosis and Treatment in Bucharest. The dogs that were diagnosed with babesiosis had an indifferent facies, photophobia with xerophthalmia and slower palpebral reflexes, the conjunctival and gingival mucous membrane being quite pale in 27 dogs (64.28 %), sub-icteric in 10 dogs (23.8 %) and icteric in 15 dogs (11.9 %). We noticed the thickened tongue with the molar tooth print in 23 dogs (54.76 %), with white-greyish accumulations of matter in 8 cases (19.04 %) and xerostomia in 19 subjects (45.23 %). The kidney and liver functional changes were biochemically confirmed by determining uraemia (between 40 and 50 mg/dl in 15 cases, between 51 and 100 mg/dl in 24 cases, over 100 mg/dl in 3 cases) and the alanine aminotransferase (between 60 and 85 UI/l in 2 cases, between 90 and 100 UI/l in 28 cases, over 110 mg/dl in 12 cases). The main urine changes referred to haemoglobinuria (between 0.2 and 1 mg/dl in 24 cases, over 1 mg/dl in 18 cases). The biochemical establishment of babesiosis was based on the determination of the haemoglobinaemia (under 5 mg/dl in 3 cases, between 5 and 6 mg/dl in 24 cases, between 6 and 7 mg/dl in 5 cases; reference values: 8-12 g/dl). The disease was confirmed by revealing the pear-shaped sporozoites in the red cells. Peripheral blood smears were done by using the May-Grünwald-Giemsa staining method. The disease was confirmed by the presence of Babesia canis.*

***Key words***: *babesiosis, ammonia halitosis, anaemia, jaundice, xerostomia*

## *Introduction*

Babesiosis was found all over the world and in 1984 Prof. Nicolae Dulceanu diagnosed it in Romania too. The spread of the disease depends on the presence of biotopes with ticks. The number of ailments reaches a peak in the interval April-October, but, as an exception, cases were also registered in the cold season. The disease appears in common dogs and improved breed dogs as well, with the secondary organopathies caused by paraclinical changes subsequent to the infestation with *Babesia canis* being amplified by immunosuppressive factors or intercurrent diseases of the infested animals [4].

After contaminating the red blood cell, *Babesia canis* sporozoites trigger the process of erythrocytholisis responsible for the appearance of haemolytic anaemia; haemoglobinuria, proteinuria, cylindruria and jaundice are the first signs of the animal falling ill [6]. The body reacts by rising temperature (39.8º-40.5ºC) due to the lipopolysaccharides released from the membranes of the sporozoites that affect the hypothalamic nervous centres. Higher body temperature is accompanied by cardio-circulatory, digestive and excretory changes, as well as by metabolic imbalances [5, 10]. The main degenerative histological changes were found in the kidneys, with degenerescence of the proximal contorted tubes and necrotic processes accompanied by the detachment of renal epithelial cells from the basement membrane. Acute tubular necrosis is probably the result of the hypoxic renal lesion caused by haemolytic anaemia and systemic hypotension. These ultrastructural renal features could explain the increase in urea and serum creatinine [8, 11].

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Kidney malfunction, frequently encountered in infections with *Babesia canis*, must be identified in due time and diagnosed correctly for the adequate prophylactic and therapeutic measures to be taken.

## *Materials and methods*

Personal research consisted of the clinical and paraclinical study on case records of the Medical Pathology Clinic of the *Spiru Haret* Faculty of Veterinary Medicine and of the Tiovet Diagnosis and Treatment Center.

Between May 2007 and February 2008, we examined and studied 42 dogs of various breeds, age between 2 and 14: 32 subjects were studied in the clinic (18 male dogs and 14 female dogs), whereas the rest of 10 (6 males and 4 females) were examined at the Tiovet Diagnosis and Treatment Center.

Using the examination methods described hereinafter, the cephalic and systemic clinical manifestations were observed, analyzed and monitored. Both the main examination methods (inspection, palpation, percussion, auscultation and thermometry) and complementary methods (blood smear staining, biochemical testing of blood and urine) were applied.

Blood samples were taken from each dog in several stages, which were used to prepare the smear and for determining and interpreting the values of the following biochemical blood parameters: ureic nitrogen, creatinine, haemoglobine, alanin aminotransferase, aspartate aminotransferase and total bilirubin. The Revlovet Plus device was used for the biochemical blood testing, and the smears were prepared by using the May-Grünwald-Giemsa method.

Urine analysis consisted of the physical (quantity, colour, smell, specific weight, and viscosity), chemical (albuminuria, haemoglobinuria, glycosuria, ketonuria, bilirubinuria, nitrite content, urobilinogen and pH) and microscopic examination (examination of the urine sediment). The physical examination was both visual and olfactory, whereas the chemical test was performed with the Pocket Chem Arkay UA-PU 4210 automatic analyzer for the determination of urinary concentrations.

## *Results and discussion*

The dogs were diagnosed between May 2007 and February 2008, in 29 cases (69.04%), the infection with *Babesia canis* being caused by failure to perform external prophylactic disinfesting of the dogs and by massive tick infestation following strolls on the Bucharest outskirts in 13 cases (30.96%).

The onset of the symptomatology was sudden, and the rapid aggravation of the dog's condition required in most cases a medical consultation in 48 hours since the owner's noticing the onset of the status of prostration.

Of the 42 subjects, 38 (90.47%) presented at the first clinical examination hyperthermia (39.9º-40.2ºC), the rest of 4 subjects (9.52%) having subfebrile temperature (39.6º-39.8ºC). Body temperature was monitored all through the period of investigation, with the day-time oscillations of body temperature presenting the specific see-saw pattern.

The anamnetic investigation and clinical examination revealed that all the subjects, irrespective of their corporal thermal condition, were in a state of prostration, with lack of appetite, indifferent expression, exaggerated calm, photophobia with xerophthalmia and slower palpebral reflexes; pallor of conjunctival and gum

mucous membranes was found in 27 dogs (64.28%), sub-icteric colour was found in 10 dogs (23.80%) and icteric colour in 15 dogs (11.90%).

The olfactory examination of halitosis in 34 subjects (80.95%) revealed the ammonia smell specific to uraemic intoxication. Accompanying cephalic manifestations were noticed in 29 cases (69.04%), the ammonia halitosis being perceived both in icteric dogs and in those with pale mucous membranes.

Thickened tongue with molar tooth print was noticeable in 23 dogs (54.76%), with white-greyish coating found in 8 cases (19.04%); xerostomia was found in 19 subjects (45.23%). The dry, chapped and discoloured nose was noticed in 12 subjects (28.57%).

Previous to performing the biochemical blood test, urine samples were collected, as the results of the chemical analysis of urine give clues to the configuration of the blood profile. Urine was collected by urethral catheterism, and only in few cases by spontaneous miction; the second is a disadvantageous variant because of urethral, vaginal or prepuce secretions potentially contaminating the urine. The values obtained following the biochemical urinary analysis were monitored in table 1.

Urine with macroscopic turbidity was centrifuged (500 rot./min.) and the sediment thus obtained was examined under the microscope. The microscopic examination tracked and established the structure of the organized sediment represented by epithelial cells, leukocytes, red cells and urinary cylinders in 29 subjects (69.04%). Unorganized sediment was found only in 4 dogs, consisting of calcium oxalates in 3 cases (7.14%) and ammonia-magnesian phosphate in one case (2.38%).

Table 1

Values of the parameters investigated by urine biochemistry

| **Investigated parameters** | **Reference values** | **Dogs with icteric mucous membranes** | **Dogs with pale mucous membranes** |
|---|---|---|---|
| Glycosuria | negative | negative in 15 dogs | negative in 27 dogs |
| Albuminuria | ≤ 30 mg/dl | negative in 8 dogs<br>above 100 mg/dl in 7 dogs | negative la 5 dogs<br>below 100 mg/dl in 22 dogs |
| Urobilinogen | negative | between 2 and 4 mg/dl in 5 dogs;<br>above 4 mg/dl in 10 dogs | negative in 27 dogs |
| Bilirubinuria | negative | between 1 and 3 mg/dl in 5 dogs;<br>above mg/dl la 10 dogs | negative in 20 dogs;<br>below 1 mg/dl in 7 dogs |
| Haemoglobinuria | negative | below 1 mg/dl in 3 dogs;<br>above 1 mg/dl in 12 dogs | below 1 mg/dl in 4 dogs;<br>above 1 mg/dl in 23 dogs |
| Ketonuria | negative | negative in 15 dogs | negative in dogs |
| Density | ≈ 1.030 | normal limits in 15 dogs | normal limits in 27 dogs |
| pH | 5,5 – 6,5 | normal limits in 15 dogs | normal limits in 27 dogs |
| Nitrites | negative | negative in 15 dogs | negative in 27 dogs |
| Leukocyturia | ≤ 75 leu/µl | 75 leu/µl in 9 dogs | 75 leu/µl in 22 dogs |

The results obtained led to the conclusion that the main urinary biochemical changes involve haemoglobinuria (42 cases – 100%), albuminuria (29 cases – 69.04%), bilirubinuria (22 cases – 52.38%) and urobilinogen (15 cases – 35.71%).

The biochemical analysis of the blood was used to confirm the diagnosis. In the first stage, the haemoglobine, ureic nitrogen and alanin aminotransferase (ALT) blood content was determined. The parameters were selected according to the

clinical manifestations, colour changes perceived in cephalic membranes, the presence of ammonia halitosis and urinary biochemical results; the values obtained are presented in tables 2, 3 and 4.

The values obtained confirmed for all the subjects the condition of anaemia, retention of ureic nitrogen, and enzymatic hepatic changes. Creatinine, aspartate aminotransferase (AST GOT) and total bilirubin were determined only in some of the dogs, the values obtained confirming the presence of kidney and hepatic failure.

Table 2

Synopsis of ureic nitrogen values in examined dogs

| Parameter | Reference value | Between 30 and 50 mg/dl | Between 51 and 100 mg/dl | › 100 mg/dl |
|---|---|---|---|---|
| Ureic nitrogen | 8-26 mg/dl | 15 cases (35.71%) | 24 cases (57.14%) | 3 cases (7.14%) |

Table 3

Synopsis of values of alanin aminotransferase (ALT) in examined dogs

| Parameter | Reference value | Between 50 and 60 mg/dl | Between 60 and 70 mg/dl | › 70 mg/dl |
|---|---|---|---|---|
| ALT GPT | 8.9-48.5 mg/dl | 12 cases (28.57%) | 28 cases (66.66%) | 2 cases (4.76%) |

Table 4

Synopsis of haemoglobine values in examined dogs

| Parameter | Reference value | Between 6 and 7 g/dl | Between 5 and 6 g/dl | Below 5 g/dl |
|---|---|---|---|---|
| Haemoglobine | 8-12 g/dl | 5 cases (11.90%) | 34 cases (80.95%) | 3 cases (7.14%) |

The correlation between renal necrosis, the cause of deficient blood filtration and systemic hypotension caused by anaemia, could be assessed and inferred to a certain extent from the study of the values obtained in the determination of ureic nitrogen and haemoglobinemia [1, 2, 3, 7, 12]. In other words, the amount of seric urea is directly proportional to the degree of anaemia. In the present work, three cases confirm this hypothesis (see tables 2 and 4). The hepatic enzyme diagnosis was changed mainly in subjects with haemoglobinemia below 6 g/dl (see tables 3, 4).

The anamnetic data point to a fulminating and galloping evolution of the disease, in absence of a medical record regarding the hepatic-renal pathology in the examined subjects. The fast feverish reaction maintained on the background of a hepatic-renal failure, the sad expression, the state of prostration, anaemia and haemoglobinuria were decisive in the reinterpretation of the diagnosis. To this purpose, blood smears were prepared with a view to confirming the infestation with *Babesia canis*. The examination of the smears highlighted the presence of pear-shaped sporozoites in the red cells (fig. 1).

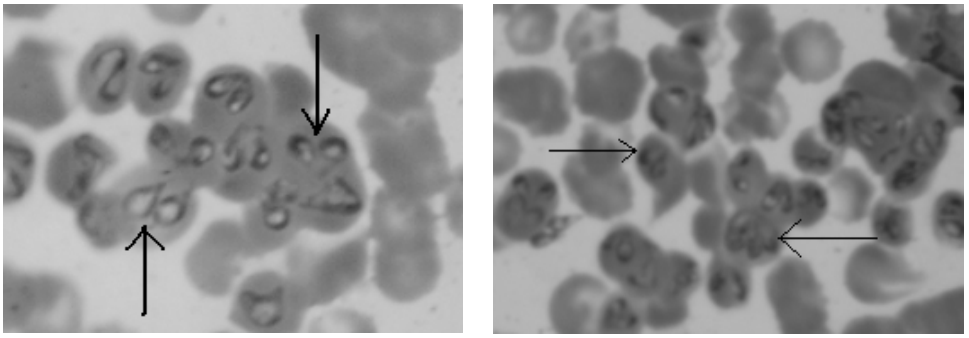

Fig. 1. Pear-shaped sporozoites in dog red cells. MGG staining method, lens immersion.
(Photo: Răzvan Condruț)

Their size was larger than the radius of the red cell and they were placed in twinned arrangements. Based on this data, the cause of the dogs falling ill was identified as infestation with *Babesia canis*, and hepatic-renal failure was considered as complication from babesiosis [9, 12].

## *Conclusions*

1. The feverish reaction maintained against the background of organ functioning failure, the sad expression, the state of prostration, anaemia and haemoglobinuria are all symptoms that lead to the suspicion of an infection with *Babesia canis*.

2. Confirmation of babesiosis is rendered by tracking the pear-shaped sporozoites inside the red cells, on the peripheral blood smears (May-Grünwald-Giemsa staining method, examination by lens immersion).

3. The cephalic manifestations were mainly determined by erythrocytholisis (pallor of cephalic mucous membranes, icterus, changes of the nose) and retention of ureic nitrogen (ammonia halitosis, coated tongue, photophobia, xerophthalmia, xerostomia, slower palpebral reflexes). These are not manifestations specific to babesiosis, but their presence can lead to the suspicion of the disease.

4. The main blood and urinary biochemical determinations whenever a *Babesia canis* infection is suspected refer to haemoglobinaemia and haemoglobinuria.

5. The correlation between the kidney necrosis and systemic hypotension could be assessed and inferred from the study of the values of ureic nitrogen and haemoglobinaemia determinations, the quantity of serum urea standing in direct proportion to the degree of anaemia.

6. The hepatic enzyme diagnosis was changed especially for the subjects with haemoglobinaemia below 6 g/dl.

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# ASPECTS REFERRING TO RECIDIVIST AGGRESSIVE BEHAVIOUR IN ROTTWEILERS

**A. SALLAY*, S. BARDAN[*]**

***Abstract***

*Eleven dogs of the Rottweiler breed were studied in order to notice the evolution of the submission-aggressiveness ambivalent behaviour conditioned by the master (temperament, time allotted to the dog, etc.) and by the environment they live in. All the dogs were trained in similar conditions for six months by specialists. Watchdogs were finally tested and proved to have the qualities for these activities in point of psycho-motive aspects. Eight months after the training came to an end, they checked the behaviour of the dogs and noticed that four dogs had the characteristic features acquired during the training session (watching something and attacking somebody), three had a delated reaction to orders and four had major behavioural deviations, which were conditioned in time, consciously or unconsciously, by their masters' physiological and nervous features. The behavioural changes were more obvious in the dogs trained for watching a building (three subjects) than in the ones for entertainment (one subject). Taking this into account, the owners or temporary holders of watchdogs must have minimum knowledge of the ambivalent behaviour brought about by the submission-aggressiveness motivational conflict.*

***Key words***: *Rottweiler, aggressive behaviour*

## *Introduction*

Any behaviour is a chain reaction, which comprises several phases that are carried out in an order imposed by the organization of the biological nature and the stimulus intensity and the conditions in which the behavioural act takes place [11, 12]. The first noticeable moment of behaviour is marked by the relationship between a receiver and the stimuli. The information is received, codified and transferred to the central nervous system. Here the second moment takes place, the brain cortical elements decoding and analyzing the information that was received. Further on, either the information is stored, enriching the experience with new data, or is transferred subcortically. Thus, the third moment is prepared: the motive answer representing the final behavioural act or its form of expression [10].

The behaviour is conditioned by the native, innate qualities (strength, agility, flexibility) and the ones the dog acquired in its lifetime, possibly as a result of the training [5]. The ambivalent submission-aggression behaviour is genetically influenced (inborn qualities), on the one hand, and, on the other hand, it is intensified and determined by the acquired qualities (training), the preponderant manifestation of a behaviour feature being conditioned and based on their interference [2, 3]. Speaking of something else, mention should be made of the fact that behaviour extremes do not except each other, on the contrary the manifestations of the two conducts overlap, the resultant of the habits of a body depending not only on their genetic dominant character, but also on the qualities acquired in its lifetime (training, bad habit, tic, etc.) [4]. In this respect, one can say that one can prevent the offensive behaviour of the dog at a young age, through specific pre-training and training techniques. This is possible through the act of learning which, under the influence of the external environment, can induce long-term changes in the innate behavioural mechanisms [8]. However, there is an age limit up to which the animal shows such availability, the ability of animals to learn is inversely proportional to their age. Finally it should be said that the animals may

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learn to carry out a particular programme, a sequence of movements, provided they can be entered as a parental model in their genetic structure [1, 5].

### *Material and working methodology*

The study was conducted on 11 dogs of the Rottweiler breed, age between 6 and 8 months, they were divided into two groups. The first group, consisting of 7 dogs (5 males and 2 females) was prepared in order to defend the public buildings and institutions, the second group, 4 dogs (3 males, 1 female), was meant for the family environment.

The dogs used for training were consulted from a medical point of view (disinfected and vaccinated, a dysplasia test was made on them, their teeth were checked), being able to work and be trained. The dogs were registered with A.C.H.R. and the territorial police station.

Working methods applied to the dogs over a period of two months included different training techniques depending on the aims that were imposed. The training for guard and protection used the motivational method (response reaction to the deliberate action); stimulating the hunting instinct, for this purpose specific working tools being used (a bag with seaweed, sack cloth, string, soft muff) and food as a reward (the dog's reaction to the moving objects) [7]. The techniques of training for entertainment and discipline included stimulation by playing (exploiting the dog's retrieving reflex); food as a reward (stimulating innate reflexes) and stimulating affectivity (development of the master-dog relationship) [9].

The end of the training was marked by the working test, the dogs being asked to guard a person, an object or perimeter decided on in advance (especially for the dogs in the first group), speed of responding to the order, testing the dog's capacity to react and the capacity to fulfil the order whatever the obstacle.

### *Results and discussions*

After eight months from the end of the training the dogs' behaviour was checked and the following things were noticed: 4 dogs showed the characteristic features acquired during the training (guarding the object and attacking the person), 3 had belated reactions to orders that had been given to them, and 4 had behavioural major deviations, which were conditioned in time – either consciously or unconsciously – by their masters' physiological and nervous features.

Behavioural changes were more obvious with the dogs that had been trained to guard a building (3 cases) than with those for entertainment (1 case).

Guarding an area in a perimeter aimed at starting the attack when the demarcation line was crossed and supporting it until the trespasser leaves the perimeter. Continuing the attack outside the perimeter established before marked the aggressive behaviour in this situation. The failure of the owner or the temporary holder of the dog to fuel the skills acquired by the dog during the training may be the cause of the attack outside the perimeter.

Guarding an object. The aim of this objective was the subject of guarding against possible offenders. In such situations the dog must only react when the object is threatened directly. The aggressive behaviour in this situation refers to the

uncontrolled reaction of the dog without the object being directly threatened. By training a dog, one can remove the expectative condition of the dog that is insufficiently accustomed to this test.

Guarding a person. The purpose of this test relates to the defence of the owner against possible aggressors. The dog must only react after the aggression was committed and it was ordered to attack. The aggressive behaviour in this situation is determined by starting the attack without reason or without being ordered. Applying inappropriate training elements leads to a more aggressive character of the dog.

The ability to fulfil the order regardless of the obstacle. The goal is to determine the dog to observe the order. The dog should not deviate from the order, regardless of the situation created by the aggressor. The aggressive behaviour in this case refers to changing the target of the attack. The owner must have full control of the dog at any time and be aware of his holding a dangerous ”weapon”, which can be triggered by merely transmitting a state of agitation.

## *Conclusions*

1. The failure of the owner to fuel the skills acquired by the dog during the training can lead to uncontrolled attack.

2. By training a dog one can remove the expectative condition of the dog that is insufficiently accustomed to the guard and protection service.

3. Applying inappropriate training elements can lead to a more aggressive character of the dog.

4. The owner must have full control of the dog at any time and be aware of his holding a dangerous ”weapon”, which can be triggered by merely transmitting a state of agitation.

5. Eight months after the end of the training the dogs' behaviour was checked and the following things were noticed: four dogs had the characteristic features acquired during the training (guarding the object and attacking the person), three had belated reactions to the orders that had been given, and four had major behavioural deviations, which were conditioned in time – either consciously or unconsciously – by the physiological and nervous features of the masters.

6. The behavioural changes were more obvious in the dogs trained for watching a building (three subjects) than in the ones for entertainment (one subject).

7. The failure of the owner to fuel the skills acquired by the dog during the training leads to a more aggressive character of the dog by merely transmitting a state of agitation regardless of the conditions that were imposed.

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