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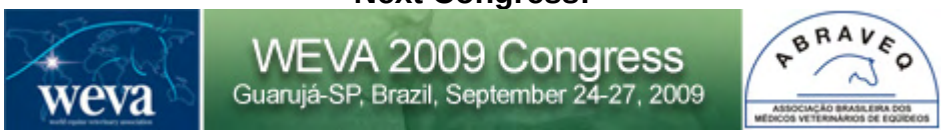

SEASONAL PROFILE OF LEPTIN LEVELS AND SOME IMMUNE PARAMETERS IN ARAB HORSES

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Leptin receptor is expressed in many tissues of the horse such as lungs, liver, gonads, brain, and subcutaneous adipose tissue, and leptin blood concentration reflects a significant influence of body fat. Leptin is identified and localized leptin receptors are shown in the the horse.

Although reports in other species concerning plasma leptin and its effect on, or response to, various hormonal systems are numerous; there is few deal with leptin secretion in the horse. The involvement of leptin in signaling the brain of the body's energy and nutritional status make it an important regulator of growth, reproduction, pregnancy, and immunity. The existence of daily rhythms of serum leptin in the horses are also shown. Further, an understanding of how different hormonal systems affect concentrations of leptin in the horse may increase our ability to better manage the horse through different phases of production and use. The goal of this study was to determine the seasonal profile of leptin concentrations, some immune parameters such as total T lymphocyte, total B lymphocyte counts,T lymphocyte subpopulation (cytotoxic, helper, natural killer cells [NK]), tumor necrosis factor (TnFα), Interleukin (IL)-2, IL-4, IL-6 in Arab horses.

MATERIALS AND METHODS: Ten Arab stallions and ten Arab mares were included in this study. Before and during experimentation, horses were maintained at ambient light and temperature. Blood samples (10 ml) were collected via jugular venipuncture at 0800 in the morning in December (short day light) and end of June (long day light). Serum was harvested immediately and frozen at -20°C for leptin analysis. Heparinized blood samples were prepared according to manual of kits. Serum leptin concentrations were determined in duplicate using a multi-species leptin RIA kit (Linco Research XL-85K), as previously used in horses, (McManus and Fitzgerald, 2000). Lymphocyte subtypes were analysed by Mouse antiequine CD4, anti CD8 (monoclonal antibodies MCA, Serotec Ltd, Kidlington,UK) using flow cytometer. Interleukin levels were analyzed using IL-2, IL-4, IL-6 ELISA kits (Endogen Ltd,Pierce Biotechnology, Inc,USA).

RESULTS: All values are given as mean +/- SD; the significance of differences between groups was assessed by Student's t-test. $P<0.05$ was considered significant. We did not observe any significant seasonal difference in immunological values of mares. However leptin concentrations were elevated in summer $p<0,05$. Mean leptin concentrations were, 2.40 +/- 1,17 (stallions) and 2.36 +/- 0.67 ng/ml (mares) in summer, 2,28 ± 1,52 (stallions); 1,86± 0,44 (mares) in winter ;No significant change was observed in leptin levels of stallions. Leptin levels appeared to be influenced by season in mares. When lymphocyte subpopulations count in periferal blood was considered, T cytotoxic and natural killer (NK) lymphocytic cells were increased significantly ($p<0,05$) in stallions during summer. No differences were found ($p>0.05$) for total B cell and T cells and helper Tcells between both seasons.

CONCLUSION: This study shows that leptin levels in blood are significantly higher in summer than winter in mares; according to previous studies, nutrient restriction resulting in low body weight in mares resulted in a profound seasonal anovulatory period that was accompanied by lower leptin. Our data agree well with those reported decrease during autumn months in mare and contribute to support the hypothesis that leptin plays a significant role in modulating of female reproductive axis. Seasonal fluctuations in immune status have been documented for avian and mammalian populations. During the late summer and early fall, immune function is bolstered to help animals cope with the more physiologically demanding

winter. The regulatory factors that mediate photoperiod-induced changes in immune status have not been fully identified. The effects of photoperiod length on the immune system appear to occur independent of reproductive status and sex steroids. Several humoral factors, including leptin, have been proposed as potential mediators of the effects of photoperiod on immune function. CD4+ (T helper) and CD8+ (T cytotoxic) T cells express the long isoform of the leptin receptor (Ob-Rb). The mice deficient in the leptin receptor (ob/ob) have impaired T cell-dependent immunity. Humans with low body weight have reduced levels of leptin and a deficient cell-mediated immune response. Leptin apparently shifts the T-cell response to favor the production of proinflammatory cytokines (interleukin [IL]-2 and interferon) over cytokines that predominantly regulate humoral immune function (IL-4, IL-6, and IL-10). The results reported here in show that concentrations of leptin in horses are affected by daylight period. Long daylight increases concentrations of leptin in mares ($p<0.05$) and stallions. Further, an increase in both cytotoxic and NK T cell counts, but not a significant increase in hepler T cells, resulted in alterations in total T cell counts in stallions.

INFLUENCE OF TRAINING ON LIPID PEROXIDATION AND ON ANTIOXIDANT SYSTEMS AT TRIATHLON HORSES

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Lipid peroxidation, or free-radical oxidation, occurs in the animal organism as chain reaction. Its products (in small amounts) are necessary for course of various biochemical reactions, but in large amounts it promotes destruction of biological membranes. There is a number of substances in an organism named antioxidants for regulation of peroxidation. They are caeryloplasmin, catalase, α-tocopherol, etc. Free-radical oxidation plays an important role in the pathogenesis of many diseases, including cardiomyopathia of horses, caused by overtraining. Physical loadings influence the intensity of peroxidation. However, the character of such influence at horses is investigated insufficiently.

The purpose of work was to investigate the intensity of free-radical processes and the condition of antioxidant systems of triathlon horses during a training season (from November till April) and in dynamics «rest - work - rest».

Methods. The objects of researches were 12 half-bred horses in the age of 6 years (4 stallions, 4 mares, 4 geldings). The experience has consisted of three periods. In the first (the beginning of a season) loading made 15 minutes at trot and 5 minutes at canter, in the second (the middle of a season) - 15 minutes at trot and 10 minutes at field gallop, in the third (the end of a season) - 25 minutes at trot and 15 minutes at field gallop with 15 jumps through obstacles. At the end of each period the tests of blood were taken before work, right after work and after 45-minute rest. The maintenance of an intermediate product of peroxidation was determined in plasma of blood – conjugated diens (CD) and final product – malon dialgehyde (MDA), and also the level of α-tocopherol, the activity of caeruloplasmin and catalase.

Results. On the average the maintenance of the CD of all groups measured before work, was changing doubtfully during the periods, in limits from 87,2 $\pm$ 3,1 up to 109,4 $\pm$ 8,9 standard units. The maintenance of MDA in the second period in comparison with the first has increased from 1,94 $\pm$ 0,31up to 3,22 $\pm$ 0,34 microM ($p < 0,001$), and in the third - up to 3,60 $\pm$ 0,29microM. After work in the first and second periods the level of CD was changing insignificantly, but in the third it was growing in 2,17 times ($p<0,05$). After rest it usually tended to decrease. The concentration of MDA raised after work: in the first period on 57,7 % ($p<0,05$), in the second - on 46,2 %, in the third - on 16,7 %. After rest it was reducing: in the first period on 42,8 % ($p < 0,01$), in the second - on 33,0 %, in the third - on 1,7 %. The distinctions between stallions, mares and geldings on the level of CD were always insignificant. But the maintenance of MDA at mares before work was lower, than at stallions, and at geldings, as a rule, was above.

The initial level of caeruloplasmin in the second period in comparison with the first has decreased from 0,89 $\pm$ 0,12 up to 0,79 $\pm$ 0,16 microM·second, and in the third has increased up to 1,16 $\pm$ 0,21 microM·second. In the first and second periods its activity tended to increase after work, and to decrease after rest. In the third period it grew both after work and after rest. However all these changes were doubtful.

The activity of catalase, measured in rest, in the second period has increased in comparison with the first period from 46,6 $\pm$ 7,2 up to 115,0 $\pm$ 7,7microM·second ($p < 0,001$). After work in the first period it tended to increase, in the second and in the third periods it tended to decrease, after rest it always tended to increase. The level of tocopherol, measured in rest, in the first period has made 70,4 $\pm$ 3,3 microM, in the second - 23,9 $\pm$ 2,7 microM and in the third - 10,6 $\pm$ 0,6 microM. The difference between all periods is reliable ($p<0,001$). But in the first and in the second periods both after work and after rest it was decreased, in the third period in both cases it increased. Essential distinctions between stallions, mares and geldings on a condition of antioxidated systems are not revealed.

Discussion. During a training season in blood of horses MDA is collected - it is a final one, toxic product of free-radical oxidation. The level of primary stages of the oxidation, bringing to the formation of CD, varied not so essentially. Thus the activity of caeruloplasmin

by the end of the experience has changed insignificantly, and the activity of catalase has decreased. Probably, the reserves of biosynthesis of catalase were reduced. The reserves of tocopherol in plasma have decreased as a result of decreasing of its maintenance in forages by the spring.

The concentration of MDA, in an exercise time raised, and after rest was reduced as a result of activation of antioxidant systems. One of the major roles in it usually belongs to catalase, but in this case by the end of the experience it has passed to other systems as a result of exhaustion of reserves for its biosynthesis. Thereof in the third period the mobilization of tissue reserves of tocopherol has begun, that was expressed in the increasing of its level in plasma both after work and after rest. The most intensive peroxidation usually was at geldings, and the least intensive at mares. At the same time the differences in the work of their antioxidant systems were not found. Obviously, sexual distinctions are caused by antioxidant properties of steroids**.**

Conclusions.

1. Training loadings influence the lipoperoxidation in the organism of horses. The most informative parameter for an estimation of its level is the maintenance of MDA in plasma.

2. By the end of a training season activity of catalase is reduced. Thus the level of freeradical oxidations grows.

3. At the end of a training season the mobilization of tissue reserves of tocopherol begins.

INTENTIONAL Zn PHOSPHIDE (Zn3P2) POISONING IN HORSE IN A HORSE-RACING AND RIDING CLUB AROUND TEHRAN-IRAN

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During five months (Jan. 2004 to May 2004), 13 horses were dead with a history of loss of appetite and sudden death. Clinical examinations revealed depression, anorexia, tachycardia, tachypnea, severe tremor and sweating, ataxia, abdominal pain and convulsive seizures. The affected animals were recumbent and expired within a few minutes to 24 hours after the onset of convulsion, without responding to current supportive treatments. At necropsy, visceral organs were dark and extremely congested. Malicious chemical poisoning tentatively diagnosed. Stomach and intestine contents, viscera and the cubs were chemically analyzed for pesticides, rodenticides and metals. Histopathologic examination revealed severe congestion, edema and hemorrhage of all tissues especially brain, lungs, kidneys, liver, heart, stomach and small intestine. Zinc phosphide was detected in the cubs and the liver zinc content was 38/81 mg/kg (ppm). Later guarding cleared the chemical agent of toxicity to be dark-gray cubs. An experimental study with the cubs showed it be the cause of this poisoning.

Key words: Zinc phosphide, Intentional poisoning, Horse

SEROEPIDEMIOLOGY OF EQUINE INFLUENZA VIRUS INFECTION IN TURKEY

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The aim of this study was to investigate seroepidemiology of equine influenza infection in Turkey measured using the haemagglutination inhibition (HI) assay. Serum samples were obtained from 623 equids aged older than 4 years from 5 different geographical regions in Turkey during the years 2003 to 2005. Antibodies against equine influenza virus (EIV) were found in 194 (31.1%) of the 623 sera. Seropositivity rates were detected as 41.8% (171/409), 12.8% (11/86), 9.4%(12/128) in horses, mules and donkeys, respectively. The data reveal that equine influenza virus circulates in Turkey at a relatively high level. Differences in seropositivity rates in equidae from different geographical regions probably reflects regional differences in their economic uses.

EVALUATION OF THE ANTI-INFLAMMATORY EFFECTS OF THE INTERLEUKIN-1 RECEPTOR ANTAGONIST PROTEIN AND PLASMA IN EQUINE SYNOVIAL FLUID USING FLOW CYTOMETRIC TECHNIQUES

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INTRODUCTION - Joint disease in horses, specifically osteoarthritis, is one of the most prevalent and debilitating illnesses affecting equine industry and for this reason continued research and improvements in therapeutics are needed. Osteoarthritis is a degenerative disease that can be triggered by a number of factors and where ultimately all articular tissues are affected. The hallmark of osteoarthritis is the degeneration of the articular cartilage matrix, where the most relevant and expressive events take place. In the development of osteoarthritis there is disruption in extracellular matrix homeostasis with an overall balance toward cartilage metabolism. Homeostasis of the articular environment relies on balance between anabolic and catabolic events and results in ability of cartilage to respond to molecular or mechanical cues. This apparently antagonic processes are orquestrated by soluble protein mediators, for example the anabolic insulin-like growth factor-I (IGF-I), and, on the other side, by inflammatory cytokines, which in turn, are implicated in degradative processes of articular cartilage, characteristic of osteoarthritis. They deplete cartilage matrix from collagen and proteoglycans and the classical example of such a cytokine is interleukin-1. Interleukin-1 has a central role in the physiopathologic processes of osteoarthritis and has been implicated in the genesis of a number of catabolic events when acting on chondrocytes and synoviocytes. Examples are gene induction for metalloproteinases and agrecanases production, as well as production of other inflammatory mediators like ciclooxygenase, prostaglandin E2 and oxygen-derived reactive species. Its biological effects are observed after interaction with two different but specific types of receptors and are modulated by the occurrence of a natural antagonist, the interleukin-1 receptor antagonist protein. OBJECTIVE - In the present study the anti-inflammatory effects of the commercial interleukin-1 receptor antagonist protein (IRAP®) were evaluated in synovial fluid using cytometric flow techniques. METHODS – The method proposed by Hasui et al (1985) was used. Data from 10.000 events were colleted in list mode and analysed using Cell Quest software. Discrete cell population was recognized on the basis of FSC/SSC properties and was electronically sorted to permit light microscopic evaluation of cells. Fluorescence data was collected on log scale. Green fluorescence from DCFH was measured at 530±30 nm (detector FL1). Quantification of oxidative burst was estimated by mean DCFH fluorescence/cell. Synovial fluid was collected from 92 tarsotibial joints and the tubes were immediately cooled in iced baths and centrifuged afterwards. The cell pellets obtained were washed three times in sterile ice-cold phosphate buffered saline (PBS). Cell viability was estimated using the tripan blue exclusion method. The number of cell was adjusted to $1x10^6$ cell/100 μL. The synovial fluid cells were stimulated *in vitro* by LPS and PMA. The oxygen-derived reactive species (EROs) production from synovial cells was evaluated after the addiction of plasma or IRAP®. The IRAP® was obtained using commercial kits and employed according to the manufacturer's instructions. Blood was collected from different horses and processed according to the IRAP methods. Kolmogorov-Smirnov test showed that the data concerning oxidative burst was parametric. Thus, one-way analysis of variance followed by the Tukey-Kramer test was used to comparison of cell fluorescence means. The statistic analysis was used throughout with the level of significance being set at $P<0,05$. RESULTS - It was observed that: 1- addition of IRAP to synovial fluid cells stimulated *in vitro* by LPS and PMA reduced the production of oxygen-derived reactive species ($P<0,001$); 2- plasma, used as a control, exhibited similar effects on activated synovial cells when compared to IRAP *in vitro*; 3- the anti-inflammatory effect is due, in its majority, to the variation in intensity of oxygen-derived reactive species, more than on fluctuations on the percentage of synovial fluid cells actively engaged in its generation *in vitro*. CONCLUSION - These results support the therapeutic aplicability of IRAP® and plasma for their anti-inflammatory effect observed on synovial fluid cells evaluated with flow cytometric techniques.

They also corroborate to the usefulness of cytometric flow techniques in equine synovial fluid cells; they are an invaluable tool to evaluate quantitative and qualitatively the production of oxygen-derived reactive species mediated by their activation with PMA and LPS.

REFERENCE VALUES OF HOLTER ECG IN THE HEALTHY HORSE

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Aim of the study

There are a few data only in the literature on the reference values of Holter ECG in the normal horse. Reference values are not determined at all for some parameters. The aim of the work was to obtain a set of relevant measurements from healthy, resting horses that can be used as reference values.

Materials and Methods

Twenty healthy horses (8 mares, 2 geldings and 10 stallions) were selected randomly. The animals varied in age between 3 and 9 years. The average weight of the horses was 495 kg (from 400 to 550 kg).

Measurements were performed with every individual horse at rest in the stable without the presence of the examiner causing extra stress. It was important to collect data from horses stabled in an environment as close to the normal conditions as possible. Measurements were made with an Argusys FD Holter ECG (Innomed Medical Inc., Budapest, Hungary). Each horse was monitored for one hour. The results of the examinations were then transferred onto a computer, using an Argusys 2.50 (Innomed Medical Inc., Budapest, Hungary) software program. The program allowed us to make an individual file for each horse.

A blanket surcingle was fitted tightly around the thorax and the Holter machine was securely fitted on the left side of the horse, at the level of the flank. The positive electrode was passed to the right side over the withers of the horse and fixed between the 6th and the 7th rib in the ventral region of the thorax. The negative electrode was fixed in the ventral region of the thorax, between the 4th and the 5th ribs, on the left side. The basal electrode was fixed on the left side at the withers.

Each horse was securely tied up in the box during the measurement period of one hour, in order to minimize movement and avoid risking any damage to the Holter machine if the horse should lie down.

Once the ECG measurements for one whole hour were downloaded to the computer, certain segments and intervals of the ECG curves were measured. These points were as follows: P-wave duration, P-wave peak interval, P-R segment, P-R interval, QRS interval, S-T segment, Q-T interval and S-T interval. The heart rate was also counted manually. Thirty measurements were recorded per hour of ECG monitoring, equalling one measurement in every 2nd minute. The distances were measured in milliseconds, by using a function of the computer program, where a distance between two points of the ECG curve could be measured. Also 20 measurements per hour were made of the height and depth of the P-, QRS- and T-waves using the square millimetre background on the computer. Special attention was directed to detect if there was a single or double P-wave, and a single or a biphasic T-wave in the individual horses.

Results

All ECG curves were analysed individually. Means, minimum and maximum values and standard deviations of the determined parameters are displayed in the *Table*.

Table

Mean, minimum, maximum values and standard deviations calculated form the results obtained from the ECG curves

	P-wave duration	P-wave interval	P-R segment	P-R interval	QRS interval	S-T segment	Q-T interval	S-T interval	Heart rate/min
Mean	118	67	160	278	132	221	557	423	37
Min.	21	43	65	135	54	119	245	196	20
Max.	317	142	360	525	295	448	754	628	70
SD	44,4	12,5	46,5	61,2	40,3	40,8	66,9	53,3	7,1

All values are given in msec, except heart rate (beat/min).

Discussion

A standard protocol was used on all horses to minimize the deviations of the results as much as possible. Each horse was monitored for a period of one hour, under normal environmental conditions. The measurements were relatively easy to obtain with the same accuracy in all horses.

The height (amplitude in mV) of the different characters of the curves was also measured, but during this process some problems were encountered. It turned out that there was a significant deviation in the height and form of the curves from horse to horse. When examining the P-wave, 5 horses had a biphasic curve while the other 15 horses had a simple positive P-wave. The QRS curve also showed similar differences, there were 15 horses that had a negative, while 5 horses had a positive QRS. Fourteen horses had a negative T-wave deflexion and six horses had a positive T-wave curve. Out of the 6 horses with a positive T-wave, only one had a simple curve and the remaining 5 had biphasic. These results made it difficult to analyse the values accurately.

Since there are no previously published data concerning the duration of the different segments of the ECG curve while using the Holter method, the results were compared to values obtained by using the standard-ECG method. Another problem with the comparison was that other authors did not measure all parameters. Our values differed only slightly and were within the range of previous measurements.

Conclusion

The present work provides useful reference values in the field of equine Holter ECG. Our study could serve as a basis for future examinations. It would be feasible to use a bigger group of subjects as well as comparing different lead systems over a time exceeding one hour. There are also very few studies examining the Holter ECG curve during exercise. The hope is that clinicians in the future will be able to use the Holter system as a reliable diagnostic tool with the necessary software custom made for the equine patient.

NEW DIAGNOSTIK AND THERAPEUTICAL APPROACHES OF CARTILAGE DISEASES

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Cartilage-diminishing processes of hores and humans are based on a disturbance of the cartilagie homeostasis and traumatic events. If the collagen and proteoglycan connnection in the joint is destroyed in the result of these processes. Increased tissue turgor leads to irreparable damages of the cartilage up to so-called bald head education of the joint surface.

Cartilage degrading illnesses are connected with inflammtoric reactions of the joint. Inflammation factors lead to activation of matrix metalloproteinases (MMP) in addition to the destruction of the extra-cellular matrix. MMPs belong to the group of zinc dependent proteases degrading the extracellular matrix of the cartilage. Own investigations have proved that metalloproteinases in particular MMP 9 are usable for diagnostical and therapeutical control of joint illnesses. Presently about 34% of all animals treated in horse clinics show cartilage damages. Chondrocytes are widely differentiated. In articular cartilage only a low number of multipotent progenitor cells are existing.

Basically two possible mechanisms of the natural repair of the cartilage fabric are distinguished.

1. „ intrinsic repair“ if mitotic chondrocytes division occurs to the surroundings of the defect. This kind of cartilage repair is not provable in adult horses.
2. „ extrinsic repair“ based on a metaplasia of cells from the surrounding tissue into the cartilage tissue. Nevertheless, the result of this natural repair process is mostly no hyaline cartilage, but fibrous cartilage with bad quality and a high amount of atypical collagen type 1 and a low amount of collagen type 2 which is typical for cartilage. The natice repair program is limited.

During the last years several study groups could show that „in vitro“ cultivation of cartilage and transplantation is possible. From arthroscopic taken cartilage chondrocyten are cultivatet and implanted later in the cartilage defects.

In own investigations 5 different operation procedures of autologous chondrocyte transplantations (ACT) were carried out in 23 Haflinger horses in the lateral rolling comb of the knee joint. Aim was to purpose differences in the operation technology, in the clinical course, in the macroscopic defect filling as well as in the histologic, histochemical, immune-histologic and electrons-microscopic processing.

The matrix-related chondrocyte transplantation (MACT) was compared with different absorbable carrier materials (matrixes). As carrier materials collagens I/III membranes, hyaluronic acid membranes, a combination of both scaffolds and a collagen I gel were applied. These materials are applied in the human medicine for cartilage transplantations. The research center for Medizintechnik und Biotechnologie GmbH developed a three-dimensional cartilage construct free of any artificial material.

The cartilage withdrawal occurred arthroccopic in the lateral rolling hill of the right Talocural joint. It were taken 3 small probes of cartilage. The cell vitality of the material was around > 95%. The monolayer cell cultures were supplemented with autologous serum. The cell concentration of seeding was 10^6 cells per square centimeter.

The implantation of the implants occurred under intubation anaesthesia in back position of the animals. In the lateral rolling comb of both knee joints a complete cartilage defect was prepared without opening the subchondral bone. The defective size was 2.0 x 1.5 cm (Fig 1).

Fig 1: Operation procedure of horse knee joint cartilage implantation

The transplantion occured 55 days after start of cultivation. On account of the low perioperative load of the hors joints a simultaneous operation on both sides was carried out also for the reduction of the animal number (19). The fixation of the grafts occurred with fibrin glue. Two defects were the control group not without any graft. The extremities of the horses loaded after the operation well. Almost all horses showed a superficial fissure dehiscent which healed. Clinically 2 horses pointed out at time of the euthanasia a minimal intense lamenss all theh other horses were lame-free. One horse got a not controllable joint infection and was euthanised. All the other horses were scarified about 553 days.

The preserved biologics showed a caudal shift independent of the scaffold. In the area of the defective filling a uniform structure of the preparations could be observed. A basale layer of cartilage and a predominantly matrix rich center could be found. Joint sided a cell poor fibrtotic tissue was found. The cartilage layer contained collagen II, in the superficial zone collagen I is provable. The matrix-related cartilage transplant is called hyalin-like cartilage.

Recapitulatory it can be estimated that the matrix associated cartilage transplant is applicable for covering cartilage defects. In future it has is to be worked on a better fixation technology of the scaffolds and also on an arthroscopical operation technology.

EQUINE ABORTIONS: A RETROSPECTIVE STUDY BETWEEN 1990-2007, MONITORING IMMUNOLOGY OF FETAL FLUIDS, MICROBIOLOGICAL AND PATHOLOGICAL FINDINGS

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Introduction, Equine abortions cause large economical losses to breeding farms around the world yearly. Despite the fact that vaccination programs are present as preventive medicine measures in breeding farms, abortions take place. Aetiologies of abortions in equines have enlarged with time and make mares prompt to suffer from infections during pregnancy and placentitis, as well as environmental exposures or nutritional deficiencies or excess that facilitates early or late failures of pregnancy. Some research have shown that the cause of abortions can not be studied focusing in on just the cause. It must be interpreted with other laboratory findings putting together the final diagnosis. The presence of early embryonic death, abortion at any age of gestation, premature deliveries and neonatal weakness, foetal abnormalities, retained foetal membranes, red bag and placentitis demand a multidisciplinary diagnostic workout. The assessment of immunology on fetal fluids in equines has not been consistently studied as in bovines. This research was conducted to evaluate abortion conditions in different equine breeding farms across Venezuela along the eastern-western northern states. The reproductive performance of mares during gestation was evaluated in some mares by monitoring, the risk of placentitis and the abortion causes were interpreted by the agreement of immunology of fetal fluids, microbiology, necropsies and histopathology findings.

Materials and Methods, a total of 40 breeding farms were involved in this study, 22 farms, Thoroughbreds, 10 farms, Quarter horses and 8 farms, Paso Fino breeds. A total of 390 foetuses were studied. The reproductive performance of farms during breeding season with crisis of abortions was evaluated. All abortions from a given breeding farm in one breeding season were studied. Aborted mares were cultured from the uterus, clinically evaluated and serum sample taken. Necropsies were performed in all foetuses, premature delivery or neonatal death. Fluids from Pleural-Pericardial-Peritoneal fluids cavities and stomach content plus organs (Kidneys, Liver, Spleen, Vitreous) were taken for microbiology and immunology to ***Leptospira, Brucella***, ***Mycoplasma spp. / Ureaplasma spp.,*** CEM, PHF, EVR, EVA and Toxicological agents. Statistical analyses were done by SAS ANOVA between abortion rates, causes and other reproductive performance variables.

Results, Abortion by five to seven months of gestation were associated to viral EVR between 1990 and 1995 breeding seasons. From 1996 on, there were no abortions associated to virus. Nevertheless, mares were in low percentage positive to EVA. However, Bacterial causes were the most common cause of abortions. Firstly associated to ***Leptospira spp***., Enterobacteria such as ***Salmonella spp***., ***E, coli, Pseudomonas spp.***, ***Klebsiella spp,***, ***Streptococcus*** β-haemolytic, ***Bacillus spp. and Mycoplasma spp..*** No ***Ureaplasma spp***.. nor CEM, PHF were found in the whole study. From 1998 up to now, **Leptospira** has been the number one cause of equine abortions in the country. Foetal fluids, Vitreous and Stomach content were consistently positive with high antibody titters against ***Serovars. pomona, icterohemorrhagiae, hadhjo, and canicola.*** The lesions in the placenta and in the foetuses were consistently present and characterised by chronic fibrosing necrotizing and haemorrhagic placentitis with vasculitis. The foetuses showed cataracts, nonsuppurative meningitis, lymphocytic bronchoalveolitis, lymphocytic interstitial nephritis and necrotizing hepatitis with hepatocytes dissociation, megalocytosis, megalokaryosis and lipidosis. Toxicity by Ionophores (Salynomycine, Narazine) in the diet caused abortions and neurological disease in Thoroughbred pregnant mares. Micotoxins such as Zearalenone (> 25 ppm) in the diet created an abortion outbreak in Thoroughbreds with high numbers of premature deliveries with foetal ileocecal intussusceptions.

Conclusions, The study of 390 foetuses with the same protocol allowed us to interpret the degree of agreement between aetiology versus foetal specific immune response and necropsy lesions associated to histological findings. **Leptospiral abortion** was the most common associated cause. The decrease of viral abortions found in this study after 1996 was possibly related to a better reproductive management and preventive medicine program. **Mycoplasmas** were isolated and related to chronic atrophic necrotizing placentitis without foetal lesions but the association with ***Leptospira spp***. is possible.

EQUINE HEPCIDIN: mRNA SEQUENCE AND EXPRESSION IN LIVER TISSUE

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Equine serum or plasma iron concentration drops quickly during inflammation, this test is a reliable procedure for systemic inflammatory process diagnosis. Accumulation of iron inside macrophages and reduction of the intestinal absorption of this element causes hypoferremia during systemic inflammatory processes. These mechanisms are mediated by hepcidin, a 25 amino acids peptide synthesized mainly by the hepatocytes in response to iron stores and inflammation. Increased hepcidin expression during inflammation restricts the iron availability, necessary for pathogenic microorganism growth. This increase in hepcidin expression can be particularly useful during initial periods of inflammation, before adaptive immunity occurs. The mRNA hepcidin sequence has been identified in human, rat, mouse, swine, and dog; however the equine mRNA hepcidin has not been characterized. We have cloned and sequenced equine hepcidin mRNA and also compared the expression to the housekeeping gene βactin in liver tissue. The hepcidin coding sequence was obtained from an equine liver sample collected *post-morten* from a clinically normal horse. Total RNA was extracted using RNeasy Mini Kit (Qiagen®), and the yield and quality of RNA was evaluated by spectrophotometry at 260/280 nm. The RNA product was treated with DNAse to eliminate genomic DNA from the samples. cDNA was prepared using the 3' Race kit (Invitrogen®). The complete hepcidin coding sequence was obtained and clonned into PCR II-TOPO (Invitrogen®). Colonies containing the correct size inserts were sequenced after mini preps were done (Qiagen®). To evaluate tissue expression profiles of hepcidin, tissues were obtained from healthy horses (ultrasound guided liver biopsy) approved by São Paulo State University Institutional Animal Care and Use Committee. Tissue samples were collected and immediately placed in liquid nitrogen. Total RNA was extracted, evaluated and treated as mentioned above. A two-step RT-PCR was used to detect expression of target transcripts. Briefly, cDNA was prepared with ImProm RT II (Promega®). Primers were designed using SciTools software (http://www.idtdna.com). Endogenous mRNA control was used to normalize the target gene amplification value (*Equus caballus* βactin mRNA, AF035774). Real time PCR was performed using model 7300 (Applied Biosystems®) with Power SYBR Green PCR Master Mix (Applied Biosystems®). All samples were measured in triplicate. The predicted anino acid sequence for equine hepcidin is: MALNTNIRAACLLLLLLASLTSGSVLPHQTRQLADLQTQDAAGMAGAAAGLMPGLHQLRRRD THFPICTLCCGCCNKQKCGWCCKT. This equine sequence has 74% to *Bos taurus* (AAI11659); 70% to *Sus scrofa* (NP 999282); 67% to *Homo sapiens* (NP 066998); 67% to *Cannis familiaris* (AAW82336); 52% identity to *Rattus norvergicus* (NP 445921) and 50% to *Mus musculus* (NP 115930). The deduced precursor of equine hepcidin is most homologous to *Sus scrofa* and *Bos taurus* species. The expressed profile of equine hepcidin in liver was very high, similar to the CT values obtained for βactin, indicating that this is expressed in the manner of a housekeeping gene in normal animals. This sequence will be helpful for additional equine studies on iron and the inflammatory process, and permit a better comprehension of iron metabolism in horses.

EQUINE MESENCHYMAL STEM CELLS FOR THE TREATMENT OF TENDINOUS LESIONS IN THE HORSE

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Purpose of the study: To describe the outcome of the stem cell treatment of tendon injuriy in the horse.

Study design: Retrospective clinical study.

Animals: 120 horses of different sex, breed and age, which suffered from tendon lesions.

Treatment regime: After clinical and ultrasonographic examination, a diagnosis of a tendinous lesion was made. Stem cell treatment was initiated by harvesting bone marrow from the sternum in the standing, sedated horse, followed by isolation and propagation of mesenchymal stem cells in vitro, which were subsequently injected into the tendinous defects. A rehabilitation regime was prescribed, which included clinical and ultrasonographic follow-up. The outcome was described as return to athletic training, return to athletic competition, failure or reoccurrence of the problem.

Results: Nearly two thirds of the horses were aged 6 to 12 years, with two thirds belinging to the Warmblood breed. There were nearly 50 % of geldings, mares and stallions being both inn the range of about one fourth. The use of the horses was mainly distributed amongst dressage (16 %), flat racing (18 %), general riding and sport horse (29 %), show jumping (16 %) and race trotting (9 %). The diseased structures were mainly the superficial flexor tendon (35 %) and various parts of the suspensory ligament of the fetlock (a total of 56 %). The history was indicative of an acute onset in 70 %, while chronicity was demonstrated in 30 % of the cases.

The results of the treatment were quoted good (back to intended use) in 53 % and in full training in 28 % of the animals. In most of the cases (93 %), there were no adverse reactions to the treatment modality investigated. The superficial flexor tendon was mainly diseased in race horses, while the suspensory ligament was predominantly diseased in dressage and show jumping horses. The intended use or level of full training was achieved in over 70 % of the patients suffering from problems of the suspensory ligament, while nearly 80 % of the superficial flexor tendonitis cases recovered to this activity level.

Discussion: The animal patients included in this study were of variable origin, and the size of the lesions investigated was very variable, too. Therefore, the study situation was difficult from this point of view. Treatment tolerance as well as clinical and ultrasonographic features were very favourable for the stem cell treatment regime. However, controlled long term studies are to be performed in order to provide reliable information on the value of the treatment when used for equine tendon lesions. Nevertheless, equine mesenchymal stem cells are easily available from various tissues and have high intrinsic capacity of tissue regeneration. Therefore, this cell type has a high potential especially for the treatment of equine musculoskeletal disorders. However, there is a great need for in depth investigation into the specifities of stem cells and stem cell treatments in the horse.

EQUINE THIRD METACARPAL BONE PROPERTIES AS EVALUATED BY AXIAL TRANSMISSION QUANTITATIVE ULTRASOUND AND QUANTITATIVE COMPUTED TOMOGRAPHY

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Introduction- Noninvasive bone assessment techniques are required to monitor and evaluate horses at risk for muskuloskeletal injuries (Jeffcott *et al.,* 1989). Quantitative ultrasound (QUS) and quantitative computed tomography (QCT) are noninvasive bone assessment techniques used in human medicine (Genant *et al.,* 1996). Aim of this prelimimary *ex-vivo* study was to investigate the third metacarpal bone (MC III) with axial transmission QUS and QCT.

Material and Methods- The right MC III of eight Standardbred horses was collected after euthanasia, which was unrelated with this study. MC III was divided in nine regions of interest (ROI), three aspects (lateral, dorsal, medial) and three levels (proximal, mid, distal). QUS measurements were obtained at the mid level of MC III. Measurements were obtained by using the multi-site QUS device (Omnisense™, Sunlight Ltd., Tel Aviv, Israel). 1000 x centistokes silicone oil was applied to the skin for acoustic coupling. Speed of sound values were recorded. QCT measurements- were performed with a peripheral QCT device (Norland/Stratec XCT 3000, Stratec Medizintechnik, Pforzheim, Germany) at the distal, proximal and mid level of the third of MC III. Cortical bone mineral density (CoBMD) as well as bone strength indices for bending (xBSI) and for torsion (pBSI) were evaluated. The measured bone sections at the mid level corresponded to the ROI evaluated by QUS. Statistical analysis- was performed by using a commercially available computer system (SAS, Cary, NC, USA). Significance level was set at $P \leq 0,05$.

Results- Speed of sound (SOS) values at the dorsal aspect of MC III were significantly lower than at the medial ($P \leq 0,05$) and lateral ($P<0,01$) aspect. SOS values correlated with CoBMD values ($r=0,40$; $P=0,052$). CoBMD were lowest at the proximal level and highest at the mid level ($P<0,0001$). xBSI values at the mid level were significantly lower ($P<0,0001$) than values obtained at the proximal and distal level. pBSI values were significantly lower in the mid-level ($P<0,0001$) compared to the distal and proximal level. pBSI values obtained at the proximal level were lower ($P<0,01$) than values obtained at the distal level. CoBMD did negatively correlate with xBSI values ($r=-0,74$; $P<0,0001$) and pBSI ($r=-0,83$; $P<0,0001$). xBSI and pBSI values did significantly correlate ($r=0,86$; $P<0,0001$).

Discussion and conclusion- Axial transmission QUS is described for the noninvasive *in vivo* evaluation of superficial cortical bone properties in horses (Carstanjen *et al.,* 2002). A moderate correlation of SOS- and DEXA-measurement results was described at the MC III (Carstanjen *et al.,* 2003). Data of this preliminary study show the tendency of SOS measurements to correlate with CoBMD measurements as evaluated by peripheral QCT. However, no correlation was obtained between SOS values and bone strength indices. The above study showed furthermore highest CoBMD values and lowest bone strength indices at the mid-level of MC III, when compared to the proximal and distal level of MC III. Additional studies need to be performed in the future to better understand bone properties in horses.

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PULMONARY HEMORRHAGE IN EXERCING HORSES: COMPOSITION OF CYTOLOGICAL ANALYSIS OF BRONCHOALVEOLAR LAVAGES AND SEARCH ANTIBODIES AGAINST EQUINE HERPES VIRUS

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Objective

Race horse's epistaxis syndrome induces bad performances and often restricts sportive career of athlete. It etio-pathogenic mechanism is not well elucidated in spite of many devoted works. The immuno-allergic and virus (influenza and Equine Herpes Virus) component have been evocated. Our objective is to contribute to the study of these two components. For that we study the composition of cytological analysis of bronchoalveolar lavages and search the antibodies against Equine Herpes Virus (EHV) in horses suffering from exercise induced epistaxis (EIPH) and witness healthy horses

Material and methods

Cytological analyses of broncho alveolar lavages (BAL) from 32 horses suffering from exercise induced pulmonary haemorrhage and from 10 from healthy horses were performed. The BAL is collected from the distal airway using an endoscope (Storz- Karl) according Hodgson 2006. The quantification of the total number of cells/ml is performed using an automated cell counter (Cytospin Shandon). The cytological evaluation of samples and leukocyte formula are achievable after coloration by May Grunwald Giemsa (MGG). In addition determine of the relative population of cells is performed via a differential cell count of 200-300 consecutive cells. The Perls coloration is used for determination of hemosiderophages according to WHITWELL & GREET (1984).

In the second time, we carried out serological search against Equine Herpes Virus, on 32 horses suffering from exercise induced epistaxis (EIPH) their average age was 7.7 ± 5.8 years and 42 witness healthy horses their average age was 4.95± 1.2 years. All are race horses.

The diagnosis of EIPH was obtained on the basis of commemoratives and clinical examination associated, systematically, to endoscopic examination. The witness healthy horses were horses that did not have any recent disease and have not receive drugs or treatment since 3 weeks before examination and sampling and that endoscopic examination had confirmed that no EIPH. All horses were not vaccinated against EHV.

For the serological diagnosis against EHV we used the complement fixation and serum neutralization test.

The complement fixation (FC) is from LBCF (Laboratory Branch Complement Fixation) recommanded by U.S. Department of Health, Education and Welfare was used to determine antibodies EHV according to ZIENTARA & al., (2000).

For serum neutralization test we used Kentuky EVH-1 strain. The virus was produced by cellular strain RK13 in Rollers bottle according to MURRAY & al., (1996). The title obtained was 6,2 log DECP 50 by ml.

This research was carried out between 2003 and 2006.

Results and discussion

The endoscopic examination revealed a left larynx hemiplegia in 5 diseased animals. Mild to severe inflammatory lesions within bronchus and bronchiols were also seen but haemorrhage signs were only evidenced in 3 horses with EIPH.

The results of cytological analyses of broncho alveolar lavages (BAL) from horses suffering from EIPH and from 10 from healthy horses are presented in the table n° 1.

Although the difference was not statically significant because of strong value dispersion, the average cellular density of BAL was more elevated in diseased horses and the strongest value was observed in this group. The leukocyte formula were similar between the 2 groups;

however, 4 EIPH horses exhibited a marked increase of neutrophiles (>36%) according to HODGSON (2006). In addition, hemosiderophages were evidenced by Perls coloration in 72% of diseased animals and in 30% healthy horses. Potentially pathogen bacteria were isolate from BAL in diseased horses as well as in controls**.**

Table 1: Results of cytological analysis of BAL from horses with EIPH and healthy horses.

	horse's number	cells/ml	% macrophage	% lymphocytes	% neutrophils
horses with EIPH	32	253	60,7	20,4	16,8
witness healthy horses	10	179	61,9	28,5	8

The results of serological survey of horses tested for equine herpes virus with complement fixation and serum neutralization respectively revealed these seroprevalences (12.4% and 9.3%) in horses with EIPH and (7.1% and 4.7%).in the healthy horses

Conclusion

Theses results suggest that cytological analysis of BAL may be helpful to diagnostic through hemosiderophage detection (although false positives occur in healthy horses) and that an inflammatory response would partially be involved in the aetiology of the syndrome exercice induced epistaxis. The seroprevalence of EHV1 were higher in horses with EIPH than healthy horses.

CONTRIBUTION TO STUDY OF DENTAL PATHOLOGIES IN THE WORKING EQUIDS IN TUNISIA

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I- OBJECTIVE

In Tunisia there is neither information nor medical data about equines dental pathologies. To palliate to this situation we carried out a descriptive survey among the working equids. Our objective is to study the bucco-dental pathologies in the working equids in Tunisia, on order to determine the relationship between body condition (especially debility) and dental problems and the relationship between dental problem and fecal fat and fecal starch analysis.

II- MATERIAL AND METHODS

A total number of 174 working equids were examined. They were 47 horses, 34 mules and 93 donkeys. They were coming from 3 regions: the governorship of Jendouba in the north, the governorship of Kasserine in the centre and the governorship of Kebili in the south of Tunisia. These regions are representative of working equids population.

A special Hausmann's gag mouth was used to keep animal's mouth open and a torch was used to enable the teeth to be seen in the back of the mouth. A few animals had necessitated to be sedated with Domosedan*.

The commemoratives were taken. Complete clinical examination, extra oral examination and oral examination were performed with the collaboration of ATPNE-SPANA team.

We have evaluated equid body condition by a scale of score up to 5 (Ouassat & al.1997) :

Score < 1 Emaciated. Looks like a skeleton
Score 1 : very thin. All ribs sticking out. Spinous process easy to see and feel.
Score 2 : Quite thin, ribs showing. Spinous processus just covered but easy to feel.
Score 3 : Good condition
Score 4 : Fat
Score 5 : Extremely fat.

Fecal samples were taken for coprology examination. For fecal fat analysis, we mixed fresh faeces with Soudan III strain and a smear is prepared on a microscope slide. The undigested fats appear as orange globules (Braun & al.1987).

Fecal starch analysis is performed with a 2% Lugol's iodure strain. Undigested starch granules appear as dark blue-black granules on microscopic evaluation

We use a scale score from nil to 4 according to granule wealth of smear in microscopic evaluation. A fecal analysis is considered as positive when the mean of 2 explorations (fat and starch) is higher than 1.5 (Ben Ali 2004).

As for a statistical analysis, the mean and the standard deviation were calculated for each parameter and the differences between the groups were established by Student t and Chi2 tests. A critical . . was used in statistical evaluation.

III- RESULTS AND DISCUSSION

Among these174 equids, 136 have shown dental abnormalities, 78% of examined total number. Irregular tooth (wave mouth) and traumatic lesions were the most frequent abnormalities. They represented respectively 41% (56 cases) and 19% (26 cases) of the whole observed affections. They sit on the molars in 72% of cases. The infectious pathologies (dental decay) were the lowest (0.8%).

The dental abnormalities were subdivided in 4 categories: congenital (regrouping incisor malformation, brachygnathia and polydontia), infectious (Caries), irregular tooth (Wave mouth, Hook, incisor wear, smooth mouth) and traumatic (Missing teeth, dental fractures, traumatism due to the Bit). 58.8% were irregular tooth, 25% were traumatic and 14.7% congenital and 0.7% were infectious. According to food pattern, The equids fed by date are more affected by dental abnormalities (50.9%) than those fed on barley (43%), on grass (35%) and on hey (31%).

The equids of Kebili (that are fed on date), are more affected (48%) than those of Kasserine (32%) and Jendouba (16%). According to the equid species (horse, mule and donkey), the percentage of affections are similar. However, it increases with the age of the animals, passing from 9% in equids aged less than 2 years to 71% in animals aged over 10 years.

The evaluation of equid body condition reveals that 32.14% of equids suffering from irregular teeth were in poor body condition (scores $\leq$ 2) whereas only 16.9% of equids without irregular teeth were in poor body condition (3).

The evaluation of fecal starch and fat analysis is represented in the table 1.

	Positive score	Negative score
equids not suffering from irregular teeth	19%	81%
equids suffering from irregular teeth	46%	54%

table 1: Evaluation of fecal starch and fat on equids with or without irregular teeth.

The positive score toward fecal fat and fecal starch show a significant difference between 2 groups : equids suffering from irregular teeth show a percentage 46% statically higher than those of equids not suffering from irregular teeth (19%).

IV- **CONCLUSION**

The bucco-dental pathologies is high prevalent in the working equids in Tunisia. They increase with the age of the animals and they vary according to the type of feeding. Horses, mules or donkeys treated at over 10 years of age and fed by date are the common affected equids. The Irregular tooth is the common lesion encountered but the tooth decay is rare. Equids suffering from irregular tooth are often in bad or poor body condition. The fecal composition show a difference between equids suffering from irregular teeth and equids not suffering from irregular teeth.

APPLICATION OF ONE STEP REAL-TIME TAQMAN®REVERSE TRANSCRIPTION-PCR ASSAY FOR DETECTION OF EQUINE ARTERITIS VIRUS IN BULGARIA

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Key words: equine, viral arteritis, diagnosis, laboratory methods, RRT-PCR

I. Introduction and Objectives

Equine arteritis virus (EAV) is a contagious viral disease of equids widespread in the horse population all over the world (4) The symptomatology of EAV infection varies widely from a subclinically silent form to overt clinical signs, which generally include pyrexia, conjunctivitis, lacrimation, nasal discharge, and edema of the limbs and ventral body (3). In infected pregnant mares, abortion is common. Because EAV-infected asymptomatic stallions shed the virus in their semen and, thus, act as a reservoir for future disease outbreaks, semen samples are screened for EAV by sensitive but time-consuming virus isolation attempts in cell cultures. PCR is a rapid technique by which the nucleic acid in any sample can be specifically amplified Moreover, over the past few years, a reverse transcription (RT) step has been used in conjunction with the PCR amplification, in the so-called RT-PCR, for the detection of the viral RNA genome in biological samples. In our study we took the advantage of that technique and applied one step Real-Time taqman®reverse transcription-PCR assay developed from Udeni and Balasuriya et all. (1) for detection of equine arteritis virus in Bulgaria. To this moment in Bulgaria the disease is established 7 years ago. The aim of this study is to detect the virus from nasal swabs and semen plasma of horses with clinical signs by iusing the one step real Time Tagman®Reverse Transcripsion PCR Assay.

II Material and methods

Virus isolation : Nasal swabs of horses for virus isolation were set into clean centrifugetubes with cell culture medium 199, containing antibiotics. First, we mixed them briefly in the vortex. Then, we obtained the supernatant fluids of suspensions through clarification by centrifugation at 1000×g for 10 min. We inoculated PK 13 cell culture with 0.2 ml of the clarified homogenate and incubated for 2-4 days 37° C. The fluids were then tested with ELISA for virus activity.

Samples: Reference Strains (Bucyrus and ARVAC), nasal swabs from animals with clinical sighs and semen plasma samples are used in our studies. ining at least 200 viral RNA copies per ml.

All of these samples had been confirmed previously to contain EAV by virus isolation in RK-13 cells, and subsequent characterization of each virus isolate by serum neutralization assay using polyclonal and/or monoclonal antibodies to EAV.

RNA extraction and RRT-PCR

Viral RNA was extracted from all samples or TCF by using QIAamp® Viral RNA kit (Qiagen, Germany).

For amplification, we used TaqMan Master Mix and - Forward Primer EAV7.53F GGCGACAGCCTACAAGCTACA; Reverse Primer EAV7.256R CGGCATCTGCAGTGAGTGA and Probe EAV7.92P 6FAM-TTGCGGACCCGCATCTGACCAA which are described by Udeni and Balasuriya et all (1) All thermocyclings were performed in AB7300 Real Time PCR thermocycler (Applied Biosystems).

25 µl reaction contained: 12.5 µl TaqMan Universal PCRmaster mix (2X) (AppliedBiosystems, Foster City, CA), with including - ROX, 0.6 µl -MoloneyMurine Leukemia Virus (MuLV) and RNase inhibitormix (40X; Applied Biosystems, FosterCity, CA), 80 nM from probe (0.2 µl), 800nM from each primer (0.5 µl), free water (0.7µl) and 10 µl RNA, isolated from each sample and from standart.

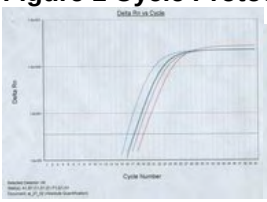
Temperature range:
35 min in 48 °C,
10 min in 90 °C,
50 cycles: 95 °C - 15 sec and 60 °C - 1min

III. Results and Discussion

We are investigated 18 samples – 6 - from nasal swabs, 5 from semen plasma, 3 – suspension from internal organs of foetus and 4 cell cultures with reference strains.

From the nasal samples and semen plasma we isolated arteritis virus with positive reaction in ELISA and cell culture. The same isolates we tested by RRT-PCR. The results showed that all of them are the arteritis.(figure1 and 2).

Figure 1 Cycle Protocol for EAV



AI type A FAM TAMRA

Well	Sample Name	Detector	Task	Ct
A1	1	AI type A	Unknown	19.08
B1	2	AI type A	Unknown	18.04
C1	3	AI type A	Unknown	17.33
D1	4	AI type A	Unknown	16.02
E1	5	AI type A	Unknown	17.23
F1		AI type A	NTC	Undet.

Figure 2 Ct of the samples

The samples which have the border concentration after the amplification were visualized in 2% agarose gel stained with 1% ethidium bromide.

All received fragments with size arouind 200 base pair which coresponded to expected lenght of amplified fragment.

1 2 3 4 5 6

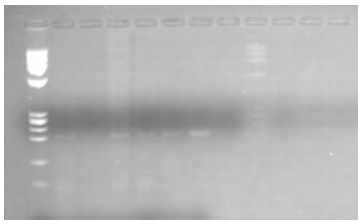


Figure3.Agar gel after amplification of EAV with primers EAV7.53F и EAV7.256R

The samples we have obtained until now are from 5 different premices with clinical signs for viral arteritis. The Equine Viral Arteritis was estimated in last 5 years in Bulgaria (2). After confirmation of our isolates from reference laboratory of EU we has become more intensive studies and we develop the prophylactic programs against this disease according to Mumford,(3) and we are made rather wide serological investigation in equine population in Bulgaria.

Conclusion

The main conclusion of our studies is that we have devepoped and used in laboratory work the RRT-PCR for detection of equine arteritis vurus. We confirmed the positive results of RRT-PCR in samples of nasal swabs, semen plasma and cell culture fluids. The other aim in next one – two years will be check the veterinary low against EAV in Bulgaria which is necessary to equal the veterinary measures in EU and our countries.

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EQUINE DIGITAL VENOGRAM IN RELATION TO THE BIOMECHANICS OF THE FOOT

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INTRODUCTION

There exist many scientific studies around the equine foot centre of pressure or mass; many techniques and instruments have been used find it (Xu H et al. 1999, Arabian AK et al. 2001 and Leveillard D. 2005) and this has helped to understand the biomechanics and the dynamic effects on gait analysis related to trim and shoeing (Willemen et al. 1996, Wilson AM et al. 1998, Xu H et al. 1999, Van Heel MC et al. 2004, Van Heel MC et al. 2005).

Many studies have been oriented to evaluate the relation between the body weight load and the anatomical changes of the foot (Crevier-Denoix N et al. 2001, Hobbs SJ et al. 2004, Burn JF et al. 2001, Colles CM et al. 1989 and Redden RF. 2003).

Olivier A et al. (1989) underlined the effect of weight loading on the coronary band interstitial fluid pressure in horses and Pietra M et al. (2004) highlighted the influence of weight bearing and hoof position on Doppler evaluation of lateral palmar digital arteries in healthy horses.

The aim of this research is visualizing with digital venography the fine and detailed variations of the vascular bed related to the biomechanics effects of changing the Palmar Angle (PA) to the ground line and weight-bearing position.

MATERIALS AND METHODS

The X-ray examinations were performed at the Veterinary Clinical Sciences Dep. of Padua, on bare feet thoroughly cleaned of six horses not referred for foot problems - 3 chronic sub-clinic laminitis and 3 normals, aged between 5 and 14 years, body weight between 400 and 550 kg-.

A wood-iron podoblock specially designed and realized with Moreau X. for this study (height 8,9 cm, length 16,4 cm, with 16,4 cm, able to change PA between –15° and +15° with a step every 5°) was placed under the frontfoot weight bearing examined with the sagittal line of the foot used to identify the principal tubulus corneus (Caudron I. et al 1997) along the dynamic podoblock's sagittal wire, the neck was maintained along the sagittal axis and at the same level of the withers during the x-ray examination, in order to distribute equally the horse weight.

The static centre of pressure was identified following the technique described by Leveillard D. (2005) with the horse standing squarely on four feet (4CPS) and we also have marked it with the horse standing on three feet (3CPS). We positioned the 4CPS or 3CPS, the weight force application point, under the barycentre of the dynamic podoblock.

In order to evaluate the difference of the weight force in different postures we calculated the load on each front foot in both postures with a digital balance.

On the solar face of the podoblocks there is a radiopaque wire, placed along the sagittal axis (length 20.5 cm and 15 cm, thickness 0.3 cm). The foot to be X-rayed was placed medially in contact with the X-ray cassette in order to reduce as much as possible the magnification phenomenon. Over the dorsal profile of the hoofwall was traced a sagittal line of radiopaque substance (barium sulphate) for visualizing the hoofwall. Konica x-ray films and radiological apparatus (Pollux 700 "Odel srl") were used. X-ray beam was oriented in the middle of solar aspect of PIII perpendicularly to the film. X-ray images were taken in latero-medial projection at 80 cm distance in order to reduce and evaluate the magnification effect (D'Arpe L. et al. 2006).

Seven x-ray images were performed moving the dynamic podoblock's angle of 5° every 30 sec. and without moving the x-ray machine from the ground. For each foot were performed two venography examinations in order to relatively reduce the importance of perivascular diffusion and blood dynamic effects correlated with the venography technique (D'Arpe 2004). The first injecting the contrast liquid (Iopamidolo 150 mg)* with the weight bearing foot upon the dynamic podoblock positioned at –15°, progressively modified to +15°. The second, one week later, vice versa injecting at +15° progressively modified to –15°.

*Iopamiro 300, fl 50ml (Bracco SpA Milano)

RESULTS

Resuming table of results after injecting at –15° and +15°

-15°	Absent vascularization of the coronary bend and the dorsal laminar vessels
-10°	Poor vascularization of the coronary bend and the dorsal laminar vessels
-5°	Still poor but improved
0°	Close to normal
+5°	Still poor but improved
+10°	Poor vascularization of the bulbar vessels
+15°	Absent vascularization of the bulbar vessels

CONCLUSIONS AND DISCUSSION

During the primary phases of this study we realized that the 30” gap time between one x-ray and the next was necessary in order to leave a significant adaptation time for vessels: a shorter time did not show any evidence of vascularization changes.

We have not noticed any significant variation in the examinations injected at –15° and the examinations injected at +15°.

According to Pietra M. et al. (2004) we noticed a considerable difference between tripodal and quadripodal posture. We have correlated this to the weight load; in quadripodal posture each front foot is charged of 115-155 kg and in tripodal posture of 220-310 Kg.

We observed, according to Thompson KN et al. (1993) and Crevier-Denoix N (2001), that the deep digital flexor tendon (DDFT) tension decreased with positive PA, in the standing horse. Furthermore according to Redden RF. (2003) DDFT tension played an important role for the laminae of the dorsal hoof wall to fill with the contrast agent.

The results obtained, relatively to vascular changes related to the PA and consequently DDFT tension, showed that the negative PA induced compression of coronary bend and dorsal laminar vessels; and the positive PA induced the compression of bulbar vessels.

This study permitted to visualize how the vascular foot bed could be modified by little changes of PA correlated to DDFT tension and the weight-load force of the three feet standing posture.

PARAMETERS INFLUENCING YEARLING PRICE AT ITALIAN TROTTERS SALES

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The aim of this work is to investigate the factors influencing the price of yearlings at sales and the relationships between the paid price and future performances. A dataset of 4605 yearlings (2400 colts and 2205 fillies), sold in 33 Italian Trotter auctions from 1999 to 2006, were examined : the mean sale price was 14,450 euros for colts and 10,194 for fillies . Price distribution was normalized by logarithmic transformation. Official harness racing results starting from 1992 was used for the evaluation of racing careers (time/km, earnings, starts, wins and shows) and also to calculate breeding values by Animal Model BLUP.

The price logarithm was analysed by a linear model including the fixed environmental effects of gender, birth month, year and type of sale: this model showed a determination coefficient (R^2) of 46%; gender and type of sale were significant for $P<0.001$, birth month and year for $P<0.01$; the birth month affected yearling value, which decreased regularly from January to July. A model including only the effect of the 239 sires showed a R^2 equal to 56%: this value raised to 63% adding the 435 maternal grandsires; if sire or both sire and maternal grandsire effects were added to the previous environmental effects model, R^2 raised to 72% and 75% respectively.

The pedigree index of the yearling for average racing time and for record in career resulted to be correlated with the sale price by –0.59 and –0.57 respectively; the pedigree index for normalized earnings/start and for overall earnings in career were correlated 0.62 and 0.45 respectively with yearling price; also, the pedigree index for the Elo-type underlying performance and for Elo final rating showed linear correlation of 0.62 and 0.63 with price of yearling at sale. The parents indexes were less correlated with price than the pedigree index, and sire's breeding value for earnings/start and for overall earnings seemed to be more important than dam's ones; the parent's breeding value more correlated with price was sire's earnings/start (0.56), whilst the less correlated was dam's earnings in career (0.29). The dam sire indexes were correlated between 0.17 and 0.30 with prices. If pedigree indexes were added to the environmental effects model, the R^2 raised to 64%; since record in career index is correlated with average racing time index, and Elo performance index with Elo rating one, only 4 genetic indexes were statistically significant in the full model.

There were 2,565 yearlings which did not have any start at 2 years of age: they had been sold at an average price of 12,056 euros; the average price of yearlings which had starts at 2 years resulted 12,861 euros; the average price of 1,502 yearlings with shows at the same age was 13,860, and the one of the 755 winners 16,177 euros. At 3 years of age, there still were 1,597 yearlings without a start, and they had an average price at sale of 10,901; the average price of yearlings with at least a start within 3 years of age was 13,215, whilst the 2,587 with shows had been priced 13,710, and the 1,950 ones with wins averaged 14,898 euros. The correlation coefficients between the sale price and the number of wins, shows and starts at 2 years of age were 0.15, 0.08 and -0.02 respectively, while they were 0.22, 0.11 and 0.007 at 3 years of age. The correlation coefficients between yearling sale price and earnings was 0.15 at 2 years of age and 0.20 at 3 years.

There were 2986 yearlings with no valid record at 2 years of age: their average price was 11,835 euros; the yearlings with a valid record at 2 years of age had an average price of 13,477 euros, and the correlation coefficient between price and time/km was –0.38. At 3 years of age, the number of yearlings with no record reduced to 1923, with an average price of 10,814 euros; the yearlings with a valid record at this age had an average price of 13,559 euros, and their correlation between price and time/km was –0.36.

It can be concluded that information on relatives ranking results, time/km and earnings play a key-role in determining the yearling price at an auction, especially for sire's earnings; however, the price paid at sale will be more related to time/km record than to wins or earnings.

APPEARANCE AND SPREAD OF EQUINE VIRAL ARTERITIS (EVA) IN REPUBLIC OF SERBIA

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Equine viral arteritis (EVA) is acute infective viral disease of horses, characterized by septicemia and manifested with fever, inflammation of respiratory and digestive system, eyelid edema, abdominal and extremities swelling and abortions. Disease is caused by virus from Togaviridae family, Arteryvirus strain. EVA virus was isolated in year 1953. in USA, and clinical manifestations of disease were described in 1957. (Doil et al.) as severe, flu-like disease with mortality rate about 30% and 50%-80% of abortion rate in gravid mares. Bryans et al. (1967.) implies that earlier described typhoid fever in horses is clinically identical to viral arteritis, on which may be concluded that the disease was present in Europe and earlier. Until the year of 1965, disease was described in USA only, and later in Switzerland (1966.), France (1978.), Austria (1969.), Poland (1979.), India (1965.), and Australia (1969.). According to serological tests (SNT) it can be presumed that disease is disseminated and present in countries of African continent, also (Moraillon, 1978.). During last ten years, disease was both clinically and serologically registered in other parts of the world and Balkan peninsula, also.

In Serbia, for the first time EVA was serologically registered in sport faucets, during year 2001. (Djuricic et al, 2002.), and later described as clinical manifestations. Implemented serological testing within receptive population (in horse farms, horse clubs and individual horse owners) shows that disease is spreaded trough whole territory of Republic of Serbia. Testing, that included 250 sport faucets, indicates that number of positive specimens was in range between 0,2%-30%, where antibody titers were from 1:20 up to 1:2560.

Based on gathered data, proposed measures are: It is necessary to train specialized veterinary settings for routine serological EVA diagnostics and discovering true virus secretors. More detailed researches about EVA spread in Serbia territory in all horse categories, with special accent on studs, would provide more realistic epizootiologycal insight in disease and manifestations. To exclude studs-virus secretors from breeding programs, this is according to EU laws.

THE ANALYSIS OF POSITIVE SEROLOGIC EQUINE GLANDERS CASES IN LATVIA WITH 1995 ON 2004

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The aim of this work was to investigate glanders epizootic situation in the country with 1995 on 2004, having generalized and having analysed received positive results to estimate sensibility and specificity CFT , comparing diagnostic kits the different countries and spending interlaboratory comparative diagnostic.

During work it was necessary to answer following questions:

1.How much glanders problem is actual for Latvia ?

2.How many serologic positive cases diagnose every year?

3.What results are given with interlaboratory diagnostics?

Key words: Malleus, Glanders, Burkholderia mallei, equine infectious diseases.

Introduction

Equine glanders are contagious, usually fatal disease of horses and other equine species, caused by the bacterium *Burkholderia mallei*, and symptomized by swollen lymph nodes, nasal discharge, and ulcer of the respiratory tract and skin.

Burkholderia mallei is a gram-negative, non-acid-fast, non-sporing, non-motile, non-capsulated bacillus, it is obligately aerobic and oxidase-positive.

For diagnostic use intradermal- palpebral test, ophaltamic test, subcutan test guinea pig contamination, serology (ELISA (Enzyme-Linked ImmunoSorbent Assay), CFT(Complement fiksacion test), indirect hemaglutination, PCR (polymerase chain reaction). CFT do not use for donkey and mule, because these animals has high anticomplement level, defect of reaction – criss-cross reaction with melioidosis. At clinically sick horses for diagnostics use purulent discharges.

The following diseases must be considered in the differential diagnosis:

-Epizootic lymphangitis

-Fungal infectious caused by Blastonyces, Sporotrictium etc

-Melioidosis

-Strangles

-Ulcerative lymphanginitis

The illness caused by Burholderia mallei, is especially dangerous to veterinaries, grooms, workers of laboratories as to be infected it is possible airborne by, and also contacting with sick animals and infected material. Infection occurs through small wound, grazes. Lethality up to 95%. It is necessary to consider , that the infectious agent is the biological agent. If has developed septicemia treatment is inefficient because the bacterium develops strong toxin. Sick horses destroy, accepting all safety measures, burn a corpse not removing a skin.

Material and methods.

Materials:

Equine glanders diagnostic spent using serological and bacteriological methods.

Serology: CFT.

Bacteriology: classical cultures cultivation.

In research has been used blood of 14 horses for the period from 01.05.2004 till 01.05.2005. Analyses of serum of horses **-** Reversa (horse Nr.1), Lusia (horse Nr.2), Relikvia (Horse Nr.3), were positive and these samples were used for further interlaboratory diagnostics.

The analysis of positive results from 1995 till 2004 were based on the routine work of the archives and annual reports.

Positive serums was stored -22°C.

Methods:

Work was developed in the National Diagnostic Centre, Animal Disease Diagnostic Laboratory Serology Division. CFT it was spent with use by the antigen made in Russia, seropositive results were checked by antigen made in the Netherland. Each suspect sample was checked repeatedly and, ascertaining positive result diagnostics continued a bacteriological method. For definition and specification of results it has been lead interlaboratory comparative diagnostic in the Estonian National Veterinary Diagnostic Laboratory – analysis has been lead with use of an antigen made in the USA. Diagnostic continued in the Netherland using macro CFT.

Results and discussion

RESULTS OF EQUINE GLANDERS SEROLOGIC AND BACTERIOLOGIC DIAGNOSTICS WITH 1995 ON 2004

Years	Quantity of sample of serum of horses	Serologic suspect/positive causes (CFR)	The percentage of positive cases to the total number of horses tested	Bacteriologic positive causes
1995	6989	4/3	0,04	0
1996	**6789**	**20/2**	**0,03**	**1**
1997	3747	12/5	0,13	0
1998	3602	4/-	0	0
1999	2656	8/4	0,15	0
2000	2815	122/4	0,14	0
2001	2714	68/4	0,14	0
2002	3206	17/4	0,12	0
2003	3560	12/2	0,05	0
2004	4968	13/3	0,06	0
In total	41046	280/31	0,075	1

Comparative testing between laboratories

In 2004 was conducted comparative diagnosis between the National Diagnostic Centre, the Estonian Veterinary Laboratory and the Central Institute for Control of Animal Diseases in the Netherlands.

Generalization of results:

Results of comparative interlaboratory diagnostics have shown, that horse Nr.1 (diagnostics was spent with the Russian antigen, using macro CFT) has titration 1:160 (positive), using USA antigen, micro CFT- 1:128 (positive), using Netherlands antigen (macro CFT) titration was been 1:80 (positive); horse Nr.2-respectively - 1:80, 1:16, 1:20(positive); horse Nr.3 –respectively - 1:10, 1:4, 1:10 (suspect). Diagnostics lead in the Netherlands has shown following results: horse Nr.1- 1:100 (positive), horse Nr. 2 - 1:100 (positive), horse Nr.3 - 1:50 (suspect).

At diagnostics were used different diagnostics methods and different antigen, but results of all laboratories coinside - 2 horses were serological positive.

Having lead bacteriological diagnostics of serological positive cases, has been drawn the conclusion, that all samples are negative.

Discussion

During work have been drawn following conclusions-for of specification of the diagnosis have been made is necessary to spend bacteriological diagnostics as presence in blood of antibodies against Burkholderia pseudomallei can give positive reaction. According to the table it is possible to see, that exact diagnostics of glanders is actual for Latvia. At presence concerning a plenty serological positive cases (perhaps crossreactions to pseudomalleus, Streptococcus equi or other), bacteriological one sample in 1996 is positive only. All the horses used in research, were not clinically sick.

Conclusion

1. In Latvia glanders is not very actual problem, but it is necessary to reveal serologic positive horses to prevent flash of an infection.

2. The number of serologic positive cases each year randing from 1 to 4.

3. It is necessary to develop more exact differential diagnostics of Burkholderia mallei (like PCR) to reveal an infectious agent of melioidosis.

MALOCCLUSIONS TREATMENT IN HORSES

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Introduction

The principles of orthodontics and functional orthopedics in the equine are based on the experiences of these treatments in humans. Today, very little information exists of this science in horses and it is much more difficult to perform these procedures on equines because they tend to be uncooperative.

Similar to the treatment of humans we always want to begin these procedures in very young horses. At an early age the bone is very "plastic" and easier "to brake" or to stimulate the growth of the bone. The malocclusions associated with these treatments have many causes such as the difference in size and degree of hardness of the dental batteries, malnutrition, and trauma. There is also a large genetic influence when the grade of consanguinity is high.

Actual cephalometric studies in horses would be of tremendous value in improving the treatment of malocclusions.

Malocclusion

Faulty occlusion occurs in horses because of the inability of the cheek teeth arcades or incisors to occlude or fit together properly. This may be due to dental malposition, lack of contact, or faulty alignment causing decreased efficiency during the excursive movements of the jaw or the inhibition of normal jaw movements for problems of the growth skeleton increase or decrease.

Orthodontics

Orthodontics is the study of the growth and development of maxillaries and the face, specially. Orthodontics can be used in horses to correct abnormal bites that are created by malocclusion, which is the malposition of the teeth, resulting in the faulty meeting of the teeth and jaws.

Brachygnathism: "Parrot mouth"

"Parrot mouth" malocclusions may be caused by a growth deficient of the mandible, maxillary with excess of growth, or a combination of both. Other cause of this problem are the position of teeth, vestibularitation or palatinitation. In young, growing horses "parrot mouth" malocclusion correction is often accomplished by growth modification using headgear or functional appliances.

Prognathism: "Monkey mouth"

This malocclusion may be caused by a growth deficient of the maxilla, growth mandibular increased, or a combination of these. Also can be cause for dental malposition. These horses usually present with anterior crossbites. In young horses with "monkey mouth" malocclusions the correction is obtained by growth modification using headgear (extraoral force appliances).

Mode of Action and Goals of Treatment with Headgear

Headgear intended for growth modification is designed to deliver an adequate extraoral orthopedic force to compress the maxillary sutures, modifying the pattern of bone apposition at these sites. The posterior and superior extraoral orthopedic forces primarily are intended to

inhibit anterior and inferior development of the maxilla. The goal of this treatment is the restriction of maxillary growth while mandibular growth continues.

The headgear appliance can be effective in the treatment of “parrot mouth” malocclusions. The ideal indication for the use of extraoral force to correct skeletal malocclusion is anteroposterior maxillary excess. The headgear restricts anterior and inferior maxillary growth while growth in other areas progresses. Therefore, the maxillary anteroposterior excess (maxillary protrusion) appears to be the most suitable skeletal problem for this treatment.

Headgear is a common term for an appliance that is used for delivering a posteriorly directed extraoral force to the maxilla. The headgear is an adjustable halter attached extraorally to an occipital area with a metal device attached intraorally on the incisors. The extraoral force affects the elastics on both sides of the headgear. The intraoral device is made of very thin stainless steel and is affixed to the incisors with acrylic resin. This device removable and must be taken off every night.

Conclusion

The early identification of malocclusions by the dental practitioner will increase the probability of successful treatment and lead to a better quality of life for the horse. Also, remember that many dental problems can be solved or prevented by maintaining the horse in as natural of an environment as possible.

“The best orthodontic treatment…is the one that it is not necessary to make.”

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FAILURE TO DETECT SITES OF SEGMENTAL INTRAPLULMONARY BLOOD INOCULATION USING MULTIPLE DIAGNOSTIC IMAGING TECHNIQUES IN CONSCIOUS HORSES

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BACKGROUND: The detection of subclinical episodes of Exercise-Induced Pulmonary Haemorrhage (EIPH) remains a problem for specialist equine veterinarians. The inability to detect the amount of intrapulmonary haemorrhage that may occur during fast exercise limits decision-making with regard to treatment regimes, and prevents monitoring of responses to treatment or as a consequence further bouts of intense exercise. Because horses were available for study, and each animal had known volumes of blood instilled into defined regions of each lung, we investigated whether these segmental, experimentally induced lesions were detectable using modern diagnostic imaging techniques.

AIM: To examine whether radiography, ultrasonography, and scintigraphy using $^{99}Tc_m$-labelled leukocytes could be used to detect pulmonary lesions induced at known sites within the lung.

METHODS: Seven adult horses of mixed age, sex, and breed (six Standardbred, one Thoroughbred) were used in this study. Animals were purchased and brought to the University of Melbourne Veterinary Clinic and Hospital and allowed to acclimatise for a minimum of 14 days. At the conclusion of the acclimatisation period, thoracic ultrasonography and thoracic radiography were performed on all horses. Thoracic ultrasonographic examination was performed using an Acuson ultrasound system with a multifrequency 5–7 MHz micro-convex transducer, while the animals stood quietly in stocks. Thoracic radiographs were taken on a Toshiba KXO-1250A X-ray machine with a Toshiba Rotanode DRX-2903HD tube head. The Fujifilm FCR Capsula computed radiography system was used to capture and display radiographic images, which were reviewed by the radiologist (MM). Horses were lightly sedated with detomidine during the radiographic examination. Blood inoculation into the lungs of each horse was carried out using the following procedure. A two metre flexible endoscope was passed via the nares into the trachea, and used to visualise the lower airways. Mapping of the airway branching patterns was done by recording continuously using a videocassette recorder during the endoscopic procedure. Horses were tranquilized with detomidine and butorphanol prior to beginning endoscopy, and airways sprayed with mepivacaine to prevent coughing on passage of the endoscope. Eight sites suitable for inoculation were identified for each horse, four in each lung (left and right). A total of four paired inoculations were carried out for each animal. Each week two sites, one in each lung, were inoculated with 35 ml of autologous blood (without anticoagulant) at sites determined from previous mapping of airways. The inoculations were carried out six days apart. One day following the final inoculation, thoracic ultrasonography and thoracic radiography were repeated. Scintigraphy using $^{99}Tc_m$-labelled leukocytes was conducted on four of the horses. Blood (150 ml) was withdraw from the jugular vein and leukocyte-rich plasma was prepared by erythrocyte sedimentation for 45 min. $^{99}Tc_m$- stannous fluoride colloid was mixed with the leukocye-rich plasma for 20 min at room temperature to achieve highly efficient radiolabelling of equine leukocytes (determined in previous *in vitro* studies of efficiency of radiolabelling), after which the radiolabelled leukocytes were washed prior to injection. For two horses, radiolabelled leukocytes were injected via the jugular vein. For the remaining two horses, radiolabelled leukocytes were injected via an arterial catheter placed in the external carotid artery and advanced to the heart. Lateral and dorsal images of the lungs were then captured via a gamma camera (Argus Epic, ADAC Labs) at 1, 3, 5 and 24 hr post injection of radiolabelled leukocytes. Six days after the final intrapulmonary blood inoculation the horses were euthanized and post mortem examinations conducted.

RESULTS: Although able to illustrate a range of minor lesions, most of which were identified as areas of mild pleural fibrosis at post mortem examination, ultrasonography was not able to assist in the identification any of the blood inoculation sites. Inoculation sites typically had dimensions of 3-4 cm across, and the most recent inoculation sites were readily

visible at post mortem, but the older inoculation sites were not obvious grossly. Thoracic radiographs were unable to distinguish any of the sites of blood inoculation. Scintigraphy to detect radiolabelled leukocytes did not identify any of the sites of blood inoculation. Injection of radiolabelled leukocytes was, however, associated with a very high level of non-specific radioactivity in the lungs, presumably because of leukocyte sequestration in the pulmonary microvasculature. This was particularly evident following intravenous re-injection of radiolabelled leukocytes. Intra-arterial injection of the radiolabelled leukocytes also demonstrated a high level of non-specific radioactivity in the lungs although this appeared to be less severe than following intravenous re-injection.

DISCUSSION: Diagnosis of EIPH is a major challenge for equine racehorse practice and there is a need for non-invasive methods of detection of subclinical cases, to aid the diagnosis and treatment of this condition. Current diagnostic imaging techniques were ineffective at detecting discrete areas of inoculated lung containing 35 ml of blood. Scintigraphy using radiolabelled leukocytes may be of benefit to identify inflammation associated with areas of recent pulmonary haemorrhage, but in this study, the radiolabelling of equine leukocytes using stannous fluoride colloid was unsatisfactory due to a very high degree of non-specific uptake of the radiolabelled leukocytes in the lungs. The reason for this phenomenon remains uncertain, but it was not prevented by systemic arterial injection of radiolabelled leukocytes.

CONCLUSION: Using modern techniques of diagnostic imaging suitable for specialist equine practice, these studies demonstrated that the presence of blood in the lungs was undetectable. We conclude that these methods are insensitive for the detection of the presence of blood at single sites in the lungs for volumes in the order of 35ml, Therefore use of diagnostic imaging methods to detect cases of subclinical EIPH is likely to be highly inaccurate with under-detection of small areas of haemorrhage.

EFFECTS OF A FATTY ACID-ENRICHED ANTIOXIDANT SUPPLEMENT ON MARKERS OF CELLULAR DAMAGE, OXIDATIVE AND INFLAMMATORY STRESS IN ENDURANCE HORSES

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Oxidative stress is described as a syndrome in which pro-oxidant elements overwhelm the organism's antioxidant capacity. This imbalance results mainly from the formation of reactive oxygen species (ROS) within the organism, which have harmful effects on membrane lipids, proteins, carbohydrates and deoxyribonucleic acids. Evidence of exercise-induced oxidative stress in horses during intense and endurance exercise has been described and recent research suggests that oxidative stress could play a role in the physiopathology or genesis of several diseases. Some imbalances have been associated with a deficit in antioxidant elements.

Endurance is one of the most demanding equestrian disciplines. Horses have to run 20 to 160 km in a day, on irregular terrain and in various weather conditions. Competing horses can be subject to many disorders such as dehydration, muscular damage, oxidative and inflammatory stress. The origin of inflammatory stress still remains unclear and could be multifactorial. The anti-inflammatory properties of N3 polyunsaturated fatty acids have been demonstrated and it has been suggested that these fatty acids could be used to prevent the occurrence of some inflammatory conditions. Furthermore,

N3 polyunsaturated fatty acids could also contribute to improving vascularisation and performance.

In this work, we have tested the effect of an antioxidant and fatty acid-enriched feed supplement on the markers of oxidative stress, muscular damage and inflammation in horses competing in endurance races ranging from 100km to 160km. Nine national and international endurance horses were subdivided in two groups in a partial crossover design: one group received a commercially-available balanced supplement (CS), and the other group a supplement enriched with antioxidants and selected fatty acids (ES). Each group received its daily supplement during the 4 weeks that preceded the "test" race. Afterwards the supplements were inverted and the same supplementation scheme was followed. All the horses' diets were standardized throughout the protocol. Blood samples were taken for each horse and each supplement, at rest before the supplementation period and after 4 weeks of supplementation prior to and after an endurance race.

The following parameters were analysed:

- cellular damage markers (CK, LDH, AST, GGT),
- oxidative stress markers (GPx, SOD, GSH, GSSG, plasma lipid hydroperoxides (Pool), plasma oxidised proteins (Protox), plasma lipophilic antioxidant capacity (ACL), plasma hydrophilic antioxidant capacity (ACW)),
- inflammation markers (fibrinogen, haptoglobin, white blood cells count, myeloperoxidase, matrixmetalloprotease),
- haematology, red cell membrane fatty acids, total plasma proteins, urea and glycaemia.

In the ES supplemented group, ACL significantly increased ($p = 0.026$) while ACW showed a non-significant increase. A raise in the N3 profile of the erythrocytic membrane was also observed in the ES group, particularly in eicosapentaenoic acid (EPA) which showed a significant raise ($p = 0.03$). The ratio of arachidonic acid on EPA (AA/EPA) (also considered as a platelet agregability index) was significantly decreased in the supplemented group ($p = 0.02$). There were no variations in markers of muscular damage or inflammation prior to racing (see Table), however post endurance race analyses are currently running.

The ES supplement has been shown to improve the horses' antioxidant status and to modify the N3 fatty acids profile of red cell membranes. A relation between these

modifications and markers of inflammation and muscular damage could be established from the ongoing post-race laboratory tests.

In the future, the effects of such a supplement on performance and recovery of both healthy and diseased horses could be investigated. The effects of the N3 fatty acid supplements on cellular membrane fluidity and integrity (erythrocytes, muscular cells,…) and perfusion could also be studied as well as its correlation to heart rate during exercise and recovery.

Table - Muscular enzymes and inflammation proteins (means ± SD) in endurance horses before (T0) and after (T1) 4 weeks of administration of a classical supplement (CS) or a supplement enriched with antioxidants and selected fatty acids (ES) and the significance of time and supplement's effects on these parameters.

		t0		t1		Significance	
Analysed parameters		**CS**	**ES**	**CS**	**ES**	**Time effect**	**Supplement effect**
Muscular enzymes	CK (UI/L)	259 ± 121	230 ± 120	246 ± 58	198 ± 24	p = 0,558	p = 0,808
	LDH (UI/L)	465 ± 83	433 ± 84	565 ± 222	458 ± 102	p = 0,146	p = 0,371
	γGT (UI/L)	13,2 ± 1,8	13 ± 1,3	15,7 ± 6,6	17,3 ± 10,2	P = 0,256	p = 0,529
	AST (UI/L)	393 ± 176	364 ± 194	360 ± 131	311 ± 60	p = 0,445	p = 0,862
Inflammation proteins	Fibrinogen (g/L)	1,87 ± 0,22	1,84 ± 0,21	1,82 ± 0,39	1,92 ± 0,23	p = 0,86	p = 0,386
	Haptoglobin (mg/L)	690 ± 292	709 ± 82	864 ± 206	835 ± 170	p = 0,008	p = 0,613
	MPO (ng/ml)	341 ± 159	286 ± 64	340 ± 94	377 ± 215	p = 0,223	p = 0,544
	MMP 2	3781 ± 2370	2822 ± 1925	3477 ± 2164	2901 ± 2216	p = 0,275	p = 0,076
	MMP 9	2889 ± 1541	2101 ± 817	2789 ± 1551	1668 ± 621	p = 0,156	p = 0,363
	Pro-MMP9	2833 ± 966	1960 ± 848	2806 ± 1120	2018 ± 826	p = 0,883	p = 0,691

CK : creatinekinase ; LDH : lactate dehydrogenase ; γGT : gamma-glutamyl-transferase ; AST : aspartate aminotransferase

MPO : myeloperoxydase ; MMP : matrixmetalloprotease

HOW TO MAKE A SUCCESS OF AN INFILTRATION ANAESTHESIA IN AMBULATORY DENTISTRY?

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The use of analgesia at the time of potentially painful procedures develops quickly these last years in veterinary medicine. The extraction of wolf teeth for example is an intervention of routine. However, it is a relatively painful if one judges some by the reactions of the even sedated patients. Analgesia remains however little practised, for reasons related on the practice, time necessary or sometimes ignorance... however the technics exist. At the time of dental extraction in the stranding horse, analgesia can be practised by general way, nerve blocks or infiltration anaesthesia. The latter appears effective but its realization can present some difficulties. The latter makes it possible to decrease the anesthetic requirement, the time of onset and the complications. It appears effective but its realization can present some difficulties.

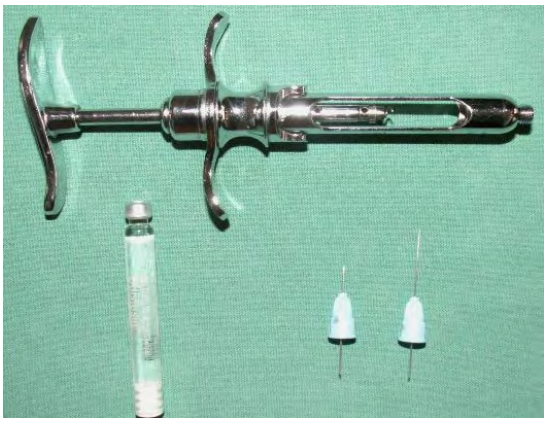
It is true that the first attempts at realization of an infiltration anaesthesia can be disappointing: realization seeming as painful as the extraction itself, “escapes" of anaesthetic out of the site of injection or rather low effectiveness is with go... We will show how to mitigate these difficulties in particular by the use of a syringe with cartridge dedicated to this use.

Two points are important, the site of injection and the way of injecting the anaesthetic. The injection should be submucosal just proximal to the mucogingival junction. In practice, it should be remembered that the infiltration anaesthesia results from the diffusion of the agent not only towards soft tissues but also towards the periostum and the bone; thus, it is indicated for the extractions of deciduous incisor and wolf teeth but not cheek teeth. It is often necessary to inject buccal side and palatine or lingual side by holding account the position the root (the ideal being also to be able to evaluate its length by radiography). The infiltration anesthesia is theoretically practiced in two steps: one anaesthetizes the surface of the mucosa to desensitize it for injection: a described method consists in placing a drop or two of the injectable anesthetic on the surface of the mucosa; we moreover tested the use of a cream for dental use to 7,5 % of lidocaïne Xylocontact ™, of a spray of anaesthetic of contact Pressicaïne fluid ™ to 12,5 % of lidocaïne and of an aerosol for cryoanaesthesia Friljet ™ (drugs of human use); then one carries out the injection under the mucosa itself : the difficulty is here in the fact that the mucous tightly adheres to the periostum and that the injection must be carried out with a strong pressure especially at oral side. This often led to a loss of product. The use of a syringe with an end allowing the locking of the needle (Luerlock) is recommended; we also tested a syringe for dental use for cartridges of 1,8 DC with aspiration (standard 2a Anthogyr instruments) on which specific needles (triple bevel, silicon coating) are screwed. The penetration must be progressive and tangential.

The use of the syringe of dental use allows a pressure injection without loss of product. Gotten analgesia is generally sufficient to carry out an extraction of milk incisor or wolf teeth without reaction on behalf of the patient. In general a cartridge is enough for a wolf tooth (less as 2ml) for the buccal injection and the possible palatine recall. The time of appearance of analgesia is a very important point and impatience is one of the principal causes of failure. It is 1 to 2 minutes for the insensibilization of the mucosa except with the cryoanaesthesia (the effect is immediate) then 5 to 10 minutes for the anaesthesia itself. It is thus advisable to carry out the anaesthesia then to proceed with other dental procedures before carrying out the extraction. In practice we do not carry out any more the phase of insensibilization; indeed, with the proviso of using the dedicated material and of introducing the needle delicately and tangentially, there is only little even no reaction to the needle introduction. Concerning the equipment of injection, a syringe with locked needle is very useful; the advantage of the syringe for dental use is for us a better ergonomics and to have at disposal a variety of anaesthetic solutions (various agents, with epinephrine or not...). The analgesia is generally sufficient to carry out an extraction of milk incisor or wolf teeth under good conditions; the rare failures and the relative failures seem rather due to a bad site of injection or an insufficient

quantity of anaesthetic really injected. We did not test the duration of analgesia but it is sufficient in ambulatory dentistry. In human practice the duration time is about 30 minutes.

Since we proceed in this way of carrying out the infiltration anaesthesias, we make some for each extraction of wolf teeth and the ratio cost, time needed vs. efficacy is positive and improves with the experiment. Remain that best comfort so much in the realization than in the effectiveness would be the use of blocks of the maxillary or mandibular nerve; if those remain essential for the analgesia of the cheek teeth, in the case of the extractions of routine last time seems too long (preparation of the site of injection, time of appearance of analgesia) and the possible complications (dependent on the injection itself and possible wounds due to the insensibilization of a wider territory) even if they seem anecdotic. The infiltration anaesthesia seems well adapted to the routine dentistry. It brings more comfort both for the patient and the operator. The techniques described here make it possible to optimize the realization and the result of it.



RADIOGRAPHIC AND ULTRASONOGRAPHIC EVALUATION OF THE PODOTROCHLEAR APPARATUS IN AMERICAN QUARTER HORSES SUFFERING FROM NAVICULAR DISEASE

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The objective of this study was to evaluate the podotrochlear apparatus using a transcuneal approach in American Quarter horses suffering from navicular disease. 22 horses from the area of Botucatu and Bauru in São Paulo State were evaluated. On those patients, radiographic and ultrasonographic evaluations were performed. On radiograph, different kinds of synovial invaginations, calcifications of the podotrochlear bursa and deep digital flexor tendon, enthesiophytes, osteophytes, and alterations in the cortical-medullary margin were observed. On the ultrasound scans, alterations of the podotrochlear bursa, adherences and irregularities at the surface of the deep digital flexor tendon, diminution of the digital cushion, calcifications of the impar distal sesamoideal ligament, and irregularities of the flexor surface of the distal sesamoid bone were observed. Radiographic and ultrasonographic alterations compatible with navicular disease were present in all of the patients. Ultrasonography using transcuneal approach was a practical and efficient method for evaluating podotrochlear apparatus injuries in equine, thus being a complementary method to radiography.

KEY WORDS: transcuneal ultrasound, navicular disease, lameness, podotrochlear apparatus.

THE EFFICACY OF A COMMERCIALLY AVAILABLE GASTRIC SUPPLEMENT FOR THE TREATMENT AND PREVENTION OF EQUINE GASTRIC ULCER SYNDROME (EGUS)

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Introduction

Equine Gastric Ulcer Syndrome (EGUS), is a widely prevalent problem in many animals (Murray *et al.,* 1989; Hartmann and Frankeny, 2003; Nieto *et al.,* 2004; Chameroy *et al*., 2006). It has been suggested that prevalence of the condition amongst racing Thoroughbreds may be as high as 90% (Murray *et al.,* 1989), and up to 51% of non-racing horses have been classed as suffering from EGUS (Bullimore *et al.,* 2001). Clinical signs of the disease include weight loss, diarrhoea, decreased appetite, behavioural changes, decreased performance and colic (Murray *et al.,* 1989; Murray *et al*., 1996; McClure *et al*., 1999; Vatistas *et al*., 1999; Bullimore *et al.,* 2001; Nieto *et al.,* 2004).

Purported reasons for the development of EGUS are wide ranging. Bullimore *et al.,* (2001) suggest that ulceration arises from imbalances between defensive mechanisms and aggressive factors within the stomach. Aggressive factors are assumed to be excess acid secretion, however, investigations into human ulceration have shown many patients display near normal acid secretion (Grossman *et al*., 1963).

Mucus secretion is acknowledged as the main defensive agent within the stomach and can protect against autodigestion, bacterial infection etc (Quigley and Turnberg, 1987; Bullimore *et al.,* 2001). Mucus contains the glycoprotein mucin and it is postulated that abnormal variations and molecular characteristics of mucins can compromise permeability of mucus gels, and therefore mucosal defence (Jass and Roberton, 1994; Filipe and Ramachandra, 1995; Bullimore *et al.,* 2001).

It has recently been noted that a gene homologous to the human $MUC5_{AC}$ is expressed within the equine stomach in both glandular and non-glandular regions (Bullimore *et al.,* 2001). $MUC5_{AC}$ is responsible for the production and expression of neutral mucins, and abnormal production of the gene may lead to reduced defence, as highlighted in humans (Bullimore *et al.,* 2001). This could indicate an area for possible manipulation, initially through the synthesis of mucins.

The aim of this study was to investigate the efficacy of a commercially available gastric supplement, purported to increase mucin production and combine with natural bicarbonate production to produce defensive gels within the stomach.

Materials and Methods

Animal Management

26 National Hunt Thoroughbred Racehorses were used in the study. All were aged between 4 and 10 years, and all resided on the same yard. All horses were maintained on their original diet, and all horses remained in their normal training routine. Horses were housed in stables, and were each turned out to grass for 1 hour per day. No additional supplements or conflicting medical treatments were given throughout the duration of the trial.

Animal Recruitment to the Trial

All animals used in the trial were routinely scoped for veterinary purposes. All horses recruited to the trial were to be scoped regardless of the trial, at the request of the trainer. Horses were initially scoped and divided into three categories depending on their ulcer score; Clear (ulcer score 0), n = 5, Mild to Moderate (ulcer score 1-2), n = 14 and Severe ulceration (ulcer score 3-4), n = 7, within which horses were further divided into supplemented (n = 14) and non-supplemented (n = 12) groups. Each group (treatment and control), were blocked to allow equal numbers of each ulcer score to be allocated to either treatment or control. Blocked animals were then assigned to treatments groups randomly.

Gastroscopy

Gastroscopic examination was performed after a fasting period of 20 hours and water was withheld for 4 hours prior to scoping. A panel of veterinarians scored each horse

according to the ulcer scoring system as set out by EGUS Council, and an average score was then given. Horses were re-scoped and graded after six weeks.

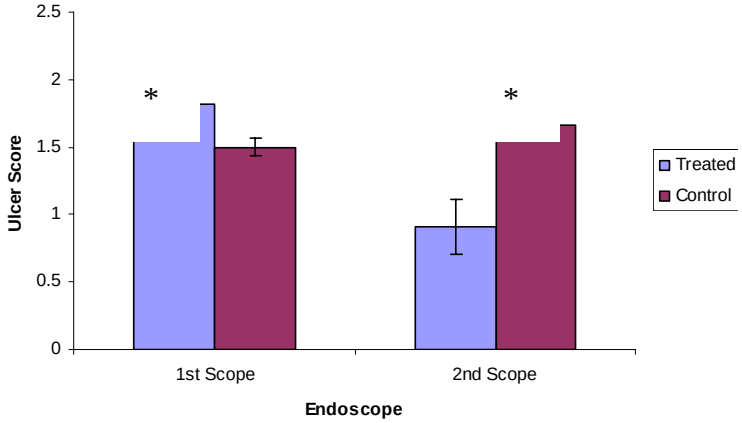
Supplementation

The commercially available supplement, GNF™ was administered to treated horses at recommended levels of 100g/day, split between three feeds, for a period of 6 weeks.

Results

The data was tested for normality using the Kolomorgorov-Smirnov Test and found to be significant (P<0.05). It was therefore assumed that the data was not normally disturbed, thus a non-parametric test was used. Data were statistically analysed in SPSS using a two tailed Wilcoxon test. Overall scores (regardless of category) showed a significant reduction over the trial in supplemented horses, compared to control horses (p<0.05) (See figure 1). Statistical analysis on individual groups was not possible due to resultant low numbers in each, although supplemented horses showed a trend towards reduced ulcer scores.

Figure 1 shows differences in ulcer score between the first and second gastroscopy exams, in both supplemented and control groups. * indicates significant differences between the two groups (P<0.05).



The average ulcer score for the supplemented group decreased from 1.82 to 0.91, whilst the average score for the control group increased from 1.5 to 1.66.

Conclusion

This study has proven the effectiveness of GNF™, as a nutritional adjunct in the management of equine gastric ulceration. A significant difference (P<0.05) in ulceration score was recorded after 6 weeks of supplementation, with the treated group showing significant improvement in comparison to the control group.

It is postulated that several of the ingredients of the supplement would have contributed to the observed results. Glutamine supplementation has proven to increase intestinal performance (Yan and Qiu-Zhou, 2006), and has been found to decrease over-expression of pro-inflammatory genes, thus leading to a reduction in intestinal damage of rats receiving acetic acid supplementation (Fillmann *et al*., 2007). Threonine is an essential amino acid and studies have shown that restriction of this nutrient may limit intestinal mucin synthesis and reduce gut barrier function (Hamard *et al.,* 2007; Faure *et al.,* 2005). GNF also contains both calcium and magnesium which are recognised as alkaline providers, and have been shown to increase intestinal mucosal integrity (Wang, 2000).

Due to the exceptionally high prevalence of EGUS in the thoroughbred racehorse sector and the limited availability of clinically proven nutritional feed supplements on the market, the results of this research will provide the industry with an effective nutritional tool in the management of EGUS.

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ABOUT CHONDROIDS

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Introduction

Streptococcus infections, mentioned as far back as the 13th century (5.6), are described by Solleysel as affecting most of the young horses.

They are accompanied with fever,nasal discharge, oedema of the sub mandibular lymph nodes, developing into abcess with cough. Morbidity ranges from 30 to 100% depending on the authors, mortality is estimated at 10% (6)

Complications are possible (19 to 42 % of the cases) mainly bronchopneumonia, or less common metastatic abcesses, guttural pouch empyema, chondroids, myocarditis, endocarditis, purpura haemorrhagica.

We're going to describe a chondroid case resulting from a chronical bilateral empyema of the guttural pouches as well as its treatment.

Case history

A two-year-old mare from a group that had been affected 6 months ago and cured with trimethroprim-sulfamides per os (AVEMIX®) is presented to find out the origin of a bilateral purulent and sporadic discharge that has been affecting the mare for 4 months resisting to the administration of cefquinome (COBACTAN®) and TM-S (7 kg in all !)

Clinical examination

Temperature, cardiac and respiratory frequencies are normal. The submandibular zone, the throat, the Viborg triangle don't show any distortion.

Tracheal and thoracic auscultations are normal.

Further examinations

The profile x-ray of the Viborg zone shows an opaqueness of the guttural pouches. The level ligne is irregular which is compatible with an empyema of the guttural pouches.

The endoscopy of the upper respiratory tract reveals a moderate ventral displacement of the upper part of the pharynx, some pus on the pharyngeal ostiums, no abnormality in the trachea.

The endoscopy of the guttural pouches shows a bilateral empyema. The pus is liquid and lumpy on the surface. The bacteriological examination of a sample reveals a streptococcus equi souche.

The blood examination shows a neutrophilic leucocytosis (GB /12/900 uL).

Treatment

-A guttural pouches lavage is made with 1,5 of physiological solution, shows chondroids that is to say pus concretions, smooth, disk-shaped and mobile. The cavity wall is smooth and bright.

-Through the lavage, only a few chondroids are evacuated, particularly thanks to the induced cough.

-An antibiotic therapy -peniciline-procaÏne twice a day (DEPOCILLINE® 20 000 UI/kgIM) is started.

-A N-acetyl-cysteine solution (EQUIMUCIN® 2g dissolved in 20ML of physiological solution) is instilled into each of the guttural pouches to fluidify the condroids. This treatment soften them but at the same time it creates a large inflammation of the guttural pouches wall, which appears rough, thick and inflamed 48 hours later. It is stopped.

-A tripode extractor (OLYMPUS POLYGRAG FG 600U) is used to extract the chondroids. Unfortunatly, due to their consistency it only succeed in reducing their size without extracting them. It is therefore stopped.

-Eventually, refering to Dr GUIGUERE (AVEF 2005 Angers), we introduce an endotracheal tube 14mm wide into the guttural pouches under endoscopic control and water is flushed through with a reflux pump. The diameter of the tube is too small to allow the

chondroids through, however the water flow together with the induced cough evacuate a fair amount of them.

Three washes are then conducted two days apart; they are not sufficient to empty the guttural pouches, but because of the inflammation of the parotid areas, submandibular zone and the pouches wall, complicated with respiratory noises, we have to interrupt all act for 10 days. Dexamethasone (dexadreson® 0,06 mg/kg) is administered for three days running and to limit the inflammation, the antibiotic therapy is carried on.

After those 10 days the cardiac and respiratory frequencies, temperature are normal. The mare however suffers from a submandibular lymph nodes hypertrophy and a serous sweatting begins.

An echography shows the presence of a phlegmon, and liquid in the guttural pouches with dense elements. This area is then massed for 5 days with a revulsive (CAPSIGEL®) in order to help the phlegmon to mature.

An endoscopy confirms that pus and chondroids remain in the pouches, the wall however is clearly less inflamed.

Two further lavages allow to evacuate all chondroids. The antibiotic therapy is stopped when the absence of pus in the pouches is confirmed 48 hours later.

Development

The distortion of the parotid area has been reduced by half within a fortnight : three weeks later a fistula about level with the right Vibord triangle appears : it heals up within a month.

Meanwhile the mare doesn't present any discharge, fever or dysphagia.

Discussion

The chondroids described here come together with a chronic empyema of the guttural pouches.

Their presence in the guttural pouches is reported as being rare in the litterature.

After a survey carried out on 91 horses suffering from empiema of the guttural pouches Carter concludes that the persistence of an empyema in the gutturale pouches is not a predisposing factor to the formation of chondroids which are significally associated with swollen guttural pouches or lymphatic nodes (33% of the cases). He mentions a dysphagia in 9% of the cases and an affection of the cranial nerves in 2% of the cases.

According to Seahorn, the presence of chondroids is often associated with discharge but no exterior sign of the affected pouches.

A local treatment has been started for its propriétés mucolytiques in order to help the drainage of the chondroids. The dissolved crystalline form used in this case led to a severe inflamation of the wall of the guttural pouches, hence its abandonned. The use of an injectable solution of (10 to 20%) depending on the authors, is reported in local instillation (Cadoré,Sweeney JVIM, Verheyen). However the outbreak or aggravation of a rash of the guttural pouches wall have been mentionned.

Aggressiveness and repetition of the lavages led to the evacuation of all the chondroids through the natural routes.

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DYNAMICS OF FREE RADICAL PEROXIDATION PROCESS IN PREGNANT MARES

Larisa Karpenko, E.Selimova, A.Bakhta, A.Andreeva
(abstract is submitted in Russian)

ДИНАМИКА ПРОТЕКАНИЯ ПРОЦЕССОВ СВОБОДНОРАДИКАЛЬНОГО ОКИСЛЕНИЯ У ЖЕРЕБЫХ КОБЫЛ

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По данным ряда исследователей период беременности сопровождается усилением процессов перекисного окисления липидов, что отражает повышенная концентрации продуктов перекисного в сыворотке крови беременных животных. Наряду с этим отмечают увеличение активности антиоксидантных ферментов, благодаря чему происходит компенсирование более интенсивно протекающих процессов свободнорадикального окисления.

Однако при различных патологиях беременности наблюдается некомпенсированное возрастание перекисного окисления липидов и недостаточность антиоксидантной системы, что приводит к развитию гипоксии, вплоть до внутриутробной асфиксии плодов.

Нами на кафедре биохимии СПбГАВМ было проведено исследование, целью которого являлось определение концентрации в сыворотки крови продуктов перекисного окисления липидов жеребых кобыл. Исследование проводили на клинически здоровых жеребых кобылах (первая половина беременности) в возрасте от 6 до 12 лет (n = 5). Забор крови осуществляли с соблюдением правил асептики и антисептики. В крови определяли концентрацию диеновых конъюгатов и диенкетонов по методу Плацера с соав., концентрацию малонового альдегида тестом с применением тиобарбитуровой кислотой. Результаты исследований представлены в таблице.

Содержание продуктов перекисного окисления липидов (малонового диальдегида, диеновых конъюгатов, диенкетонов) в крови жеребых кобыл (M ±n)

Таблица.

Показатель	Ед.из.	Содержание продуктов ПОЛ в крови здоровых лошадей	Содержание продуктов ПОЛ в крови жеребых кобыл
Диеновые конъюгаты	едА /мл	0,23 ±0,065	0, 35 ±0,03
Диенкетоны	едА /мл	0,12±0,05	0,21±0,06
Малоновый диальдегид	мкмоль/л	17,5±1,46	39,4±2,05

Из данных таблицы следует, что при беременности наблюдается усиление процессов перекисного окисления липидов, на что указывает повышение в крови жеребых кобыл концентрации диеновых конъюгатов, диенкетонов, малонового диальдегида - маркеров интенсивности процессов перекисного окисления липидов. Это необходимо учитывать при содержании жеребых кобыл.

Summary

Dynamics of course of processes of processes peroxide of oxidation fats at pregnant mares.

In article are given experimental according to processes peroxide of oxidation at pregnant mares.

PERCENTAGE OF SELEN AND THYROID HORMONES IN SERA OF HEALTHY HORSES

L. Karpenko, R. Selimov
(abstract is submitted in Russian)

СОДЕРЖАНИЕ СЕЛЕНА И ТИРЕОИДНЫХ ГОРМОНОВ В СЫВОРОТКЕ КРОВИ ЗДОРОВЫХ ЛОШАДЕЙ

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Селен - это биологически активный микроэлемент, входящий в состав большинства гормонов и ферментов и связанный таким образом со всеми органами и системами. Его поступление наряду с другими микроэлементами необходимо для поддержания нормальной жизнедеятельности организма. В естественных условиях Se поступает в организм животных главным образом в виде селенсодержащих аминокислот - селенометионина (Se-Met) и селеноцистеина (Se-Cys). Искусственное снабжение организма селеном при его алиментарном дефиците может осуществляться в форме селенита или селената натрия. Как органический, так и неорганический селен легко всасывается в желудочно-кишечном тракте.

Известно, что селен является одним из важных пищевых антиоксидантов, то есть агентом, способствующим детоксикации реакционноспособных производных кислорода в организме. Во-первых, селен является антиоксидантом непрямого действия, то есть те его соединения, которые поступают с пищей, сами по себе свойствами антиоксидантов не обладают. Более того, некоторые из соединений селена, особенно при их передозировке, могут проявлять прооксидантное действие. Активными биоантиоксидантами являются только селенопротеины, синтезируемые в организме. Во-вторых, наряду с антиоксидантным действием, ряд селеноэнзимов обладает и другими, весьма важными видами биологической активности.

Селен участвует в синтезе кофермента Q10, имеющего большое значение для функционирования сердечной мышцы, защищает от кислородной недостаточности. Селен предотвращает разрушение печени; соединяясь с солями тяжелых металлов, селен выводит их из организма. Кроме того, в сочетании с витамином Е и β-каротином восстанавливает и укрепляет иммунную систему.

При дефиците селена в рационе питания в организме могут возникать следующие изменения: снижение иммунитета, повышение склонности к воспалительным заболеваниям, снижение функции печени (анемия, некроз), болезни кожи, ускорение атеросклероза (как следствие кардиопатия, инфаркт), катаракта, репродуктивная недостаточность, замедление роста, онкологические заболевания (рак желудка, простаты, толстого кишечника, молочной железы).

Беломышечная болезнь, мышечная дистрофия характерны при дефиците селена, а также витамина Е. В скелетных мышцах и сердце развивается некроз и дегенерация тканей. У больных животных наблюдаются скованность движений, хромота и сердечная недостаточность.

Другие симптомы - вялость, потеря веса и диарея - могут быть связаны с гормонами щитовидной железы. Щитовидная железа производит два тиреоидных гормона, отличающихся лишь наличием или отсутствием одного дополнительного атома йода в молекуле — тироксин (Т4) и трийодтиронин (Т3). При этом тироксин является относительно малоактивным тиреоидным гормоном, фактически — прогормоном, и слабо связывается непосредственно с рецепторами тиреоидных гормонов в тканях.

Перед тем, как оказать действие на клетки органов-мишеней, большая часть тироксина непосредственно в клетках конвертируется в биологически активную форму — трийодтиронин. Этот процесс происходит при участии ферментов дейодиназ. Селен как раз способствует активации дейодиназ в щитовидной железе, печени, почках, гипофизе и тем самым превращению Т4 в Т3. При дефиците микроэлемента селена в организме или при генетическом дефекте монодейодиназы, предопределяющем её пониженную активность в тканях, развивается состояние недостаточности гормона щитовидной железы, несмотря на кажущийся нормальным уровень Т4 в плазме крови. Что касается антиоксидантной защиты щитовидной железы, дефицит селена способствует действию свободных радикалов (перекиси водорода, образующейся в избыточном количестве в гиперплазированной щитовидной железе) на липофильные мембраны, приводя к развитию некроза, фиброза, атрофии железы.

Нами было проведено исследование группы жеребят, а также группы лошадей в возрасте 4-6 лет на содержание тиреоидных гормонов и селена в сыворотке крови (таб 1)

Таблица 1

Содержание тиреоидных гормонов и селена в сыворотке крови клинически здоровых лошадей

	T_3, нмоль/л	T_4, пмоль/л	Se, мкг/мл
Группа 1 (жеребята)	8,2±1,07	16,3±0,34	0,267±0,07
Группа 2 (лошади 4-6 лет)	7,9±0,81	15,8±0,40	0,292±0,076

При данном исследовании не была выявлена зависимость уровней гормонов щитовидной железы и селена в сыворотке крови от возраста животных. Однако предполагается, что селенодефицитные состояния у лошадей могут биохимически проявляться не только пониженным содержанием собственно селена, но и изменением уровня T_3 в сыворотке крови; это может иметь значение при диагностике недостатка селена в организме. Помимо этого, результаты исследований могут иметь значение для надлежащего нормирования рациона кормления по селену и другим микроэлементам.

INFLUENCE OF "HELAVIT" USING TO PERCENTAGE OF THYROID HORMONES IN HORSES

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(abstract is submitted in Russian)

ВЛИЯНИЕ ПРЕПАРАТА «ХЕЛАВИТ» НА СОДЕРЖАНИЕ ТИРЕОИДНЫХ ГОРМОНОВ В ОРГАНИЗМЕ ЛОШАДЕЙ

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Биологическое действие тиреоидных гормонов распространяется на множество физиологических функций животного организма. В первую очередь, тиреоидные гормоны обеспечивают постоянство температуры тела, регулируя поступление кислорода в клетки. Кроме того, они регулируют интенсивность углеводного обмена (усиливают образование глюкозы), жирового обмена (усиливают распад липидов), белкового обмена (усиливают синтез белковых молекул). В частности, рост и развитие клеток половых желез, костной ткани, нервной системы во многом зависят от действия тиреоидных гормонов.

Известно, что двумя основными гормонами щитовидной железы являются трийодтиронин (Т3) и тироксин (Т4). Следует отметить, что большее биологическое значение имеет Т3, активность которого превышает активностьТ4 в 4-5 раз. Т4 можно считать предшественником Т3, так как в норме Т4, в особенности в периферических тканях, переходит в Т3 под воздействием дейодиназ. Если же конверсия Т4 в Т3 затруднена, то это приводит к гипотиреозу различной степени тяжести.

Препарат, примененный нами в опыте, - «Хелавит» - представляет собой комплекс микроэлементов (марганец, цинк, кобальт, селен, железо, медь, йод), связанных в виде хелатных соединений. Преимуществом этого вида соединений является их лучшая усвояемость и биологическая доступность.

В 2007 г. мы проводили испытания препарата «Хелавит». В данном опыте группа из пяти лошадей в течение месяца получала препарат - 20 мл на голову ежедневно. Перед началом и после окончания опыта у лошадей взяли пробы крови. В числе прочих провели исследование показателей функционирования щитовидной железы (Таблица 1)

Таблица 1

	До применения препарата	После применения препарата
Т3, нмоль/л	8,0±2,1	1,1±0,22
Т4, пмоль/л	16,0±0,68	13,57±1,3
ТТГ, ММЕ/л	0,15±0,056	0,065±0,01

Спектр действия препарата «Хелавит» весьма широк, в этой же статье мы обратим внимание непосредственно на влияние препарата на функционирование щитовидной железы. Как упомянуто выше, превращение Т4 в Т3 осуществляется под действием дейодиназ. Активация же дейодиназ происходит под влиянием селена. Мы пришли к выводу, что селен, поступивший в организм в составе препарата, вызвал снижение уровня Т4. Что касается снижения концентрации Т3, то это может быть связано с тем, что первичный отбор проб крови производился в более холодное время года, нежели вторичный.

Итак, комплексный препарат «Хелавит» может послужить средством профилактики нарушений функции щитовидной железы. В последующих публикациях планируется осветить и другие эффекты от действия данного препарата.

THE DISTRIBUTION AND PROPHYLAXIS OF HELMINTHOSES OF HORSES IN LATVIA

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The horse helminthoses are widely spread in many countries and they cause serious losses in horse-breeding. Due to their impact, the foals fall behind in growing and development, the working and sporting abilities of horses are decreased, they become more susceptible to other diseases and even may result in death of the animal. In order to restrict the spread of helminthoses, different control programmes are suggested where an important role plays animal welfare, a regular control of the epizootic situation, and dehelmintisation is carried out in due time.

The goal of this work was to investigate the distribution of horse parasitoses in Latvia, and to determine the efficiency of some anthelmintic preparations in the cases of strongylatoses and parascaridosis in horses.

The distribution of horse helminthoses was determined according to the results of investigations performed at the Laboratory of Parasitology of the Faculty of Veterinary Medicine of the Latvian University of Agriculture, as well as the survey data of the diagnostic examinations the state veterinary labarotories during the period from 1965 till 2007 were analysed.

The efficiency of anthelmintics was determined by the results of helminthocoprological examinations on spontaneosly infected animals. McMaster or the standartised Fuelleborn methods were used to examine the horse faeces before and several times after the dehelmintisation of animals. The preparations were administered orally in doses accordingly the instruction in the form of solution (ivomec), bolus (piperazine adipinate), or paste (febantel, equalan, tridon-g, telmin + trichlorfon, vermitan) one or two days in succession in the morning before the animal feeding, or individually to every animal in the morning feeding time mixed in the concentrated feed (rintal, panacur, moranteltartrat). During the time of the experiment, the animals were observed clinically.

It was found out that strongylatoses are the most widespread horse helminthoses in Latvia. This infection is registered in all regions of the state. Its average extensity varied in the range of 49.7 - 82.5%. The small strongyles of the *Cyathostomidae* family were found more often. Parascaridosis was diagnosed in 1.2 – 8.2%, oxyurosis < 3.3%, anoplocephalidosis and strongyloidosis < 0.5% of horses. Some horses were infected with fasciolosis, dicrocoeliosis, echinococcosis, cysticercosis, probstmayriosis, chabronemosis, draschiosis, onchocercosis and setariosis.

In the cases of the horse strongylatoses, most effective (96.6%-100%) were the following anthelmintics: febantel, equalan, ivomec, tridon-g, panacur and vermitan, but in the cases of parascaridois most effective were febantel, ivomec and telmin+trchlorfon. The efficiency of moranteltartrate and piperazine adipinate was a little lower.

EFFECTS OF LONG TERM USE OF ANTHELMINTICS ON NEMATODE POPULATIONS IN WORKING EQUINES

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Introduction

In Morocco, working equids are heavily infected with gastrointestinal helminths and bots. Anthelmintic treatments are rarely implemented by owners due to their unaffordable cost and ignorance of their benefit. Apart from free health care exclusively provided by a welfare association, SPANA (The Society for the Protection of Animals and Nature) in limited regions, the rest of equids remain exposed to heavy parasitic infections and a nutritional deficit. Infected equids may suffer from low weight gains and reduction of performance at work, which result in the early demise of animals. The majority of equines slaughtered to feed zoo carnivores in Rabat showed signs of parasitic infestation and concomitant pathologies.

Over the last decade, SPANA has used anthelmintic treatment systematically in any equid showing clinical symptoms that might be related to parasitic infestation. These include signs of colic, weight loss, cough, diarrhoea and poor body condition. Recently, a special concern was expressed about the impact of use of anthelmintrics in working equids, over the long term. A comparative study was conducted to evaluate the infection level with gastrointestinal nematodes of horses in souks where SPANA provide health care and in others where it is not represented.

Material and Methods

A fecal survey was performed in three regions of Morocco (Chemaia, Khemisset and Midelt) where equine husbandry and practices are similar. As SPANA does not operate in the entire country, two villages of each region were selected, one a part of their action zone and another where health care is not provided. At the weekly market, fecal samples was taken directly from the rectum of animals and stored at +4 °C, until they are processed in the laboratory. A total of 217 equids of different species (horses, mules and donkeys) took part in this survey. All animals were systematically treated with ivermectin and controlled 15 days later to evaluate efficacy of treatment.

Fecal egg count was evaluated using the Mc Master technique. The results are given as number of eggs per gram of feces (epg).

Results and Discussion

In a non SPANA covered zones, prevalence of infection with gastrointestinal nematodes was 100% in working equids with a mean epg of 2900 to 5200. In Chemaia, egg counts reached maximum of 17200 epg (Table I).

Table I. Nematode egg counts (epg) in a non SPANA covered village.

Region	nb. animals	nb infected	% infection	epg	
				mean	range
Chemaia	21	21	100	5200	700 - 17200
Khemisset	19	19	100	3200	400 - 16200
Midelt	31	31	100	2900	300 - 14500

In villages where SPANA operates, the prevalence was significantly lower with a mean egg count of 680 to 1050 epg. Nematode egg output was low, except in Midelt where few animals had high egg counts (Table II).

Table II. Nematode egg counts (epg) in a SPANA covered village.

Region	nb. animals	nb infected	% infection	epg	
				mean	range
Chemaia	22	21	95	1050	100 - 3800
Khemisset	24	23	96	680	400 - 4250
Midelt	100	85	85	1000	300 - 17000

This survey showed that among 217 equids, 93% were infected with digestive strongyles, 26% with ascarids and only 8% with *Oxyuris*. The mean egg output of *Parascaris* was 622 epg and only 133 epg for *Oxyuris*. Infection with pinworm was encountered in all age classes, whereas *Parascaris* was limited to horses below 5 years old.

Strategic treatments organized by SPANA in these regions helped in maintaining low infection level in their zones of action, while the infestations remained high in regions where parasite control is not provided. Ivermectin used in this program had an efficacy over 99% against the digestive strongyles and ascarids, and 100% against *Oxyuris*. It was assumed that, at present resistance to ivermectin has not been yet established.

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LYME BORRELIOSIS AND ANAPLASMOSIS IN HORSES IN AUSTRIA: A SEROPREVALENCE STUDY

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The aim of this retrospective study (2000 –2007) was to determine the prevalence of *Borrelia burgdorferi.* and *A. phagocytophilum* infection in austrian horses. Therefore we examined the existence and level of IgG-antibodies against *Borrelia burgdorferi* and *A. phagocytophilum* in blood samples by an indirect immunofluorescence assay (IFAT) using *Borrelia burgdorferi* and *A.. phagocytophilum* as antigen. We examined blood samples for antibodies against *Borrelia burgdorferi* in 396 horses, for antibodies against *A. phagocytophilum in* 148 horses and in 45 samples we were looking for antibodies against both causal agents. A microscopic examination for *A. phagocytophilum* inclusion bodies in granulocytic neutrophils was done in 71 cases. In blood samples antibodies against *Borrelia burgdorferi* were found in 172 horses by IFAT. As 44 horses had an titer (1:32) below the cut off (1:64) we considered 128 horses as seropositiv. Antibodies against *A. phagocytophilum* were found in 59 blood samples by IFAT. Twelve horses had an titer (1:40) below the cut off (1:80),so that 47 horses were considered as seropositiv. In the case where we examined blood against both causal agents, we were able to find in 11 animals antibodies against *Borrelia burgdorferi* and *A. phagocytophilum*. By microscopic examination we found in 6 of 71 horses inclusion bodies of *A. phagocytophilum* in circulating neutropihls granulocytes. All these 6 horses showed an anemia and a marked thrombocytopenia. The majority of the horses affected with *Borrelia burgdorferi* and *A. phagocytophilum* lived in, or near by, regions, known as ticks areas. Serological tests are often used to diagnose both. infections. However, patients in the acute phase of the disease are frequently seronegative. Therefore, serological tests always should be done on paired serum samples collected during the acute phase and 14 to 20 days later. Lyme disease and Anaplasmosis are diagnosted with increasing frequencyin Austrian horses since 2000. Tick-borne diseases normally occur in restricted areas, where infected ticks live. In the last years an enlargment of the endemic areas where *Ixodes ricinus* lives has been observed, this may be a possible reason that, in recent years, *Borrelia burgdorferii* and *A. phagocytophilum* infection has increased in incidence and clinical importance. Horses as well as dogs may represent a good biological indicator for the presence and distribution of tick-borne diseases

STUDY OF VARIABLES COMMONLY USED IN EXAMINATION OF EQUINE ACUTE COLITIS CASES TO ASSESS PROGNOSTIC VALUE

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Abstract

In a retrospective study, the prognostic value of clinical and laboratory variables, used in routine examination on admission in the hospital of 311 horses with acute colitis, were tested. The following variables proved to be significant in discriminating between horses which survived and those which died.

1. No significant ($p>0.05$) differences between the survived and died horses with acute colitis were determined regarding to age, race and sex, as well as the body temperature, respiration rate and total plasma protein.

2. High-significant ($p<0.001$) differences between the survived and died horses with acute colitis were determined regarding to heart frequency, capillary filling time, hematocrit, base excess, hydrogen carbonate, pH value, leukocyte number and HCT/TPP ratio. The hematocrit value is the most valuable from all examined clinical and laboratory parameters regarding its prognostic importance.

3. The research on statistic dependence between the individual parameters shows that in not survived colitis horses exists generally a higher correlation than in survived patients.

PREVALANCE, RISK AND THERAPY OF EQUINE POSTOPERATIVE PARALYTIC ILEUS

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Abstract

The retrospective analysis comprised 156 horses with a post-operative paralytic ileus developing after colic surgery. The clinic data and laboratory parameters of these horses were compared with the data from 1216 other colic horses that underwent surgery, but did not develop a postoperative ileus.

The incidence of the postoperative ileus was 11.3 % among colic surgery horses. It was found that horses with a postoperative ileus have significantly increased their packed cell volume and heart rate as well as have frequently showed other signs of endotoxemic shock before surgery. Postoperative ileus appears most frequently in horses with hernia foraminis omentalis and lipoma pendlulans. Horses with distinctive distension of the small intestine and especially horses with a resection and anastomosis of the small intestine show a significant increase risk of developing a postoperative ileus.

A shortening of surgery time, performing an enterotomy of pelvic flexura with colonic evacuation and postoperative peritoneal lavage are procedures which may decrease the probability of developing of a postoperative ileus in horses.

The survival rate was significantly lower among horses that developed a postoperative ileus (67.3 %), compared with horses that did not (96.1 %).

A combined use of three prokinetics: neostigmine, metoclopramide and domperidone shows a significant increase in survival rates compared with an ordinary use of one of prokinetics. Therefore, the prophylactic use of these three drugs should be considered rather than waiting for the development of distinctive clinical signs of the disease. Relaparotomy with manual decompression and massage of the small intestine is not an alternative to combined medicamentous therapy.

A COMPARATIVE STUDY OF AEROBIC BACTERIA IN UPPER RESPIRATORY TRACT OF HORSE AND DONKEY

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A comparative study of normal microflora inhabiting the upper respiratory tract of horses and donkeys belonging to organized farms and those engaged in draught work was carried out. For this purpose samples were taken from upper respiratory tract i.e. deep nasal swabs from 50 horses belonging to organized farm and 68 horses engaged purely in draught work. Similarly, samples were taken from 30 donkeys belonging to organized farm and 20 engaged in draught work. The samples were processed for the cultivation and identification of aerobic bacteria using established procedures.

The results reveal that in horses belonging to organized farm the most prevalent organism was *Streptococcus equi*,(46%), followed by *Staphylococcus aureus*(32%), *Rhodococcus eq*ui (24%), *Micrococcus* sp. The less frequent organisms were found as *Aerococcus, Escherichia coli, Proteus*(14%), *Pasteurella* (12%) etc.

In horses that are kept for draught purpose not in organized farm, the occurrence pattern of bacteria in respiratory tract was found different. *Bacillus* sp were present in 52% of the animals followed by *Streptococcus*(50%), *Staphylococcus* (44%), *Pseudomonas* (33%), *Rhodococcus equi* (27%). Less frequent organisms belonged to genera *Aerococcus* (17%), *E.coli* (14%), *Salmonella*(11%), *Proteus* (10%), *Pasteurella* 7.3%) etc.

In donkey population belonging to organized farm the occurrence of bacteria in upper respiratory tract was found as Staphylococcus (100%), Streptococcus (83.3%), Rhodococcus equi (66.6%), Proteus (60%), Bacillus (56.6%), Pseudomonas (56.6%), E.coli (56.6%), Enterobacter(43.3%), Pasteurella(40%) Salmonella(33%), Aerococcus(33.3%) etc.

The bacterial population found in donkeys meant for draught purpose not in organized farm was found as *Bacillus, Staphylococcus, Streptococcus equi*(100%), *Staphylococcus* and *Enterobacter* (95% each), *E.coli* (85%), *Pseudomonas* (65%), *Pasteurella* (50%), *Proteus* (35%), *Aerococcus and Micrococcus* (33% each), *Salmonella* (25%) etc.

CONTENT ANALYSIS OF FREE-TEXT CLINICAL RECORDS: THEIR USE IN IDENTIFYING SYNDROMES AND ANALYSING HEALTH DATA (RESEARCH ABSTRACT)

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Take Home Message

Content analysis allows the extraction and analysis of data from free text clinical records. This enables clinicians to retain the richness of free text in clinical recording and facilitates the detection of new clinical syndromes.

Introduction

The Hong Kong Jockey Club database, established in the early 1970s, holds health and performance records for over 6000 horses. However, the reasons for retirement are recorded as free text. Here we use context analysis, a method more frequently used in social sciences, to extract and classify free text descriptions to describe the retirement pattern of Thoroughbred racehorses in Hong Kong.

Materials and Methods

The content analysis software package- WordStat and SimStat, (Provalis Research, Quebec, Canada) was used to provide a replicable technique for compressing many words, ungrammatical phrases and unstandardized abbreviations of clinical text into categories based on explicit rules of coding.

Results

Automated categorization based on a user-defined dictionary allowed over 95 % classification of 3727 free-text clinical records taken from a 12-year period (1992-2004). Twelve groups were identified.. The system demonstrated inherent standardisation amongst words and phrases used for clinical description, including patterns of related clinical signs. By associating different clinical descriptions it also allowed syndromes to be identified in the data.

Discussion

The use of context analysis to categorise “free text” clinical records offers a rapid and reliable alternative to coding systems in clinical data recording. It allows clinicians the opportunity to continue to use free text to describe disease. This offers more flexibility to discover new clinical syndromes as well as to develop a veterinary lexicon for standardization of data entry.

Acknowledgement

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CONTROL OF TICKS *AMBLYOMMA CAJENNENSE* AND *DERMACENTOR* (*ANOCENTOR*) *NITENS* AT PARASITIC PHASE IN HORSES IN PAN-AMERICAN GAMES RIO-2007, RIO DE JANEIRO, BRAZIL

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Amblyomma cajennense is a tick found in Brazilian Southern region and Rio de Janeiro State and it's usually in horses, but can parasite bovines, lambs, goats, mules, wild animals and even the man. This ixodide is considered the most important vector of spotted human fever caused by *Rickettsia*, especially *R. rickettsii*. *Dermacentor* (*Anocentor*) *nitens* is commonly found in Brazilian Northern and Southern, having recognized predilection to ear, nostrils, perineum, neck hair and tail. Besides that, this ixodide is considered biological vector of *Babesia caballi* in America. The present study aimed to evaluate the control of *A. cajennense* and *D*. (*A*.) *nitens* in horses at Sporting Complex of Deodoro (SCD) in Pan-American Games Rio-2007, Rio de Janeiro, Brazil. The control program was based on weekly horses aspersion with cypermethrin 15% at SCD, in a total of 460 semi-stabled horses and 11.040 applications during 180 days before games. Treatment period had duration of 24 weeks, from January to June of 2007 and horses were weekly examined through visual and manual inspection and maintained at semi-stable management. All animals were maintained inside their stalls during the aspersion. Efficiency of ticks' control was observed from June to July of 2007 as during this period is commonly observed high infestations of *A. cajennense* and *D*. (*A*.) *nitens* larvae in horses at Rio de Janeiro State. After spraying any reaction were observed and ticks control was fully satisfactory, since after first two months, from March and April of 2007, ticks were rarely found, and since June, any ticks were observed on horses. Thus, cypermethrin aspersion as part of strategy control of ticks is efficient to eliminate ticks' infestation in horses, decreasing parasitism risks to animals and humans, besides to avoid the appearing clinical cases or horses infections by *B. caballi* and brazilian sppoted fever caused by *R. rickettsi* in humans.

USE OF FLANNELS DRAG, CO_2 TRAPS, AND SENTRIES HORSES TO EVALUATE AND CONTROL TICKS *AMBLYOMMA*, *DERMACENTOR* AND *RHIPICEPHALUS* IN PAN-AMERICAN GAMES 2007, RIO DE JANEIRO, BRAZIL

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Characterization and identification of infected areas constituted the initial phase of ticks control program accomplished to Pan-American Games 2007, Rio de Janeiro, Brazil at Sporting Complex of Deodoro (SCD). *Amblyomma cajennense*, *Dermacentor* (*Anocentor*) *nitens*, *Rhipicephalus* (*Boophilus*) *microplus* and *Rhipicephalus sanguineus* are the main species of ticks that occurs in this area. This work was realized in SCD areas subject to ticks presence, from January to June of 2007, and ticks represent one of the main obstacles to accomplishment of Pan-American Games Rio 2007, due to the risk of direct parasitism on the horses and people, besides the pathogenic agents transmission risks as *Rickettsia rickettsii*, *Neorickettsia* spp. and *Borrelia* spp. for humans; *Babesia equi*, *Babesia caballi* for horses and *Ehrlichia* spp. for both. This study aimed to identify risk areas to presence of ticks by flannels drags, CO_2 traps and sentries horses and to determine the need for treatment the land areas with agricultural acaricide Karate Zeon 50 CS – Lambdacyalithrin microencapsulate suspension (Alfa-ciano-3-fenoxibenzil-3(-2-cloro-3,3.3-trifluoroprop-1-enil)-2,2-dimetil-ciclopropano carboxilato-1:1((X)-(-1R,3R),(Z)-(1S,3S),R-e) and the Demand 10 CS product (Lambdacyalithrin microencapsulate) during Pan-American Games Rio 2007. To evaluate larvae in pastures, cotton flannels drags were used. Flannels measured 1,0 m^2 and were dragged in several areas of SCD, twice a week, about 30 minutes, being visually inspected to identification of tick species phases. Larvae were collected and maintained in alcohol 70% and identified by stereoscopic microscope. Besides flannels drags method, several CO_2 traps with 250g of dry ice on 1.0 m^2 of white cotton's flannel were putted in this area. Traps distribution was made randomly at morning and afternoon and inspected at one hour regular intervals, staying on the areas about three hours. This method made possible to capture of nymphs and adults of *A. cajennense*, which pass part of its free life in soil and in pastures. Ticks found on traps were collected, conserved in alcohol 70% and specifically identified by stereoscopic microscope. The methodology was able to identify infected areas, and made possible the application of Karate Zeon 50 CS and the Demand 10 CS products in an efficient and safe way, and with analysis of different phases of tick's occurrence up to complete elimination, verified by weekly evaluation. Only *A. cajennense* was captured by these methods. Flannel drag method made possible to collect just larvae of *A. cajennense*, as expected. Dry ice traps were efficient to capture nymphs and adults *A. cajennense*, mainly at afternoon. Overall of 10 nymphs and 62 adults ticks were captured during the whole studied period. At beginning of program, sentries horses were infected with different evolutionary phases of *A. cajennense* and *D.* (*A.*) *nitens*. After 180 days of the control program any phase of ticks were observed on horses. Identification of infected areas by these methods made possible to establish efficient procedures through pulverization of acaricide Karate Zeon 50 CS and Demand 10 CS products. Flannels drag method demonstrates highly effective for capture *A. cajennense* larval phase, while CO_2 traps method demonstrates high efficiency to capture nymph and adults *A. cajennense*. Association among flannels drag, CO_2 traps and acaricides pulverization was able to control tick's population in Pan-American Games area.

TICKS CONTROL OF *DERMACENTOR (ANOCENTOR) NITENS* IN HORSES BY TOPICAL APPLICATION OF TICKCIDE PASTE

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Dermacentor (Anocentor) nitens is an endemic tick at Rio de Janeiro State, Brazil and responsible to direct damages, mainly blood spoliation of horses; and indirect by transmission of *Babesia caballi* to horses. The control of this tick became necessary to the biosecurity of equestrian activities at Pan-American Games RIO-2007. The equestrian activities of the Games were accomplished at Sporting Complex of Deodoro (SCD), Rio de Janeiro, Brazil (22^{o} 52' 00" S and 43^{o} 24' 00" W) with humid tropical climate. In this place, 460 horses are maintained at semi-stable management. These horses are used in military activities, Dressage, Eventing, Pole and Equoterapy. The tick *D. (A.) nitens* parasites mainly the ear, nostrils, perineum, neck hair and tail of the horses. Some of those sites are difficult to be accessed, mainly when ticks control were made with powder's topical products and carrapaticide solutions as bath form. These presentations usually had low residual effects and news infestations in short periods, maintaining ixodide population in pastures and animals. This work aimed to study a experimental acaricide formulation as paste form composed by thriclorfon + coumaphos + cyfluthrin in a specific concentration against to *Dermacentor (Anocentor) nitens*. For that, 460 horses were used and maintained in semi-stabled management at SCD. Monthly, one topical application of the paste by manual way were accomplished in ears, nostrils and tail's base, following all individual safety's norms. Applications were accomplished during 24 weeks, from January to June of 2007, and horses were weekly examined through visual inspection to verify the efficiency and permanence of paste in the sites. During the paste applications, any aggressive or defense attitudes by horses were observed. Treatment with tickcide paste was able to eliminate progressively all the evolutionary phases of *D. (A.) nitens* resulting in its total elimination 120 days after the beginning of applications. Residues of paste were observed during 14 days after application, without any collateral reactions which demonstrated repellent and acaricide action against all of life's phases of *D. (A.) nitens.* Paste presented higher easiness application way when compared with other application forms. The success of ticks control depends on association of procedures, that impossibility introduction of ticks from others areas, as well as of those that make possible ticks population reduction minimizing ticks infections in horses.

SEROLOGICAL MONITORING OF EQUINE INFLUENZA IN IRAN

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Equine influenza is an acute, contagious respiratory disease caused by two distinct subtypes (H7N7, formerly equi-1, and H3N8, formerly equi-2) of influenza A viruses within the genus Influenzavirus A of the family Orthomyxoviridae. In fully susceptible animals, clinical signs include pyrexia, and a harsh dry cough followed by a mucopurulent nasal discharge. Diagnosis of influenza virus infections is based on virus isolation from horses with acute respiratory illness by sample (nasopharyngeal swabs or nasal and tracheal washes) inoculation in Embryonated hens' eggs and/or cell cultures, or on the demonstration of a serological response to infection that identified by HI and single radial haemolysis (SRH) and ELISA. Infection may also be demonstrated by detection of viral antigen in respiratory secretions using an enzyme-linked immunosorbent assay. In this study to screen horses for anti influenza virus antibody, serum samples were randomly collected from 100 horses Fars and Khorasan provinces and were tested by compitative ELISA kit (Anigen AIV Ab kit). Twenty two samples (22%) were positive for anti influenza antibody. To identity the involved subtypes, HI test is on going.

UNUSUAL CLINICAL MANIFESTATION OF STRANGLES IN ISRAEL

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Strangles is caused by *Streptococcus equi* subsp. *equi*, a beta haemolytic, Lancefield group C streptococcus. The syndrome was described in early veterinary science literature and first reported by Jordanus Ruffus in 1251. It occurs worldwide and causes heavy economic losses in terms of cost of treatment, quarantine measures, and occasionally, even loss of animals.

Strangles is a highly contagious disease that occurs mostly in young horses less then 24 month of age and affects primary unvaccinated animals. When *S. equi* subsp. *equi* is introduced in to a naïve stable, morbidity may be as high as 100% whereas case mortality is low, between 2 and 5%.

The upper respiratory tract is regularly involved, with mucopurulent nasal discharge and gross swelling and abscessation of the mandibular lymph modes. Sinus tracts are usually formed and rupture through the skin

Some *S. equi* subsp. *equi* infections result in metastatic abscessation, often in adult horses. Metastatic abscesses, “bastard strangles”, may present with abscesses in the thorax, abdomen, lungs, nervous system, eye and fleshy part of the hindquarters. Strangles can last 3 to 4 weeks.

During the period from 1998 till 2003, only 11 samples (0.95% of the samples submitted to the KVI from horses) have been found positive for S*. equi* subsp. *equi*.in Kimron Veterinary Institute (KVI). All those samples were taken from horses with classical manifestation of strangles; half of them were adult horses.

In contrast, during the past 3 years, 38 severe cases (5.24% of the samples submitted to the KVI from horses) of strangles were reported to the KVI Israel, 35 of which were shipped to KVI for *S. equi* subsp. *equi* confermation.

Metastatic abscessation in sick animals that were reported by clinicians is presented in table 1.

As is shown in table 1, 23 out of 30 reported cases (76%) affected adult horses whereas 4 out of 7 aberrant clinical cases were reported in young horses less then 24 month of age.

The aberrant clinical cases were very severe, and the affected horses were sick for periods of up to 2 month, while the classic cases last from 3-4 weeks.

It is important to emphasis the augmented prevalence and the severity of the clinical manifestation of strangles in the past 3 years in comparison to the years before 2004.

The data presented in table 1 together with the severity of the clinical cases and the data from earlier years lead us to suspect an unexplained increase in the microorganism's violence. Insufficient preventative biohazard measures may have caused the steep increase in the incidence of the infection.

Table 1.**Strangles clinical manifestations, in Israel from 2004-2007**

Age	Classical* manifestation	Neck abscessation	Aberrant** manifestations	No information	Total
< 24 month	3	0	4	0	7
≥24 month	14	4	4	1	23
No information	3	2	1	2	8
Total	20	6	9	3	38

* Submandibular abscessation and nasal discharge were referred as, classical manifestation

**Aberrant manifestations were observed in one affected anatomical site: hind leg abscessation; aborted fetus; mesenteric lymph nod; eye; pectoral muscles; testicul; shoulder; fetlock, and in few cases up to three aberrant affected sites were observed: eye and shoulder and neck abscessation; eye and jaw abscessation.

THE USE OF AUTOLOGOUS MESENCHYMAL STEM CELLS FOR THE TREATMENT OF EXPERIMENTALLY-INDUCED EQUINE TENDINITIS: CLINICAL, ULTRASONOGRAPHIC AND POWER-DOPPLER EVALUATION.

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Introduction

The superficial digital flexor tendon (SDFT) injury is an important cause of lameness and loss of use in horses because of its hight incidence, long periods of rest and low efficacy of the current treatments. Natural healing is often slow and of poor quality, resulting in long layoffs and a predisposition to reinjury.

In the late 90´s, the medical interest has been directed to the stem cells therapy of degenerative diseases and in cases of deficient healing process. These cells are involved in the growth, remodeling and healing process (Richardson, 2005). Many tests have been taken in equine tendinopathy (Young *et al*., 1998; Smith *et al*., 2003; Thomas, 2003). The mesenchymal stem cells can give rise to osteoblasts, condrocytes, tenocytes, fibroblasts, adipocytes and myofibroblasts.

Objective

This study aims to evaluate the efficacy of intralesional mesenchymal stem cells implant, obtained from bone marrow aspiration, for the treatment of induced tendinitis in horses. For this purpose, parameters assessed included clinical evaluation, ultrasonographic (US) exams, and power-doppler

hiper-sensibility.

Materials and Methods

A total of six animals, between 2 and 3 years of age, were subjected to the induction of tendon injury through the administration of collagenase (1 ml, 2,5mg/ml) in the superficial digital flexor tendon on both forelimbs. After twelve days, one of the limbs was treated by intratendíneous implant of the mononuclear fraction, containing the mesenchymal cells, collected from the bone marrow, in the region of the 5th sternebra. When led to the laboratory, the cells were separated through the use of Ficoll Hypaque and 0,5 ml of autologous serum was added, so the implant could be performed, under ultrasonographic guidance into the core lesion of the damaged tendon (treatment). The animals were evaluated weekly, from the moment of the injury induction , up to being complete 120 days, through clinical examinations, ultrasonographic and power-doppler exams. The control group consisted on the contralateral members.

Results & Discussion

Differences were not observed between the groups to the clinical evaluation, not presenting increase of local sensibility, temperature or lameness to the end of the experiment. However, there still was a small increase of volume presented by all limbs. The animals showed a small discomfort after the implant, which disappeared in a few days. At the ultrasonographic examination , the period of 4 months was not sufficient so that the images could present a normal echogenicity, and a perfect delimitation of the injuried area was not possible at that time. Also at the US examination, differences were not noticed between the groups, being that they were presenting very similar results of echogenicity, percentage of harmed area and percentage of injury reduction. The power-doppler US evaluation allowed to observe hyper-sensibility in both groups to 30 days after the injury induction, reaching his very point around 45 days, remaining constantly up to 60 days, when blood flow signs started to be less evident, and its identification was not possible when 120 days was completed. The signs of blood flow were firstly observed peripheral to the tendon, both in the treated group and the control group. However, with the passing of the days, signs of this blood flow appeared in greater intensity on the inside of the tendon in the treated group. This characteristic was seen in less intensity in the control group.

Conclusion

The use of mesenchymal stem cells did not appear to accelerate the regenerative process, but have been showing promising results in the structure and maybe the function of the tendon after injury. There was no differences between control and treatment groups related to clinical or US evaluation until 120 days after the collagenase induction. But at the US power-doppler, the implanted group appeared to have a better blood perfusion all over the injuried tendon. There is still several questions that must be answered and new researches should revel new techniques for applying mesenchymal stem cells implants to new opportunities in the regeneration of soft tissues.

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CLINICAL EFFICACY OF A MIRACLE DRUG CALLED DIMETHYL SULPHOXIDE: A RETROSPECTIVE STUDY

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Dimethyl sulphoxide (DMSO) has many characteristics which makes it a challenging drug in equine practice. It protects cellular components from the effects of ionising radiation, increases the diffusion of oxygen through the tissues. It has anti-inflammatory, analgesic and diuretic actions. DMSO protects the integrity of endotoxaemic vascular endothelium, either by scavenging free oxygen radicals released from neutrophils under the influence of endotoxin, or by inhibition of platelet aggregation resulting from blockade of prostaglandin biosynthesis and the subsequent formation of thromboxane A2 (1, 2, 3). Although dimethyl sulfoxide has not been approved for intravenous application in horses in Europe, many equine practitioners use it in the treatment of endotoxaemia and in other pathologic conditions (4, 5). The efficacy of DMSO in equine endotoxaemic cases is still controversial due to insufficient data originating from experimental and/or clinical trials. The objective of this study was to determine with the help of a retrospective analysis whether intravenous DMSO is effective in the treatment of endotoxaemia. Cases with gastrointestinal compromises, grain overloads or pathologic conditions associated with foaling were regarded as suspected endotoxaemic patients. Endotoxaemia was established on the bases of leukopenia, neutropenia, hyperaemic mucus membranes, tachycardia and fever. At least three of the aforementioned five findings had to be present to incorporate the cases into our study. The selected cases were divided into two groups according to the treatment regimen with (n=37) or without (n=67) dimethyl sulphoxide. The DMSO infusion was administered within 24 hours after admission and repeated 2 to 3 times on the following days in the dosage of 1 g/kg at a concentration of 10% in 5% dextrose or saline solution. Outcome and duration of hospitalisation were compared between the two groups with Fisher's exact test and t-test. Changes in the clinical and laboratory values as pulse rate, respiratory rate, body temperature, packed cell volume, thrombocyte, white blood cell and neutrophyl granulocyte number at admission and on the 3rd day were evaluated by analysis of variance. There was no significant difference between the duration of hospitalisation when compared the two groups. The odds of a positive outcome were worse (OR=0,36) concerning the DMSO treated patients. There were no significant differences in the improvement of the clinical and laboratory values of the treated and non-treated groups. Along with the statistical analysis it became apparent that the clinical and laboratory values at admission were more pathologic of the DMSO treated group. Before treatment there were significant differences between the two groups regarding packed cell volumes (P=7,55e-0,6), pulse rates (P=0,04) and respiratory rates (P=0,024) but there were no significant differences between leukocyte, neutrophyl granulocyte and thrombocyte numbers. It was not possible definitively to evaluate the efficacy of DMSO infusion as a treatment for endotoxaemia using the retrospective data analysis. Not any remarkably positive effect of dimethyl sulphoxid was detected maybe due to inadequate timing, duration, dosage, concentration and/or the way of administration used. The main problem of this retrospective study is the significant difference between the two groups at admission. The clinician's decision determining the use of the drug was greatly influenced by the clinical and laboratory parameters of the animal. It can be suspected that as a result of missing data describing the clinical advantages, the efficacy and the exact way of administration, DMSO is not the drug of choice when facing endotoxaemia. On the other hand when clinical data suggest serious endotoxaemic case the clinician, who believes in the miracle drug having a lot of positive anti-endotoxaemic effect according to the published theories, rather decides to put the horse on DMSO infusion as a last chance. The study highlights the need for further prospective studies using randomised clinical trials to evaluate accurately the efficacy of dimethyl sulphoxide.

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GENETIC ANALYSIS OF MOROCCAN BARB HORSE: APPLICATION TO INDIVIDUAL IDENTIFIVATION AND PARENTAGE VERIFICATION

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Moroccan equine population is estimated to 160.000 horses. About 80 % of them are Arab-Barb, 10% are Barb horses; the remaining 10 % are comprised of Arabian, Thoroughbred, Anglo-Arab-Barb and saddle horses. The improvement of this breeding requires its rationalization and, therefore, the use of modern techniques for its management. In order to meet these requirements, the National Stud Farms have developed a computerized system, called MINISIRE, similar to the SIRE system (system identifying and indexing horses) adopted in France. In the same way of the implementation of the MINISIRE, modern identification means and parentage verification have been introduced. Thus, Veterinary Genetics Laboratory (LAGEV) was created at the Hassan II Institute of Agronomy and Veterinary Medicine in 1990. Since its creation, LAGEV is institutional member of the International Society for Animal Genetics (ISAG). At the beginning, LAGEV used traditional gene markers for blood typing. Since 1998, new genetic markers have been introduced. One of the newer testing methods in identifying horses is a DNA-based test using microsatellite marker analysis. Thus, and since 2000, typing by microsatellite markers was applied to Arabian, Anglo-Arab and Thoroughbred horses. Since 2005, DNA typing was generalized to the whole equine production in Morocco. The objective of this work was to evaluate the efficacy of this new technology in individual identification and parentage testing of Barb horse.

The analyses were carried out on blood samples from a total of 89 horses, of both sexes, throughout different regions of Morocco We used PCR to amplify microsatellites using StockMarks for Horses Equine Paternity PCR Typing Kit (Applied Biosystems, Foster City, CA, USA) which includes 17 previously reported loci : ASB2, ASB17, ASB23, AHT4, AHT5, HMS1, HMS2, HMS3, HMS6, HMS7, HTG4, HTG6, HTG7, HTG10, VHL20, LEX3 and CA245 (Dimsoski, 2003). Amplification reactions were performed in one multiplex PCRs. Reactions were performed with reagents supplied in the kit and according to manufacturer instructions. Electrophoresis was carried out on an ABI PRISM 310 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) using the recommended protocols. Size analyses of DNA fragments separated were performed with Genotyper software. Then number of alleles per locus and allelic frequencies were estimated by direct counting from observed genotypes. Heterozygosities values (He and Ho) were computed using the GENETIX programme (Belkhir *et al*. 2000). Theoretical identity probabilies (PI) and of exclusion probability (PE) were estimated according to the formulas of Hanset (1976) and Rendel and Gahne (1961).

All of the primers amplified well, with the exception of HTG10 which sometimes exhibited low peak heights not suitable for automated scoring. This marker was excluded. The 16 microsatellites used were highly polymorphic in Barb horse (Table 1). The total number of alleles was 132. The number of alleles per locus varied from 4 (HTG7) to 13 (ASB17) and the average number of alleles was 8.062. The observed heterozygosity (Ho) and the expected heterozygosity (He) ranged from 0.3939 to 0.8030 (mean 0.6269), from 0.4572 to 0.8475 (mean 0.7281), respectively. The total PI and PE values of the 16 microsatellite loci analyzed were 2.823×10^{-17} and 0.9999 respectively. These are higher than the values obtained with classical gene markers: $PI=0.11 \times 10^{-6}$ and PE=0.986 (Ouragh *et al.,* 1994). Compared to the study carried out in Thoroughbred, Arabian and Anglo-Arab (Ouragh, 2003) PI and PE values were also higher in Barb horses.

These results indicate that the test using microsatellite marker analysis constitutes a highly efficient and reliable alternative for individual identification and paternity testing of individual Barb horses. Thus, since 2005, competent authorities of equine industry in Morocco and the World Barb Horse Organisation (WBHO) have initiated DNA typing for individual identification and parentage verification of Barb horse.

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Table 1. Number of alleles per locus, Heterozygosity, PI, PE, He and Ho of microsatellite markers in Barb horse.

Locus	Nb of Alleles	PI	PE	He	Ho
HTG6	8	0.0807	0.7597	0.7805	0.7273
VHL20	11	0.0386	0.8763	0.7805	0.7273
HTG7	4	0.3411	0.3865	0.4572	0.4394
HTG4	8	0.1182	0.6999	0.7043	0.7424
AHT5	6	0.1291	0.6539	0.7167	0.6667
AHT4	7	0.1068	0.7000	0.7443	0.7273
HMS3	9	0.0512	0.4887	0.8284	0.6061
HMS6	7	0.1351	0.6814	0.6688	0.6667
HMS7	8	0.0913	0.7556	0.7429	0.7576
HMS2	11	0.0931	0.7609	0.7304	0.3939
ASB2	10	0.0541	0.8334	0.8188	0.8030
CA425	7	0.1954	0.5743	0.6010	0.4091
ASB17	13	0.0218	0.9241	0.8910	0.6818
LEX3	9	0.0428	0.8609	0.8439	0.3939
HMS1	5	0.3080	0.3961	0.5142	0.6364
ASB23	6	0.0910	0.7399	0.7593	0.5909
TOTAL	**129**	**2.8233×10^{-17}**	**0.9999**	**0.7281**	**0.6269**

PI: Probability of identity, PE: Exclusion probability, He: Expected heterozygosity, Ho: Observed heterozygosity

CHROMOSOMAL ABNORMALITIES IN MOROCCAN HORSES: PRELIMINARY INVESTIGATION

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Chromosomal abnormalities in horses can be congenital or may appear during the gametogenesis or embryogenesis. The direct consequences are essentially affections in the reproductive tract resulting in sterility, reduced fertility or abortion. The consequences can be also a modification in the attitude and conformation of the animal. These chromosomal abnormalities can thus be suspected on the basis of clinical examination in particular with persistence of fertility disorder. However, the realization of a cytogenetic examination remains imperative for the confirmation of such a diagnosis. The establishment of a karyotype can be supplemented when is necessary by molecular approaches (chromosomal painting: FISH and/or PCR using specific primers).

This work represents the first part of a research project aiming to establish a diagnosis of the principal chromosomal abnormalities in Moroccan horses by cytogenetic study in collaboration with the Laboratory of Animal Cytogenetic (National Veterinary School, Toulouse). This first part aims to identify animals showing signs of suspicion of possible chromosomal abnormalities on the basis of detailed clinical examination. Thus, epidemiological investigations were realized within 1244 horses attending the regional Stud farms of Meknes (397), Bouznika (605) and El-Jadida (242). A card of investigation including description of the animal, clinical examination and principal symptoms being able to evoke a chromosomal anomaly was established. Animals answering to the following criteria were selected:

- Mare with an abnormal attitude and an abnormal body conformation or with anatomical abnormalities of the genital tract.
- Unfertile mares unscathed of any genital infection or any disease affecting the reproductive tract
- Mares giving birth to foal with an abnormal conformation or attitude
- Stallions with fertility disorders resulting from aetiologies other than genital infections or bad control of breeding.
- stallions with azoospermy or oligospermy
- Stallions with a low rate of success

The results obtained were analyzed statistically using Stat Box software. Factorial analysis of correspondences (FAC) and χ^2 tests were calculated to determine the existence of dependences between stud farms and causes of infertility.

Informations collected through the investigation made it possible to isolate 75 animals candidates to chromosomal abnormalities. These animals were divided into eight groups according to the clinical signs:

- Group A: mares with vulvar deformation and vacuum after two cycles
- Group B: mares with antecedents of abortion not explained by an infectious disease.
- Group C: mares with abnormal attitude and infertile
- Group D: mares with body malformation.
- Group E: vacuum mares after two cycles or more
- Group F: mares in heat more than 12 days
- Group G: stallions with fertility disorders
- Groupe H: mares with not functional ovaries

Mares of group E were most frequently met in all the stud farms, and 42 mares were isolated in total. (Fig. 1)

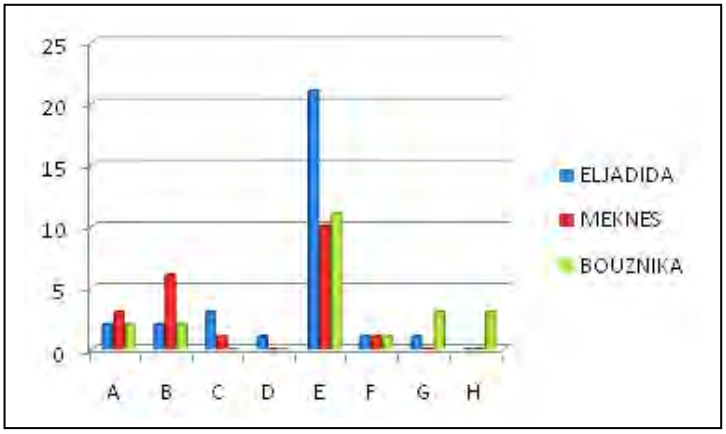

Figure 1: Percentage of suspected horses by group and Stud farm

Statistical analysis permits to conclude that the distribution of the causes of infertility is completely independent of the area factor or Stud farm factor. The study also shows that the Arabian horses present a high predisposition of a possible chromosomal abnormality (48%), followed by Thoroughbred horses (27%) and Arab-barbs horses (23%).

While trying to assign to each group of suspected animals the most probable chromosomal anomalies according to the clinical signs, we notice that monosomy of X chromosome (63X0) would be the most frequent anomaly within the Moroccan equine population with 20%, followed by trisomy of X chromosome (65 XXX) with a percentage estimated at 16 %. According to Darré *et al.* (2000) and Power (1990), monosomy of X chromosome (63 X0) is described as being the most frequent chromosomal abnormality in horses. On the other hand trisomy of X chromosome (65 XXX) is not very frequent in horses (Klunder *et al*,. 1990; Nie et al,. 1993). These assumptions of frequency of X monosomy and trisomy must be confirmed by a cytogenetic analysis.

This investigation allowed identifying suspected animals with chromosomal abnormalities. A cytogenetic examination of the 75 animals identified is now imperative to carry out a diagnosis of certitude of the presence of chromosomal abnormality. It will be the objective of the second part of the project.

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EQUILIS RESEQUIN IS A NEW COMBINED VACCINE AGAINST RHINOPNEUMONIA (HERPESVIRUS OF THE 1ST AND THE 4TH TYPE) AND INFLUENZA IN HORSES

Sergey Polishchuk

Intervet, Moscow

Horse viruses of herpes and influenza are registered very often and nearly in all counties of the world including Russia. According to number of characteristics two subtypes of herpes virus can be distinguished – respiratory and fetal. Respiratory strains cause lesion of respiratory organs and single abortions, fetal cause abortions and single cases of development respiratory diseases. Infection with EHV-1 appears rarer, clinical signs are similar to EHV-4 but in addition it brings on a large quantity of abortions, death of newborn foals and myeloencephalopathy. The most widespread form of EHV is highlycontagious infection of respiratory tract and also abortions which has an economic significance.

Horses and ponies are susceptible to this disease so the source of causative agent are ill animals and animals which have had the disease. Herpesvirus infection brought once to the stud farm persist there for many years bringing on clinically apparent or concealed clinical course.

Horses of any age are susceptible to horse influenza virus but young unvaccinated animals are infected more often.

- The most important feature of horse influenza virus is that the virus is able to spread in population of susceptible animals significantly faster than other infectious agents bringing on respiratory diseases in horses.
- American types of horse influenza strains are spread in horse population all over the world in last decade. They can be considered precinctive along with strains of European types.
- At this time American types of cultures are causes for most of influenza cases in vaccinated and non-vaaccinated horses in Europe.

In connection with this the International Epizootic Bureau and the World Health Organisation recommend: vaccine against horse influenza must contain both European and American types of strains.

General vaccination is the best method to decrease clinical presentation of disease and virus spreading.

Use of Equilis Resequin

The 1st combined virus-vaccine against rhinopneumonia and horse influenza contains inactivated horse herpesvirus of 2 types plus 3 mostly typical inactivated strains of horse influenza.

Studies demonstrated that use of Equilis Resequin lead to fourfold decrease of virus spreading after control infection by horse herpes virus of Type 1 which is the most pathogenic of two existing types. It was attended by significant decrease of clinical presentations of disease such as heat, nasal outflow and cough.

PANACURE IN HORSE BREEDING

Sergey Polishchuk

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Invasion diseases are widespread overall affecting all species of animals including horses. In number of invasion diseases especially in helminthiasis even though there's no mortality and the disease progresses subclinically productivity of animals reduces and also their normal physiologic development retards.

Besides that it is important to take into consideration that by no means all antiparasitic preparations can be used on horses. Majority of antihelmintics having certain medicinal effect also have a number of side actions. In such situation it is necessary to have highly effective and at the same time safe preparation.

Particularly, all over the world Panacur Pasta is a popular, appropriate, highly effective and safe preparation. Panacur can be used even on pregnant mares in lactation period.

Even low doses of Panacur have antihelmintic action which is aimed to kill adult forms, larvae and eggs of nematodes in gastrointestinal tract and lungs (ascaridiasises, strongylatosises, metastrongilosises, dicticaulosises etc.). Preparation has only antihelmintic action on organism and don't affect vital organs, system of organs and functions of organism.

Right after 12 hours after injection of Panacur approximately 90-100 % of eggs stop there development and, thus, invasion larvae of the 3rd developmental stage don't appear.

Long-term use also doesn't cause undesirable side effects and for this no special diet or other precautions are needed.

BUTOX EFFICACY IN HORSE RHINESTROSIS AND GASTROFILESIS

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It is well-known that ectoparasites significantly affect all horse-breeding, both livestock and sport. It is especially important to remember that ectoparasites transfer causative agents of many dangerous diseases such as babesiosis, piroplasmosis, anaplasmosis, and also tuberculosis, anthrax, mastitis, infectious keratoconjunctivitis.

Anyway, the paramount task of veterinary service is exactly prophylaxis of any disease, and if this task isn't performed in future you'll have to spend much more means (pharmacotherapy) that is unprofitable neither for owners nor for animals.

In connection with this it is necessary to use preparation which can insure reliable and effective protection of horses from ectoparasites with low costs and which could be highly safe for animal and human, and could be easy to use.

Intervet company offers preparation meeting all these requirements – BUTOX is an antiparasitic medicine of new generation working against ticks, lice, flies and other insects.

Sanitization should be conducted in period of active botfly flight once a month. Dosage: 1 litre of 0.0025 % Butox solution for one adult horse. Efficacy of horse protection is 97.2-100 %. No deviations in animals' organism were registered. Hair-covering didn't loose its lustre. Dermatitises and skin alopecia weren't observed. Visible mucous tunics had natural pale pink colour.

HIGHER LIPID PEROXIDATION INDEXES IN HORSES ELIMINATED FROM ENDURANCE RACE BECAUSE OF SYNCHRONOUS DIAPHRAGMATIC FLUTTER (THUMPS)

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This study reported the high lipid peroxidation activity in horses eliminated from a long distance race due to Synchronous Diaphragmatic Flutter. Eighteen racing horses from a total of 85 horses participated in (120km) long distance race were involved in this study. Nine horses were eliminated from the race because of Synchronous Diaphragmatic Flutter (SDF). The other nine horses were randomly selected and enrolled in another control group (CT). All horses in both groups have run a similar distance between 60 and 90 km while plasma sodium (Na^+), potassium (K^+) and chloride (Cl^-) concentration was decreased but not significantly. At the end of the race significant decrease was seen in ionized calcium ($p \leq 0.05$). Lipid peroxide and Malondialdehyde concentration in plasma were higher compared with values obtained before the start of the race. In thumps-eliminated horses these parameters were higher than in the control horses. Glutathione peroxidase activity was significantly lower at the end of the race, while creatine phosphokinase was severely and similarly increased in four folds in both groups. A significant increase in lactate concentration was seen in both groups; however this parameter was significantly higher in the thump-eliminated horses than the control group. A positive correlation was reported between LPO and calcium ions; MDA and calcium ions. This article establishes elevated lipid peroxidation products level as one component of biochemical characteristic of Synchronous Diaphragmatic Flutter (Thumps). The low level of Ca^{++} in plasma of horses is very much associated with high levels of lipid peroxidation indexes.

PHYSICOCHEMICAL EVALUATION OF FAECES AND GASTROINTESTINAL TRACT CONTENTS IN HORSES SUBMITTED TO CARBOHYDRATE OVERLOAD

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This work was carried out to evaluate the effect of carbohydrate overload in pH and bufferinging capacity in faeces and at different segments of gastrointestinal tract of horses. Six crossbreed mature horses were used and adapted to experimental diet composed by grass hay and concentrate, in a 60:40 proportion and 2.5% body weight (BW) of intake. They were submitted to carbohydrate overload, with gastric infusion of 17.6 g starch/kg BW and slaughtered after 24 (n = 3) and 36 hours (n = 3), while two horses just received experimental diet as control group. Faeces were collected from ampulla recti of horses submitted to overload in four hours intervals, until euthanasia, according to experimental group. Immediately after euthanasia, intestinal segments were separate and samples of contents were collected. Faeces and intestinal segment contents were submitted to pH and buffering capacity (BC) determination, immediately after sampling. The bufferinging capacity was determined as titration acidity with 0.25 M acetic acid of 80 mL of faeces-water-mixture (mmol/L) proportion 1:1 and 100 mL of digesta filtrate, respectively. The bufferinging capacity refers to the acetic acid volume used in the reduction of current pH to pH 5. There was a reduction ($P<0.05$) in fecal pH of horses submitted to carbohydrate overload from 20 hours up to 36 hours post-overload, compared to base line values, with pH 5.63, 4.84 and 6.09, respectively. Linear reduction ($r^2 = 0.93$) was observed in faeces bufferinging capacity at zero to 28 hours after carbohydrate overload, varying from 16.46 to 8.52 mmol/L. Decrease of BC accompanied decline of pH values, justified by large amount of hydrolyzed carbohydrate fermented in large intestine, exceeding the horses's capacity buffering secretion. Considering the linear model $\hat{Y} = 16.16 - 0,32X$, each four hours following carbohydrate overload there was a reduction in BC of 1.28 mmol/L and at end of 28 hours post-overload, the reduction in BC was of 8.96 mmol/L. The Table 1 shows the mean values and standard deviation of digest content pH in segments of gastrointestinal tract and faeces of horses slaughtered in time zero, 24 and 36 hours after carbohydrate overload. At small intestine, only the duodenum presented decrease ($P<0.05$) on pH in horses at 36 hours after carbohydrate overload, probably due to stomach digesta reflux during the necropsy. At large intestine, there wasn't difference ($P>0.05$) between cecal pH at 24 and 36 hours post-overload and control animals, with mean values of 6.46.

However, there was reduction pH at right ventral colon, left ventral colon, right dorsal colon, transverse colon and descending colon 24 hours after overload, not differing of the pH values 36 hours post-overload. Carbohydrate overload promoted pH reduction at large intestine due to lactic acid producer bacteria proliferation, which increases at low pH, together with increase the volatile fats acids production by bacterial fermentation. Small intestine presented larger bufferinging capacity compared to large intestine segments, due to bicarbonate buffering production through pancreas and liberation for duodenum together with phosphate buffering from diet, both contributing for the best bufferinging capacity of small intestine and caecum. In relation to large intestine, caecum BC was larger compared to other segments, independent of starch overload. However, in control animals the BC caecum was larger compared to animals submitted to overload and evaluated at 24 and 36 hours post-overload, with mean values of 96.19, 80.25 and 79.50 mmol/L of content, respectively. After 24 hours post-overload, the right dorsal colon digesta, transverse colon and descendent colon wasn't bufferinged in an efficient way for maintaining the pH above 5.0, being horses prone to mucosa lesions due to acidity. Horses adapted to rich hydrolyzed carbohydrate diet and submitted to dietary carbohydrate overload were able to buffering the cecal content in an efficient way, maintaining pH above sub-clinic threshold of acidosis. Carbohydrate overload in horses promote a reduction in pH and bufferinging capacity in large intestine contents and faeces, and could promote mucosa lesions and systemic acid-base disturbances in horses.

Table 1. Mean values and standard deviation of digest content pH in segments of gastrointestinal tract and faeces of horses slaughtered in time zero, 24 and 36 hours post-overload.

Intestinal Segments	Time Post-overload (hours)			
	Control (n = 2)	24 hours (n = 3)	36 hours (n = 3)	VC (%)
Stomach	2,96±0,77^{Ad}	5,04±0,49 ^{Ade}	3,60±1,34^{Af}	29,56
Duodenum	6,04±0,26^{Bc}	6,63±0,25^{Aab}	5,38±0,30^{Ccde}	5,88
Jejunum	7,21±0,22^{Aa}	7,05±0,36^{Aa}	7,38±0,12^{Aa}	3,89
Ileum	7,52±0,03^{Aa}	6,89±0,71^{Aa}	7,06±0,53^{Aab}	5,01
Caecum	6,74±0,00^{Ab}	6,11±0,90^{Abc}	6,52±0,79^{Ab}	11,26
Right ventral colon	6,49±0,03^{Ab}	5,43±0,90^{Acd}	5,48±0,66^{Ac}	13,44
Left ventral colon	6,49±0,08^{Ab}	5,37±0,87^{Ad}	5,27±0,8^{Acd}	15,19
Right dorsal colon	6,41±0,10^{Aa}	4,62±0,21^{Be}	4,49±0,21^{Be}	4,16
Transverse colon	6,50±0,05^{Ab}	4,63±0,23^{Be}	4,71±0,21^{Bde}	3,84
Descendent colon	6,57±0,03^{Ab}	4,56±0,17^{Be}	4,81±0,32^{Bcde}	4,21
Faeces	6,60±0,13^{Ab}	5,60±0,42^{Bcd}	4,84±0,72^{Ccde}	3,89
VC (%)	4,40	10,14	11,93	

Means in the same line followed by different capital letter differ by Student t test ($P<0.05$).

Means in the same column followed by different normal letter differ by Student t test ($P<0.05$).

CV – Coefficient of Variation.

APPARENT DIGESTIBILITY AND GASTROINTESTINAL TRACT KINETICS IN HORSES FED DIETS FED HIGH FAT DIETS

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This work aimed to evaluate apparent digestibility of nutrients and kinetics of passage at gastrointestinal tract of horses fed high fat diets. The assay carried out at Riding School of Brazilian Army and Equine Health Laboratory of Universidade Federal Rural Rio de Janeiro and at Veterinary School of Universidade Federal of Minas Gerais. Fifteen horses with of 478.85kg body weight were used in a completely randomized design with three diets and five repetitions. Diets were composed by concentrate and coastcross hay (*Cynodon dactilon*). Soybean oil was included at 7 and 14% of control diet, and ether extract level of diets were 5% (control diet), 13 and 21%. To maintain similar concentrate:roughage proportions of 67:33 and digestible energy levels in the diets with inclusion of soybean oil, there were a reduction of concentrate and hay supply (Table 1). Soybean meal was included in diets to maintain similar protein levels among diets. Trial had duration of 34 days with 30 days to adaptation and four days to faeces collection. Passage kinetics of intestinal contents were estimated by LIPE® (Isolated Lignin Purified Enriched) as external marker. LIPE® was given only one time and faeces samples were collected at 0, 2, 4, 8, 12, 16, 20, 24, 30, 36, 42, 48, 54, 60, 66, 72 and 78 hours after. All data were submitted to variance analysis and averages were compared by Student Newman-Keuls test ($P<0.05$). Apparent digestibility of protein and ether extract increased in high fat diets with values of 83,3 and 91,2%, respectively, however, cellulose digestibility decrease in diet with 21% of ether extract (Table 1). Apparent digestibility of others nutrients and Mean Retention Time (MRT) and Rate of Passage (RP) weren't affect ($P>0.05$). High fat diets were palatable and colic or diarrheas weren't observed. Soybean oil can be used in diets for horses, reducining dry matter intake and increasing energy density without affecting kinetics of passage int gastrointestinal tract that is interesting to athletic horses.

Table 1 – Average values of daily intake of nutrients, nutrients apparent digestibility coefficients and kinetics of passage in hores fed diets with soybean oil

	Diets			
	5% EE	13% EE	21% EE	CV(%)
Dry Matter Intake (Kg/day)	9.02[a]	7.11[b]	6.15[c]	7.37
MRT (Hours)	34.3[a]	40.4[a]	32.3[a]	11.84
RP (%/Hours)	2.9[a]	2.5[a]	3.1[a]	12.29
	Digestibility Coefficients			
Dry Matter	62.3[a]	62.6[a]	68.8[a]	8.0
Crude Protein	70.5[b]	80.3[a]	83.3[a]	6.3
Ether Extract	71.8[b]	89.7[a]	91.2[a]	7.7
Neutral Detergent fiber	53.9[a]	48.8[a]	41.2[a]	13.3
Acid Detergent fiber	41.3[a]	35.9[a]	30.7[a]	19.5
Cellulose	50.1[a]	42.9[a]	31.8[b]	18.8

Means in the same line followed by different capital letter differ by SNK test ($P<0.05$).
CV = Coefficient of Variation.

HOW TO INCORPORATE PHYSICAL THERAPY INTO EQUINE VETERINARY PRACTICE

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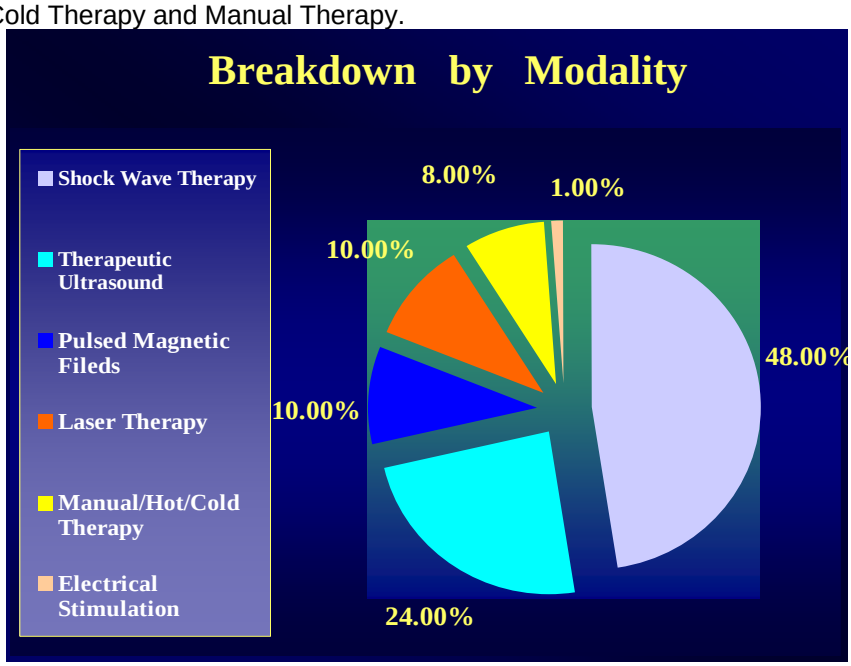
The use of physical therapeutic modalities is a well-established and widely used component of human sports medicine practice. Despite its obvious advantages in improving the quality of tissue repair and reducing convalescence, its routine employment in equine veterinary practice remains uncommon. This may be largely due to the fact that equine veterinarians generally have limited positive exposure to the uses and benefits of physical therapeutic modalities as related to the equine patient and perhaps underestimate the financial viability of incorporating these modalities into their practice.

This paper serves to review, from an economic approach, the process of establishing a physical therapy program within an equine veterinary practice and to provide several "model" programs that when properly instituted not only result in enhanced veterinary care but are financially viable as well.

The data collected through analyzing two equine physical therapy programs in two equine referral practices over a ten year period provide valuable insight with respect to case selection, capital cost and fee structures as well as choices in therapeutic equipment, and overall program organization. Additional information gathered from referring practitioners' individual use of physical therapeutic modalities further contributes to understanding how to successfully incorporate physical therapy into equine practice.

Through a simple evaluation process of the practice caseload, client base and personnel and facility several models for incorporating physical therapy into a practice will be presented. The size, scope and organization of the program being based on several factors including the correlation between case categories and therapeutic modality, cost and availability of equipment versus rate of return on investment and the structure of the practice i.e. ambulatory versus hospital.

In the course of analyzing the two referral practice programs, cases were categorized and correlated by diagnosis and treatment modality. Cases were grouped in the following categories; Tendon and Ligament Injuries, Back and Sacroiliac, Navicular and Caudal Heel, Wounds and Lacerations, Fractures and Osteopathies, Nerve and Muscle Injuries, Joint Contracture, Bursitis, Lymphangitis and Cellulitis, and all Other. The therapeutic modalities discussed and evaluated include; Extracorporeal Shock Wave Therapy, Therapeutic Ultrasound, Laser Therapy, Pulsed Electromagnetic Field Therapy, Electrical Stimulation, Heat and Cold Therapy and Manual Therapy.



Revenue generated from each modality as well as the frequency of use was tracked separately (see graph above) and juxtaposed to its investment and maintenance costs to evaluate its overall cost effectiveness and its rate of return on investment. Considerations such as the practicality and safety of the modality, the cost and quality of labor required for the modality and the realistic demand for such treatments must also be taken into account when designing a program that is safe, effective and capable of generating revenue.

Among other findings of the modality cost effectiveness analysis, it was apparent that although Extracorporeal Shock Wave Therapy was consistently the highest contributor to overall program revenue its high capital cost necessarily results in a slower rate of return on investment. Therapeutic ultrasound on the other hand with a relatively low capital cost and high frequency of use contributes significantly to overall program revenue and offers a rapid rate of return on investment. Both modalities however require a caseload with a significant number of tendon and ligament injuries to be most beneficial.

A breakdown of each of the referral practice caseloads will be presented as well as the figures representing cost of equipment and each modality's contribution by percent to the overall revenue generated by each program. A fee structure for treatments and therapeutic equipment rentals will also be discussed and four uniquely designed "model" programs will be outlined and offered as guides to incorporating physical therapy into a variety of types of equine practices. The strengths and potential drawbacks of each will also be discussed as will the goals of each model.

Capital outlay in establishing such a program can, but need not be significant. The cost, usefulness and adaptability of therapeutic equipment does vary widely and must be carefully considered along with the specific nature of the practice caseload. Goals in establishing any equine physical therapy program must be realistic and equal weight should be given to what may be potentially gained medically and financially from such a program. Ultimately the goals of any program no matter the size or scope should be to improve the rate and quality of healing, to offer better palliative care for the equine patient and to provide clients with enhanced follow-up treatment and communication. Based on the results of this review, we feel that these goals can be achieved through the implementation of several types of programs and submit that physical therapy if employed correctly can be a valuable asset to an equine veterinary practice both medically and economically.

CLINICAL PROBLEMS OF EQUINE PATIENTS IN INDIA AND ANTIMICROBIAL DRUG USE

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Considering the lack of information regarding equine patients, their problems and antimicrobial drug uses in equines, the survey study was conducted with objective to identify most commonly encountered clinical problems of equids and antimicrobial drug (AMD) use by equine veterinarians in different parts of India.

Three hundred fifty eight (358) equine clinicians of 14 states (Haryana, Uttar Pradesh, Himachal Pradesh, Punjab, Gujrat, Maharashtra, Delhi, Jharkhand, Rajasthan, West Bengal, Uttrakhand, Karnataka, Manipur and Andhra Pradesh) of India responded to survey through filling of proforma (with objective and subjective questions) posted to them. Of 1360 proforma sent to veterinarians in different parts of India during July 2006 to December 2007, 358 filled proforma returned by 31St March 2007. A total of 15 questions were specifically asked about their equine patients, common diseases/ syndromes of equines and common AMDs used in equines.

Of the 358 Vets 276 were treating animals at civil veterinary hospitals (CV) and patients belonged to general public while 34 were providing their services at organized equine farms (OFV), equine breeding stud farms or equids maintained on Farms of big Farmers. Fifty-five Vets were engaged in equine practice treating high number of equids year after year (HV) at both types of sectors (public as well as large farmers).

Equine vets in India identified >60 diseases or syndromes affecting equids (haemoglobinurea, nasal catarrh and nasal granuloma, retention of placenta, debility, urinary tract infections, digestive problems, bronchitis, pneumonia, skin carcinoma, equine encephalitis, abscess, hoof problems, canker, eye worms, actinomycosis, anemia, fractures, corneal opacity, muscular atrophy, hebronemiasis, colic, lameness/ laminitis, mineral deficiency, babesiosis, surra (trypanosomiasis, capped knee, oral and vaginal thrush, infertility, pyometra/ edomemtritis, pyrexia of unknown origin (POU), jaundice, metabolic disorder, anorexia, monday morning sickness endoparasitic infestation, ectoparasitic infecstation, strangles, urolithiasis, dystokia enteritis, diarrhea, urine retention (mainly in mules), cellulites, impaction, joint ill/ polyarthritis, guttural pouch infection, sprain, deformities, abortions, Herpes virus infection, salmonellosis, aspergillosis melanoma, barsati, mange, tetanus, dogbite, teeth irregularities, nervous disorder, poisoning, wound/ injury, mastitis, otorrhea, maggot wounds, frog, ocular parasites, mycotic maxillary sinusitis, naval ill, equine Influenza, stomatitis, accidents, pica, eye infection, rheumatism, allergy, foot rot, heat stroke, cold stroke, lung worms and glanders), of these 20 most common are colic, lameness, babessiosis, surra (trypanaosomiasis), infertility, pyometra, pyrexia of unknown origin (PUO), debility, infestation of endoparasites and ectoparasites, strangles, urinary tract infections (UTI), diarrhoea/enteritis, urine retention, impaction/ indigestion, polyarthritis and Tetanus. Common diseases reported by CVs, OFVs and HVs had good correlation (r>0.91).

Dystokia, infertility, abortions, thrush, fractures, canker, foal diarrhoea, strangles, lameness and babesiosis were the diseases mainly (*P*, 0.10) reported by the vets treating either large number of equine patients (HVs) or by the vets working on organized farms (OFVs) than common vets (CVs). However, some problems appeared to be area specific (P, <0.10) and need more investigations viz., eye infections and actinomycosis (Haryana and Uttar Pradesh), ocular parasites (Bareilly, Aligarh and Bijnor in UP), capped knee and urolithiasis (western Uttar Pradesh), melanoma and melanocarcinoma (Rajasthan, Uttar Pradesh and Haryana).

Almost similar drug/ drug combinations were used by all three classes of vets and use of AMDs by CVs, HVs and OFVs had strong (*P* <0.01) statistically correlation (r>0.89). Antimicrobial drugs are commonly prescribed by Vets to >70% equine patients. Trend of using AMDs in treatment was more common with OFVs and HVs than CVs. About 60 AMDs and their combinations are prescribed by equine vets, of the 60 ten are commonly used all over India (Fig.1). However, OFVs and HVs were significantly inclined (*P*<0.05) for use of penicillins, tetracyclines, gentamicin, cotrimoxazole, cefotaxime, ampicillin+ gentamicin and metronidazole than CVs.

Although intramuscular (IM) route of AMD administration remained the most preferred one (>83%), alternates routes (oral, intravenous, subcutaneous, subconjunctival, intrauterine and intra-articular) were mostly followed by OFVs and HVs (P, <0.02) only.

Most of the equine vets (>82%) expressed the acceptability of AMDs marketed for human use only for use in equine patients whenever required. The perception was more intense in OFVs (P, 0.05) than in CVs and HVs.

More than 50% vets expressed that AMDs adversely affect the health of equines patients after treatment and to counter adverse effects of AMDs >55% prescribe supportive therapy to their >50% cases. Among the AMDs, adverse effects were more common with use of penicillins, tetracyclines, fluoroquinolones, sulphdimidine, gentamicin, chloramphenicol and metronidazoles. Adverse effects of sulphdimidine, gentamicin and metronidazoles were more often observed by OFVs than CV and HVs.

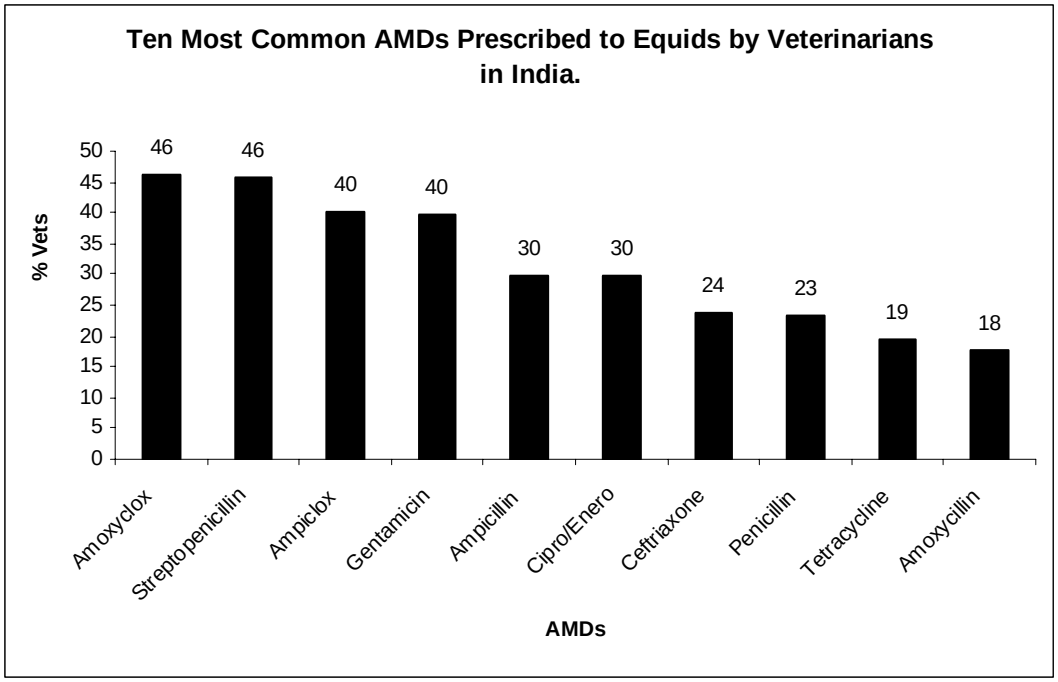
Most of the vets (> 50%) treating equines reported that to counter bad effects of AMD therapy supportive treatment should be given in >75% cases. Most common supportive drugs administered by vets to equine patients are antihistaminics, liver tonics, vitamins, probiotics, cortisone and mineral mixture preparations. To counter adverse effect of sulpha drugs some of the vets also used sodabicarb and fluid therapy.

Use and availability of probiotics was more common with OFVs and HVs (P, 0.05), while CVs were more inclined to use of antihisaminics and cortisone than OFV and HVs.

For prescribing AMDs 78-89% vets gave more weightage to diagnosis, 9-12% to prognosis, 18-26% to clients' economy, 11-13% to animal age, 9-12% to animal use and only 2% to treatment history.

More than 75% vets expressed the need of AMD sensitivity testing of bacterial isolates at least in selected cases for better treatment. Less number of HVs (75%) considers utility of AMD sensitivity than OFVs (87%).

In column of suggestions for development, 100 vets offered their views and expressed that scientist working on equids might pay attention towards development of: vaccine for control of trypanosomiasis (22), treatment and management of colics (18), better cure for infertility and urogenital infections (12), equine specific probiotics (7), treatment of babessiosis (5), vaccine for control of *Salmonella* and EHV induced abortions (5) and respiratory infections (4).



EVALUATION OF FUNCTIONAL ACTIVITY OF LYMPHOCYTES DURING MEDICAL IMMUNOREHABILITATION OF ATONY HORSES

Olga Romanova, Oksana Kryachko, Niels DUSEK
(abstract is submitted in Russian with a summary in English)

ОЦЕНКА ФУНКЦИОНАЛЬНОЙ АКТИВНОСТИ ЛИМФОЦИТОВ ПРИ МЕДИКАМЕНТОЗНОЙ ИММУНОРЕАБИЛИТАЦИИ ЛОШАДЕЙ-АТОПИКОВ

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Целью нашей работы являлась оценка функциональной активности Т-лимфоцитов лошадей при иммунореабилитационном применении рекомбинантного интерлейкина-2.

Лошадей для эксперимента выбирали из числа страдающих ХБО, но находящихся в стадии ремиссии. 6-ти лошадям двукратно, с интервалом 2 дня, вводили внутривенно ронколейкин в дозе 250 тыс. Ед, остальные 6 служили контролем. Взятие крови осуществляли в вакутейнеры с ЭДТА в начале эксперимента, через 3 дня после последней инъекции препарата и на 30-ый день опыта. Необходимым условием получения достоверных результатов являлась срочная доставка крови в лабораторию: менее 1 часа с момента взятия. Исследования проводились на базе кафедры патофизиологии СПбГАВМ.

Для оценки функционального состояния использовали реакцию торможения миграции лейкоцитов (РТМЛ) по методу Дж.Бендиксена и соавт.(1980) в модификации В.Г.Морозова и В.Х.Хавинсона (1980). В основе реакции лежит феномен подавления миграции лейкоцитов фактором, который выделяют сенсибилизированные лимфоциты при взаимодействии со специфическим антигеном (митогеном). В качестве Т-клеточных митогенов использовали белки растительного происхождения конканавалин А (конА) и фитогемагглютинин (ФГА).

Влияние рекомбинантного интерлейкина -2 на пролиферативную активность Т-лимфоцитов зрелых форм представлено на рис.1.

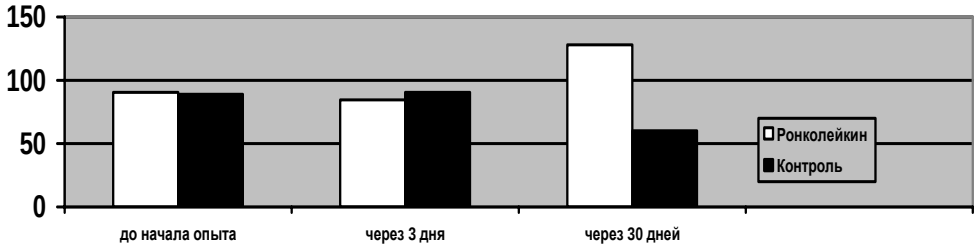

Рис1.Влияние Ронколейкина на функциональную активность Т-лимфоцитов в РТМЛ с Кон А, %

Из представленных данных видно, что на третий день после последней инъекции ронколейкина в крови лошадей опытной группы процент миграции лейкоцитов незначительно снизился (на 5,8%) по отношению к первоначальным показателям. Через месяц эксперимента наблюдалось увеличение процента миграции лейкоцитов в РТМЛ с Кон А у лошадей опытной группы (на 37,6%) тогда, как в контрольной группе этот показатель снизился по сравнению с предыдущими периодами исследований.

Сходную картину мы увидели в РТМЛ с ФГА (Рис.2).

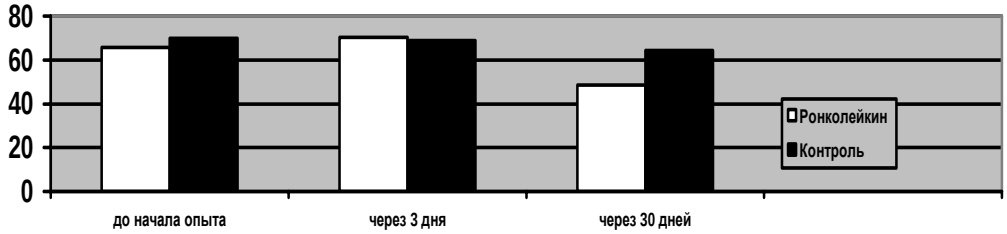

Рис. 2. Влияние Ронколейкина на функциональную активность Т-лимфоцитов в РТМЛ с ФГА, %

На третий день после повторного введения ронколейкина процент миграции лейкоцитов с ФГА увеличился по сравнению с исходным показателем. В то же время через 30 дней эксперимента наблюдалось его значительное снижение и по сравнению с первым днём исследований, и по сравнению с теми же значениями в контроле. На рисунке 2 хорошо видно, что процент миграции лейкоцитов с ФГА в контрольной группе животных имел тенденцию к снижению на всём протяжении эксперимента.

Полученные в ходе эксперимента данные согласуются с результатами исследований по изучению влияния рекомбинантного интерлейкина-2 на функциональную активность Т-лимфоцитов при наличии острого воспалительного процесса у лошадей (Крячко О.В., Романова О.В., 2004). В то же время проведённый эксперимент наглядно продемонстрировал возможности рекомбинантного интерлейкина-2 в качестве эффективного иммунореабилитационного средства. Наблюдаемый нами рост функциональной активности молодых форм лимфоцитов в реакции с Т-клеточным митогеном ФГА на третий день исследований коррелирует со снижением процента миграции зрелых форм. С одной стороны это может свидетельствовать о наличии остаточного воспалительного процесса в период ремиссии при ХБО и необходимости локальной лимфоцитарной активности. С другой стороны, полученный эффект демонстрирует реализацию потребности организма лошади-атопика в коррекции реакций бластной трансформации лимфоцитов с помощью рекомбинантного интерлейкина-2. Высокая пролиферативная активность лимфоцитов в первые несколько дней после применения ронколейкина стабилизировалась на 30 день эксперимента, что мы считаем благоприятным признаком для пролонгации периода ремиссии за счёт уже собственной Т-клеточной регуляции. Снижение процента миграции лимфоцитов, сенсибилизированных ФГА, также, указывает на необходимую регуляцию иммунологических механизмов, способных обеспечить острые проявления атопии.

Таким образом, по результатам проведенного эксперимента можно сделать следующие выводы:

1. Применение Ронколейкина лошадям, страдающим ХБО, в период ремиссии способствует регуляции реакций бластной трансформации Т-лимфоцитов.
2. Ронколейкин способствует корректному повышению пролиферативной активности зрелых форм Т-лимфоцитов в период ремиссии при ХБО в течение 30 дней после двукратного внутривенного введения.
3. Применение Ронколейкина в качестве иммунореабилитирующего средства способствует снижению процента миграции лимфоцитов, сенсибилизированных Т-клеточным растительным митогеном ФГА.

Summary

Estimation of lymphocyte functional activity has been accomplished in atopic horses within period of remission. As the immunorehabilitation remedy horses received recombinant interleukin-2 twice at therapeutic dose with interval of 48 hours. The examples of blood in experimental and control groups of horses were obtained before beginning of experiment, in three and thirty days after the last drug injection to put on RTML. In results of conducting experiment it has been revealed the increase of proliferative activity of mature T-lymphocytes and decrease of migration percentage of FGA sensitized cell under Roncoleukin action.

MYCOTOXICOLOGICAL QUALITY OF EQUINE FEED IN ARGENTINA

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Fungi able to produce mycotoxins are widespread contaminants of agricultural commodities and feeds. Fungi and mycotoxin present in raw materials could contaminate the final compound, lead to nutrient losses and produce detrimental effects on animal health and production. It is necessary to control the mycotoxicological quality. Aflatoxins are a group of secondary metabolites produced by *Aspergillus flavus* and *A. parasiticus* mainly. Horses are more susceptible to the adverse effects of aflatoxins than other species. The disease is characterized by decreased feed intake, body weight loss, liver damage, centrilobular hepatic disease, and brain, kidney and heart damage. *Fusarium verticillioides* is the most important fungus on corn. It produces fumonisins; a group of mycotoxins implicated in equine leukoencephalomalacia, porcine pulmonary edema and cancer promoter in rats (Harrison et al. 1990, Marasas et al. 1988). The purposes of this study were: 1) to investigate the mycobiota of raw materials and 2) to determine the aflatoxin B_1 (AFB_1), zearalenone (ZEA) and fumonisin B_1 (FB_1) natural contamination in equine feed in Argentina.

A total of 50 equine feed samples (maize, oat, ensiled alfalfa), 3 kg each, were collected at random during one year from hippo centers (pure race, pole, sport mares and racially mixed horses) located in Córdoba province, central Argentine. Quantitative enumeration of fungal propagules was done on dichloran rose bengal chloranphenicol agar (DRBC) to estimate total culturable mycobiota and dichloran 18% glycerol agar (DG18) for xerophilic fungi. Results were expressed as frequency (percentage of samples in which each genus was present) and relative density (percentage of isolation of each species among strains of the same genera). Thin layer chromatography (TLC) was applied to the separation and quantification of AFB_1 and ZEA (Official Methods of Analysis, 1995). High-performance liquid chromatography (HPLC) was applied to the quantification of FB_1 (Shephard et al. 1990 modified by Doko et.al. 1995). *A. flavus* strains were evaluated for the ability to produce AFB_1 (Geisen 1996).

Total counts (DRBC) ranged between 2.2×10^4 to 7.3×10^5 CFU g^{-1} with a mean value of $8.7 \times 10^4 \pm 1 \times 10^3$ CFU g^{-1}. Counts of xerophilic fungi (DG18) ranged between 1.3×10^4 to 1×10^6 CFU $g^{-1}$with a mean value of $4.8 \times 10^5 \pm 1 \times 10^4$ CFU g^{-1}. Mycological examination of the samples is shown in Table 1. Six fungal genera were identified. The most frequent genus isolated was *Aspergillus* spp. and Mucorales followed by *Fusarium* spp. Yeasts were isolated at a frequency of 100% (Figure 1.a.). From *Aspergillus* genus, four species were isolated. The most prevalent was *A. flavus* (43%) (Figure 1.b.). From *Fusarium* spp., two species were isolated and *F. verticillioides* was the predominant (Figure 1.c.). All *A. flavus* strains from oat samples were able to produce AFB_1 at levels between 20 and 35 ppm. Thirty-five percent of the samples were contaminated with AFB_1 at levels that varied between 5 and 64 ppb. The 28.6% of the samples were positive to ZEA contamination at detectable non-quantitative levels. Fumonisin B_1 was not detected (Table 2). Oat samples were contaminated with AFB_1 and 20% of them showed levels over the recommended limit (20 ppb). All samples were contaminated with ZEA: maize (100%), oat (40%) and ensiled alfalfa (10%). The co-occurrence of AFB_1 and ZEA was observed in 40% of the analyzed samples.

High levels of colony counts were found, which exceed the feed hygienic quality limit (1×10^4 CFU g^{-1}) (GMP 2005). These results are in agreement with recent studies that found counts over 1×10^5 CFU g^{-1} (Accensi et al. 2004, Rosa et al. 2006) and contrast with those found in other animal feed in Argentina (Dalcero et al. 1997, Magnoli et al. 2004). In the present study, a percentage of aflatoxin producers were found and important levels of AFB_1 were detected in oat samples. ZEA was present in lower proportion and FB_1 did not occur. These results agree with Dalcero et al. (1997) and Magnoli et al. (1999) in poultry feed. The present study has shown the simultaneous occurrence of two important mycotoxins, AFB_1 and

ZEA in feed intended for equine consumption. A synergistic toxic response is possible in animals on simultaneous exposure. The results of this research indicate that the high prevalence of potential toxin producers is indicative of mycotoxin problems in equine feeds. Studies of the mycotoxins natural occurrence and efficient prevention methods during feed processing should be encouraged and conducted simultaneously to avoid animal production impairment and hazards to animal and human health.

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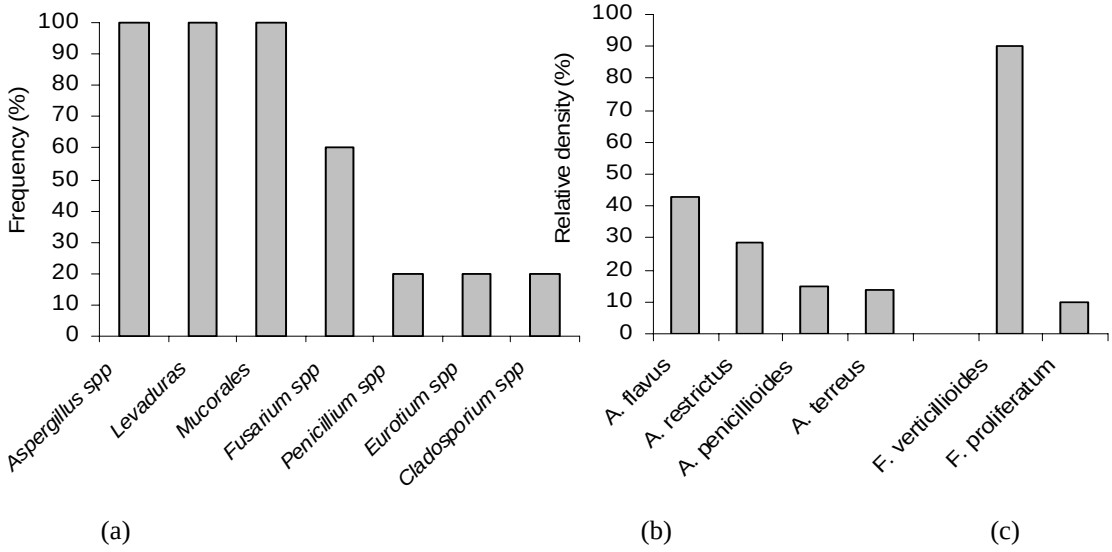

Figure 1: Percentage of genera (a), *Aspergillus* spp. (b) and *Fusarium* spp. (c) isolated from equine feed.

Table 1: Mycotoxin levels from equine feed.

Mycotoxin	Levels (ppb)	Methodology
AFB_1	5,36 – 64,40	TLC
ZEA	DNC*	TLC
FB_1	ND**	HPLC

* = Detectable, non-quantificable
** = Not detected
Quantification limit 5 ppb.

STUDY OF MYCOTOXICOGENIC MYCOBIOTA AND MYCOTOXINS IN EQUINE FEEDSTUFFS

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Most feedstuffs, cereal crops and other agricultural commodities are very susceptible to contamination by molds able to produce mycotoxins. Mycotoxins are toxic secondary metabolites that can cause adverse effects such as carcinogenesis, teratogenesis, nephrotoxicity and immunosuppression, leading to numerous pathologies and consequent economic losses (Hussein and Brasel, 2001). *Aspergillus* and *Fusarium* are the most frequently genera involved in animal and human cases of mycotoxicoses. In equines, mycotoxicoses are mainly related to corn based feedstuffs consumption contaminated with fumonisins (FBs) produced by *F. verticillioides.* These mycotoxins are responsible for equine leukoencephalomalacia (ELEM), an acute and fatal neurological disease characterized by neurotoxic symptoms — including loss of feed consumption, lameness, ataxia, oral and facial paralysis, head pressing, and recumbence (Marasas et al., 1988). More than one mycotoxin may exist simultaneously in a particular commodity or ingredient. Generally, the effects of these toxins tend to add up in synergic response, increasing the risk and hazard to animal health and productivity (D'Mello et al., 1999). Aflatoxins (AFs) are mycotoxins produced by *A. flavus* and *A. parasiticus*. Aflatoxin B_1 (AFB_1) is the most frequently detected and it has been described as the strongest biologically synthesized hepatocarcinogenic substance that can affect humans and animals (Rosa et al., 1996). Checking the mycological and mycotoxicological quality, control of feedstuffs and commodities destined to equine consumption is critical for improving animal production and performance. The purposes of this study were: 1) to determine the natural incidence of *Aspergillus* spp. and *Fusarium* spp. and the distribution of potential mycotoxin producers, 2) to detect and quantify FBs and AFs in equine feedstuffs.

Thirty samples of 21 different commercial feeds were randomly collected from five different studs located in Rio de Janeiro, from October 2005 to March 2006. Feeds were subdivided as intended for light effort, middle effort and intense effort horses. Analysis of the mycobiota was made by the plate dilution spread method onto dichloran rose bengal chloranphenicol agar (DRBC), dichloran glycerol 18% agar (DG18) (Pitt & Hocking, 1997) and Nash-Snyder culture media. Total fungal counts were expressed as CFU/g. The isolation frequency (%) of fungal genera/species was determined. Taxonomic identification of total fungal genera and *Aspergillus* and *Penicillium* species was done according to Pitt and Hocking (1997), Klich (2002), Samson et al. (2000) and Nelson et al. (1983). Mycotoxins determination was done using commercial ELISA kits (Beacon Analytical Systems Inc.)

Total fungal counts were similar on both DRBC and DG18 media. They ranged from not detected (ND) to 3.8×10^6 in light effort, ND to 2.2×10^4 in middle effort and from ND to 1.0×10^3 CFU/g in intense effort feed. *Penicillium* (48%) and *Aspergillus* (37.3%) were the most frequently isolated genera, followed by *Monilliella* spp. (5.33%), *Cladosporium* spp. (5.33%), *Fusarium* spp. (1.33%), *Rhizopus* spp. (1.33%) and *Mucor* spp. (1.33%). Three *Aspergillus* species were identified. *Aspergillus niger* was the prevalent species (39.29%) followed by *A. flavus* (14.29%) and *A. ustus* (3.57%). Eigth *Penicillium* species were identified as *P. fellutanum* (13.89%), *P. corylophilum* (8.33%), *P. minioluteum* (5.56%), *P. raistrickii* (2.78%), *P. citrinum* (2.78%), *P. commune* (2.78%), *P. miczynskii* (2.78%) and *P. brevicompactum* (2.78%). The telomorphic phases *Eurotium* spp. (28.57%) and *Eupenicillium* spp. (2.78%) were also isolated. A percentage of 63.33 of the samples showed FBs contamination with an

average level of 1.44 ± 1.93 ppm. All samples (100%) showed AFs contamination with an average level of 8.36 ± 9.80 ppb.

For animal feed, the FDA advisory guidelines (2001) recommend that contaminated corn or corn by-products be limited to no more than 20% of the diet for equids. In this work, three samples had higher AFB1 and FB1 levels than the established limit (20 ppb and 5 ppm). Even if the amount of mycotoxins produced is not enough to cause adverse effects in animals, it is a sign that the feed will be less nutritious (Dalcero et al., 1997). It is also necessary to establish maximum limits of fungal counts for potential AFs and FBs species since the presence of toxin-producing fungi is a possible risk. Further methods are required to reduce infection by mycotoxin-producing fungi in equine feed and procedures for treating heavily contaminated feed lots are also important to avoid animal production impairment and hazards to animal and human health. These data from Brazil should be of interest worldwide.

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RUPTURE OF SMALL INTESTINE INTO THE MESENTERY WITHOUT EVIDENCE OF OVERLOAD OR OBSTRUCTION IN 4 HORSES

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Introduction: Although diverticula of the small intestine are largely described in the horse[1-3], to our knowledge, there is only one report[4] of rupture of the small intestine (ileum) between the 2 layers of the mesentery without a pre-existent diverticulum. The aim of the present study is to describe 4 cases of spontaneous rupture of the small intestine into the mesentery (1 located on ileum and 3 on jejunum) and to compare our data with those of the literature.

Material and Method: The clinical data and post mortem findings of 4 horses admitted at the University of Liège with a diagnosis of spontaneous perforation of the small intestine into the mesentery were reviewed. One of these horses (case 1) was found dead at home and was directly referred to the Department of Pathology. The 3 other horses underwent a complete physical examination including naso-gastric intubation, transrectal palpation and abdominocentesis, as well as a complete blood count and biochemistry on admission. Transabdominal ultrasound examination was also carried out. Two horses underwent an exploratory laparotomy whereas for the third (case 4) the owner elected euthanasia.

Results: Horses were warmblood type: 2 geldings and 2 mares, with a bodyweight ranging from 450 to 520 kg and aged 15, 13, 16 and 21 years respectively. Horses were depressed with intermittent severe pain, poorly responding to any NSAIDs administered. Two horses (cases 3 and 4) presented recurrent colic and one of them (case 3) also suffered from chronic weight loss. On admission, all horses showed severe signs of endotoxic shock and profuse sweating. There were no gut sounds in all cases. One horse (case 2) presented a light bilateral abdominal distension whereas the others were lean-flanked. By nasogastric intubation reflux was recovered varying between 1 and 11 litres. Transrectal palpation revealed 1 or 2 loops of slightly distended small intestine and pain in the right flank in case 2, a vacuum sensation in case 3 and only one very slightly distended loop of small intestine in case 4. The transabdominal ultrasound showed some small intestinal loops with thickened wall in all cases. Some slightly distended small intestinal loops and an increased amount of abdominal fluid were also found in cases 2 and 4. The peritoneal fluid was orange to dark red in all cases. Blood analysis showed a haemoconcentration: PCV were respectively of 56, 50 and 48 percent for cases 2, 3 and 4. White blood cells were diminished to normal (respectively 5.7, 2.4 and 7.6 $x10^3$/ mm^3). When measured, blood gas values, creatinin, urea and ions were in the normal range or not significantly modified. Cases 2 and 3 were subjected to euthanasia during surgery because of peritoneal contamination. Post mortem examination of all 4 horses revealed a rupture of the small intestine on the mesenteric border (ileum in case 2 and jejunum in the other cases) without evidence of obstruction, leading to the formation of an alimentary pouch (approximately 30-40 cm diameter) between the 2 layers of the mesentery. In all cases the rupture was longitudinal and located on the attach of the mesentery. The sizes of the intestinal rent varied between 2 and 5 cm. In these 4 horses the site of intestinal rupture did not show any evidence of diverticula,. A thickening of the intestinal wall centered on the zone of the rupture was noticed in all horses. This thickening included all the length of the small intestine in case 1. Histological examination of the small intestine (realised in cases 3 and 4 only) revealed an eosinophilic enteritis with a hypertrophy (or more correctly hyperplasia) of all muscular layers including muscularis mucosae, more pronounced near the ruptured zone.

Discussion: Rupture of the small intestine into the mesentery without evidence of overload or obstruction is a rare pathology in horses. Clinical symptoms are close to those of a bowel rupture: fast evolution towards severe endotoxic shock, little abnormality on transrectal palpation and thickening of the small intestine visualised by ultrasonography. In our study the horses were middle aged to old, which could let suppose a degenerative phenomenon. The hypertrophy of the smooth muscular layer was not caused by a (sub)obstruction and thus seems idiopathic, as previously described[1]. However, muscular hypertrophy observed here is

less important than in other reports[1,4] and did not lead to a significant narrowing of the intestinal lumen. Because of similarities (muscular hypertrophy, some intestinal ruptures and occasional history of recurrent colic or weight loss) with other reports,[1] it is possible that the rupture of the small intestine into the mesentery belongs to the same pathological entity as idiopathic muscular hypertrophy, associated or not with the formation of diverticula.

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EFFECT OF TYPE OF WORK ON SUB CLINICAL EXERTIONAL MYOPATHY (SCEM) IN WORKING EQUINES

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ABSTRACT

Sub clinical exertional myopathy (SCEM) is one of the major problems of working equines as symptoms are not diagnostic, hence owner remains worried for underperformance but does not provide rest or treatment to animal, which is important under such conditions. The present study was carried out to assess the impact of type of work on the development of SCEM in working equines. The serum samples were collected from 240 animals. The animals were divided into 3 groups A,B & C with each group consisting of 80 animals. Gp. A & B animals were working as pack and cart animals respectively in brick kilns while Gp. C animals were working as cart animals in city to carry passengers and goods. In gp. A & B, serum samples were collected before, during and at the end of brick kiln working season and in gp. C before and after work. Serum samples were subjected to enzyme estimation for creatinine phosphate (CK), lactate dehydrogenase (LDH), aspartate transaminase (AST) to know the extent and duration of muscle damage supported by total serum protein value. The study revealed absence of SCEM before start of brick kiln season in gp. A & B while before work in gp. C, but there was a significant increase in AST ($p<0.01$), CK ($p<0.00$) & LDH ($p<0.01$) and total protein ($p<0.01$) during working season in brick kiln as well as after work in gp. C. there was non significant increase in CK, AST, & LDH enzyme level between pack (gp. A) and cart animals (gp. B). There was significant ($p<0.05$) difference between gp. B & gp. C animals after work enzyme status suggesting more muscle damage in cart animals working at brick kilns. Persistent high enzymatic activity was observed in brick kiln animals (A & B) up to end of working season. There was significant increase in enzymatic activity of CK, LDH, AST & TP after work in gp. C animals. However exceptional high enzymatic activity of CK was observed in gp. C animals after in few cases which may be due to absence of work on previous days. It was concluded that enzyme level does not crosses the clinical level in serum activity. But persistent increase in enzyme level in serum is indicative of SCEM in working equines & the severity of condition increases towards the end of season although animals does not exhibit any typical signs of myopathy. The comparison of 3 work types i.e., brick kiln pack, brick kiln cart & cart animals carrying passengers in city revealed that brick kiln cart animals suffers most from SCEM as revealed by serum activity of muscle enzymes & total serum protein value.

INFLUENCE OF DIFFERENT INTENSITY TRAINING TO ELECTRICAL ACTIVITY OF HEART IN SPORT HORSES

A.N.Shestakova
(abstract is submitted in Russian)

ВЛИЯНИЕ ТРЕНИНГА РАЗЛИЧНОЙ ИНТЕНСИВНОСТИ НА ЭЛЕКТРИЧЕСКУЮ АКТИВНОСТЬ СЕРДЦА У СПОРТИВНЫХ ЛОШАДЕЙ

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Высокий уровень тренированности лошадей характеризуется способностью организма сохранять хорошую работоспособность в условиях большой кислородной недостаточности. Об уровне физической работоспособности, который зависит от деятельности кардио-респираторной системы, можно судить по данным статической электрокардиографии. Целью наших исследований являлось изучение изменений параметров ЭКГ у лошадей в зависимости от вида тренинга. В период с 2000 по 2007 годы нами было зарегистрировано 346 электрокардиограмм у спортивных лошадей с различными видами тренинга, принадлежащих Кировскому ипподрому, лаборатории коневодства ВГСХА, конно-спортивным клубам Кирова и Сыктывкара, а также цирковым труппам из разных городов России.

Методы исследования. ЭКГ регистрировалось посредством трехканального электрокардиографа KARDIOVIT AT-1 (SHILLER, Швейцария) по методике сагиттальных туловищных отведений по М.П. Рощевскому. При использовании данной методики регистрируется ЭКГ хорошего качества, животные испытывают меньше неудобств в момент исследования, обеспечивается плотный контакт электрода с кожей (рис.1).

Электроды при данной методике накладываются следующим образом :

- красный – краниальная часть грудной кости
- желтый – нижний склон холки
- зеленый – точка пересечения перпендикуляра, опущенного от 13-го ребра с белой линией живота
- черный – в области левой локтевой складки

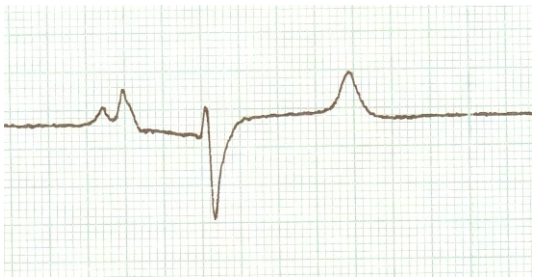

Рис. 1 Нормальная ЭКГ у спортивной лошади (по методике сагиттальных туловищных отведений).

Результаты исследования. Нами было установлено, что изменения электрической активности миокарда у подопытных спортивных лошадей были пропорциональны интенсивности физической нагрузки. Так, амплитуда зубцов у хорошо тренированных лошадей значительно выше, чем у нетренированных, особенно это касается зубцов R, S, T . Среднее значение амплитуды зубца R в I группе (интенсивный тренинг) составило 0,7 ± 0,13 мВ, а во II группе (прокат) – 0,3 ±0,09 мВ; зубца S в I группе составило 2,4 ± 0,11 мВ, а во II группе – 1,3 ±0,08 мВ; зубца T – 1,1 ± 0,06 мВ и 0,4 ± 0,01 мВ соответственно. Следовательно, амплитуда зубцов у спортивных лошадей увеличивается в разы: а именно – зубца R в 2,3 раза, зубца S в 1,8 раза, зубца T в 2,8 раза. Этот факт свидетельствует о более высокой ЭДС миокарда в результате тренинга

(разница по амплитуде зубцов желудочкового комплекса достоверна $p<0,01$). Повышение ЭДС миокарда свидетельствует о формировании спортивного сердца с развитием его тоногенной дилатации и гипертрофии, что в свою очередь приводит к увеличение силы сердечных сокращений согласно закону Франка-Старлинга.

Что касается продолжительности интервалов, то здесь также четко прослеживается разница значений между животными опытных групп. Средняя продолжительность интервала P-Q у лошадей I–ой опытной группы (тренированные) составила 0,3 ± 0,03 сек., у животных II–ой группы (прокатные)– 0,24 ± 0,02 сек., т.е на 25 % больше. Продолжительность желудочкового комплекса QRS у лошадей I-ой группы была равна 0,06 ±0,007сек., а II–ой группы – 0,08 ± 0,016 сек. Средняя продолжительность интервала Q-T у хорошо тренированных лошадей составляла 0,44 ±0,04 сек., а у плохо тренированных – 0,52 ± 0,06 сек. Из полученных данных видно, что временные характеристики ЭКГ отражают сокращение продолжительности электрической систолы желудочков у лошадей, испытывающих интенсивные физические нагрузки, что является свидетельством высоких функциональных способностей спортивного сердца.

При анализе **ЧСС** в покое наблюдается значительное уменьшение показателей у высокотренированных животных (25 ± 1,1 уд./мин) – 1,9 раза по сравнению с прокатными лошадьми (48 ± 3,4 уд./мин). Уменьшение ЧСС у спортивных лошадей является компенсаторно-приспособительной реакцией, позволяющей поддерживать величину сердечного выброса, и свидетельствует об относительном преобладании парасимпатической активности (ваготония). Такое снижение ЧСС отражает оптимальный уровень нейровегетативной регуляции тренированного сердца, в частности существенное ограничение в покое симпатической активности. Наличие тахикардии у прокатных лошадей является свидетельством того, что при нерациональном тренинге в сердце преобладают адренергические влияния.

Что касается функциональных характеристик работы сердца, то здесь наблюдалась следующая картина: СПП (систолический показатель предсердий) в I–ой группе равнялся 16 ± 0,6 %, во II–ой группе – 23 ± 1,1 %, СПЖ (систолический показатель желудочков) – 29 ± 2,5 % и 41 ± 3,3 % соответственно, ДСК (диастоло-систолический коэффициент) – 1,0 ± 0,1 и 0,65 ± 0,05 соответственно. Анализируя полученные данные можно смело утверждать, что функциональные способности миокарда у животных 1-ой группы находятся на высоком уровне (чем меньше значения СПП и СПЖ, тем лучше миокард адаптируется к нагрузке). Что касается диастоло-систолического коэффициента, то по его величине судят о достаточности процессов восстановления миокарда. Только у хорошо тренированных животных мы наблюдаем преобладание диастолы над систолой в покое.

Динамика изменений ЭКГ при нерациональном тренинге :

1. Гипертензия в малом круге кровообращения – при конкуре и выездке тренинг лошадей связан с частыми задержками дыхания, что может вызвать повышение давления в малом круге. Гипертензия малого круга в свою очередь вызывает гипертрофию правых отделов сердца: повышается электрическая активность, замедляется проведение импульса, развивается дистрофия миокарда. ЭКГ-признаки : отклонение оси сердца вправо, перегрузка и гипертрофия правого предсердия - деполяризация возросшей массы правого предсердия требует большего времени, вследствие этого восходящее колено зубца P записывается дольше – формируется остроконечный и высокий зубец – P-pulmonale, увеличение P-Q , т.к. миокард предсердий быстрее реагирует на появление гипертензии, развивается артериальная гипоксемия и дистрофия – инверсия сегмента S-T.

2. Ишемия (гипоксия) миокарда – при любой достаточно интенсивной нагрузке развивается так называемая рабочая гипоксия, когда потребление кислорода в реакциях энергетического обмена превышает возможности его доставки к клеткам. Ишемические проявления у лошадей с гипертрофией миокарда связаны с патологией микроциркуляции, что проявляется увеличением массы миокарда, и отставанием роста капиллярной сети. Она характеризуется кратковременным снижением кровоснабжения и гипоксией участков миокарда. Ишемия в первую очередь сказывается на процессе

реполяризации (восстановление готовности к очередному возбуждению, т.е. реставрация положительного заряда внешней стороны клеточных мембран), замедляя выход клеток из состояния электровозбудимости. Нарушение реполяризации на ЭКГ при развитии гипертрофии (или дилатации) сердца обусловлено увеличением времени прохождения волны деполяризации от внутренних слоев к наружным слоям за счет увеличения объема мышечной массы сердца. На ЭКГ формируются «коронарные» зубцы Т (глубокие отрицательные), особенно при субэпикардиальной локализации ишемии.

3. Дистрофия миокарда – поражение миокарда, характеризующееся обратимой дистрофией мышечных волокон. Распознается только по ЭКГ по смещению сегмента S-T вверх или вниз от изолинии более, чем на 0,2 мВ. Субэндокардиальная дистрофия – смещение вниз, субэпикардиальная – вверх. Наиболее часто поражаются субэндокардиальные слои, которые в силу особенностей интрамурального ветвления венечных артерий, даже в здоровом сердце находятся в наихудших условиях кровоснабжения. Рабочая гипертрофия миокарда только усугубляет дефицит их питания – «относительная коронарная недостаточность». Дистрофические изменения неизбежно вызывают расстройство сократительной способности миокарда: удлинение Q-T больше, чем на 10 % от нормы (в лучшем случае) или электрическую альтернацию желудочковых комплексов – QRS разной конфигурации и полярности (при тяжелой степени дистрофии).

Выводы :

1. Полученные данные свидетельствуют о том, что у высокотренированных лошадей происходит адаптация кардиоваскулярного аппарата к физической нагрузке за счет экономизации работы сердца и повышения его функциональных способностей.
2. Временные характеристики ЭКГ (продолжительность интервалов) отражают сокращение длительности электрической систолы желудочков и удлинение электрической диастолы у лошадей, испытывающих интенсивные физические нагрузки, что является свидетельством высоких адаптационных способностей спортивного сердца.
3. Последствиями нерационального тренинга могут быть серьезные нарушения в сердечно-сосудистой системе: гипертензия в малом круге кровообращения, гипоксия и дистрофия миокарда, неполная А-В блокада.

LAMINITIS: EGYPTIAN VET'S PRACTICE
CLİNİCO- RADİOLOGİCAL AND TREATMENT- TRİALS OF EQUİNE LAMİNİTİS İN EGYPT

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Abstract

Seventy four laminitic, mixed breed, drafting and riding horses were incriminated in the present study. They were gathered from Surgery Clinic, Faculty of Veterinary Medicine, Cairo University, various stables in the pyramids area and Equestrian army club. Seven apparently healthy horses were chosen as a control group. The laminitic and control horses were of different sexes (53 male and 28 female) and ages (6 to 19 years). Their weight varied from 250 to 450 kg. The study was conducted as 2 main steps; to determine the clinical, laboratory and radiological changes occurred during different stages of laminitis and to evaluate the suggested useful treatment for the disease.

The aim of the present study was to evaluate different specific treatment regimes on different forms of laminitis in equines. The adopted treatment regimens included traditional therapy i.e. initial treatment regimen, radial vein phlebotomy and also modern medicines i.e. pentoxifylline (Trental®) to increase the digital blood flow, nifedipine (Epilat®) as an antihypertensive or virginiamycin (Founderguard®) as L-lactate antagonist for treatment of acute and subacute laminitis. Additionally, anterior hoof wall resection and corrective shoeing for treating chronic laminitis were also used.

Thirty nine radiographs of chronic laminitic horses and five of normal control horses were undertaken in attendance study that aimed to provide a radiological data about the picture of the sequences that occurred in hoof with chronic laminitis and the rotation degree of the third phalanx in different stages by a simplified computerized method (Adobe Photoshop CS 2). The measurements and calculation of different distances and angles of the hoof that would be useful as a prognostic indicator of the disease and useful also in assessment of the treatment efficacy of laminitis were advocated for evaluation. The study concluded that there were some important points should be put in consideration during manipulation and treatment of chronic equine laminitis cases according to that radiological evaluation

Treatment of chronic laminitis was done by anterior hoof wall resection, hoof trimming but the horn of the quarters and heels was kept and preserved to fit the heart bar shoe onto solid wall with application of soft silicon in order to fix the shoe and raise the heel, in addition to the use of methionine and biotin in daily ration of affected horses to promote hoof growth. The study found that treatment of chronic laminitis needs more time (up to 6 month) in addition to excessive cost for treatment and farriery fees.

LAPAROSCOPIC TECHNIQUES FOR UNILATERAL ABDOMINAL CRYPTORCHIDECTOMY IN STANDING HORSES

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INTRODUCTION - Laparoscopy is a safe and minimally invasive technique currently used to locate and remove abdominal testes in horses. When compared to more invasive techniques, it presents minimal morbidity and provides earlier horses' return to activities. Laparoscopic cryptorchidectomy can be done by flank access in standing patient or by ventral access in dorsal recumbency. Left or right flank access avoid the use of general anesthesia and its associated risks, allowing adequate inspection of the inguinal region and associated structures. Many techniques have been described for standing laparoscopic cryptorchidectomy, and the main difference between them is the haemostatic method applied at the spermatic cord. In our routine, recumbent laparoscopic procedure is restricted for cases of bilateral abdominal testes retention or for unilateral abdominal cryptorchidism when the horse has an unruly behavior.

OBJECTIVE - The purpose of this abstract is to present a modified technique and compare with other two current laparoscopic cryptorchidectomy techniques in standing horses.

METHODS – Twenty three horses with unilateral testis retention were submitted to flank laparoscopic procedure. Fourteen animals were submitted to technique "A", three to technique "B" and six to technique "C" (modified technique). All the techniques were accomplished by withholding food from the animal for 18 hours and water from 12 hours. The horses were restrained in standing position in a stock and were sedated with xylazine (0,6 mg/kg, iv) and butorphanol (0,03 mg/kg, iv). The correspondent flank was prepared for aseptic surgery and lidocaine was infiltrated directly at the laparoscopic portal sites. Two laparoscopic portals were done in the paralombar fossae on the same side of testis retention; the first one for laparoscope insertion and insufflation, and the second for grasping forceps insertion. After the carbon dioxide pneumoperitonium establishment using 8 mmHg pressure, the abdominal testis was located and grasped, and its excision done using one of the three techniques.

Technique A: After grasped, the abdominal testis was dislocated to the abdominal wall direction. The second trochar was retracted and the portal was enlarged allowing partially testis exposure. Lidocaine was then injected into the testis using a long needle. After traction and complete testis exposure an emasculator was applied at the spermatic cord, out of abdominal cavity. The spermatic cord and epididymis were transected, releasing the testis. The stump was introduced into the abdominal cavity and inspected for hemorrhage.

Technique B: A third portal was created and an encircling ligature using size 2 polypropylene prepared in a knot pusher was inserted into the abdomen. The ligature was applied over the mesorchium including spermatic cord and a part of the epididymis, and this loop was tightened. The knot pusher was removed and a laparoscopic scissor was inserted through the third portal to cut the spermatic cord and epididymis. The stump was inspected for hemorrhage and the testis was exposed after enlargement of the second portal.

Technique C: A third portal was created for insertion of a laparoscopic scissor, and the gubernaculum testis and mesorchium were then transected. The second portal was enlarged, the testis and epididymis was completely exposed and an emasculator was applied, out of the abdomen, as described on technique A. After testis excision using one of the three techniques, the abdominal cavity was decompressed, laparoscopic instruments were removed and skin were closed using size 2-0 nylon in an interrupted pattern. On the enlarged portal the external abdominal oblique muscle were closed using size 0 polyglactine 910 in a continuous pattern. Horses received antibiotics and nonsteroidal anti-inflammatory drugs for 3 days. The contra lateral descend testis was removed by routine orchiectomy in standing position.

RESULTS AND DISCUSSION - The advantage of technique “B” is that previous exposure of the testis is not necessary to ligate the mesorchium, preventing insufflation loss and gubernaculum traction, but the disadvantages are the need of 3 laparoscopic portals, remainder suture material into the abdomen and part of the epididymis attached to the gubernaculum, the need of surgeon ability and appropriate material for laparoscopic ligature to make the knot. Technique “A” advantages is that only 2 portals are need for testis grasping and exposure and the fact of no suture materials are leaved into the abdominal cavity, but as disadvantages the testis exposure is difficult because excessive traction in gubernaculum testis is applied and part of the epididymis usually remains into the abdomen. Technique “C” presents as advantages the ease to exposure the testis due to previous transection of gubernaculum testis, reducing traction in structures and less discomfort of the patient compared with the technique “A”, allowing complete epididymis removal. Compared with technique “B”, technique “C” is less complicated, faster and less expensive, avoiding the presence of suture material into the abdominal cavity. Disadvantage of technique “C” is the need of 3 laparoscopic portals.

CONCLUSION - In the authors’ opinion, technique “C” eliminates the disadvantages observed on technique “A” and “B”, combining simplicity and efficiency for laparoscopic testis removal in cases of abdominal unilateral cryptorquidism, mainly in horses with hipoplasic testis and restrictive gubernaculum.

A CASE OF PERSISTENT HYPERAMMONEMIA POSSIBLY DUE TO A UREA CYCLE ENZYME DEFICIENCY

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Introduction: In the horse, the gastrointestinal tract is a major site of ammonia production due to the hydrolyzation of urea by microbial urease. Ammonia diffuses into the portal circulation and is transported to the liver to be converted by the Krebs-Henseleit urea cycle within the hepatocytes into urea and glutamine. Blood urea is eliminated by renal excretion. Hyperammonemia is poorly documented in horses. In this species, it is often related to liver insufficiency or to intestinal problems with overproduction of ammonia by the bacterial flora overwhelming the hepatic metabolism. In most cases this leads to an acute rise in ammonia and acute cerebral dysfunction (hepatoencephalopathy). Hyperammonemia can however also be related to compromised liver function due to acquired or congenital portosystemic shunting or to inborn errors of metabolism including urea cycle enzyme deficiencies. The latter are congenital disorders due to complete or partial functional deficiency in one of the enzymes in the urea cycle which leads to a complete or partial insufficiency to metabolize ammonia.

Case: A 13 year old Selle Francais gelding was admitted at the equine clinic of the University of Liège with the complaint of apathy, loss of appetite, weight loss, muscular fasciculations and weakness since 6 months. His body condition was poor. The horse presented a slight depression, showed regularly muscular fasciculations and showed a grade 1/5 ataxia and weakness in the 4 legs. A rectal examination, complete haematology and biochemistry including serum vitamin E dosage, abdominocentesis, coprology, gastroscopy, dental examination, urine analysis, radiography of the cervical vertebral column, echocardiography and a glucose absorption test were performed, but no significant abnormalities were found. An exercise test (lunging) was performed, and serum muscular enzymes measured before and 6 hours after the test didn't increase. Abdominal ultrasonography showed bilaterally enlarged kidneys with loss of visibility of the medulocortical junction. Histology of a renal biopsy showed a subacute, multifocal, interstitial nephritis with a membranous glomerulopathy and a slight tubular degeneration. However, these lesions were thought to be aspecific and were not considered to be related to the clinical signs. During his 17 days of hospitalization, the serum level of ammonia was measured 3 times and systematically compared to the same healthy mare. The blood samples were immediately put on ice and were analysed within 30 minutes of sampling. The ammonia was each time significantly increased in the sick horse (111µmol/L versus 24µmol/L in the control horse; 175µmol/L versus 35µmol/L in the control horse; and 187µmol/L versus 19µmol/L in the control horse). Because of this persistent hyperammonemia, a bromsulphalein (BSP) elimination test was performed which evidenced a prolonged BSP retention time ($T_{1/2}$: 8,85 minutes, normal value : $\leq$ 4,5 minutes). The ultrasonographic window to visualise the liver was too small and furthermore limited by peripheral large vessels to perform a safe hepatic biopsy. Serum and urine were analysed for urea cycle defects in a human hospital. Blood samples were centrifuged and serum was separated within 15 minutes of sampling. Serum and urine samples were placed at -20°C until analysis. Plasmatic and urinary levels of amino acids and their derivatives, urinary organic acids and urinary purines and pyrimidines were measured in the affected horse and in a healthy control mare. Compared to the control horse, the affected horse showed an increase in citrulline both in urine (17 mmol/mol compared to 1 µmol/L in the control horse) and plasma (100µmol/L compared to 64 in the control horse and 15-55µmol/L of human references). There was a tendency for decreased plasma amino acids in the affected horse. The total of plasma glutamic acid and glutamine was not increased. The profiles of urinary organic acids and purines and pyrimidines, including orotic acid, were similar in both horses. A muscle biopsy of the triceps and of the sacrocaudalis dorsalis medialis muscle was performed which showed a neurogenic degeneration of muscle fibres, without inflammatory infiltration.

Discussion: In the reported case, the persistent hyperammonemia could explain all clinical signs and the muscle fibre degeneration evidenced on muscle biopsy. To explain the persistent hyperammonemia in this case, a portosystemic shunt was excluded since bile acids were within the normal range. Intestinal problems which could lead to increased intestinal production of ammonia were neither present. The horse formed normal faeces and showed no signs of colic during the duration of the hospitalization. It was not clear whether the hyperammonemia was due to a liver insufficiency or to a metabolic defect. It was strongly indicated to perform a liver biopsy to confirm or exclude a liver disease, but the owner of the horse did not agree considering the risk taken by this ancillary test. The increased BSP retention time could have been caused by a liver insufficiency, but also by biochemical abnormalities as seen in humans with urea cycle enzyme deficiencies. Moreover, no blood parameter or abnormality on liver ultrasonography was indicative of a liver insufficiency in this horse. The urine and blood analysis to test the urea cycle were not compatible with the hyperornithinemia, hyperammonemia and homocitrullinuria (HHH) syndrome, reported in a few young horses. However, the hypercitrullinemia could indicate a deficient function of one of the following urea cycle enzymes: argininosuccinate lyase (ASL), or argininosuccinate synthetase (ASS). Argininosuccinate synthetase deficiency in humans (known as argininosuccinicaciduria) is typically accompanied by much higher citrulline concentrations (1000-5000µmol/L) than argininosuccinate lyase deficiency (known as citrullinemia) (100-300µmol/L), favouring the latter diagnosis in our horse. However, argininosuccinate, which accumulates in human citrullinemia, was not detected. Both deficiencies have been described as late-onset deficiencies in human medicine, which results in more moderate signs compared with the severe neonatal-onset disease.

Conclusion: this presentation reports a case of persistent hyperammonemia possibly due to a urea cycle defect.

BILATERAL LASER VENTRICULOCORDECTOMY AS A TREATMENT OF LARYNGEAL STENOSIS DUE TO EXTERNAL COMPRESSION

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Abstract

THE AIM OF WORK: A 4-years-old Quarter horse mare with laryngeal stenosis was treated by bilateral Nd-YAG laser ventriculocordectomy in order to reduce airway obstruction, improve the hypoxaemia and prolongate the life of the pregnant mare. The horse initially presented with dorsal displacement of the soft palate and soft palate cyst – the cyst was removed by surgically. The site of the operation healed by second intention and the retraction of the wound generated a mild cleft palate. The horse represented four month later with dyspnoe, dsyphagia, partial dorsal displacement, aspiration pneumonia, anorexia and a three-month-old pregnancy. We decided to treat symptomaticly with antibiotics, mucolitics, vitamins. After three more months the condition of the horse worsened she could not have opened the vocal cords. The decision was made to perform a transendoscopic bilateral Nd-YAG laser ventriculocordectomy to preserve the life of the mare till parturition.

MATERIALS AND METHODS: the endoscopic findings were laryngeal stenosis due to impossibility to open the vocal cords and compression of the larynx by bilateral perilaryngeal tissue proliferation seen well from the trachea. On the laterolateral view radiographic evidence of perilaryngeal tissue calcification was seen by X-ray. Azary-tracheostomy tube was inserted into the trachea as a first step and both vocal cords and laryngeal vetricules were removed by transendoscopic Nd-YAG laser using in contact mode (15W, continious mode) then. The tracheostomy tube was left in place for 6 days and the horse was under antimicrobial and antiinflammatory therapy for 10 days.

RESULTS: the progression of the condition of the mare was evident and parturition was uneventful resulting a healthy foal. Keep the contact with the owner by phone one year after the ventriculocordectomies the mare is in a stable good condition and the goal to be a breeding mare is fulfilled.

DISCUSSION: in humans the most common primary lesions of the laryngeal cartilages are chondroma and chondrosarcoma but the symmetrical developement and the improving condition of the horse make the possibility of tumor not likely. In humans there is presumption that repetitive microtrauma related to muscular overuse probably led to inflammatory changes at tendinous insertion on the laryngeal cartilages resulting calcification. In horses there is no available data.

CONCLUSION: bilateral ventriculocordectomy can improve the condition of a horse with laryngeal stenosis due to external compression by increase the diameter of the airway and presumably decrease the degree of microtrauma of the larynx caused by dyspnoe.

EFFECTS OF İNTRAVENOUS LİDOCAİNE İN HEALTHY ADULT HORSES

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Lidocaïne is frequently used as a procinetic drug in horses suffering from ileus. However, little is known about the pharmacokinetic and the pharmacologic effects of this drug in healthy and in sick horses. The purpose of this study was

(1) to non invasively evaluate, using transparietal ultrasonography, the effects of a lidocaïne perfusion in healthy horses at a dosis commonly used in horses suffering from an ileus.

(2) to establish the plasmatic level of lidocaïne and of monoethylglycinexylidine (MEGX), one of its metabolite, obtained by the use of this dosis of lidocaïne perfusion.

Five healthy adult halfblood horses were used in this study. Each horse was studied twice, once during administration of lidocaïne (LID group), and once during administration of a placebo (PLAC group). Lidocaïne was given as a 1.3 mg/kg IV bolus within 10 minutes followed by a perfusion at 0.05 mg/kg/min during 110 minutes. The placebo consisted of a perfusion of 0.9 % of NaCl given during 120 minutes at the same rate than during lidocaïne perfusion.

A clinical exam (including an evaluation of behaviour, frequency of gut sounds (FGS) on abdominal auscultation, heart rate (HR) and respiratory rate (RR)), an ECG, a non invasive measurement of the systolic and the diastolic systemic arterial pressure (SAP_s and SAP_d, respectively), a blood sample, and a videotape recording of ultrasonography of the duodenum were performed before (T0) and 10 (T10), 15 (T15), 30 (T30), 45 (T45), 60 (T60), 75 (T75), 90 (T90), 105 (T105) and 120 (T120) minutes after the beginning of the lidocaïne or the placebo administration. Moreover, blood samples were taken 15 (TPI15), 30 (TPI30), 45 (TPI45), 60 (TPI60) and 90 (TPI90) minutes, and 2 (TPI2h), 3 (TPI3h), 5 (TPI5h), 7 (TPI7h), 9 (TPI9h), 12 (TPI12h) and 24 (TPI24h) hours after cessation of the lidocaïne perfusion. Ultrasonography was performed using an Aloka SSD 900 echograph and an abdominal convex UST-990 beam, and SAP_s and SAP_d were measured using a digital blood pressure monitor (Model KH 8088, Kompernass, Bochum). Blood samples were centrifugated and serum was freezed at -20°c within 1 hour of sampling and until analysis. Serum lidocaïne and MEGX concentration (LID_{ser} and $MEGX_{ser}$, respectively) were measured using high-performance liquid chromatography combined with electrospray ionization mass spectrometry.

On duodenal videotapes recordings, the following parameters were measured : frequency of duodenal contraction (FDC), duodenal diameter during contraction and during relaxation (DD_c and DD_r, respectively), and duodenal wall thickness (DWT).

The mean values of the parameters obtained (1) at T0 and during the perfusion in the PLAC group and in the LID group, and (2) at T0 and under perfusion within each group, were compared using an ANOVA for repeated measurement with the SAS program (SAS Institute Inc., Cary, USA).

The effects of the lidocaïne perfusion on behaviour were moderate and consisted of a slight excitement with trembling in 4 horses. The FGS, HR, RR, SAPd, DDr, and DWT were not significantly modified during perfusion of lidocaïne or placebo, and were not significantly different between the LID and the PLAC group. No cardiac arrhythmia was detected during lidocaïne or placebo perfusion. The SAP_s did not show significant changes during perfusion in the 2 groups, but was significantly lower in the LID than in the PLAC group at T10 and T30. The FDC significantly increased at T15 to T90 in the PLAC group, and at T10 and T15 in the LID group, but was not significantly different between groups. The DD_c significantly decreased at T30 as compared to T0 in the LID group and was significantly lower in the LID group than in the PLAC group at T10.

The LID_{ser} reached a mean value of 813 ± 144 ng/ml at T10 and maintained a steady state value ranging from 712 ± 95 and 816 ± 116 ng/ml until the end of the lidocaïne perfusion. It started to decrease 45 minutes after cessation of the perfusion, and was undetectable from 5 hours after cessation of the perfusion.

The $MEGX_{ser}$ progressively increased from T10 (44 ± 19 ng/ml) to T120 (264 ± 158 ng/ml) during the lidocaïne perfusion. It progressively decreased from 15 minutes after cessation of the perfusion and was undectable from 7 hours after cessation of the perfusion. In previous studies performed in horses with an ileus, an analgesic effect of lidocaïne was obtained at LID_{ser} ranging from 701 to 1222 ng/ml, and reduction of gastric reflux was obtained at LID_{ser} ranging from 1000 to 2000 ng/ml. Side effects were reported in horses with LID_{ser} ranging from 1850 to 4530 ng/ml.

In conclusion, lidocaïne given as a 1.3 mg/kg IV bolus within 10 minutes followed by a perfusion at 0.05 mg/kg/min during 110 minutes (the dosis most commonly used in horses suffering from an ileus) in adult healthy horses was well tolerated, induced a transient significant decrease in SAP_s and in DD_c at the beginning of the perfusion, allowed to obtained a steady state value of LID_{ser} ranging from around 700 to around 800 ng/ml, and was associated with a progressive increased $MEGX_{ser}$.

DIAGNOSTICS OF EQUINE INFLUENZA IN ELISA

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ABSTRACT:

ELISA is a wide spread method of diagnostics of several infections of animals and human owing to high sensitivity, specificity and opportunity of automation of reaction. Standard method of retrospective diagnostics of equine influenza is haemagglutination ingibition test (HI). At the same time use of HI is limited by factors negatively influencing on specificity of results. There are termostable and termolable ingibitors from sera, nonspecific haemagglutination and complement. Physical and chemical influence (heat, processing CO2, adsorption using caolin, destruction by oxidizers (Kalii periodat, etc.) used for destruction of sera ingibitors. This complicates performing of reaction and negatively influences on results. Use of ELISA allows to exclude the described above negative factors and to increase fast of reaction.

In this study two straines of equine influenza virus: "A/equi1/Prague/56" and «A/equi2/New - Market /93» were used. Viruses were cultivated in 9 up to 10 day old SPF - chicken embryos. Antisera to virus strain «A/equi2/New - Market /93» were received by intranasal virus application to seronegative foals. Antisera to virus strain "A/equi1/Prague/56" were received by immunization of rabbits by purified virus. In HI the titres of antibodies to viruses has accordingly 1:128 and 1:256.

Results. This method based on indirect ELISA. Purified virus antigene was adsorbed in cells of polystyrene micropanel and binded to specific antibodies present iin sera therefore the complexes antigen - antibody is formed. The received immune complexes were binded with specific conjugate (purified by low presure chromatography antibodies to equine IgG, labeled by horseradish peroxidase). Soluble colored product was formed after addition of substrate solution. Intensity of solution's color is proportional to level of antibodies in probes.

Using homological sera titres of antibodies up to 1: 100000 in ELISA were detected. Cross reactions between sera to equine influenza viruses of 1 and 2 types were not detected.

ELISA was allowed to reveal positive seroconversia to equine influenza virus type 2 at 18 horses from 42 investigated in several farms. Probes of sera were received from horses with symptoms of sharp respiratory disease: temperature up to 39,5 - 42 °, serosous - mucous nasal discharges and periodic cough. At 1 - 3 day postinfection horses developed specific antibodies to equine influenza virus type 2 in diagnostic titre of 1 : 6400 or less specified value. Correlation between a level of the antibodies revealed in HI and ELISA, terms from the beginning of disease and weight of illness was detected. At retrospective research after 3 weeks the titres of antibodies were increased up to 1 : 51200 - 204800. High sensitivity of reaction (the diagnostic titre of antibodies 1:6400 and is higher) excludes negative influence nonspecific factors.

So ELISA for diagnostics of equine influenza, alternative to haemagglutination ingibition test and single radial haemolysis, authorized Office International Epizootics was developed. Due to high sensitivity and specificity ELISA may be used as trouble-shooting test of equine influenza antibodies, and for control of intensity of immunity that allows to correct terms of vaccination and plan preventive actions.

STUDY OF ACTIVITY PATTERNS OF SUPERFICIAL HINDLIMB AND BACK MUSCLES IN CLINICALLY SOUND AND LAME HORSES USING SURFACE ELECTROMYOGRAPHY

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Aim of the study:

Malfunction of equine movement apparatus is an active veterinary research area. Available diagnostic techniques aim more for underlying structural abnormalities, but are usually silent about muscle function disorders. In such situations, Electromyography comes up as a useful tool. Among the techniques in practice, surface electromyography (SEMG) has the advantage of being non-invasive. Its ability to provide reproducible data can be paired up with other motion analysis techniques to provide reliable integrated knowledge about underlying phenomenon. As yet, no identifiable attempts have been made to establish diagnostic potential of the technique, which is partly due to absence of approved protocols for SEMG in veterinary research.

Keeping in mind this sensitivity of SEMG to activity generated by superficial muscles, this study was designed to explore activity patterns of 5 such equine back and hindlimbs muscles (Longissimus dorsi, Semitendinosus, Biceps femoris, Gluteus medius, and Long digital extensor) in clinically sound and lame horses. The aim was not only to verify the sensitivity of SEMG to appreciate variations in muscle function, but also to compare the functional characterization of these muscles in horses with different soundness status.

Materials and methods:

The study was performed on ten clinically sound, and ten chronically hindlimb lame (1-2 degrees out of 4) horses. After 3 training sessions on treadmill, the horses were measured in trot for 3 x 20 seconds. Neurodata TelemyoMini-16 system was used to record EMG data. The data thus obtained were analyzed with SPSS 14.0. To understand the distribution of activity of muscles during a stride cycle, 0-4 quartile values of their mean activity were calculated. The maximum (4^{th} quartile) was brought to 100, so that the values of 0-3^{rd} quartiles could be taken as percentage of the maximum thus making the data from different horses comparable with each other. First, 2^{nd}, and 3^{rd} quartile values of all the muscles were compared statistically (P=0.05). The groups were described as Sd (Mean of values from both hindlimbs of a sound horse), L (Mean of values from both hindlimbs of a lame horse), SL (Sound side of a lame horse), LL (Lame side of a lame horse). Within a group, the 3 quartile values of all the 5 muscles were compared with each other to account for their intragroup relationship and ranking (P=0.05).

Results and Discussion:

Significant differences in the muscle activity, when compared among the 4 groups, were observed only for second and third quartile values of Semitendinosus. Significantly higher Q2 value in 'SL' (43.60 ±8.68) as compared to 'Sd' (36.50 ±4.49) indicates higher activity around transition between rest and activity phases. With progression of activity phase, this increase reached to an extent that Q3 values of both 'SL' (58.51 ± 8.83) and 'L' (56.34 ± 5.87) became significantly higher than that of Sd (48.21 ± 5.2).

Additionally, the three quartile values of each muscle were compared with those of the other 4 muscles within a group (Sd, L, SL, or LL). The resultant ranking of the muscles within a group, in order of decreasing magnitude of the quartile values, was found as in Table 1.

Table 1: Ranking of the muscles within a group

Sound (Sd)	Lame (L)	Sound Side of Lame Horses (SL)	Lame Side of Lame Horses (LL)
Ld* Se, Gl, Lde** Bi	Ld Se Gl, Lde Bi	Ld Se Gl, Lde Bi	Ld Se, Gl, Lde Bi

*__Ld__=Longissimus dorsi, __Se__=Semitendinosus, __Gl__=Gluteus medius, __Lde__=Long digital extensor,

__Bi__=biceps femoris.

**Two or more muscles without any significant difference between their quartile values were ranked together

Indicates significant difference between all the 3 quartile values of the muscles at the two ends of the line, unless otherwise indicated

Indicates significant difference between one or two quartile values of the muscles at the two ends of the line

Sd was the only group where mutual significant differences among muscles had the same trend for all the 3 quartile values. In all other groups inconsistencies were observed which could be due to different degrees of adaptation over the period of time. In LL group, the ranking order was essentially the same as that of the Sd group, which indicates that sound side of the lame horses undertook more of the compensatory activity. This is further supported by the observation that muscle activity pattern of L group is heavily influenced by that of SL group.

Conclusion:

The observed differences of muscle activity in horses of different health status are not only suggestive of the underlying compensatory mechanisms but also of the potential of SEMG to point them out. These mechanisms have led to a compensatory higher muscle activity of the sound side of the lame horses. This increase, however, is not coupled with a significant decrease in the activity of the lame side. Together these two observations indicate an overall increase of muscle use in lame horses for an otherwise less demanding gait pattern. This increased activity not only represents the underlying pain, but can also lead to a vicious cycle where overuse leads to pain, and the resulting pain leads to further overuse.

This difference of muscle activity among groups was better understood when their mutual relationships were first studied within a group, and the resultant ranking then compared among groups (Table 1). The subtlety of the differences could be due to adaptation resulting from the chronic nature of the lameness coupled with the fact that horses with variable history of lameness were included in the study.

These findings indicate the potential of SEMG as a useful clinical tool. Nevertheless, it is imperative that a series of studies be carried out in compliance with a set of procedural protocols before a diagnostic value can be assigned to the technique.