Veterinary European Equine Meeting of the Year 2008

XIV SIVE/FEEVA CONGRESS

Lido di Venezia
Palazzo del Casinò
January, 25th-27th 2008

Organized by

PROCEEDINGS

Traduzione a cura di / Translated by MAURIZIO GARETTO, Med Vet, Torino
VALUTAZIONE QUANTITATIVA E QUALITATIVA CON METODO DOPPLER DUPLEX DEL FLUSSO EMATICO DIGITALE IN CAVALLI SANI ED IN ALTRI CON MALATTIE PREDISPOSENTI ALLA LAMINITE O CON LAMINITE MANIFESTA

QUANTITATIVE AND QUALITATIVE DUPLEX DOPPLER EVALUATION OF DIGITAL BLOOD FLOW IN HEALTHY HORSES AND HORSES WITH DISEASES PREDISPOSING TO LAMINITIS AND OVERT LAMINITIS

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Introduction. Clinical evaluation of digital blood flow by palpation (digital pulse) has been a very used subjective technique for years in horses to analyse the hoof condition. A number of non-invasive methods have been developed to assist in this procedure but only duplex-Doppler ultrasonography (DDU) has been well established as sensitive, non-invasive and relatively inexpensive method. In equine practice there are numerous studies supporting the use of this technique to evaluate the digital blood flow but unlike humans, there are not well established scoreboard diseases. We hypothesized that DDU may be used to differentiate palmar digital artery flow in healthy horses, in horses with disease that predispose to laminitis and in horses with overt laminitis.

Material and Methods. In 42 adult Pure Breed Spanish horses, complete clinical examination, blood tests, systemic non-invasive pressure measurements and hoof radiographies were performed. According to results, horses were classified in 3 groups: group I, healthy horses (n=9); group II, ill horses with endotoxemia (n=19) and group III, horses with overt laminitis (n=14). These groups subdivided in: group IIA, ill horses without digital pulse (n=9); group IIB, ill horses with digital pulse (n=10); group IIIA, laminitic horses without rotation or sinking of the third phalanx (n=8) and group IIIB, laminitis horses with rotation or sinking of the third phalanx (n=6). For the DDU exam a 7.5 MHz linear array ultrasonographic probe was used and situated at the latero-palmar aspect of the metacarpophalangeal joint in both forelimbs in order to obtain blood flow spectrum Doppler (qualitative and quantitative study). Factors like temperature in the scan room, timetable for the scan, ultrasound system, examiner (blind) and all legs bearing weight were under control.

Results. Qualitatively the Doppler spectrum consisted of a non laminar blood flow characterized by a systolic peak velocity, followed by at least two diastolic waves and end-diastolic plateau velocity in horses of groups I and II. However, most horses of group IIIA (n=6), showed laminar blood flow spectrum with greater systolic deceleration velocity drop nearly reaching the base line followed by one to three diastolic waves and end diastolic plateau velocity. In the rest of horses of group IIIA (n=2) and horses of group IIIB Doppler spectrum showed a triphasic pattern characterized by a prominent systolic peak, early diastolic retrograde flow and late diastolic antegrade wave. Regarding to quantitative results, there were significant differences (p<0.05) for heart rate (p=0.009) between groups I and IIIB; diameter of the vessel (p=0.03) between groups IIA and IIIB; acceleration time (p=0.012) between group IIA and the other groups; velocity time integral (p=0.04) between group IIIB and the other groups and blood flow (p=0.012) between groups IIA and IIIB.

Discussion. This is the first study showing a triphasic pattern, as in high resistance vessels, in horses affected by severe laminitis. However, we agree with previous studies that quantitative Doppler parameters are very variable and not very representative of health, disease or laminitis condition.

Conclusion. Spectra Doppler patterns are more significant to assess digital blood flow in horses than DDU quantitative parameters. Additional studies are necessary in order to evaluate if qualitative Doppler parameters are useful to monitor the efficacy of treatment.
References


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ECOGRAFIA ED ISTOLOGIA DEI MENISCHI EQUINI: STUDIO COMPARATIVO DEL MENISCO MEDIALE
ULTRASONOGRAPHY AND HISTOLOGY OF THE EQUINE MENISCI: A COMPARATIVE STUDY OF THE MEDIAL MENISCUS

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Introduction. Paired menisci are found within the stifle joint. They are semilunar and composed of hyaline cartilage, fibrocartilage and fibrous tissue in proportions that vary with animal and age. They provide congruence of incompatible articulating surfaces (two condyles of the femur directed caudodistally and separated by a deep intercondylar fossa and the tibial plate with a central eminence and two condyles). They play an important role in shock absorption, stability and power transmission. In horses, ultrasonography is routinely used to assess soft tissue structures and bony surfaces of the limbs. Meniscal lesions have a high prevalence and can be described as central degeneration, horizontal tear, partial oblique tear of the distal angle, combined horizontal and oblique tears, or complex tear (1). Central hypoechoic areas are commonly seen in the equine medial meniscus at ultrasonography but the exact clinical significance is unknown because no complete comparative study of ultrasonographical and histological findings is reported. In horses, hypoechoic areas have been associated with fibre disruption and collapse, edema, or degenerative processes such as fibroplasias or necrosis (2, 3) but only histological studies of human menisci have been described (4, 5).

Objectives. This study aimed to compare ex-vivo ultrasonographic and histological features of the equine medial meniscus.

Materials and methods
Ultrasonography. Medial menisci were examined post-mortem in situ and after excision in a water bath with a 7.5 MHz linear transducer.

Histology. Three different embedding medium were tested: paraffine for classical microscopy, methylmetacrylate for cutting with a vertical diamond wire saw and tissue tek cryo for cutting with a cryostat. Vertical sections were made. These were plane perpendicular sections to a given horizontal plane (Baddeley et al., 1986). The isolated menisci were either cut from the cranial to the caudal horn resulting in radial sections (the sections were triangular in shape, with thin axial border and thick abaxial border), or cut into quarters that were then cut tangential from the abaxial border to the axial border resulting in sections perpendicular to the radial sections. Sections were stained with toluidine blue.

Results
Ultrasonography. Thirteen medial menisci of 12 warmblood horses aged from 9 month to 15 years were examined. On radial ultrasonographic images, the medial meniscus was triangular with a concave proximal border and a slightly concave distal border. Two menisci showed a normal homogeneous echogenicity. Eleven menisci contained either a central hypoechoic area with irregular margins and/or a linear, horizontal hypoechoic zone that looked like a fissure.

Histology. Cartilage matrix was blue, collagen fibres were purple-coloured and fibro-chondrocytes nucleus were dark blue. The type of cells and the proportion of collagen fibres and matrix varied from the centre to the periphery. In the 2 sound menisci, dense collagen fibres were found in the middle of the meniscus with cells that looks like fibrocytes; more matrix and cells that looks like chondrocytes were seen in the periphery. The hypoechoic defects observed in 11 menisci were mainly associated with internal architectural changes: modified orientation and/or increased quantity of col-

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lagen fibres and/or accumulation of matrix with more chondrocytes. The periphery showed signs of
degeneration with fibrillation, erosion and formation of chondrocyte clusters. One central lesion
was associated with thick trabeculae with increased cellularity and increased amount of matrix.
One meniscus presented edema and one horizontal lesion corresponded to very dense fibrous tissue
with important neovascularization. Normal menisci were avascular structures. Blood vessels may
be found at the periphery, near the cranial and caudal attachment zones of the horns and in asso-
ciation with the medial collateral ligament, but the central zone of the menisci was avascular.

Discussion - Conclusion. This study demonstrates that hypoechoic areas in the medial meniscus
correspond to different types of degenerative or regenerative lesions with architectural changes.

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Information on the purpose of the work. Equine long bone fractures continue to be one of the most challenging conditions for equine surgeons to manage, with generally poor survival, reportedly as low as 8%. One major problem that contributes to poor survival for adult equine fracture patients is a slow rate of bone healing. Over the last decades, a great deal of research has focused on therapies for enhancing bone regeneration. Bone morphogenic protein (BMP) can cause molecular and cellular impulses and induce rapid bone healing process. Therefore, it is good for cases of delayed or non-union fractures as well as filling up of intervertebral gaps by osteogenic activity.

Used methods. The bovine long bone were collected, and processed through defating, grinding, decalcification, degreasing, protein extraction, dialyzing, purification and lyophilization. The extracted BMP was kept in the refrigerator. Ten adult horses of both sex were used and divided randomly into two equal groups. The right metatarsus was prepared and anesthesia was induced by xylazine, diazepam and ketamin HCl and maintained with halothane 2%. All horses were handled according to Shiraz University regulation for animal rights. Under general anesthesia, a window defect was made through midmetatarsal region by electric osteotom in all horses. The horses of the first group received BMP that was injected at the window site of metatarsal bone. The wound was closed, bandaged and splinted in both groups. They were monitored clinically for 10 weeks and histomorphometric evaluation was performed after 10 weeks.

Obtained outcomes and conclusions. Clinically the horses in BMP group were using their legs slightly from 28th and completely on 42nd postoperative day but the horses in control group did not use their legs even on 42nd postoperative day. Radiologically the callus bridged the defects up to 75% of the defect in some cases at 6 weeks and consolidated and fill the defect completely at 10 weeks. But in the control group the callus didn’t appear until 6 weeks and in the best situation only 50% of the defect was filled at 10 weeks. Histomorphometric data showed better thickness of cortical and trabecular bone in treatment group and periosteum was present and intact at the window site in patients of treatment group compared to control group. It can be concluded that the extracted protein was BMP and it had accelerated the bone healing process by osteogenic activities.
MALFORMAZIONE OCCIPITOATLANTOASSIALE (OAAM)
IN UN PULEDRO DI PURA RAZZA SPAGNOLA (PRE):
DIAGNOSI RADIOGRAFICA E TOMOGRAFICA
OCCIPITOATLANTOAXIAL MALFORMATION IN AN ANDALUSIAN FOAL:
RADIOGRAPHIC AND TOMOGRAPHIC DIAGNOSIS

*Libero professionista, Alicante - Spagna

Segnalamento. Puledro Pura Razza Spagnola (PRE) di quarantacinque giorni d’età.
Anamnesi. Giumenta con laminite cronica. Partorisce il puledro senza complicazioni. Dal giorno
della nascita il proprietario nota che l’animale mostra segni di incoordinazione in determinate posizioni,
soprattutto in quelle di suzione.

Segni clinici. All’esame clinico si presenta vigile, attivo, con un peso di 120 kg. Temperatura rettale,
pulso e frequenza respiratoria sono normali. L’ispezione del collo rivela una massa di consistenza ossea sul lato sinistro a livello delle prime vertebre cervicali. All’esame neurologico, la testa e il collo sono mantenuti deviati verso destra e manifesta una moderata atassia con deficit propriocettivo delle estremità anteriori. Succhia normalmente dalla madre però con movimenti incoordinati.

Diagnosi. Lematologia e l’analisi del liquido cerebro spinale non danno risultati rilevanti. Si realizzano radiografie digitali* in proiezione ventro-dorsale e latero-laterale** nelle quali si nota una anormalità nell’articolazione tra l’osso occipitale e la C1 consistente nella fusione tra le due strutture. Le ali dell’atlante si presentano asimmetriche, lo spazio articolare tra C1 e C2 è aumentato, il dente dell’epistrofeo ipoplasico e leggermente dislocato ventralmente. Si può apprezzare che le proporzioni tra asse longitudinale e trasversale dell’epistrofeo sono anormali con i processi trasversi simili a quelli di una prima vertebra cervicale. Lo studio tomografico*** rivela un’anomalia della posizione del dente dell’epistrofeo nella sua articolazione con la C1 e a questo livello si riscontra la compressione del midollo.

Diario. L’ematologia e l’analisi del liquido cerebro spinale non danno risultati rilevanti. Si realizzano radiografie digitali* in proiezione ventro-dorsale e latero-laterale** nelle quali si nota una anormalità nell’articolazione tra l’osso occipitale e la C1 consistente nella fusione tra le due strutture. Le ali dell’atlante si presentano asimmetriche, lo spazio articolare tra C1 e C2 è aumentato, il dente dell’epistrofeo ipoplasico e leggermente dislocato ventralmente. Si può apprezzare che le proporzioni tra asse longitudinale e trasversale dell’epistrofeo sono anormali con i processi trasversi simili a quelli di una prima vertebra cervicale. Lo studio tomografico*** rivela un’anomalia della posizione del dente dell’epistrofeo nella sua articolazione con la C1 e a questo livello si riscontra la compressione del midollo. Nella ricostruzione del TAC, possiamo apprezzare la compressione del midollo tra il tetto dell’atlante e il dente dell’epistrofeo. La diagnosi definitiva è di malformazione occipitoatlantoassiale (OAAM) associata a compressione dinamica del midollo spinale.

Trattamento. Nessuno, considerata la prognosi sfavorevole.

Discussione. La malformazione occipitoatlantoassiale (OAAM), è una rara patologia congenita riportata nei bovini, cani, gatti, ovini ed equini.1 I segni neurologici associati a questa condizione includono tetraparesi, atassia, deficit propriocettivo, scoliosi cervicale e lateralizzazione della testa. Sono state definite tre categorie per questa patologia: 1) occipitalizzazione dell’atlante con atlantizzazione dell’epistrofeo del cavallo arabo 2) malformazione occipitoatlantoassiale congenita e asimmetrica, 3) fusione asimmetrica occipitoatlantoassiale. Il puledro descritto in questo lavoro appartiene alla prima categoria sebbene non sia un cavallo arabo. A nostra conoscenza è il primo caso di OAAM in cavalli PRE riportato in letteratura, così come non abbiamo riscontrato studio tomografico di questa patologia in animali in vita.

Conclusioni. Consideriamo questo caso rilevante in quanto a nostro parere è bene includere la OAAM nelle diagnosi differenziali di giovani puledri con problemi neurologici. Per questo è doveroso effettuare un buon riconoscimento clinico dell’animale inserendo nel nostro protocollo un’accurata e minuziosa palpazione delle strutture cervicali associando lo studio radiografico della zona occipito-atlanto-assiale. La tomografia computerizzata ci permette avere un’idea più completa delle relazioni articolari presenti nei nostri pazienti.

Categorica l’esclusione dell’animale da qualsiasi programma di riproduzione.

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**Collimar, X-ray Collimator
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Proceedings of the European Equine Meeting of the Year 2008 - XIV SIVE - FEEVA Congress, Venice, Italy
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ESPERIENZE CLINICHE SULL’UTILIZZO DELL’ASSOCIAZIONE LIDOCAINA-BUTORFANOLO-DETOMIDINA NELL’ANESTESIA EPIDURALE CAUDALE DEL CAVALLO

CLINICAL EXPERIENCES IN THE ASSOCIATION OF LIDOCAINE-BUTORPHANOL-DETOMIDINE FOR THE CAUDAL EPIDURAL ANALGESIA IN THE HORSE


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Introduction - Caudal epidural injection of local anaesthetic is a common method to produce analgesia and anaesthesia of the caudal and perineal regions in the standing horse. In man and in animals, addiction of opiates and \( \alpha-2 \) agonists to the local anaesthetic epidurally administered can improve and prolong analgesic effects, without altering motor functions (1). In this way doses can be reduced in order to minimize the possibility of loss of motor control. Epidural administration of the association of lidocaine and butorphanol (2) or of detomidine alone(3) are reported but not of the three agents together. According to dosages reported (4), epidural administration of detomidine alone induces analgesia of relative short duration that extends more cranially but results in profound sedation and significant decreases in both cardiac output and heart rate, increase in arterial blood pressure and ataxia similarly to the systemic administration.

Aim of the work is to refer about the efficacy and safety of a local anaesthetic (lidocaine), an opioid (butorphanol) and an \( \alpha-2 \) agonist (detomidine) epidurally administrated in low doses in 8 mares for surgery of the lower uro-genital tract.

Materials and Methods - Eight mares 8-13 years of age, weighing 435±38 Kg ASA physical status 1 and 2 were tranquillized with acepromazine e.v. 0,02 mg/Kg. With the horse held in stocks the skin over the region was clipped and surgically prepared. After location of the first intercoccygeal vertebral space, the skin and subcutaneous tissue above the space was desensitized by administration of 2 ml of lidocaine 2%. Thirty minutes later an association of lidocaine (0,30 mg/Kg), butorphanol (0,02 mg/Kg) and detomidine (0,006 mg/Kg) brought to 15 ml in saline solution was epidurally injected at the Co1-Co2 level, with Quincke 20 G spinal needle. After onset of analgesia, surgery for reparation of recto-vaginal laceration was performed. Onset and duration of analgesia were evaluated by clipping perineal skin and vaginal mucosa. Onset and duration of loss of anal reflex and tail relaxation were also recorded. During surgery, which lasted 90' ± 38', at 0, 5, 10, 15, 30 minutes from the injection and successively every 20', systemic arterial blood pressure, respiratory rate, SpO2 and heart rate were also recorded.

Results - Onset time for somatic and visceral analgesia sufficient for surgery was 25' ±6' and 24' ±8' respectively (Fig. 1). In all the animals tail flaccidity and loss of anal sphincter reflex occurred in 7' ±2' after inoculation. Duration of somatic and visceral analgesia was 135' ±19' and 158' ±13' respectively (Fig. 2). Recovery of muscular function of anal sphincter and tail tonicity occurred together with the end of cutaneous analgesia, whilst recovery from visceral analgesia took place 20' later. At 200' after surgery, three subjects showed the typical way of walking with high steps and pointed hoof that lasted 20', described from other authors when butorphanol 0,04 mg/Kg was epidurally injected (2). No mare demonstrated sign of excitation or sedation, besides that derived from previous premedication with acepromazine. No important variations were observed in the cardiac and pulmonary parameters and no mares showed urinary retention.

Conclusions - Loss of rear motor control is the main limiting factor in the use of caudal epidural analgesia in the horse. The presence of butorphanol and detomidine in association with lidocaine allowed to reduce the dose of the last one, minimizing risks of ataxia and related problems.
Fig. 1

Onset Times

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Mares</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>somatic analgesia</td>
</tr>
<tr>
<td>2</td>
<td>visceral analgesia</td>
</tr>
<tr>
<td>3</td>
<td>loss of anal and tail reflex</td>
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</tbody>
</table>

Fig. 2

Duration of analgesia

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Mares</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>somatic analgesia</td>
</tr>
<tr>
<td>2</td>
<td>visceral analgesia</td>
</tr>
</tbody>
</table>

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With the doses of butorphanol and detomidine utilized in this study, no CNS nor cardiac or respiratory effects have been observed. Epidural association of lidocaine-butorphanol-detomidine is safe, improves the quality and prolongs duration of analgesia, making surgery of the caudal regions of the horse easier.

References

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Pregnancy is a physiological state that implies several mother adjustments, both at circulatory and metabolic levels, in order to guarantee a constant energetic contribution to the growing fetus. The integrated activity of the components of the renin - angiotensine - aldosterone system (RAAS), with the release of renin from the kidney, acts in the control of renal sodium excretion and internal homeostasis. Furthermore, at cardiovascular level, RAAS acts in the defense of arterial pressure. At endocrine level, mainly because of the action of the aldosterone, RAAS assures a suitable exchange of oxygen and nutrients between the mother and the fetus. As a consequence, RAAS influences the viability of the fetus and partially could be determinant of the weight to the birth.

Objectives. The main purposes of this research were: 1) to establish reference range for serum renin concentrations (SRC) in pregnant Spanish Purebred mares; 2) to analyze changes related to pregnancy in SRC and 3) to assess SRC changes linked to the age of the pregnant mare.

Materials and Methods. A total of 33 Spanish Purebred broodmares, aged between 4 and 17 years was studied. Jugular venous blood samples were extracted every month during pregnancy, always in the morning. Immediately after withdrawal, blood was centrifuged and serum was harvested. SRC were measured by competitive immunoassay.

Results. Mean SRC were 4.18 pg/ml, with maximum and minimum values of 9.26 and 1.44 pg/ml respectively. SRC during the second half of pregnancy were significantly higher than those of the first half. Mean values in the first five months of pregnancy ranged in a narrow interval, between 2.56 and 3.31 pg/dl. They increased progressively from 6th month of pregnancy (3.26 pg/ml), achieving maximum mean values, three-fold higher than basal values, at 10th (7.20 pg/ml) and 11th months (7.23 pg/ml). No significant differences existed in relation to the age of the mares.

Discussion. Previous researchers regarding the different components of the RAAS have reported lower SRC in horses than in other animal species (Guthrie et al., 1982). In fact, SRC were lower in the pregnant Spanish mares than the mean values reported in women and laboratory animals (Bentley-Lewis et al., 2005). On the other hand, SRC in our mares were higher than the values found for growing Spanish foals (Rovira, 2007), adult horses (Guthrie et al., 1982) and adult ponies (Broughton Pipkin et al., 1982; Forhead et al., 2000). Furthermore, in the pregnant Spanish mares, SRC showed marked interindividual differences, as showed by the wide range of variation. This result could have been linked to the effect of pregnancy in this hormone. On contrary, thoroughbred (McKeever et al., 1992) and Standardbred horses (Andersson et al., 1987) seem to have higher SRC in comparison to the pregnant Spanish mares. In these last researches, both groups of animals were in active training and it has been established that daily exercise increases RAAS activity (McKeever et al., 1992).

The observed hormonal dynamic during pregnancy in the pregnant mare seems to be different from that described for women. In humans, the maximum SRC is found during the first period of pregnancy, with a “plateau” at 5th month and without significant changes until delivery (Bentley-Lewis et al., 2005). By contrast, the maximum SRC in Spanish pregnant mares were found at the end of pregnancy, reflecting significant differences in the RAAS response to pregnancy between species.

Conclusions. In conclusion, SRC in pregnant Spanish Purebred broodmares are within the reference range established for horses. Furthermore, the dynamic of SRC during pregnancy in Spanish broodmares is characterized for “iperreninemia”, without clinical signs related to diseases.
results might be the consequence of the interaction between several metabolic and hormonal factors during pregnancy.

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INFLUENZA DELL'ETA E DEL SESSO SUI PARAMETRI EMATOLOGICI DEI CA VALLI ANDALUSI

THE INFLUENCE OF AGE AND GENDER ON HAEMATOLOGICAL PARAMETERS IN ANDALUSIAN HORSES

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Purpose of the work. The purpose of the work was to determine the reference values and the influence of age and gender on haematological parameters in Spanish horses between 1 to 7 years of age.

Material and Methods. Haematological parameters were determined in 160 healthy, unrelated Spanish horses, 80 males and 80 females divided in four groups of age of forty animals each, (group A: 1 to 2 years old; B: 2 to 3 years old; C: 3 to 4 years old; D: 4 to 7 years old). Each group was in turn divided in two groups of twenty animals, according with gender. All animals belong to farms located in Valencia and Murcia, on the east of Spain.

Whole blood was collected into 10-ml EDTA vacutainer tubes and transported to the haematology laboratory within 3 hours of collection. All samples were processed the same day of collection. The following parameters were determined using an electronic cell counter (Sysmex-F 820) calibrated for equine blood: red blood cell count (RBC), Haemoglobin concentration, Packed cell volume (PCV), Mean corpuscular volume (MCV) Mean corpuscular haemoglobin (MCH), Mean corpuscular haemoglobin concentration (MCHC), White blood cell count (WCC) and Platelet count.

Results. The mean RBC, WBC, haemoglobin concentration, PCV, MCV, MCH, and MCHC are presented in table 1. All parameters are within published reference ranges. Statistically significant age-related changes (p< 0.05) have been observed in WCC, RBC, MCV, MCH and platelets. WCC and RBC decrease with age, whereas MCV and MCH increase with age. All age-related differences were present in animals of both sexes. Group B (2 to 3 years old) show the highest counts of haemoglobin concentration, PCV and RBC among the age groups. Only haemoglobin concentration shows a significant difference between males and females, males showing a higher count than females. Females showed a statistically significant higher WCC than
males in age group D. In addition, males present a significant higher haemoglobin concentration than females in age group B.

**Conclusion.** This is the first work done on haematological parameters on Spanish horse of this age. A variety of factors can affect the haematologic parameters in resting horses, including breed, age and gender. Therefore, reference values should be adjusted, if possible, for specific age, breed and gender.

**Table 1**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean ± SD</th>
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</thead>
<tbody>
<tr>
<td>WCC (cel/μl)</td>
<td>9819.5 ± 2421</td>
</tr>
<tr>
<td>RBC (10⁶/mm³)</td>
<td>8 ± 1.4</td>
</tr>
<tr>
<td>HB (gr/dl)</td>
<td>13.6 ± 1.7</td>
</tr>
<tr>
<td>PCV (%)</td>
<td>38.8 ± 6.2</td>
</tr>
<tr>
<td>MCV (fl)</td>
<td>48.5 ± 4.2</td>
</tr>
<tr>
<td>MCH (pg)</td>
<td>17.2 ± 2.1</td>
</tr>
<tr>
<td>MCHC (g/dl)</td>
<td>35.4 ± 3.9</td>
</tr>
<tr>
<td>PLAT (10³/mm³)</td>
<td>142.4 ± 74.2</td>
</tr>
</tbody>
</table>

**References**


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Proceedings of the European Equine Meeting of the Year 2008 - XIV SIVE - FEEVA Congress, Venice, Italy
VALORI NORMALI DELLE SOTTOPOPOLAZIONI LINFOCITARIE NEI CAVALLI ANDALUSI
NORMAL VALUES FOR LYMPHOCYTE SUBPOPULATIONS IN THE ANDALUSIAN HORSE

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Purpose of the work. The aim of this work was to obtain reference ranges for lymphocyte subsets in Spanish horses from 1 to 7 years of age, as well as to characterize age- and gender-associated changes in lymphocyte subpopulations.

Material and Methods. 160 healthy Pure Spanish breed horses were divided in four groups of age of forty animals each, (group A: 1 to 2 years old; B: 2 to 3 years old, C: 3 to 4 years old; D: 4 to 7 years old). Each group was, in turn divided in two groups of twenty animals, according with sex. All animals belong to farms located in Valencia and Murcia, in the east of Spain.
Whole blood was collected into EDTA vacutainer tubes and transported to the haematology laboratory within 3 hours of collection. All samples were processed the same day of collection.
Lymphocyte subsets were analyzed on an EPICS XL-MCL Flow Cytometer (Beckman Coulter) using a single platform procedure with the following fluorochrome-labelled monoclonal antibody combinations CD2-CD4, CD2-CD8, CD2-B lymphocytes. Lymphocytes were identified by analysis of forward- and side-angle light scatter. For each sample, 20,000 events were recorded. Absolute quantification of cells was performed by means of Flow-Count Fluospheres® (Beckman Coulter). Helper T cells were identified by the expression of CD2 and CD4 antigens on their surface, Cytotoxic T cells by CD2 and CD8 coexpression. The total of T cells (CD3+) was the sum of CD2+CD4+ and CD2+CD8+ (T Cytotoxic). NK cells were calculated by subtracting the total of T cells from the total of CD2+ cells, being this epitope expressed on the surface of all T lymphocytes and NK cells. Since the Mab for B-Cell identification did not perform properly, this cell population was estimated by subtracting from 100% the percentage of both T and NK cells (CD2+).

Results. Absolute counts of total leukocytes, lymphocytes, T cells, CD4+ and CD8+ T cells and B cells are presented in Table 1. The number of leukocytes and lymphocytes are within reference range, somewhere in between published values of cold- and hot-blooded breeds.
Number and percentage of total T cells, CD4+ and CD8+ T cells are close to the lower range of previously published results. Number and percentage of B cells are within reference range, although result varies enormously between published data, and there is very little information about equine NK cells.
The absolute counts of leukocytes, lymphocytes and of all their subsets decreased significantly with age (p<0.05), except for CD4+ T cell counts in group B, that were higher than group A (Fig 1).
The percentage of total T cells and CD4+ T cells increase significantly with age, although in group C, the percentage of lymphocytes was lower than in group B. Percentage of CD8+ T cells also show a tendency to increase with age, although no significantly. The CD4:CD8 ratio shows no differences between age groups, although there is a slight tendency to increase with age (Fig 2).
The percentage of B cells and NK cells decreased significantly with age, this tendency being steady among all age groups.
There were no significant differences in the absolute counts and the percentage of leucocytes, lymphocytes or any of its subsets between males and females.

Conclusion. To our knowledge, this is the largest study of reference values of lymphocyte subsets in healthy horses, and the first study of lymphocyte subsets of any kind done in this breed. There is a

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steady decrease of leukocytes, total lymphocytes count and lymphocyte subset cells with age that mimics that seen in other species and may contribute to an age-associated decrease in immunocompetency. The absolute counts of lymphocyte subsets vary according with age and breed, therefore, reference values must be adjusted for both parameters. Furthermore, there are important methodological differences between published data, especially about the use of single- versus double platform, what is arguably the most important source of error.

References
Smith R III, Chaffin K, Cohen N, Martens RJ. Age-related changes in lymphocyte subset in Quarter Horse foals. AJVR 2002(63) 531-536

Fig 1. Absolute counts of lymphocytes and their subsets (*= significant differences between age groups)

Fig 2. Percentage of lymphocyte subsets (*= significant differences between age groups)
Table 1

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leukocytes/μl</td>
<td>9743.4 ± 3071</td>
</tr>
<tr>
<td>Lymphocytes/μl</td>
<td>4241.8 ± 1451.3</td>
</tr>
<tr>
<td>Lymph %</td>
<td>44.8 ± 9.9</td>
</tr>
<tr>
<td>T cells/μl</td>
<td>2451.5 ± 722.2</td>
</tr>
<tr>
<td>T cells %</td>
<td>58.5 ± 8</td>
</tr>
<tr>
<td>Th/μl</td>
<td>1808.7 ± 580.4</td>
</tr>
<tr>
<td>Th %</td>
<td>43 ± 6.8</td>
</tr>
<tr>
<td>Tc/μl</td>
<td>660.8 ± 2560.7</td>
</tr>
<tr>
<td>Tc %</td>
<td>15.4 ± 4</td>
</tr>
<tr>
<td>B cells/μl</td>
<td>452.1 ± 197.1</td>
</tr>
<tr>
<td>B cells %</td>
<td>10.5 ± 2.8</td>
</tr>
</tbody>
</table>

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Cell population from equine small airways is essentially uniform in the normal horse and those with diffuse lung inflammation. Bronchoalveolar lavage (BAL) is considered representative of whole lung condition (HOFFMAN, 1999; HEWSON & VIEL, 2002; PICKLES et al, 2002a), being considered the preferred cytological technique for the evaluation of horses with small airways inflammation (VIEL & HEWSON, 2003). The purpose of the present study was to investigate the effects of stable environment and exercise in the small airways cell population. The studied subjects were thoroughbred colts divided in three groups, being the Natural Condition group (NC) (n=18) - comprehending yearlings in natural farm condition -, Environment Challenge group (EC) (n=24) - colts stabled for 30 days in wood shavings bed and receiving grains and hay -, and Exercise Challenge group (EX) (n=12), constituted by colts that experienced a 1.000m workout of 15-16 m/s in a racetrack 24 hours before the evaluation. Colts were sedated with acepromazine (0.03 mg/kg, IM), xylazine (0.3-0.5 mg/kg, IV) and butorphanol (0.05 mg/kg, IV). Bronchoalveolar lavage fluid (BALF) was recovered using a Cook® bronchoalveolar lavage catheter blindly introduced till lodged in a bronchus, and infusing 300 ml of sterile phosphate buffered saline solution (PBS), divided in 60 ml aliquots, and gently aspirated. BALF was maintained in sterile bottle and conserved in ice while transported to the laboratory. BALF was centrifuged in 340 g for 6 minutes in 4°C, and after preparation the slides were colored with Romanowski technique (Pan aspi®), and 500 cells were counted in 1000X magnification. Total nucleated cell count was 40.6% and 42.3% higher in group EX compared to groups NC and EC respectively, being a significative difference (P<0.05). Group NC presented differential cell count in accordance to values accepted for normal horses, as reported by HOFFMAN (1999), VIEL and HEWSON (2003) and ROBINSON (2003). Differences in differential cell count between groups, observed in lymphocytes, neutrophils, multinucleated macrophages and hemosiderophages counts. In relation to lymphocytes, there was a 27.5% decrease in group EX in comparison to group NC (P<0.05). On the other hand, group EX evidenced a 76.6% and 90.2% increase in neutrophil percentage in relation to groups NC and EC respectively (P<0.0001). There was a decrease of 57.9% in neutrophil count in group EC compared to group NC (P<0.05). Multinucleated macrophages were 80.0% more frequent in group EC compared to group NC (P<0.05). Group EX was the only group to show hemosiderophages, representing 2.75% of the BAL fluid cells. In the present study, 30 days of stabling management was not responsible for airway inflammation in normal TB colts, evidencing even a decrease in neutrophil count, although the increase in multinucleated macrophages could represent an early Th-2 inflammatory response. Exercise could be seen as causing airway inflammation or, as the BAL collection was proceeded 24 hours after the exercise, it could be suggested that group EX could already represent an IAD group, and already evidencing EIPH before initiating racing campaign.
References


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IMPIEGO TRANSENDOSCOPICO DI UN LASER A DIODI NEL TRATTAMENTO DI ALCUNE AFFEZIONI OSTRUTTIVE DELLE VIE AEREE SUPERIORI IN 16 CAVALLI TROTTATORI (2006-2007)

TRANSENDOSCOPIC USE OF A DIODE LASER FOR THE TREATMENT OF SEVERAL UPPER AIRWAY OBSTRUCTIVE DISORDERS IN 16 STANDARDBRED TROTTERS (2006-2007)


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In sport horses, transendoscopic laser treatment of upper airway obstructions (UAO) presents several advantages over traditional surgical treatment (Tulleners, 1998; Parente, 2007). In fact, the procedure may be performed without general anesthesia and without skin incision, allowing a much shorter recovery period for the patient. Besides, the physical action of laser light has a positive effect on tissues, by stimulating fibroblasts and vascular proliferation, promoting a more rapid regenerative process. Among different lasers available, the diode laser is particularly desirable, since it is portable and less expensive. A disadvantage is that diode lasers have a maximum power setting of up to 30 W when compared to Nd:YAG (100 W), although such power appears high enough for most contact procedures. Aim of the present study is to report the results of a transendoscopic diode laser technique in 16 standardbred racehorses with different upper airway disorders. The patients of the study were 16 standardbred trotters, 8 males and 8 mares, from 2 to 8 years old (mean 4.5 years) with poor performance and/or production of respiratory noise during exercise. Each patient underwent a thorough evaluation protocol including history, with registration of the best racing time recorded (BRTR) before the onset of UAO, physical examination, laboratory evaluation, endoscopy of the upper airways at rest and - in negative or suspicious cases - under strenuous exercise on a high speed treadmill (SATO I). According to these procedures, the diagnosis of epiglottic entrapment (EE) was achieved in 12 patients, dynamic dorsal displacement of the soft palate (DDSP) in 3 patients and axial deviation of the aryepiglottic folds (ADAF) in 2 patients. In one patient DDSP and ADAF were present in association. All animals with DDSP had been treated previously by traditional surgery (myectomy of sternothyroideus and staphylectomy). To perform the laser procedure, the patient was restrained in stocks and sedated with intravenous administration of detomidine (0.01-0.02 mg/kg) and butorphanol (0.044-0.066 mg/kg). The pharyngeal mucosa was desensitised by transendoscopic nebulisation of a 2% lidocaine solution. At this point, the laser fibre (diameter 600 µm, length 4 m) connected to the 30 W laser source (Velure S9/30, Lasering s.r.l.) was passed into the biopsy channel of the endoscope (Olympus), and the laser power was set at a variable range of intensity from 12 to 15 W under continuous emission. The laser procedures were conducted according to Parente (2004) for EE, Hogan et al (2002) for DDSP and King et al (2001) for ADAF. After laser treatment, a “throat spray” containing DMSO, dexamethasone and rifampin was irrigated through a nasal cannula into the nasopharynx twice daily for the first week and once daily during the second week. Besides, flunixin (1.1 mg/kg i.v., s.i.d. for one week), prednisone (400 mg per os s.i.d. for the first week, 200 mg s.i.d. for the second week and 100 mg every other day for 5 further treatments) and ceftiofur (2.2 mg/kg i.m., b.i.d. for two weeks) were also administered. Serial endoscopic controls were performed every other day for the first two weeks after treatment. All patients returned to training after two weeks and 15 horses returned to racing after 45 days. In 5 horses the racing time returned to the BRTR level before treatment. Of these, 4 after 2 months and 1 after 10 months. 2 horses improved their BRTR, one after 2 months and the other after 4 months. 3 horses did not have a BRTR both before and after treatment (2 of them were debutant). 5 horses showed a mild worsening of their BRTR (1 second on average). The diode laser pro-

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procedure was simple to perform and well tolerated by the patients, and no serious complications were observed during the recovery period. The latter was particularly short, since after 2 weeks all patients (100%) had returned to training. Furthermore, the racing activity after treatment was satisfactory since 15 out of 16 horses (94%) returned to racing and 7 (47%) of these returned to either previous or improved BRTR level.

References
Parente, E.J. (2002): Transendoscopic axial division of epiglottic entrapment. Clinical Techniques in Equine Practice, 1, 9-12

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DISTENSIONE CON GAS AD ALTA PRESSIONE IN ARTROSCOPIA EQUINA:
425 CASI
HIGH PRESSURE GAS DISTENSION IN EQUINE ARTHROSCOPY:
425 CASES

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Introduction. The aim of this study is to underline the suitability of high pressure (250-300 mmHg)
joint distension by use of CO₂ for arthroscopic procedures and to evaluate and identify technical
advantages and disadvantages that might be associated with this technique in horses. The choice of
the medium used to obtain joint distension has been critically reviewed and for each distension
medium advantages and disadvantages have been detected. Fluids and gases can be used to the pur-
pose and currently Ringer’s sterile solution is mostly used. Main advantages in using gases vs fluid-
s as distension media have been described more in human than in veterinary medicine literature.
They are due to 3 major physical principles: better dioptic effect, evenly distributed wall pressure,
and gravity without floating effect. In all of the papers describing gas arthroscopy techniques it is
suggested not to increase the gas pressure above a threshold of 100-150 mmHg depending on the
experiences of the authors to minimize the risk of both venous gas embolism and subcutaneous em-
physema.

Materials and methods. In four hundred and twenty five horses (112 males, 115 females and 198
geldings) 636 joints were scoped on the period January 2004-December 2006. There were 369 Ger-
man Warmbloods and the remaining 56 horses belonged to six other breeds. Routine monitoring in-
cluded end-tidal carbon dioxide (EtCO₂) which was measured using a capnometer connected to the
breathing circuit through a non-disposable silicon tube. CO₂ was delivered by a pressure regulator
allowing to maintain the pressure in the chosen range; this was connected to the arthroscopic sleeve
through a non-disposable silicon tube without a bacterial CO₂ filter. At the end of the procedure the
gas supply was shut off, and 500-5000 ml of saline solution (NaCl 0.9%) were used to irrigate the
joint.

Results. All the 636 high pressure gas arthroscopic surgeries included in this study were effective,
easy to perform and the operative timing was comparable with the other techniques previously re-
ported. Joint capsule penetration and visualization of the intrarticular space, fragments and instru-
mental movements was better in many joints. No anaesthesiological complications were ob-
erved and there were no changes either in EtCO₂ or on the heart rate throughout the surgical pro-
cedures. Subcutaneous emphysema was always present after surgery, slight swelling of the joint oc-
curred in 28 horses (4.4%), suture infection in 14 (2.2%), no joint infection occurred. No venous
gas embolism was clinically observed and subcutaneous emphysema resolved itself within a period
of 6-24 hours of recovery without further treatments.

Conclusions. The characteristics of the most commonly used distending media have been reviewed
and discussed in literature, yet a comparison is needed about the delivery pressure of gases to per-
form arthroscopies. In the authors’ opinion the pressures that have been so far used and suggested
as being suitable to perform arthroscopies (80-100 mmHg) do not exploit the true potential of gas
arthroscopy itself. High pressure gas arthroscopy increased the articular operative space minimiz-
ing therefore the risk of iatrogenic lesions especially in small joints. For each joint there are spe-
cific indications for using a 250-300 mmHg distending pressure. Indications for 250-300 mmHg dis-
tention pressures are summarized in table 1. The use of 80-100 mmHg of pressure for arthroscopic
procedures has always been suggested to avoid the risk of subcutaneous emphysema and venous gas
embolism. Regarding the venous gas embolism, in the present study continuous monitoring of the
EtCO₂ revealed that no changes follow the high inflating pressure and that no other parameter such as heart rate and breathing rate would be influenced in any way. Pressures of 250-300 mmHg cause subcutaneous emphysema in 100% of the cases but this does not imply any other kind of complication. It seems that the emphysema does not increase post operative pain as horses do not show any sign among those usually recognized as signs of acute pain in horses. In our study there were no anaesthetic complications such as changes in ETCo₂ or heart rate throughout the arthroscopic procedures and no intra- or post-surgical complications so severe to set limits for the applicability of the technique. This gives evidence that the intra-articular delivery of high pressure CO₂ does not cause negative effects of any significance throughout the anaesthesia, the surgical procedure or the recovery.

Tab. 1: Indications for 250-300 mmHg distension pressure and comparison with 80-100 mmHg.

<table>
<thead>
<tr>
<th>JOINT</th>
<th>Penetration</th>
<th>Visualization</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distal inter-phalangeal joint (dorsal approach)</td>
<td>Much easier</td>
<td>Improved</td>
<td>Much wider operative space providing to minimize damage to articular cartilages while moving</td>
</tr>
<tr>
<td>Fetlock joint (dorsal approach)</td>
<td>Easier</td>
<td>Improved</td>
<td>Improved visualization of the metacarpal-tarsal median ridge</td>
</tr>
<tr>
<td>Fetlock joint (palmar/plantar approach)</td>
<td>No difference</td>
<td>No difference</td>
<td>Penetration and visualization effectively improved in case of hypertrophic synovia and fibrosis</td>
</tr>
<tr>
<td>Hock joint</td>
<td>Easier</td>
<td>No difference</td>
<td>Improved visualization especially for the femoro-patellar compartment, No difference for the other compartments</td>
</tr>
<tr>
<td>Stifle joint</td>
<td>No difference</td>
<td>No great difference</td>
<td></td>
</tr>
</tbody>
</table>

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VALIDAZIONE DELL’ANALIZZATORE PORTATILE LACTATE SCOUT
NEL PULEDRO NEONATO SANO E IN QUELLO RICOVERATO
IN TERAPIA INTENSIVA

EVALUATION OF THE PORTABLE ANALYZER LACTATE SCOUT IN HEALTHY
AND CRITICALLY-ILL NEONATAL FOALS

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Alessandro Pirrone, Med Vet

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La determinazione della lattatemia è divenuta pratica comune in medicina veterinaria e la possibilità di utilizzare analizzatori portatili ha reso questo parametro economico, rapido e facile da valutare anche in campo. Alcuni analizzatori portatili (Accusport, Accutrend e i-STAT) sono stati validati nell’equino adulto. Scopo di questo studio è valutare l’accuratezza dell’analizzatore portatile Lactate Scout (Senslab, Leipzig, Germany) che utilizza un metodo diverso dagli altri analizzatori e che è stato fin’ora validato solo nel cane adulto. Lo studio è stato effettuato su 26 puledri neonati sani e 89 puledri di età inferiore ai 7 giorni ricoverati in terapia intensiva. La lattatemia è stata determinata nei puledri sani ogni 12 ore dal momento della nascita fino alle 72 ore di vita e in quelli malati fino a 72 ore dal momento del ricovero. Una aliquota di sangue prelevato dalla vena giugulare è stata raccolta in provette contenenti fluoruro di sodio come anticoagulante e utilizzata per la determinazione mediante Chemistry Analyzer AU400 (Olympus Diagnostica GmbH, Ireland) con metodo enzimatico colorimetrico, ritenuto il metodo di referenza. La lattatemia rapida è stata ottenuta da una goccia dello stesso prelievo tramite l’analizzatore Lactate Scout, che utilizza un metodo enzimatico amperometrico. L’indagine statistica è stata effettuata mediante la correlazione lineare secondo Pearson, la regressione lineare e il metodo di Altman-Bland, come descritto in bibliografia per gli altri analizzatori portatili. Inoltre, l’effetto del valore ematocrito (Hct) sulla misurazione della lattatemia ottenuta con Lactate Scout è stato valutato utilizzando la correlazione lineare secondo Pearson. È stato possibile ottenere un totale di 201 coppie di determinazioni. Il coefficiente di correlazione (r) tra i valori misurati con i due metodi è risultato pari a 0,95 (p<0,01). Il grado di accordo tra i due test è stato misurato per un range compreso tra 0,8-16 mMol/L. La regressione lineare ha rilevato una buona correlazione tra i due test (R² = 0,89; pendenza = 0,79, intercetta = 0,44, intervallo di confidenza al 95% = 0,31-0,57). Il grado di correlazione valutato con il metodo di Altman-Bland ha confermato l’accuratezza del Lactate Scout (media delle differenze = -0,09 ±0,76, intervallo di confidenza al 95% = -1,57-1,39), infatti quasi tutti i valori rilevati sono compresi nell’intervallo tra il valore medio delle difference ±1,96xs, ad eccezione dei valori più alti per i quali l’analizzatore diventa meno attendibile. Comparando infatti i due metodi per valori di lattatemia ≥ 5 mMol/L, la correlazione rimane comunque elevata (r = 0,94; p<0,01), ma la regressione lineare mostra un aumento dell’intercetta e un aumento dell’intervallo di confidenza (intercetta = 1,68; intervallo di confidenza al 95% = 0,01-3,35). Anche il grado di correlazione rileva un aumento della media delle difference ±1,96xs, con intervallo di confidenza al 95% = -3,583-1,464. Per verificare l’accuratezza del Lactate Scout per valori ≥ 5 mmol/L, sono necessari ulteriori studi, in quanto solo 12 dei 201 campioni analizzati avevano valori superiori a questo limite. Non è stata osservata alcuna correlazione statistica (r = -0,04; p = 0,63) tra la concentrazione di lattatemia ottenuta con Lactate Scout e l’Hct (mediana 39%; range 35-41%) misurato su 58 campioni. In questo caso, la mancanza di correlazione suggerisce che l’analizzatore portatile può essere utilizzato efficacemente anche in pazienti anemici o policitemici. I risultati di questo studio di comparazione indicano che le concentrazioni di lattatemia ottenute con l’analizzatore Lactate Scout sono comparabili a quelle ottenute con la metodica di riferimento. Inoltre l’analizzatore Lactate Scout necessita di una quantità minima di campione (0,5 µL), è rapido,
può operare a temperature ambientali molto variabili (5-45°C) e ha un ampio range di misurazione (0,5-25 mMol/L). Queste caratteristiche lo rendono non solo facilmente utilizzabile in campo e da operatori non specializzati, ma anche un valido strumento per la valutazione prognostica e per il monitoraggio del pulcino neonato ricoverato in terapia intensiva. La lattatemia infatti rappresenta uno dei parametri più utili per valutare lo stato emodinamico del paziente in risposta alla terapia effettuata.

Bibliografia

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BETA-CATENINA E PECAM-1: POSSIBILE RUOLO NELLA PATOGENESI DEL TESSUTO DI GRANULAZIONE ESUBERANTE DURANTE LA GUARIGIONE DELLE FERITE NEL CAVALLO

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**Introduction.** Healing of wounds located on the limb of horses is often complicated by the formation of exuberant granulation tissue (EGT) clinically referred to as proud flesh. Treatments that prevent or resolve this condition are unavailable such that wound management can be labor-intensive and expensive. This may relate to insufficient knowledge of the underlying molecular mechanisms.

**Aim.** This study aimed to clone full-length equine PECAM1 and β-catenin (CTNNB1) cDNAs and to study the spatio-temporal expression of mRNAs and corresponding proteins during wound repair in horses.

**Methodology.** Cloning was achieved either by screening a previously-derived size-selected cDNA library or by a PCR-based cloning technique. Expression was studied in intact skin and in 1, 2, 3, 4 and 6 week-old wounds of the body and the limb. Temporal gene expression was determined by RT-PCR while protein expression was mapped immunohistochemically.

**Results.** Full-length equine PECAM1 cDNA consisted of 3381 bp encoding a 738-amino acid protein (theoretical MW 82.3-kDa) while CTNNB1 cDNA consisted of 2382 bp encoding a 781-amino acid protein (theoretical MW 85.5-kDa). The temporal expression pattern of the two genes was similar: wounding up-regulated mRNA expression which did not return to baseline and over-expression was noted in body compared to limb wounds. Immunostaining for both PECAM1 and β-catenin was especially pronounced in wounds with EGT.

**Conclusions.** This study is the first to characterize equine cDNA for PECAM1 and CTNNB1 and to document that these genes are differentially expressed during wound repair in horses. Our data suggest that these molecules contribute to the excessive proliferative response seen in horse limb wounds.

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PREVALENZA DI ULCERE GASTRICHE IN CAVALLI COLD-BLOOD IN POLONIA

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Objectives. Equine gastric ulcer syndrome (EGUS) is a common health problem in horses and foals, and may have an impact on their condition and performance. The prevalence of EGUS in Thoroughbred racehorses in training has been shown to be up to 82-91% and up to 58% in recreational horses. High intensity training and racing are closely associated with a high prevalence of gastric lesions in both Thoroughbred and Standardbred racehorses, as well as nonsteroidal anti-inflammatory drugs (NSAIDs) and corticosteroids overuse, intermittent feeding and fed with a high-protein and high-calcium diet. A great number of studies can be found that deals with prevalence of gastric ulcers in sport and show horses, however, with different focus. The aim of our study was to establish the prevalence of gastric ulcers in cold-blooded horses in Poland and classify it by Number/Severity (N/S) grading system.

Material and Methods. The study was carried out from November to December 2006 on a group of 349 slaughtered cold-blooded horses. Directly after the horses were slaughtered, the stomachs were flushed with water and visually controlled for the occurrence of ulcers. Prevalence, distribution and severity of gastric ulcers were estimated. Lesions involving the squamous mucosa and the glandular mucosa of the antrum and pylorus were graded and recorded by camera. Samples of representative gastric ulcers were then placed in the solution of 10% formalin and processed routinely for histopathology. Number/Severity (N/S) gastric lesion scoring system was used. That is, to estimate number of a lesions, we used five grade system, where numbers from 0 to IV indicated quantity of lesions, namely; 0: no lesions, I: 1-2 localised lesions, II: 3-5 localised lesions, III: 6-10 lesions, IV: > 10 lesions or diffuse (or very large lesions). Severity of lesions was estimated by the use of six grade system, where 0: no lesions, I: lesions appears superficial (only mucosa missing), II: deeper structures involved (greater depth than No. I), III: multiple lesions and variable severity (I, II and/or III), IV: same as II and has active appearance (active = haemorrhage or adherent blood clot). Hyperkeratosis or mucus hyperaemia was classified as I.

Results. 125 (36%) of the horses had no lesions in both the squamos mucosa and the glandular mucosa of the antrum and pylorus. Evidence of gastric ulcers was detected in 224 (64%) of the horses. Number of the lesions in both parts of a stomach was respectively- I: 29 (8%), II: 56 (16%), III: 41 (12%), IV: 98 (28%). The squamous mucosa around the margo plicatus was most commonly affected, and severity of lesions was respectively 0: 180 (51%), I: 27 (8%), II: 43 (12%), III: 44 (13%), IV: 30 (9%), V: 25 (7%). There was no significant difference between squamous mucosa and glandular mucosa of the antrum and pylorus, and severity of lesions was respectively 0: 165 (47%), I: 40 (11%), II: 37 (11%), III: 55 (16%), IV: 27 (8%), V: 25 (7%).

Results and Conclusions. Gastric ulceration was present in a large proportion of cold-blooded slaughtered horses (64%). This result is relatively lower in comparison with sport and show horses, where depending on a study present respectively: 86% (Begg LM et al.) and 88,3% (Bell RJ et al.). Although our result is significant higher as opposed to the study of Sandin A et al., where only 7% of the cold-blooded was affected with the gastric ulcers. No association was found between presence of lesions of the squamous mucosa and those of the pylorus. Further studies are needed to determine the aetiology of the syndrome in cold-blooded horses, and to find ways to reduce, if possible, the frequency of gastric ulcers. References are available at presenting author’s e-mail address.

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Introduction. Mesenchymal stem cells (MSCs) have recently attracted a great deal of interest as potential protagonists in cell and gene-based therapeutic applications. They were firstly isolated from bone marrow and subsequently recovered from several other tissue sources, including periosteum, trabecular bone, adipose tissue, synovium, skeletal muscle, skin, etc. MSCs display extensive self-renewal capacity in vitro and are multipotential, being able to differentiate into osteoblast, chondrocyte, adipocyte, and myoblast lineages. In addition, their capacity to adopt neural, hepatic, cardiomyocyte-like, endothelial-like and insulin secreting cell phenotypes has been recently demonstrated. Much of the interest in the veterinary medicine is focused on the equine orthopedic field, with the use of MSCs in the experimental treatment of tendon and ligament injuries. Although several cell sources could be considered, clinical use has limited the choice to MSCs isolated from bone marrow and fat. As adipose tissue allows the easy extraction of a large volume of tissue with limited donor-site morbidity, it is considered a particularly exciting stem cell source. Its stromal component has been demonstrated to contain, in humans and rodents, an especially high concentration of MSCs which are also easy to isolate and to expand in vitro. The results obtained in experimental clinical studies employing adipose-derived MSCs in the therapy of horse tendon and ligament injuries generated great excitement in the animal-owning public. However, the lack of studies of characterization of these cells in the horse makes empiric their current clinical use. The purpose of this preliminary study was to isolate the stromal-vascular cell fraction (SVCF) from equine adipose tissue and to verify the existence of a cell population showing the same biological features of adipose-derived MSCs described in other species.

Material and methods. Equine adipose tissue samples were obtained from subcutaneous fat during surgeries. The tissue was finely minced and digested with 0.075% collagenase type I (Worthington Biochemical - Lakewood, NJ). After centrifugation, the supernatant was discarded, while the pellet- ed stromal vascular cell fraction (SVCF) was resuspended, plated in tissue culture flasks and incubated at 37 °C/5% CO₂. Cells were maintained in basal medium (DMEM, 10% FBS, 1% antibiotic/antimycotic solution) until passage 12. At passage 3 cells were analyzed for their clonogenicity by means of a colony forming unit-fibroblast assay (CFU-f-assay). To demonstrate their multipotentiality, cells were then differentiated along the adipogenic and osteogenic lineages. They were induced with a combination of adipogenic factors (dexamethasone, 1-methyl-3-isobutylxanthine, insulin and indomethacin) and differentiate in culture for a 21 days period. The osteocitic phenotype was induced by culturing cells in a specific induction medium containing a mixture of osteogenic factors (dexamethasone, ascorbic acid, β- glycerophosphate).

Results. Processing of 1g of adipose tissue routinely yielded SVCF samples of 1 x 10⁶ cells. When maintained in basal medium, this heterogeneous population easily expanded in vitro forming a monolayer of spindle-shaped cells exhibiting a fibroblast-like morphology consistent with that of MSCs from other species. A homogeneous and proliferating adherent cell population was obtained after 10-12 days of culture. CFU-f assay showed a high frequency of fibroblastic colonies. Within 14 days after induction of adipocyte differentiation, a high percentage of the cells accumulate intracellular lipids as multiple droplets which can be stained for neutral lipid with Oil Red O dye.
When grown in osteocyte-specific induction medium, the formation of clusters of calcium-producing cells was observed. Calcium deposition was demonstrated by Alizarin red S and Von Kossa stainings.

**Discussion and conclusion.** Several factors provide convincing evidence that the cells described in this study are MSCs: published methods for MSCs isolation from other species were used to isolate equine cells; the cells are very similar to their rodent and human counterparts based on morphology and growth traits; finally, the cells are multipotential, capable of differentiating into adipocytes and osteocytes in vitro.

**Suggested literature**

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OSTEOCONDROMA DELL’OSSO NASALE IN UN CAVALLO
OSTEOCHONDROMA OF THE NASAL BONE IN A HORSE

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Purpose. To describe a case of osteochondroma of the nasal bone in a colt.

Clinical data. A 18-months old warmblood colt was referred for the presence of a single median mass deforming the nasal profile. The lesion was present from six months before the examination and it was gradually increasing in size. There was no history of trauma.

The lesion was 6cm x 4cm x 3cm in size, smooth, bony hard, cold, indolent and fixed to underlying bone. No other bony growths were evident at the physical examination.

Methods. Radiographies showed a proliferation of osseous tissue, arising from nasal bones, with areas of irregular radiolucencies.

Cytological examination of fine-needle aspirates of the lesion showed few scattered, large- to medium-sized mesenchymal cells. Moderate anisocytosis and anisokaryosis were present. Individual cells appeared round to spindle-shaped to stellate and often had wispy trails of cytoplasm. Cytological interpretation was a mesenchymal neoplasm, probably an osteoma or chondroma.

Anesthesia was induced with acepromazine (0.0125 mg/kg e.v), romifidine (0.05 mg/kg e.v.), guaifenesine (100 mg/kg e.v.) and ketamine (2.2 mg/kg e.v.) and maintained with isoflurane and oxygen in a semiclosed system. The periosteum coated mass was removed with a bones scalpel. The edges of the periosteum and of the fascia were apposed with 3-0 Poligrecapone 25 (Monocryl® Ethicon, Sommerville, NJ) in a simple continuous pattern; the skin was sutured in an interrupted pattern with 2-0 nylon (Ethilon®, Ethicon, Sommerville, NJ) and a Penrose drainage applied to prevent seroma formation. The Penrose was removed 5 days after the surgery and the skin suture was removed 10 days later.

Results. The gross specimen was smooth, ivory white in appearance, ovoid in shape. Histologically was composed of multilobulated neoplastic tissues consisting of irregular islands of well defined bone and cartilage. The hyaline cartilage covered most of the underlying trabecular bone formed by endochondral ossification. The overlying chondrocytes were hypertrophied near developing bony trabeculae. Mitotic figures were rare (< 1 mitosis per 45x field of view). An histological diagnosis of osteochondroma was made.

Follow-up. One month later, the nasal profile presented an irregular fibroelastic mass, protruding of 1,5cm, in the area of the previuos surgery. Radiographies show an area of irregular radiodensity with a peripheral soft tissue opaque outer rim, resembling a periostal reaction.

One year later, the change of the nasal profile persisted with the same features at the clinical and radiological examination.

Conclusions. The osteochondroma is a benign cartilage-capped tumor-like exostosis adjacent to a physis or subarticular growth cartilage (1). It could be more exactly classified as a skeletal dysplasia, arising from the margins of growth plates or from focal disturbances of the periostium. In horses, the lesions tend to be present at birth and to be bilaterally symmetrical. Common sites are metaphysis of long bones, ribs, scapula and vertebrae (2, 3, 4, 5). In literature, the first publication of solitary osteochondroma of the nasal bone in a horse was made in 1994 by Adair HS et al.; since then, no other cases of this benign tumor arising from an intramembranous bone have been published and no information relative to the follow-up, however, are reported in the previous report (7). In this case the 1 year follow-up confirms that in the horse the behaviour of solitary ostheocondro-
ma involving flat bone is benign, unlike that of the cat. Prognosis after surgical excision appears good, except for aesthetic reasons, relevant in show horses.

Further, the occurrence of osteochondroma in an intramembranous bone supports the hypothesis that this neoplasm can originate from metaplasia of cambial periosteum (7).

The cause of osteochondroma has still not been defined. According to congenital theory, presence of embryonic cartilage results in intensified bone growth after puberty (8,9). In human medicine, some authors suggested trauma with consequent periostitis as a predisposing factor for benign bones tumors. Hormonal theory in which there is increase of periosteal osteoblast activity, stimulated by endocrine mechanisms which results in increased bone growth has also been suggested. Most authors feel that it originates from the pre-osseous connective tissue (10).

Clinical presentation and radiological and histological features of osteochondroma of the nasal bone in the horse appeared to be characteristic but differential diagnosis should include granuloma, ossifying fibroma, osteoma, condroma, osteosarcoma and chondrosarcoma. Histological evaluation is needed for definitive diagnosis (2).

References


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Purpose of the work. Aim of this work is to present two cases of hepatic biopsy under laparoscopic guide performed in two horses affected by hepatic diseases.

Methods.

1st clinical case
A 9-year-old bay Anglo-arabian neutered horse was referred for anorexia and loss of weight. The physical examination showed slimness and congested mucosae. Preoperative bloodworks reported GGT and bile acids, dramatically, increased. The ultrasound examination showed hepatomegaly with hyperechoic areas, following by distal acousting shadowings, spreaded into the parenchyma. After the echography a hepatic biopsy under laparoscopic guide has been suggested. A standing horse laparoscopy was performed. The horse was sedated and restrained in well padded stocks. A regional anesthesia with lidocaine 2% in the site on skin incision was performed. The optic port was created 8-centimeters cranially to the iliac crest and 20 centimeters distally to the transverse processes of lumbar vertebrae to introduce a 30° laparoscope. The laparoscopic exploration was made on left and right side, starting on the left one, after carbon dioxide insufflation. On the left side was possible to observe: the spleen, bladder, left kidney, descending colon and renosplenic ligament. The spleen appeared increased in size and pale-red. On right side was observed the right lateral lobe and caudate process of the liver, right dorsal colon, right kidney, duodenum, base of the cecum. The liver revealed a pleated and yellowish-green surface. To complete the liver examination a second 10mm-port was created to introduce a laparoscopic ultrasound probe. A third instrumental port, created 5cm cranially and ventrally to the optic port, was made to introduce a biopsy forceps. Two samples of hepatic tissue were acquired. The hystological diagnosis was: severe perilobular and intralobular hepatic fibrosis.

2nd clinical case
A 19-year-old bay Quarter Horse female was referred for weight loss, sensory depression, dysorexia. The physical examination showed slimness and congested mucosae. Preoperative bloodworks reported anaemia, leukocytosis and an increased levels of GGT and bile acids. A PCR test of urine resulted negative for leptospirosis. Ultrasound examination showed hepatomegaly, evident biliary tracts with hyperechoic biliary walls. A standing horse laparoscopy was performed. The horse was sedated and a regional anesthesia with lidocaine 2% in the site on skin incision was performed. The optic port was created 8-centimeters cranially to the iliac crest and 20 centimeters distally to the transverse processes of lumbar vertebrae to introduce a 30° laparoscope. The laparoscopic exploration was made on left and right side, starting on the left one, after carbon dioxide insufflation. On the left side a film of fibrin created a slight adhesion of the spleen to the abdominal wall, but no other macroscopic alteration on the spleen were observed. On the right side the hepatic right lobes appeared increased of volume, with rounded margins covered by a biliary exudate. An instrumental 5-mm-port was created cranially and ventrally to the optic port to introduce a biopsy forceps. Three withdrawals of hepatic tissue were acquired. The hystological diagnosis was: cholestasis and chronic cholangio-hepatitis.

Outcomes. The hepatic samples gave in both cases an accurate diagnosis and no complications after surgery were observed. The use of paralumbar fossa for the placement of optic and instrument-
tal ports has provided a good and easier manoeuvrability of the instruments. The surgical time was 30 minutes in the first case and 20 min in the second one. The longer surgical time in the first case was due to use of laparoscopic ultrasound probe for choosing the correct site of withdrawal.

**Discussion.** The laparoscopic guide in standing horse has presented two relevant advantages comparing the ultrasound guide: 1-the visualization and magnification of the pathological organ; 2-the possibility to control immediately the bleeding in the site of biopsy, avoiding subsequently complications. Furthermore, the use of a laparoscopic ultrasound probe in the first case has permitted to examine the surface and the internal structure of the liver, increasing the sensibility of the exam.

**Conclusions.** Our positive experience in the use of lapascopy as biotactical guide is confirmed by results reported by other authors in veterinary literature. Moreover, the only disadvantage of lapascopic technique represented by the only surface examination could be avoided using a laparoscopic ultrasound probe during the procedure.

**References**


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Introduction. In the last decades the improvement of specific hardware and software allowed the traditional method of evaluation to be supported by computer assisted sperm analysis, thanks to which more precise and objective results have been gained in comparison with those reached by semen evaluation using optical microscope. In this study both methods have been employed to value the most important parameters by which it’s possible to obtain an assessment of the sperm quality, to use it at best in case of artificial insemination.

Materials and methods. During the 2006 reproductive season, the semen of 8 standardbred stallions has been collected and evaluated by using traditional method and computer assisted sperm analysis. A phantom and a Colorado artificial vagina have been used to collect the ejaculated in a stud farm (fig. 1). After measuring the volume of the gel-free ejaculated, the spermatozoa concentration (C) has been calculated by using a spectrophotometer: after this, using the formula \( Vs=60/C-201 \), we calculated the semen volume required to have in all the samples a standard number of spermatozoa equal to 20x10⁶, considered optimum for the computer assisted sperm analysis. This volume has been put in 3 different test tubes, containing an extender composed by skimmed milk and antibiotics, conserved at 37°C: these samples have been used to evaluate semen at T0 (within 1h from the collect), at T1 (after 24h) and at T2 (after 48h) after conserving the test tubes at 4°C. Besides 20µl of not diluite semen have been added to 1ml of dilute formalina, to evaluate spermatozoa morphology. Spermatozoa vitality and morphology have been evaluated by traditional method, using an optic microscope to observe specimens stained with the eosina-nigrosina colouring in case of the first parameter and fixed in formalina for the second one. The employ of the Hamilton Thorne Computer Assisted Sperm Analyzer-CEROS, version 12 has permitted to value: VAP (Average Path Velocity), VSL (Progressive Velocity), VCL (Track Speed), ALH (Amplitude of Lateral Head), BCF (Beat Cross Frequency) (fig. 2), total and progressive motility. The averages of these data have been submitted to statistic analysis using the One-Way ANOVA model to compare the results related to the time and Pearson’s linear correlation test to obtain a comparison between the stallion.

Results. The differences about total (fig. 3) and progressive (fig. 4) motility in relation to the time have been strongly significant \((p<0.001)\), as those regarding BCF, VAP and VSL, while about VCL there was an important variation only between T1 and T2 \((p<0.05)\) and about ALH only between T0 and T1 \((p<0.01)\) and T0 and T2 \((p<0.05)\). Not important differences have been observed about the percentage of vital (fig. 5) and abnormal (fig. 6) spermatozoa during T0, T1 and T2. The most important differences between the stallion have been observed about the percentage of spermatozoa total and progressive motility, vitality and morphology.

Discussion. The differences between total and progressive motility in relation to the time show a reduction of these parameters since the ejaculated has been collected up to 24 and 48h, as already asserted by other Authors. Spermatozoa vitality and morphology are not influenced by the time: this fact, already described in bibliography, suggests that there isn’t a strong correlation between...
these 2 parameters and total and progressive motility, as believed in the past. Besides the differences between the stallions about spermatozoa vitality, motility and morphology suggest that there is an individual variability able to influence semen quality, even if the subjects are breeded and used at the same conditions.

**Conclusion.** Computer assisted sperm analysis assures extreme precision and objectivity if the hardware is set rightly, anyway it must be supported by vitality and morphology evaluation, performed by an operator or using specific software. The results show that there isn’t a correlation between spermatozoa vitality, morphology and motility, so a semen sample with a low percentage of motility is not necessarily unfertile and using a deep artificial insemination it’s possible to increase the possibility to have a pregnancy. Moreover using both the methods it’s possible to have a complete evaluation of the considered semen, that permit to use it at best, fresh or cooled.

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PARAMETRI EMATOBIOCHEMICI NELLA FATTRICE DURANTE IL PERIPARTUM: RISULTATI PRELIMINARI

HAEMATOLOGICAL AND BIOCHEMICAL FINDINGS IN PERIPARTUM MARES: PRELIMINARY RESULTS


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In bibliografia sono presenti pochi studi riguardanti l’andamento dei parametri ematobiochimici nella fattrice durante l’ultima parte della gestazione ed il postpartum. La gravidanza e la lattazione infatti provocano un’aumentata richiesta metabolica ed il parto rappresenta ovviamente un cambia-
mento improvviso che coinvolge l’intero organismo. Quindi lo scopo del nostro lavoro è quello di individuare eventuali variazioni rispetto ai range di riferimento per l’equino adulto e di analizzare l’andamento fisiologico dei parametri ematobiochimici durante l’ultima fase della gestazione ed il primo periodo dopo il parto. Lo studio è stato effettuato su 25 fattrici gravide di razza Standardbred riferite per il monitoraggio della gravidanza e l’assistenza al parto. I seguenti parametri ematobio-
chimici sono stati valutati a venti (T-20) e dieci giorni (T-10) prima del parto, al momento del par-
to (Tp) e 7 giorni dopo (T+7): Hgb, Hct, RBC, WBC, Plt, MCV, MCH, MCHC (CELL-DYN 3500R, Abbott laboratories, CA, USA); fibrinogeno, lattato, AST, CK, SAP, creatinina, ube,
bilirubina totale, diretta ed indiretta, GGT, PT, albumine, albumine/globuline, calcio, sodio, cloro, pot,
tasso, magnesio, glucosio, acidi biliari (Chemistry Analyzer AU400, Olympus Diagnostica GmbH, Ireland). L’andamento dei diversi parametri nel tempo è stato analizzato tramite il test t-Student per dati appaiati. Tutte le fattrici hanno presentato una gravidanza fisiologica con parto eutocico e nascita di puledri sani e vitali. La media di tutti i parametri è risultata compresa nei range di riferi-
tmento per l’equino adulto utilizzati dal laboratorio di riferimento, ad eccezione per la SAP (T-20: 314,5 u/l ±94,9; T-10: 329,4 u/l ±82,2; Tp: 444,1 u/l ±109,9; T+7: 400,4 u/l ±76,1) (range 127-241 u/l). I seguenti parametri aumentano in maniera significativa tra T-20 e Tp: SAP, PT, albumine, bi-ilubina indiretta, magnesio e glucosio (p<0,05), mentre diminuiscono calcio ed acidi biliari (p<0,05). Tra T-20 e T+7 si ha un aumento di CK e bilirubina diretta (p<0,05) ed una diminuzione di creatinina e WBC (p<0,05). Tra T-10 e Tp aumentano invece: Hct, RBC, WBC, AST, CK, SAP, GGT, PT, Mg, lattato, glucosio (p<0,05), albumine (p<0,01) e diminuiscono urea ed acidi bi-
liari (p<0,05). Tra T-10 e T+7 aumentano CK, albumine/globuline, calcio (p<0,05) e diminuiscono bilirubina totale ed indiretta, potassio, glucosio (p<0,05) e creatinina (p<0,01). Infine tra Tp e T+7 aumentano calcio e rapporto albumine/globuline (p<0,05); si ha invece un calo significativo di Hgb, Hct, RBC, WBC, SAP, bilirubina indiretta, GGT, albumine, sodio, cloro, lattato (p<0,05) e di crea-
tinina, proteine totali e glucosio (p<0,01). L’aumento della cortisolemia che si verifica al momento del parto potrebbe in parte giustificare l’aumento di glucosio, leucociti, Hct ed eritrociti. Il rialzo di questi parametri, insieme a quello delle PT, potrebbe anche essere indicativo di uno stato fisiologi-
co di emocostruzione. Infine anche la lieve reazione infiammatoria che precede il distacco della placenta potrebbe causare un aumento dei leucociti. I valori medi di creatininemia durante l’ulti-
ma fase della gravidanza (T-20: 1,57 mg/dl ±0,23; T-10: 1,54 mg/dl ±0,24; Tp: 1,65 mg/dl ±0,27) si sono rivelati vicini al limite superiore del range fisiologico (0,90-1,50 mg/dl), per poi calare a T+7 (1,23 mg/dl ±0,16), probabilmente per un diverso metabolismo proteico associato alla presenza del feto. Gli alti livelli di SAP, riportati anche da altri autori ed in altre specie, potrebbero essere giu-
stificati dalla presenza fisiologica dell’isozima placentare. Inoltre anche il colostro e il latte della fattrice contengono un isozima della SAP, suggerendo che la ghiandola mammaria possa esserne un’ulteriore fonte. I nostri risultati rappresentano solo dati preliminari e l’analisi di un numero mag-
giore di animali potrebbe permettere di stabilire diversi intervalli di riferimento da utilizzare duran-
te il peripartum per il monitoraggio della salute della fatrice.

**Bibliografia**


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Introduction. Equine LSA is considered to be an uncommon disease in the horse, although it is the most common neoplasm of the equine haematopoietic system (accounting for between 1.3% and 2.8% of all tumours in this species). Lymphoma has been reported in horses ranging from foetal to advanced age, and limited surveys have identified neither gender nor breed predispositions. The cause of LSA has not been determined. Clinical manifestations are non specific and extremely variable depending on the system most affected, making a clinical diagnosis difficult. Diagnosis is aided by the histology or cytology of biopsies or body fluids, from subcutaneous nodules, lymph nodes, liver or bone marrow. The prognosis is very poor. When clinical signs of a tumours become apparent the course of the disease is usually rapid and fatal. Treatment is no satisfactory and rarely attempted. Aim of this paper is to provide a description of pathological modifications in an unusual case of large granular lymphoma (LGL) involving nasal and paranasal sinuses.

Clinical Case. A case of large granular lymphoma (LGL) involving the left chonco-frontalis and chonco-maxillaris sinuses was diagnosed in a anglo-arab (a.a.) mare, five years old. Weight loss, limbs oedema, marked obstructive dyspnoea with respiratory noises, exophthalmia, bilateral eyelids swelling, conjunctivitis, submandibular lymphadenopathy were the most consistent clinical findings. The mare were normothermic, lethargic and apparently without pain. Routinely haematology and biochemistry examination were unremarkable. Thoracocentesis and abdominocentesis had not been made. Endoscopic examination allows us to obtain a biopsy from a mass occluding left nasal cavity. The course of the disease was surprisingly rapid with only one week between the onset of clinical signs and euthanasia. Complete necropsy, which is the most reliable method of confirming the existence of abdominal masses, was not possible in this case. The skull was imaged in transverse and sagittal planes with a 1,5 Tesla magnet (Philips), using T1 and T2 sequences. Macroscopic examination of the head showed, in the nasal cavity, in the maxillary, nasal and frontal sinuses of the left side, cerebroid masses with lardaceous consistency. Submandibular, retropharyngeal lymph nodes and nuchal ligament with contiguous neck muscles were seriously infiltrated. Histologic and immunohistochemical examinations revealed large-size neoplastic cells proliferation, with wide cytoplasm and PTHA – CD3 positives granules. On the basis of specimens, diagnosis of large granular lymphoma (LGL) of upper respiratory tract has been made.

Discussion. Equine lymphoma is a heterogeneous disease entity with much variations among animals in presenting signs, clinical course, laboratory data and pathologic findings. It has been classified into mediastinal, alimentary, multicentric and cutaneous forms. Large granular lymphoma is very rare in equine (in literature are described only 3 cases in horses and 1 in a mule). This case of LGL was unusual because the location of the tumour mass involving nasal and pharyngeal areas and its lymphoid tissue. This case illustrates the difficulty in making a definitive clinical diagnosis when neoplastic cells are not identified in peripheral blood or pleural and peritoneal effusion. The animal affected was presented as being acutely ill and there was a rapid
worsening in its condition in the last stage of the disease. The MRI was useful for the identification of nasopharyngeal involvement and suggestive of lymphoma tumour expansion into orbital cavity.

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STRATEGIA DIFENSIVA MESSA IN ATTO CONTRO I VIRUS DELL’INFLUENZA DA PARTE DELLE VIE RESPIRATORIE DEGLI EQUINI
DEFENSIVE STRATEGY PERFORMED AGAINST INFLUENZA VIRUSES BY EQUINE RESPIRATORY TRACT

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Introduction. Influenza virus particles infect host cells by means of hemagglutinin spikes (H1-16) binding to sialoglycoconjugates acting as receptors on the host cell surface, and by means of neuraminidase spikes (N1-9) cleaving terminal sialic acid from cell surface glycoconjugates allowing the virion to escape from the host cell during the budding process. Equine influenza viruses preferentially recognises the dimer sialic acid-α2-3-Galactose. This work aims to visualise in situ the presence of sialoderivatives in the horse respiratory tract in order to localise sialoderivatives acting as influenza virus receptors.

Materials and Methods. Nasal mucosae, trachea, bronchus and lung parenchyma were removed from 8 mature horses of both sexes, fixed at room temperature in Carnoy’s fluid for 24 h and postfixed in 2% calcium acetate-4% paraformaldehyde solution (1:1) for 3 h. Specimens were then dehydrated, embedded in paraffin wax and cut in serial sections 5μm thick. Sialic acid characterization was performed by means of lectin histochemistry in two different ways: a direct evidentiation using MAA and SNA biotinylated lectins, and an indirect method performed by sequential treatment providing mild and strong peridate oxidation and saponification combined with sialidase digestion and PNA and RCA I horseradish peroxidase conjugated lectins.

Results. No difference were shown between sexes. Direct visualisation of sialic acid residues, performed by means of MAA and SNA lectins, having nominal specificity towards two different dimers of sialic acid and β-galactose, distinguishable on the basis of α2-3 and α2-6 linkage, showed the presence of both the dimers above all in some cells and in cell coat of epithelial lining of trachea and in the cell coat of nasal mucosa. Indirect detection of sialic acid residues performed using periodic oxidation, deacetylation, sialidase digestion and lectin incubation showed that both the dimers directly evidenced are linked to galactose(1-3)-N-acetyl-galactosamine since only PNA reactivity was affected by enzymatic digestion with or without prior deacetylation, while RCA I reactivity was unmodified by the same treatments. Deacetylation with KOH did not affect sialidase/PNA reactivity of samples, showing the lack of sialic acid residues acetylated at C4. Sequential treatments demonstrate the presence of periodate labile sialic acid linked both α2-3 and α2-6 to galactose(1-3)-N-acetyl-galactosamine in some cells and in the cell coat of trachea epithelial lining. The same sialoderivatives are present in the cell coat and in the goblet cells of nasal mucosa; in addition, nasal goblet cells express great amount of C9 and/or C7,9 and/or C8,9 and/or C7,8,9 acetylated sialic acid linked α2-3-galactose.

Discussion. On the basis of obtained data we can affirm that equine influenza virus specific sialoreceptor is present on the cell coat and in some cells of the trachea epithelial lining; at these levels the dimer sialic acid-α2-3-galactose shows periodate labile sialic acid residues. Also at nasal cell coat level the same receptor is present, but in a lesser quantity. Sialoglycoconjugates showing the dimer sialic acid-α2-3-galactose, having the sialic acid residues C9 and/or C7,9 and/or C8,9 acetylated, are present in the nasal goblet cell secretion; we can hypothesise that these sialoderivatives, forming the mucous layer on the nasal mucosae, could have a specific role in the defence from equine influenza virus performed by agglutination of virus. In addition, sialoderivatives of both surface and secretion type, characterised by the dimer sialic acid-α2-6-galactose, specifically recognised by human influenza virus, are expressed by the same tissue structures; these sialoglycoconjugates likely perform an unspecific defence masking the specific receptors.

Conclusions. To have a complete picture of sialoderivative types express by different tracts of equine respiratory tract may be useful for provide new ways to treat the infectious disease and for evaluate possible new recognition sites in case of virus mutation and/or transmission among different species.

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Proceedings of the European Equine Meeting of the Year 2008 - XIV SIVE - FEEVA Congress, Venice, Italy
Tramadol (T) is a centrally acting analgesic structurally related to codeine and morphine. T has been used clinically for the last two decades to treat pain in humans following orthopaedic surgery and major gynaecologic surgeries, in addition to non surgical conditions (Close, 2005). The primary phase I metabolites, namely, O-desmethyl-tramadol (M1) and N-desmethyl-tramadol (M2) may be further metabolized to three additional secondary metabolites, namely, N,N-didesmethyl-tramadol (M3), N,N,O-tridesmethyl-tramadol (M4) and N,O-didesmethyl-tramadol (M5). In humans, the major metabolic pathways are O-demethylation (M1) catalyzed by isozyme cytochrome P450 (CYP) 2D6 and N-demethylation by CYP 2B6 and 3A4 (M2); CYP 2D6 and CYP 2B6 and 3A4 are also primary involved in M5 and M3 formation, respectively (Subrahmanyam et al., 2001).

Clinical response of T is strictly correlated to its metabolism, because of the different analgesic activity of its metabolites. M1 is reported to be the major active metabolite and is 200 times more potent at the μ-receptor binding than parental drug T (Raffa et al., 1992). Another metabolite, M5 has a higher affinity than T for the μ-opioid receptor is M5. The aim of the present study is to evaluate in the horse the pharmacokinetic profile of T and its metabolites M1, M2 and M5 following a single dose (5 mg/kg) of T administered by intravenous formulation, by modified release tablets and by an immediate release capsules, and to assess the potential effect on bioavailability of immediate release capsules formulation in fasting and fed animals. Animal treatment was divided in four randomized phases. The subjects were 24 male race-horses, aged from 9 to 13 years weighting from 450 to 550 kg. The horses were previously determined to be clinically healthy based on physical examination and haematological analyses. In the first phase, 6 animals were administered with T iv (5 mg/kg; tramadol Exal 50 mg/ml). To allow T intravenous administration a 14 G catheter was placed in the right jugular vein. In the second phase 6 race-horses were administered with T (5 mg/kg; tramadol Exal 50 mg/capsule immediate release) by naso-gastric tube. These subjects were fasted for at least 10 hours before and after dosing and deprived of water for 3 hours before dosing. In the third phase of study design 6 animals were administered with T (5 mg/kg; tramadol Exal 50 mg/capsule immediate release) by naso-gastric tube with food and water ad libitum. In the last phase 6 animals were administered with T (5 mg/kg; Contramal sustained release tablets 100 mg/tablet) via naso-gastric tube in fasting state.

Blood samples were collected via a 14G catheter placed in the left jugular vein at 0, 5, 15, 30, 45 min and 1, 1.5, 2, 4, 6, 8, and 24 h following 1-3 phases and at 0, 5, 15, 30, 45 min and 1, 1.5, 2, 3, 4, 5, 6, 7, 8, 9, 10, 24, 28 and 36 h after oral sustained release tablets (phase 4) administration. Plasma concentrations of T and M1, M2 and M5 were measured by HPLC apparatus with fluorimetric detection. Adverse effects as nausea, confusion, agitation, tremor and tachycardia were noted in all horses T iv administered although with different strength among the animals. These effects started from 3-5 min and rapidly increased to 15-20 min after drug injection but were transient and resolved by the end of the 2nd hour study-period. Horses showed any adverse symptom after os administrations.

A one-compartment model fit best the plasma concentrations of T and M2 after all the treatments. Unfortunately for M1 and M5 was not always possible to fit plasma curves, due to very low and
fluctuant concentrations (close to LOQ) of these metabolites and these data were not inserted in the study. Following iv administration plasma concentration of M2 was about 2 fold higher than the others metabolites, with a Cmax and Tmax of 0.25±0.01 μg/ml and 1.25±0.18 h, respectively. The M1 and M5 concentrations were similar (low and variable) with Cmax(s) between 1-2 hours: these plasma concentrations were similar to T and M2 concentrations about after 6 hours from the drug administration with values close to LOQ. Cmax, and T 1/2 of T were 3.59±0.20 μg/ml and 0.69±0.10 h, respectively. In fasted horses, after os immediate release administration, Cmax and Tmax of T and M2 were 1.77±0.22 and 0.40±0.09 μg/ml and 0.42±0.08 and 2.00±0.45 h, respectively. Also after this administration M1 and M5 plasma concentrations were variable and close to LOQ but quantifiable till 8th hour. Metabolites concentrations became similar to T between 6 and 8 hours. Bioavailability (F%) of T by this administration was 64.5±8.36%. In fed horses, after os immediate release administration, Cmax and Tmax of T and M2 were 3.61±0.50 and 0.37±0.25 μg/ml and 0.33±0.11 and 2.00±1.04 h, respectively. M2 concentration was two fold higher than those of M1 and M5. Metabolites concentrations were reached from T concentrations about after 4th hours from drug administration. F% of T by this administration was 84.6±18.35%. Following sustained release tablets administration, M2 was the main molecule quantifiable with a Cmax and a Tmax of 0.19±0.05 μg/ml and 3.33±0.72 h, respectively. T and M5 had similar values with Cmax of 0.057±0.07 and 0.043±0.001 μg/ml and Tmax 4.46±0.55 and 3.51±0.81 h, respectively. M5 showed a Ka and Kel of 0.50±0.10 (1/h) and 0.19±0.02 (1/h), respectively. The four molecules (T, M1, M2 and M5) reached the same concentrations after 1 day from drug administration. F% of T was 10.5±2.41%. M2 formation was fastest following iv administration Ka 2.04±0.46 (1/h) and slowest after sustained release treatment 0.58±0.22 (1/h), whereas the Kel of the four different treatments resulted not significantly changed. Also Cmax values were not significantly different. In conclusion, in horses, T could be administered orally either in a fasted or fed condition. Since T seems to be metabolised preferentially in M2 (inactive), the clinical efficacy of this drug in this animal species could be minor than in humans.

References

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Proceedings of the European Equine Meeting of the Year 2008 - XIV SIVE - FEEVA Congress, Venice, Italy
VALUTAZIONE DI DUE METODICHE PER LA DETERMINAZIONE DELLE IMMUNOGLOBULINE NEL SIERO DEL PULEDRO DI ASINO SORCINO CROCIATO DELL’AMIATA
EVALUATION OF TWO METODOLOGIES TO DETERMINE IMMUNOGLOBULINS IN SERUM OF FOALS OF AMIATA DONKEY

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Introduzione. Nella specie equina ed asinina la placenta è di tipo epitelio-coriale, quindi impermeabile alle macromolecole. Di conseguenza il passaggio degli anticorpi tra madre e feto può avvenire esclusivamente attraverso l’assunzione del colostro entro le prime 24 ore di vita, quando gli enterociti riescono a pinocitare le macromolecole e a trasportarle nel flusso ematico. Il mancato trasferimento dell’immunità passiva (Failure Passive Transfer, FPT) è tra le patologie più frequenti del periodo perinatale e colpisce il 3-25% dei puledri.1-6 Le cause di FPT possono essere legate sia alla madre come cattiva qualità del colostro, lattazione precoce, agalassia, sia al puledro, ad esempio per morte della futtrice, incapacità di assumere il colostro, impossibilità di assorbimento delle immunoglobuline (Ig) a livello gastro-enterico.7,8 La diagnosi precoce è fondamentale per una terapia adeguata ed efficace nel puledro neonato.

Scopo del presente lavoro è stato quello di valutare due metodiche per la determinazione delle immunoglobuline (Ig) nel siero di puledro di Asino Sorcino Crociato dell’Amiata.

Materiali e metodi. L’indagine è stata condotta su 6 puledri. Tutti i soggetti sono stati sottoposti a visita clinica entro 5 minuti dalla nascita, è stato calcolato il punteggio APGAR9 per valutarne la vitalità. Prima che ogni puledro assumesse il colostro (T0), a 24 (T1) e 48 ore (T2) di vita, è stato effettuato un prelievo di sangue dalla vena giugulare; il campione è stato centrifugato ed il siero congelato a -20 °C, quindi utilizzato per la valutazione della concentrazione delle Ig con il Test di turbidità allo zinco solfato (TTZS)10 e con Immunodiffusione Radiale Monospecifica per IgG equine (SRID Kit, VMRD, USA). Inoltre il trasferimento dell’immunità è stato valutato a 24 ore dalla nascita anche con test commerciale ELISA semiquantitativo (Plasma Foal IgG Midland Quick Test® Kit, Agrolabo, Italia). Sui risultati ottenuti sono stati calcolati la media (X) e la deviazione standard (DS). I dati ottenuti dalle analisi eseguite con le due metodiche, raggruppati per momento di campionamento, sono stati analizzati tramite il test della varianza e con il test di Tukey-Kramer per evidenziare le eventuali differenze significative tra i gruppi per p<0,05; infine è stato applicato il test della correlazione tra i valori delle densità ottiche vs le concentrazioni in mg/dl ottenute con lo SRID per ogni momento di campionamento.

Risultati. I risultati relativi a media ± deviazione standard sono riportati in tabella 1. Tutti i puledri presentavano con il test ELISA una concentrazione di Ig superiori a 800 mg/dl a T1. L’analisi della varianza ha evidenziato una differenza statisticamente significativa per il TTZS tra T0 vs T1 e T2, mentre per lo SRID una differenza statisticamente significativa tra T0 vs T2 e tra T1 vs T2. Non esiste una correlazione significativa tra le due metodiche per ogni tempo di campionamento.

Tabella 1 - Media ± deviazione standard della densità ottica (DO) e della concentrazione di Ig (mg/dl) ottenute rispettivamente con il TTZS e con lo SRID.

<table>
<thead>
<tr>
<th>Metodiche</th>
<th>T0</th>
<th>T1</th>
<th>T2</th>
</tr>
</thead>
<tbody>
<tr>
<td>TTZS (DO)</td>
<td>1,31±0,28a</td>
<td>1,83±0,42b</td>
<td>1,84±0,15b</td>
</tr>
<tr>
<td>SRID (mg/dl)</td>
<td>204,75±105,7a</td>
<td>492,25±305,3a</td>
<td>787,75±100,8b</td>
</tr>
<tr>
<td>ELISA</td>
<td>-</td>
<td>&gt;800 mg/dl</td>
<td>-</td>
</tr>
</tbody>
</table>

Proceedings of the European Equine Meeting of the Year 2008 - XIV SIVE - FEEVA Congress, Venice, Italy
Discussione e conclusioni. Studi condotti nel puledro equino dimostrano che al momento della nascita (T0) i valori di densità ottiche ottenute con il TTZS sono inferiori a 0,45, corrispondente ad una concentrazione di Ig minore di 400 mg/dl, per poi aumentare entro le 24-36 ore di vita fino a stabilizzarsi su valori di densità ottica maggiori di 0,90, riferibili a concentrazioni di Ig superiori a 1500 mg/dl.10 Nella nostra indagine le densità ottiche medie ottenute con il TTZS nel puledro asinino già a T0 risultano molto elevate e maggiori di 0,45 (DO 1,31±0,28) corrispondenti a concentrazioni di Ig superiori di 1500 mg/dl. Questo è in contrasto con il risultato ottenuto a T1 con il metodo SRID. Ciò potrebbe dipendere dalla differente composizione del latte di asina rispetto a quello della cavalla.11

Nel caso dello SRID le concentrazioni medie di IgG ottenute a T0 sono compatibili con i risultati attesi, mentre a T1 e T2 sembrerebbero identificare un insufficiente trasferimento dell’immunità passiva. In particolare nel caso delle concentrazioni ottenute a T1, queste risultano inferiori a quelle ottenuta con il test ELISA. Crediamo che nel caso dello SRID, la variabilità dei risultati ottenuti e l’apparente differenza con il test ELISA, potrebbero essere giustificate dalla non perfetta reattività crociata tra le IgG specifiche equine e quelle dell’asino amiatino.

Per i motivi discussi riteniamo che il trasferimento dell’immunità passiva nel puledro asinino debba essere ulteriormente studiato allo scopo di identificare metodi di analisi attendibili e che non è possibile utilizzare per l’asino i valori di riferimento relativi al puledro equino.

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CORRELATION BETWEEN EQUINE GASTRIC ULCER SYNDROME (EGUS) AND VARIATION OF SOME LABORATORY PARAMETERS IN SPORT HORSES


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Gastric ulcer is a complex disorder, best referred to as Equine Gastric Ulcer Syndrome (EGUS), associated with damage to oesophageal, gastric and duodenal mucosa. EGUS prevalence is reported to be extremely high in racing horses (up to 90%) (Murray, 1985; Murray et al., 1996; Dionné et al., 2003; Ferrucci et al., 2003). Although gastroscopic examination represents the preferred diagnostic tool, some workers have hypothesised that, as in human medicine, this syndrome could be associated with variations of some laboratory parameters (LP). However, so far there are only few reports with regard to equine medicine (Vatistas et al., 1999; McClure et al., 1999). Aim of the present study was to determine a possible significant correlation between variations of some LP and the prevalence and severity of EGUS in sport horses in training.

168 horses in training and competition were studied (155 Standardbred, 8 Thoroughbred, 5 warm-blood). All patients underwent a thorough protocol of evaluation, including physical examination, complete blood cell count (BCC), plasma and serum biochemistry (total protein, TP, albumin, A/G ratio, aminoaspartate transpherase, AST, alkaline phosphatase, ALP, gamma-glutamyl-transpeptidase, GGT, total bilirubin, coagulation profile and plasma fibrinogen concentration, PFC) and gastroscopic examination. The gastric lesions observed were classified according to MacAllister et al. (1997), considering their number and severity. In order to evaluate the correlation between the presence (or absence) of gastric ulcers and the considered LP, different statistical models, based on logistic regression, were built. Furthermore, to evaluate the association between the severity of gastric ulcers and the considered LP, different statistical models, based on ordinal regression, were built. Statistical analysis was conducted by means of the SPSS® v.14.0 software for Microsoft Windows®.

As far as BCC is concerned, a significant correlation (P = 0.039) was observed between haematocrit (Ht) increase and EGUS severity and that the increase in Ht was positively correlated with an increase in haemoglobin concentration (Hb), red blood cell count (RBC) and mean corpuscular volume (MCV).

Since horses in intense training and competition are at high risk of developing severe gastric lesions, and an increase in Ht, Hb, RBC and MCV is a normal physiological adaptation, aimed at increasing the oxygen transport capability to peripheral tissues, the increase in those parameters was probably a concurrent factor and not a consequence of EGUS.

As far as plasma and serum biochemistry is concerned, a decrease in TP concentration was significantly correlated (P = 0.033) with the severity of gastric lesions. An increase in PFC was significantly correlated both with severity (P = 0.039) and presence of gastric lesions (P = 0.043). These results may be explained by hypothesising that, similarly to that observed in human medicine, gastric ulcers induce an inflammatory response in the gastro-duodenal mucosa, characterised by an increase in PFC. The decrease in TP concentration may be the expression of intestinal malabsorption, consistent with the duodenal inflammation induced by EGUS. Although the variations in these
parameters should not be considered as diagnostic for EGUS - only gastroscopic examination allows one to determine the presence, number and severity of gastric lesions - the results reported here may contribute to a better understanding of the mechanism of EGUS and of the associated symptoms, which are often vague and non-specific.

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Purpose of the work. Aim of this study is to define the indication for equine ocular ultrasonography and to obtain representative ultrasonographic images of pathological clinical cases.

Methods. The ultrasonographic exam was realized in 15 horses, different for breed, age and sex, using a standard protocol. All the patients referred for an ocular ultrasound examination were previously submitted to a standard ophthalmic exam.

For ultrasound examination horses were sedated and anaesthetic eye drops were applied on the cornea.

Ocular images were obtained using a Digital Color MT Ecograph equipped of microconvex (7.5 MHz) and linear (10.0 MHz) probes. A transcorneal approach with longitudinal, transverse and oblique scans was routinely used, and a standoff pad was applied to evaluate the more superficial structures.

The ultrasonographic protocol included in order: cornea, anterior chamber, iris and ciliary bodies, lens, vitreous, ocular fundus and retrobulbar space. Moreover, the retrobulbar vasculature was studied using a color-Doppler system.

In the normal eye, the cornea appeared as a curvilinear, hyperechoic line; the anechoic area between the cornea and anterior lens was represented by the anterior and posterior chambers; the posterior one was not easy observable because in the horse the anterior chamber is normally very close to the iris and ciliary body, that appeared as echoic structures positioned anterior and laterally to the lens. Moreover, it was possible to visualize the granula iridica (corpora nigra) protruding into the anterior chamber. In normal condition, anterior and posterior lens capsule were recognizable as hyperechoic lines, but they usually were not observable at the same time. Distally to the anechoic vitreous, a hyperechoic concave line obtained by the retina, choroid and sclera was visualized. The optic nerve appeared as an hypoechoic area surrounded by hyperchoic retrobulbar tissue.

After examination, eyes were washed with sterile solution and artificial tears were applied.

Outcomes and discussion. In the fifteen patients (n = 30 eyes) underwent to ultrasound examination it was possible to diagnose cataracts (8 eyes), posterior synechia (1 eye), vitreal opacities (6 eyes), retinal detachments (3 eyes), retrobulbar mass (1 eye): in some cases different diseases were observed in the same patient.

In case of posterior synechia, the iris adhered to the cornea was visualized as an increased thickness of anterior lens line: an increase of echogenicity of lens and vitreal opacities were also visualized.

When a cataract was diagnosed, the lens presented a modification in shape and size and an increase of echogenicity.

Vitreal opacities were visualized as hyperechoic point-like lesions or linear/curvilinear echogenicities shapes and sizes: the differential diagnosis included hemorrhage, inflammatory and degenerative diseases.

The retinal detachment appeared as an hyperechoic line prolapsed into the vitreous but anchored to the optic nerve was observed (“seagull sign”).

Retrobulbar masses showed different echographic aspects: hypo-anaechoic areas for liquid collection as blood or pus, homogeneously or inhomogeneously echoic areas in case of solid mass (neoplasia, inflammatory disease). In the clinical case examined the retrobulbar mass was uniformly hy-
poechoic: a fibrosarcoma was histologically diagnosed after necropsy. As reported by the veterinary literature, the most frequent ocular disease observed by the authors is the cataract (53%), generally due to chronic inflammatory disease and traumas rather than congenital or hereditary factors. When a cataract was echographically observed, it was often associated to vitreal opacities; curvilinear vitreal opacities must be differentiated from retinal detachment. In the main, the echographic diagnosis of retinal detachment required three basics: more echogenicity comparing to the vitreal opacities, less mobility and the presence of, at least, an anchoring point to the fundus (ora serrata and/or optic nerve).

**Conclusion.** The ocular ultrasonography in horses represents a valuable imaging diagnostic tool for the diagnosis of the globe, intraocular and periocular diseases, particularly when cornea or lens opacities preclude ophthalmoscopy of deeper structures. Moreover, when retrobulbar masses occur, ultrasonography is able to provide helpful information about the structure and the vascular pattern and to guide the withdrawal.

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INDAGINE SUI DOPE TEST SUI CAVALLI DA CORSA IN IRAN:
STUDIO RETROSPETTIVO (2001-2007)
A SURVEY ON DOPE TESTS OF RACE HORSES IN IRAN:
A RETROSPECTIVE STUDY (2001-2007)

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Introduction. In sports, doping refers to the use of performance-enhancing drugs, particularly those that are forbidden by the organizations that regulate competition.

Objectives. The aim of study was to (1) determine the rate of dope and the used drugs in Horse races in Iran in last 5 years (2) evaluate current dope control management in Iran.

Material and Methods. Dope testing of horse races in Iran started at 2001. Horses are selected for dope testing by the the winner's method and just 1st & 2nd in every run are selected. Once selected, they are escorted away from the unsaddling area and taken to the dope-testing unit where they are identified from their passport examined by a Veterinary Officer. After being washed down, the horse is put into one of the dope testing unit's stables until a urine sample can be provided. To evaluate the dope status and determining the dope & the used drug's rate in Iran, data of 5 years (2001-2002 & 2005-2007) were collected by referring to Iran’s equisterial federation and the confirmed doped horses were determined and analyzed. The dope test's documents of 2003 and 2004 were not complete and the total documented runs were fewer than 250 so these years data didn't analyzed.

Results. The mean dope rate (2001-2007) were 21.9%. The dope rate of 2001-2002 & 2005-2007 were 32.2%, 34.4%, 21.7%, 10.25% & 11.14% respectively. The dope rate decreased gradually from 32% to 11.14% from 2001 to 2007. The most used drugs in all years were morphine except 2005 where cafein (53%) was the prominent. The most used drugs were cafein (21%), morphine (19%) and phenyl buthazone (10%) respectively. The results reveales that totally 58 type of drugs were used through years in which 26.7% were Combinations. Cafein was the most used drug in combinations and the morphine was the so after. The variation of used drugs decreased gradually from 2001-2007.

Conclusion. This study was the first retrospective study on horse races doping tests in Iran. The prominent feature was the high incidence of dope in Iran in comparison with developed countries. In spite of crime identity of the morphine administration, unfortunately usage of morphine as a drug didn't decreased in races through the years. It might have forsenical or historical (the history of the usage of morphine in horses is related to many years ago in Iran) origin. The decreased level of dope in 2006 & 2007 in comparison to the years before might be due to the increased level of trainer's knowledge and performing legal attention to this problem.

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La diarrea è ritenuta una delle patologie di più frequente riscontro tra i puledri al di sotto dei 6 mesi di età, con massima incidenza nel corso delle prime settimane di vita. Accanto alle noxae di natura manageriale, virale, batterica e parassitaria, ritenute comunemente responsabili di tale patologia, nell’ultimo decennio, in seguito alla crescente importanza assunta negli animali da reddito da due enteropatogeni opportunisti quali *Giardia* e *Cryptosporidium*, è scaturita la necessità di definire il ruolo svolto da tali agenti protozooi anche nella specie equina.

La scarsità dei contributi bibliografici nazionali relativi alla presenza ed al significato di tali infezioni nel cavallo, ha portato alla presente indagine con l’obiettivo di stabilire la responsabilità o corresponsabilità di *Giardia* e *Cryptosporidium* nelle sindromi dissenteriche dei puledri e di definire il loro grado di diffusione in Italia Centrale.

L’indagine è stata condotta su una popolazione campionaria di 120 puledri, di età compresa tra i 7 giorni ed i 6 mesi di vita, provenienti da 5 distinti allevamenti dell’Italia Centrale. I puledri sono stati suddivisi in 4 gruppi omogeneamente distribuiti sulla base dell’età espressa in settimane (1-2, 2-4, 4-8, > 8) e successivamente sulla base della presenza/assenza di diarrea. Campioni fecali sono stati raccolti individualmente e sono stati saggiati sia per la ricerca di uova di *Strongyloides westeri*, *Parascaris equorum* ed oocisti di *Eimeria leukarti* (ritenuti i maggiori agenti parassitari di enterite), previa concentrazione per flottazione con soluzione zuccherina di Sheather (p.s. 1,27) e Iodio Mercurata di Potassio (p.s. 1,5), sia per la ricerca coproantigenica di *G. duodenalis* e *C. parvum*, per mezzo di un kit commerciale in immunofluorescenza diretta (Merifluor Crypto-Giardia-Merdian®) altamente sensibile e specifico. Parallelamente è stata eseguita anche una valutazione quantitativa ed i campioni sono stati classificati secondo 4 distinti patterns d’escrezione (0: Negativo, 1: 1-2 cisti o oocisti, 2: 2-5 cisti o oocisti, 3: 6-7 cisti o oocisti, 4: > 8 cisti o oocisti) sulla base del numero di elementi parassitari repertati per campo ottico (magnitudo 400X). Nei soggetti con sintomatologia diarroica, infine, il materiale fecale prelevato mediante tamponi rettali è stato analizzato per l’isolamento e tipizzazione di agenti batterici del genere *Salmonella*, *Clostridium*, *Escherichia* e per la ricerca degli Enterovirus mediante TEM. A partire dai risultati ottenuti è stata calcolata la prevalenza complessiva delle due parassitosi e quella relativa ai singoli allevamenti e classi d’età. L’associazione tra le classi d’età e la prevalenza delle infezioni è stata valutata mediante modello statistico GLM. È stata calcolata la copropositività media dei soggetti affetti da turbe enteriche e di quelli asintomatici e sono state valutate anche le prevalenze degli altri agenti patogeni oggetto di indagine; infine, i patterns d’escrezione sono stati messi in relazione alla classe d’età.

Entrambe le infezioni sono risultate abbastanza frequenti tra i puledri, variando su valori compresi tra il 3,33% ed il 23,33% (prev. complessiva 15%) per *Cryptosporidium* ed il 6,66 - 26,66% (prev. complessiva 9,16%) per *Giardia*. Tutte le aziende oggetto di campionamento sono risultate infette sebbene con differenti tassi di prevalenza. L’età dei puledri si è rivelata un fattore in grado di influire sia sulla prevalenza (p<0,05) che sull’intensità d’escrezione (p<0,05). La massima prevalenza è stata osservata nei puledri di età compresa tra le 8 settimane ed i 6 mesi di vita; questo dato, già rilevato in altri studi epidemiologici, sembrerebbe confermare il ruolo svolto dai puledri al di sopra delle 8 settimane come serbatoio principale d’infezione. Relativamente ai patterns di escrezione, i puledri di età compresa tra 0 e 4 settimane di vita hanno mostrato la maggiore intensità d’escrezione (patterns 3 e 4). Negli animali oggetto di campionamento solo 21 soggetti (pari al 17%...
sul totale) mostrava sindromi dissenteriche e di questi il 23,8% risultava patente per Giardia mentre il 19,04% per Cryptosporidium. I risultati del presente lavoro sembrerebbero mostrare come tali infezioni siano frequenti nelle aziende del Centro Italia e come gli aspetti sia clinici che epidemiologici siano strettamente correlati all’età dei puledri. In puledri al di sopra delle 8 settimane di età si è evidenziata una massima capacità diffusiva delle infezioni associata ad un impatto clinico pressoché nullo, mentre negli animali al di sotto delle 8 settimane d’età ai bassi indici di prevalenza si contrapponeva un’elevata morbilità, accompagnata sempre da sindromi enteriche di lieve entità.

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Usability of the Eosin-Thiazin Staining Method in Endometrial Cytologies of Mares

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Introduction. The exfoliative endometrial cytology, within the scope of the gynaecological examination of breeding mares, is an easily implementable diagnostic method. The removal of the exfoliative cytologies can be combined with the sampling collection for bacteriological examination. The presence of neutrophil granulocytes is deemed to be an indicator of endometrial inflammation. A good correlation of bacteriological and cytological results have been described in various research and literature, so mares with uterine infections are rapidly detectable through this examination method. The necessity of a gynaecological treatment can be realised before bacteriological results are available. In the case of the bacteriological examination is presenting dubious results, the interpretation of the cell picture in endometrial smears can support the confirmation of diagnosis. Mares with chronic nonsuppurative endometritis, which show no bacterial growth, are detectable through the cytological examination. Unfortunately, in practice the endometrial cytology is not a common diagnostic procedure. One of the reasons is the so far required time-consuming staining method according to Papanicolaou-Shorr. The duration of this method is 60 minutes. The aim of this study was to test the capability of the Eosin-Thiazin staining method for endometrial cytologies, which takes only three minutes.

Material and Methods. The endometrial smears of 27 breeding-mares were taken with a guarded culture swab to avoid contamination from the caudal reproductive tract. After the smear application on two slides, one was stained according to Papanicolaou-Shorr and the second was stained by the Eosin-Thiazin method (Hemacolor®, Merck). The comparison of the presentability of the neutrophil granulocytes was carried out. All cytologies were evaluated on the basis of a score according to Couto and Hughes. Cytologies with 0 - 3% neutrophils granulocytes were classified as negative, cytologies with  > 3% neutrophil granulocytes were classified as positive.

Results. In both stainings neutrophil granulocytes could be detected doubtlessly. In the uterine smears stained with Papanicolaou-Shorr ten breeding mares (37%) showed neutrophil granulocytes. In the Hemacolor® staining method twelve probes showed neutrophil granulocytes (44%). Thus in 25 mares (95%) the results of both colouring methods corresponded. These findings demonstrate that the Eosin-Thiazin method is suitable for the quick detection of neutrophil granulocytes to diagnose an endometritis. In two cases the diagnostic accuracy of the Papanicolaou-Shorr method was even excelled.

Discussion. The Eosin-Thiazin method can substitute the Papanicolaou-Shorr staining for the detection of neutrophil granulocytes in exfoliative endometrial cytologies of breeding mares. The benefit of Eosin-Thiazin staining is the fast availability of results and a certain familiarness of microscopical picture from blood smears. For the simple demonstration of neutrophil granulocytes in the endometrial smears, that are the main indicators for endometrial inflammation, these staining method is sufficient. For a differentiated staining of inflammatory cells, or to demonstrate hormonal influences, the Papanicolaou-Shorr staining still exhibits advantages. The considerable gain of time with Eosin-Thiazin staining hopefully provides a widespread use of the endometrial cytology in breeding mares.

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Heart murmurs (HM) represent a relatively common finding in horses of different age and breed (Patteson & Cripps, 1993; Marr & Reef, 1995; Young & Wood, 2000). In young racing thoroughbreds, the prevalence of HM is extremely high (81%) although in most cases they appear to be functional or associated with mild valvular regurgitation and therefore of no clinical significance (Kriz et al., 2000). According to recent studies, such an elevated HM prevalence in racing horses, particularly of the atrioventricular valves, could be related to a profound adaptation of cardiac muscle to training and athletic activity (Young, 1999; Kriz et al., 2000). Aim of the present study was to evaluate the prevalence of HM in standardbred trotters and to determine their clinical significance. A group of 594 standardbred trotters, 330 males, 229 mares and 35 geldings, from 1 to 9 years old (mean 3.7 ± 1.5 years), presented for poor performance, underwent a thorough diagnostic protocol at rest and, whenever necessary, under strenuous exercise on an high speed treadmill. In 125 out of 183 horses, in which one or more HM were identified, echocardiographic examination (ECC) was performed (Technos MPX, phased-array 2.5 MHz, Esaote Biomedica) in order to evaluate morphology, motility and dimensions of the cardiac structures (Long et al., 1992). To evaluate transvalvular flows, color doppler ECC was performed. On auscultation, HM were detected in 183 patients. According to their characteristics, HM were classified into murmurs of tricuspid valve regurgitation, ranging from grade 1/6 to 4/6 (76.2%), murmurs of mitral valve regurgitation, from grade 1/6 to 3/6 (8.8%), murmurs of aortic valve regurgitation from grade 1/6 to 3/6 (4.2%), murmurs of pulmonary valve regurgitation from grade 1/6 to 2/6 (1%), functional systolic murmurs (FSM) from grade 1/6 to 4/6 (5.2%), functional presystolic murmurs from grade 1/9 to 2/6 (3.6%) and functional early diastolic murmurs (EDM) from grade 1/6 to 2/6 (1%). ECC showed normal morphology, motility and dimensions of cardiac structures in 92.8% horses. In one case, an increase in pulmonary valve diameter, greater than aortic root diameter, was observed. Left atrial, left ventricular and mitral valve dilatation, associated with an increase in the fractional shortening (FS%), were observed in one case. In one horse with functional atrial fibrillation, a mild decrease in the FS% was detected. Mild degenerative lesions of one or more valvular leaflets were observed in 6 horses. Colour doppler ECC showed the presence of one or more jets of valvular regurgitation in 103 patients (82.4%); the regurgitant jets were classified, according to their severity, as not significant (76.7%), mild (13.7%), moderate (8.6%) and severe (1%).

According to the results observed, it may be concluded that, even in standardbred trotters, the presence of HM on auscultation is a relatively common finding (30.8%) and that, as in thoroughbred horses, most of these murmurs are of no clinical significance. Substantial differences between thoroughbreds and standardbreds may be observed considering the characteristics of the HM detected. In fact, tricuspid valve regurgitation (76.2%) and mitral valve regurgitation (8.8%) are more frequently represented in our standardbred population than in young racing thoroughbreds (28.5% and 3.8% respectively). On the other hand, in standardbreds the prevalence of FSM (5.2%) and EDM (1%) is much lower than that reported for thoroughbreds (57.7% and 40% respectively). Finally, it may be noticed that there was an elevated correspondence between cardiac auscultation and color doppler ECC when the affected valve localization is considered (92.2%); however, there was no similar correspondence between the murmur intensity and the severity of valve regurgitation.
tion. Therefore, in order to achieve an accurate diagnosis and prognosis in a standardbred trotter in training one must rely on a thorough clinical and technical evaluation.

References

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