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SOME BIOCHEMICAL AND HEMATOLOGICAL CHANGES IN URINE RETENTION FOLLOWING MUCOUS PLUG OBSTRUCTION IN DRAFT HORSES

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The present study aimed to investigate some biochemical and hematological changes in urine retention in draft horses.

Materials and methods

Whole blood samples were collected from the jugular vein in clean, dry tubes containing disodium salt of EDTA as anticoagulant and used to determine total red blood cells count (RBCs T/l) using a haemocytometer, hemoglobin concentration (Hb g/l) using Sahli's apparatus and packed cell volume (PCV %) using microhematocrit method according to Coles (1986).

Blood samples without anticoagulants were collected from the jugular vein in clean, dry tubes. These samples were used for the determination of blood serum levels of total proteins (g/l), albumin (g/l), globulins (g/l), glucose (mmol/l), urea nitrogen (ºmol/l), creatinine (ºmol/l), sodium (mmol/l), potassium (mmol/l) and chloride (mmol/l).

Serum biochemical parameters were determined calorimetrically using kits of Boehringer Manheim (GmbH diagnostica) and by means of Digital Ultraviolet Spectrophotometer (Digital Ultraviolet spectrophotometer, CE 292, series 2, Cecil instruments, Cambridge England, Series No. 52.232.).

Blood serum electrolytes (Na+, K+) were determined by means of Flame Photometer Corning 400. Serum chloride level was measured by means of chloride analyzer.

Urine samples from normal and colicky cases were subjected to analysis according to Coles (1986).

Statistical analyses of the data were undertaken using one-way ANOVA. Statistical analysis was performed with Statistical Package for the Social Sciences for Windows (SPSS, version 10.0, Chicago, IL, USA).

Results

Horses were admitted with case history of urine retention sweating, and exhibiting signs suggestive of abdominal pain, such as kicking at the belly, and getting up and laying frequently. Clinical examination revealed tachycardia, tachypnea, congested or slightly cyanotic mucous membranes in some cases. Rectal palpation identified rectum filled with feces and that the bladder filled with urine (urine retention). Colic symptoms were relieved upon evacuation of the bladder by catheterization. Voided urine was oily in appearance and viscous. Following removal of all retained urine, animals became quite and urinated normally. Results showed insignificant changes in total red blood cells count (TRBCs T/l), hemoglobin concentration (Hb. g/l) and packed cell volume percent (PCV %).

Serum biochemical parameters revealed significant increases in serum urea nitrogen (p < 0.01) and creatinine levels (p < 0.01) with significant decreases in serum glucose (p < 0.01), sodium (p < 0.01), potassium (p<0.05) and chloride levels (p < 0.01) (Table 1).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control group</th>
<th>Colic group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total RBCs count (T/l)</td>
<td>11.19 ± 0.78</td>
<td>11.61 ± 1.57 NS</td>
</tr>
<tr>
<td>Haemoglobin Conc. (g/l)</td>
<td>13.34 ± 2.05</td>
<td>13.00 ± 1.24 NS</td>
</tr>
<tr>
<td>PCV (%)</td>
<td>35.3 ± 2.86</td>
<td>36.70 ± 1.76 NS</td>
</tr>
<tr>
<td>Total protein (g/l)</td>
<td>73.62 ± 6.32</td>
<td>75.72 ± 6.22 NS</td>
</tr>
<tr>
<td>Albumin (g/l)</td>
<td>39.75 ± 5.62</td>
<td>44.34 ± 4.86 NS</td>
</tr>
<tr>
<td>Globulins (g/l)</td>
<td>33.87 ± 7.73</td>
<td>31.25 ± 5.37 NS</td>
</tr>
<tr>
<td>A/G ratio</td>
<td>1.19 ± 0.27</td>
<td>1.45 ± 0.28 NS</td>
</tr>
<tr>
<td>Glucose (mmol/l)</td>
<td>5.62 ± 0.92</td>
<td>2.80 ± 0.67**</td>
</tr>
<tr>
<td>Urea nitrogen (ºmol/l)</td>
<td>4.75 ± 0.95</td>
<td>8.25 ± 1.53**</td>
</tr>
<tr>
<td>Creatinine (ºmol/l)</td>
<td>93.70 ± 39.78</td>
<td>292.60 ± 54.81**</td>
</tr>
<tr>
<td>Sodium (mmol/l)</td>
<td>125.30 ± 7.94</td>
<td>91.60 ± 1.89**</td>
</tr>
<tr>
<td>Potassium (mmol/l)</td>
<td>5.05 ± 1.49</td>
<td>3.73 ± 0.43*</td>
</tr>
<tr>
<td>Chloride (mmol/l)</td>
<td>168.0 ± 14.75</td>
<td>115.10 ± 9.66**</td>
</tr>
</tbody>
</table>

Table 1. Mean and standard deviation (X ± SD) values of studied parameters in control and diseased horses

*: Significant (p<0.05)
**: Highly Significant (p<0.01)
NS: Non significant
Discussion

In the present study, the case history of diseased horses was retention of urine, which was the principle cause for colic in the investigated animals. No hindrance was observed during catheterization, which exclude calculi as a cause for urine retention in these cases. The cause of urine retention may be attributed to the high viscosity of urine and to the voluminous amount of mucous, which results in the formation of mucous plug that occlude the internal orifice of the urethra and leads to urine retention (Hackett et al., 1982).

The main cause of hyponatremia, hypokalaemia and hypochloremia in the present study is the copious sweating (Carlson et al., 1997 and Radostits et al., 2000). Reportedly, the most common causes for loss of sodium is heavy sweating in horses (Kokko and Tannen, 1986). The significant decrease in serum glucose level (p < 0.01) could be attributed to decrease food intake due to severe abdominal pain. Another cause of hypoglycemia is the strong effort done by colicky horses, in order to overcome the severe pain. The increased serum creatinine (p < 0.01) and urea nitrogen (p < 0.05) levels may be attributed to decreased glomerular filtration rate and indicate kidney dysfunction (Carlson et al., 1997 and Radostits et al., 2000). In conclusion, the most significant hematological and biochemical changes in renal colic in horses were increased serum urea nitrogen, creatinine, hyponatremia, hypokalaemia, hypochloremia and hypoglycemia.

References


EXTRA CORPOREAL SHOCK WAVE TREATMENT EVALUATION IN EQUINE TARSAL OSTEOARTHRITIS


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Introduction

Osteoarthritis has been defined as a disorder of movable joints, characterized by degeneration and loss of articular cartilage and the development of new bone on joint surfaces and margins. As in human, equine osteoarthritis is probably not a single disease but reflects a common response of joint tissues to a number of potential causes. Osteoarthritis of the tarsometatarsal joint and distal tarsal joint is a common cause of hind limb lameness in horses.

Extracorporeal shock wave (ESWT) has become popular treatment modalities for equine musculoskeletal problems. Extracorporeal shock wave is pressure waves generated outside the body that can be focused at a specific site within the body.

The aim of our study was to evaluate the effects of the extracorporeal shock wave therapy (ESWT) in the treatment of osteoarthritis (OA) of tarsal joints in equine.

Materials & methods

Horses presented to the São Paulo State University Hospital, Botucatu Unit for lameness evaluation were given the options to use ESWT. Between January 2004 and March 2005 thirty-two horses with osteoarthritis and varying degrees of lameness were treated with ESWT. All horses had a history of lameness or reduced performance for at least 1 month, or had recurrence of previously diagnosed OA. Lameness was evaluated with horses walking and trotting in hand in a straight line, by lunging on hard and soft surfaces, and after flexion tests.

Results

Lameness was graded by 0 to 5 scores (Stashak, 2002). For inclusion in this study, lameness had to be improved significantly by perineural analgesia of the tibial and fibular nerves. Some of the animals had an intra-articular analgesia of the tarsometatarsal or tarsocrural joints. All horses had to have radiographic evidence of OA for inclusion in the trial. The ESWT treatment was performed on an ambulatory place with the horse standing sedated with xylazine 10%; in none of horses in this study was local anesthesia required. The ESWT procedure involved the application of 2000 shocks (0.16mJ/mm2) at the site or sites of the lesion of the tarsal joint. The selection of parameters for the shockwave therapy was based on the manufacturer's recommendations. On three occasions at three-week intervals, the horses were sedated and their lesioned hind limbs were treated with ESWT. Following treatment, horses were stall rested for 1 week, then limited to hand walking and ground work for an additional 4 weeks. Clinical and Radiography evaluations were also performed before each of the three treatments of ESWT and 30, 60 and 90 days after the last of the first treatment.

Discussion

In 81.25% (26) of the animals has shown lameness score improvement for 90 days after ESWT. Regarding athletic activity, 59.37% (19) of animals treated, return the same level of athletic performance they presented prior to establishment of the injury. Radiographically, horses with osteophyte on the dorsal or dorso medial aspect of the tarsometatarsal joint improved most consistently. The results found for bone remodeling for animals treated with ESWT at the lesions location were: 43.6% total success, 30.0% partial and 26.4% of lesions with an absent of success. The effects of ESWT on musculoskeletal injuries and diseases have not been study in details. The reduction in lameness seen in many cases suggests that the treatment may be
analgesic. No horse suffered exacerbation of lameness after treatment, indicating that, in spite of this potential mode of action, the treatment is safe. McClure et al. (2000) demonstrated that ESWT increased osteoblastic activity. Similar events were observed in our experiment with positive results for remodeling, showed by osteophyte reduction and osseous remodeling. Endocondral and intramembranous ossification were stimulated as indicated by an increased in extracellular matrix proteins. Shock wave therapy may stimulate cells by stimulating the release of cytokines such as TGF-β1 or over mediators.

Based on these results, ESWT is indicated as an alternative in the treatment of osteoarthritis tibiotarsal and tarsometatarsal in equine.

Acknowledgements

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PREVALENCE OF INTERVERTEBRAL OSTEOARTHRITIS CAUSED BY BACK PAIN IN WESTERN PERFORMANCE HORSE IN SÃO PAULO STATE - BRAZIL

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To describe the prevalence, clinic, thermographic and ultrasonographic characteristics, and severity of articular process osteoarthritis in the thoracolumbar column in western performance horses with back pain in São Paulo state - Brazil.

Materials and methods

A sample of 20 animals admitted in UNESP's Medical School of Veterinary and Zootechnic Hospital clinical complains of back pain, was submitted to a physical and thoracolumbar exam and also thermographic and ultrasonographic exams. Age, sex, and event (barrel race, cutting, rein ing) were recorded for each horse. Looking for painful sensibility points in the back, decreases or increases in lateral or dorsoventral mobility and normal gait disorders of the animal. After physical exam, animals were submitted to a thermography, looking for disorders for the thermal gauge of horse's thoracolumbar section (hot spot and cold spot) mapping and leading the ultrasonographic examination. Using linear transducer of 7,5 MHz ultrasonography, in longitudinal and transversal cuts where a soft-tissue and osseous lesion could be observed. The visualization of articular processes was done by transversal images. After clinical exams, the prevalence, clinic, thermographic and ultrasonic characteristics osteoarthritis of articular processes events were observed in animals with back pain.

Results

In 20 attended animals, 12 (60%) were diagnosis with osteoarthritis of articular processes, being 8 barrel racing horses, 3 cutting horses and 1 reining horse. The age of animals vary between 3 to 7 years and there were 5 females and 7 males. In physical exam for osteoarthritis, were mainly found: sensibility in lumbar section (12 animals; 100%); excessive response to mobilization test (10; 83%); cervical lateroflexion not performed when in circulate movement (8; 67%); ventral rotation of the pelvis during the circulate movement (6; 50%); back stiffness in circular gallop (3; 25%) and hare gallop (8; 67%). The thermographic exam showed in all 12 animals, areas in lumbar section with increase temperature, bilaterally in 7 animals (58%), mainly in left side in 3 animals (25%) and right side in 2 animals (17%), and in all events the temperature decreases in 0,2 to 0,6°C. The ultrasonography showed asymmetry of the articular process (6 animals; 50%); periarticular proliferation (2 animals; 17%); hyperechoic points around the articulation and articular irregularities (8 animals; 67%); a view with discontinuous hyperechoic line for the articulation. The articulations that were mainly affected alternated in agreement with animal activity. The barrel racing horses presents most of the L1 and L3 lesions but only 1 animal presents a L5 lesion; all the cutting horses presents osteoarthritics lesions between L4 and S1 and reining horses presents L3 and L4 lesions.

Discussion

In our studies with Western Performance Horses, the prevalence was about 60% of invertebral osteoarthritis in back pain, an increase percentual when confronted with studies in Thoroughbred horses, where the prevalence is 25%. This disagreement results by three facts: the kind of job executed by the horse, kind of saddle used in contests (larger and heaviest than the saddleback used in jumping events and animal configuration, witch together causes bigger demand in lumbar column, mostly during rotation movement and lateroflexion movement. The thermographic images in agreement with different studies where thermal decrease is observed, generality bilateral in lumbar scans of osteoarthritis horses. The thermography results were corroborated by ultrasonography, witch supplies data of lesion characteristic.

Unilateral lesions were observed mostly in barrel racing horses, where in 60% of events the left side was seized, probably caused by the fact that the animal circles the barrel twice over the left side and only once for the right side. The cutting horses present caudal articulations lesions, caused by the fact that the
animal takes over a forced ventralflexion position during almost the contest period. The reining horses present media lumbar section lesions caused by the excessive movement of rotation, which acts mostly over the lumbar caudal vertebrae. As from the L5 vertebra, the others are stabilized by the intratransversal articulations; the mostly affected will be the L3 and L4 articulations.

In this way we can notice that the articular osteoarthritis process is a disease with high incidence in Western Performance Horses with back pain and mostly of that is caused by the sport activity of these animals. The association between thermography and ultrasonography showed efficacy in these disease diagnoses.
PRELIMINARY ASSESSMENT OF THE GENETIC RELATIONSHIPS AMONG SPANISH HORSE BREEDS VIA MOLECULAR COANCESTRY INFORMATION

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Introduction

The importance of the assessment of the genetic relationships among Iberian horse breeds is more than of local interest, as they are the genetic basis from which some well known horse breeds such as Lippizan and present Native American strains of horses were formed (Lopes et al., 2005; Royo et al., 2005). Most published studies on this task have been carried out using microsatellites (Azor et al., 2005; Cañón et al., 2000; Morais et al., 2005).

Recently, Caballero and Toro (2002) have formalised the way in which it is possible to obtain coancestry coefficients from molecular information. Eding and Meuwissen (2001), using simulated data, showed that molecular coancestry has some interesting properties, namely that average kinship between populations become constant very quickly after population fission, causing between-population diversity to remain constant. However, the number of studies using molecular coancestry to assess the genetic relationships between breeds is scarce (Álvarez et al. 2005).

The aim of this work is to show preliminary results on the assessment of the genetic relationships among five Spanish horse breeds using molecular coancestry information.

Material and methods

Blood samples were collected in tubes with EDTA k3 from 197 unrelated horses belonging to 5 Spanish horse breeds. Sampled breeds and number of individuals per breed (in brackets) are: Andalusian (And; 38), Asturcón (Ast; 39), bay-Asturcón (b-Ast; 55), Mallorquín (Mal; 25) and Spanish Trotter (Tro; 40). Genomic DNA was extracted from whole blood following “Salting out” procedure (Miller et al. 1988).

Samples were genotyped for a set of 8 microsatellite (ATH4, AHT5, ASB17, HMS1, HMS3, HMS7, HTG4 and VHL20) from those recommended by the expert group of ISAG. Microsatellites were amplified following standard procedures (StockMarksÆ for horses, Equine Partenity PCR Typing Kit, PE Applied Biosystems, Foster City, CA) and analysed using an Applied Biosystems 3100 DNA sequencer. Allele sizes were determined with software packages GeneScan 3.7 and Genotyper 3.7 (Applied Biosystems) using the LIZ 500 bp internal size standard.

Parameters characterising genetic variability (such as observed and expected heterozygosity and average number of alleles per locus), and within and between-populations molecular coancestry coefficients (fij) and kinship distance (Dk) were computed using the program MolKin v2.0 (Gutiérrez et al., 2005) which is freely available at http://www.ucm.es/info/prodanim/html/JP_Web.htm. The molecular coancestry between two individuals i and j can be computed at a given locus using the following scoring rules (Caballero and Toro, 2002; Eding and Meuwissen, 2001): , where Ixy is 1 when allele x on locus l in individual i and allele y in the same locus in individual j are identical, and zero otherwise. Notice that this value can only have four values: 0, _, _ and 1. The molecular coancestry between two individuals i and j (fij) can be obtained by simply averaging over L analyzed loci . The kinship distance between two individuals i and j is Dk = [si + sj]/2L - fij, where si is self-coancestry for the individual i. The within- and between-breeds molecular coancestry and Dk are computed by simply averaging the corresponding values for all the within- or between-population pairs of individuals. Using also the program Molkin the F-statistics, FIS, FST, and FIT, and various genetic distances where computed (see Gutiérrez et al., 2005 for more details).
Results and discussion

Across breeds, the observed and expected heterozygosity and the average number of alleles per locus for the assayed set of microsatellites were, respectively, of 0.701, 0.805 and 10.1. Across microsatellites the expected heterozygosity and the number of alleles varied, respectively, from 0.748 (HTG4) to 0.884 (VHL20) and from 7 (HTG4 and AHT5) to 17 (ASB17). At breed level the highest observed heterozygosity (Table 1) was observed for the bay-Asturcón and the lowest for the Andalusian horse. The lowest average number of alleles per locus (rarefacted to 34 copies) was observed for the Asturcón breed and the highest for the bay-Asturcón. F-statistics, FIS, FST, and FIT for the whole analysed population were, respectively, of 0.046, 0.091 and 0.133. The average molecular coancestry for the whole population was of 0.194.

The between breeds molecular coancestry values (Table 1) varied from 0.420 (for the Asturian breeds pair) to 0.510 (for the Andalusian-Mallorquin pair). The average between breeds molecular self-coancestry $[(s_i + s_j)/2]$ varied from 0.623 (for the pair b-Ast-Mal) to 0.686 (for the pair And-Tro).

Most genetic distances are highly dependent on the observed allele frequencies, which are in turn highly dependent on recent evolutionary processes such as genetic drift. However, the formula used to compute the kinship distance, $D_k = [(s_i + s_j)/2] - f_{ij}$ has two terms that may be useful in assessing whether differentiation among breeds may be recent or remote in origin; the first term $[(s_i + s_j)/2]$ provides information on recent differentiation whilst $f_{ij}$ informs on the allele frequencies before separation of populations (Álvarez et al., 2005). $D_k$ is, in consequence, a genetic distance with classical properties but corrected for the identity of the breeds before separation. In this note we show separately both terms of the between breeds $D_k$. It can be graphically shown that recent differentiation differentiates to a large extent both Asturcón populations that have a common origin (Royo et al., 2005).

As a conclusion, we have shown that genetic analyses based on molecular coancestry information are useful to distinguish between ancestral and recent genetic differentiation among horse breeds, thus allowing useful information on the history of the present horse populations.

This study was partially funded by a grant from the MEC-INIA, no. RZ03-011 and two contracts between the Spanish Government through Ministerio de Agricultura, Pesca y Alimentación and the Universidad de Córdoba titled “Esquema de Selección del Caballo de Pura Raza Española” and “Esquema de Selección del Caballo Trotador Español”.

<table>
<thead>
<tr>
<th>Between breeds</th>
<th>Within breeds</th>
</tr>
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<tbody>
<tr>
<td>Ast</td>
<td>b-Ast</td>
</tr>
<tr>
<td>0.420</td>
<td>0.477</td>
</tr>
<tr>
<td>0.628</td>
<td>0.482</td>
</tr>
<tr>
<td>0.672</td>
<td>0.653</td>
</tr>
<tr>
<td>0.643</td>
<td>0.623</td>
</tr>
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<td>0.662</td>
<td>0.642</td>
</tr>
</tbody>
</table>

Table 1. Paired average molecular self-coancestry $[(s_i + s_j)/2]$ below diagonal; between breeds molecular coancestry $f_{ij}$ above diagonal and, within breeds, molecular coancestry $s_i$, molecular self-coancestry $s_i$, observed heterozygosity (Ho) and rarefacted (to 34 copies) number of alleles per locus (k). The abbreviations correspond to the following horse breeds: Asturcón (Ast), bay-Asturcón (b-Ast), Andalusian (And), Mallorquin (Mal) and Spanish Trotter (Tro).

References

BREEDING EVALUATION OF ENDURANCE HORSES IN SPAIN (PRELIMINARY RESULTS)**

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2 Department of Agroforestal Sciences, University of Sevilla. Spain.

In this work we present the results of the first genetic evaluation for the Endurance Rides in Spain. The endurance ride records for sport horses used in this study were obtained from Spanish Equestrian Federation. This type of competition is carried out across country, it consists in long distance races where not only is speed important but the endurance and the adapted physical conditions of the horse are essential. Most of competitors are Spanish Arab horses, but other breeds like Sport Spanish horse breed, Thoroughbred, Anglo-arab or Hispano-arab and other foreign breeds like Hannoverian, KWPN or Sille Français are included as well. In the consulted bibliography there are scarce published papers about genetic evaluation in Endurance Rides.

Breeding value estimation for endurance is the main selection criterion in the Spanish Arab Horse. The Spanish Ministry of Agriculture, Fisheries and Food and Spanish Arab horses breeder association’s project of genetic improvement includes in its selection the Endurance as specific breeding objective.

Data sets used in this preliminary study take into account rides from January 2000 to May 2005 of the main endurance competitions celebrated in Spain and some competitions celebrated in foreign places like Portugal and Italy, with distances between 60 and 200 kms. The Categories of Endurance Rides that were collected were National (CEN One, Two and Four stars) and International Competitions (CEI Two, Three and Four Stars). The most of registered record corresponded to level 1 (CEN one star) with a 41.4 % and the inferior percentage was for level 4 (CEN and CEI four stars).

The total number of observations comprised 1802 entries of 804 horses from 121 endurance rides. The average of number of participants in each ride was 22. Rides with less than five participants were eliminated. The genealogical information of the horses that contain four generations deep and it was obtained from the Studbook of each breed (facilitated by Ministry of Defense). The relationship matrix involved a total of 2560 animals. Ride time average were calculated corresponding to different Categories and levels, there are included in table 1.

<table>
<thead>
<tr>
<th>Categories</th>
<th>Average (hours)</th>
<th>Coefficient of Variation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CEN*(Level 1)</td>
<td>5.67 ± 1.55</td>
<td>27.30</td>
</tr>
<tr>
<td>CEN**(Level 2)</td>
<td>8.93 ± 2.56</td>
<td>28.66</td>
</tr>
<tr>
<td>CEI**(Level 2)</td>
<td>10.60 ± 4.14</td>
<td>39.07</td>
</tr>
<tr>
<td>CEI*** (Level 3)</td>
<td>12.28 ± 2.66</td>
<td>21.68</td>
</tr>
<tr>
<td>CEN**** (Level 4)</td>
<td>11.19 ± 1.20</td>
<td>10.74</td>
</tr>
<tr>
<td>CEI***** (Level 4)</td>
<td>13.10 ± 1.58</td>
<td>12.12</td>
</tr>
</tbody>
</table>

Table 1. Average time ride in levels and categories in Spanish endurance rides.

Ride time and final ranking were the considered traits. Ride time was normalized to compare the ride times of a horse over different distances, it was transformed to a deviation to the best time obtain within ride. The ranking was normalized to a score scale (100 were given to the first place and 0 points for the last) (Chico, 1994). Unplaced horses were beaten by the last-placed horses (Ricard et al 2000), estimating that a ride time for the latter depends on the average increase in ride time for the rest of participants.

Variance components and genetics parameters were estimated by restricted maximum likelihood method and a preliminary least-squares analysis of variance was realized using a general linear model (GML) procedure with a SAS program to decide the fixed effect included in the animal model.

** Financial support for this work was provided by the “Subdirección General de Medios de Producción Ganaderos” (General Board of Livestock; Ministry of Agriculture, Fisheries and Food) within a collaboration agreement with Spanish Arab purebred horse association (AECCA). The authors wish to thank the support of Spanish Equestrian Federation.
A BLUP animal model was used to estimate breeding values. The genetic analysis was performed using VCE software package v.5.0 designed by Groeneveld.

The main disadvantage of using data from endurance rides is that environmental factors have more influence in this type of competition than in others. The following available information on each ride include as fixed effect: breed (5 levels: Spanish Arab purebred, Anglo-arab and Hispano-arab, Thoroughbred, Sport Spanish horse and other breeds), sex (2 levels: male and female), breeder, rider, age (5 levels: 6-7, 8-9, 10-11, 12-14 and >14 years old), season (4 levels), geographic zone (5 levels: north, centre, south, eastern region and foreign places) and distance (4 levels: 60-99, 100-139, 140-160 and >160 kms). The random effects were the direct additive genetic effect, permanent environmental effect and residual error.

With this analysis we have developed the data collecting system for this type of competition and the best trait model to evaluate this type of performance in horse.


FIRST BREEDING EVALUATION OF SPANISH TROTTERS**

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Introduction

In Spain, the Breeding of trotter horses is located in a restricted geographic area called the Balearic Islands. Spanish trotter horses have a total breeding area of 5014 km². Annually, about 1500 horse races are celebrated in the 11 different hippodromes, with the participation of more than 4500 animals.

In 2004, the Minister of Agriculture, Fisheries and Food approved an official action for the Organization and Promotion of Equine Sector in this country. Within this official project, the beginning of equine improvement is one of the aims.

Since then, the Association of Breeders and Owners of Spanish Trotter Horses (ASTROT) has developed a Breeding Scheme for this breed. Because of this, the first breeding evaluation of Spanish Trotter horses has been made.

Material and methods

The Spanish Trotter evaluation was based on 42998 racing performances from 2003 to 2005. This information (data of timing, classification and earning) was collected systematically by the Balearic Trot Federation. The genealogic information was obtained from the Stud-book of this breed. This stud-book was officially founded in 1979 and has around 15600 animals registered. The pedigree file for the genetic evaluation was generated using four generations of the participant horses.

The genetic analysis was performed according to a repeatability BLUP Animal Model, using Groeneveld’s VCE (version 5) software program.

After preliminary work, only three of the different traits proposed for the Breeding Scheme were included in the estimation of a genetic index. The results for this breed were produced using a multivariate model.

Annual earnings (measured in euro), annual best racing time (in seconds) and proportion of races placed 1-4 (measured as the percentage in relation to the total number of an animal’s stars) were included. It was necessary to use a mathematic transformation to normalize some variables. Annual earnings were normalized by a logarithmic transformation, and proportion of races placed 1-4 by a square root transformation.

The fixed effects included in the model were Sex (in 3 classes: male, gelding and female), Year of birth (from 1984 to 2002), Year of race (from 2003 to 2005) and Total number of starts in a year (being a covariable for the earnings). The random variables of the model were individual additive effect and permanent environmental effect (there were 1580 horses with racing records: 836 males, 592 females and 152 geldings).

The breeding value was presented using a merit index. This index was calculated from a weighing of the different breeding values obtained for each variable, according to the following expression:

\[ GI = 0.1143 \times bv_{PL} + 0.1714 \times bv_{AE} + 0.7143 \times bv_{BT} \]

Where: GI is the Genetic Index; bvPL is the Breeding Value for the proportion of races placed 1-4; bvAE is the Breeding Value for annual earnings; and bvBT is the Breeding Value for annual best racing time.

Results and discussion

The genetic parameter was estimated. In this analysis, the annual earnings (euro) presented the highest heritability (0.456), whereas the proportion of races placed 1-4 (percentage) presented the lowest one
The repeatabilities of those performance traits were 0.558 and 0.565 respectively. The heritability of the other trait, the annual best racing time (seconds), was 0.355 and its repeatability was 0.565.

All the genetic parameter values obtained in this study were in a normal range. They were a little higher than the values indicated in the other trotter breeds of the world. This could be caused by the homogeneity of environment for the Spanish Trotter horses because of the limited geographic breeding area.

The average reliability of the breeding value estimation was $0.715 \pm 0.064$ for annual best racing time, $0.684 \pm 0.064$ for annual earnings and $0.562 \pm 0.064$ for proportion of races placed 1-4. There were 8 animals with positive progeny tests despite the lower number of racing performances utilized.

A genetic evaluation model has been developed by the variables: annual best racing time, annual earnings and proportion of races placed 1-4, using sex, year of birth, year of race and total number of starts in a year as fixed effects.

The analysed traits have presented an adequate heritability for the genetic evaluation. Despite these results, the proportion of races placed 1-4 have presented the lowest heritability, and because of this, the use of annual earnings is recommended. Since the repeatability is higher than 0.5 for all traits, many racing performances are not necessary to obtain adequate values.
TECHNOLOGIES APPLIED IN THE CONSERVATION PROGRAM OF THE ASTURCÓN PONY

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The Asturcón pony is considered the most ancient native horse breed in the Iberian Peninsula (García-Dory, 1980). The Asturcón individuals coincide basically with those horses described by the roman chroniclers during the Cantabrian wars. After the Spanish Civil War the Asturcón population size decreased dramatically and our horse became in high risk (Álvarez Llana, 1995).

The recovery of the Asturcón pony breed was initiated in the 70's of the XXth century. Despite the historical evidences highlighting that most of the Asturcón individuals were bred in western Asturias and were bay-coated the founders of the present Asturcón population were recovered in the black coated population managed in semi-feral conditions in the Sueve's range in eastern Asturias. The initial process of recovery of the breed is well documented (García-Dory, 1980; Álvarez Llana, 1995) and involved less than 40 founder mares. A new effort for recovery of Asturcón individuals carried out during the early 90's involved a few more females from the Sueve's areas and a little group of black-coated mares from the area of Los Picos de Europa, out of the Sueve's range. In addition to these conservation efforts, during the 90's some breeders grouped in the García-Dory association initiated a preservation program for the western (bay-coated) Asturcón.

Both Asturcón populations have been studied as two different ones in some studies (Royo et al., 2005b, Royo et al., 2005c), including a recent work about the origins of the iberian horses (Royo et al., 2005a). The major reason to differentiate them is their coat colour, being always black in Asturcón, and bay, or sometimes black, in bay Asturcón. Both populations present the chestnut allele in a low frequency (Royo et. al, 2005c).

Nowadays, breeders of these two populations are involved in the improvement of the knowledge about the degree of differentiation between the Asturcón and Bay Asturcón populations, as an important criteria for the implementation of common policies of conservation of these equine genetic stocks.

We all, as a Public Regional Centre of Research expert in Animal Genetics Resources, proposed four different approaches, in order to asses the differentiation of these populations in terms of morphology and genetics.

1- Study of morphological differences.
2- Analysis of the genetic founder matrilines in both populations.
3 - Development of new mating strategies using both genealogical and molecular coancestry information.
4- Development of a diagnostic protocol for coat colour polymorphisms in order to improve mating strategies.

1- Morphological study.

Zoometry was carried out on a representative sampling of Asturcón and bay Asturcón populations using digital techniques previously developed for cattle (Goyache et al., 2001). Three digital pictures were obtained for each individual to obtain body measures and profiles in order to further assess statistical differences. The poor zoometric differences found between the two sampled populations of Asturcón, are only due to management, regardless the coat colour. To confirm this hypothesis we need to compare a bigger sample of individuals manage in equivalent semiferal conditions.
2 - Characterisation of the founder matrilines via mitochondrial DNA (Royo et al. 2005b)

The aim of this work is to ascertain possible differences among founder matrilines in the Asturcón pony via mtDNA and compare the obtained sequences with those from the Bay Asturcón pony population. Here we analyse a 361 bp D-loop fragment in 32 individuals representing the following founder geographical areas: Borines (7), Cereceua (5), Raiceu (5), La Vita (4), La Goleta (6) and Potes (5). Most of them are common land of the Sueve's mountains, whilst La Goleta is representative of the Sueve's lowlands and Potes includes a little number of individuals recovered out of the Sueve. The obtained sequences were compared with 14 samples of the bay Asturcón population. Up to 13 different haplotypes (10 in Asturcón samples) defined by 32 variable sites were identified, seven of them being unique. Haplotype diversity was of 0.31 and 0.36 for Asturcón and Bay Asturcón samples. Two haplotypes are shared by 27 of the 46 samples. Differentiation among the Asturcón geographical populations and with the bay Asturcón population do not present a clear maternal genetic support.

3 - Molecular coancestry information.

Founder contributions have been lost in a large extent in the individuals forming at present the Asturcón pony studbook. The conservation of the genetic variability, besides the possible programming of matings between Asturcón and bay Asturcón individuals makes necessary the implementation of new mating strategies combining genealogical and molecular coancestry information in order to maximize the genetic representation of the founder alleles in the next generation. To deal with this task we have developed 2 different programs that are freely available at http://www.ucm.es/info/prodanim/html/JP_Web.htm. The first is the program ENDOG (v3.0; Gutiérrez and Goyache, 2005) that manages pedigree information and the second is the program MolKin (v2.0; Gutiérrez et al., 2005) that manages molecular information. The combination of these programs allows planning matings in order to minimize genealogical and molecular coancestry.

4 - Development of a diagnostic protocol for coat colour polymorphisms.

We have developed a diagnostic protocol, which allowed to simultaneously identify the presence of the chestnut allele (C901T) in the MC1R gene and the black allele in the ASIP gene. As a preliminary work we diagnosed 45 individuals belonging to Asturcón population and 60 individuals belonging to bay Asturcón populations. Obviously the frequency of black recessive allele in Asturcón was 1, only black-coated animals are allowed in the stud-book. In bay Asturcón, where bay coat is predominant, the frequency of the black allele was 0.42. The presence of the chestnut allele was 0.07 in Asturcón, and 0.12 in bay Asturcón (Royo et al, 2005c).

Conclusions.

We have demonstrated that the differentiation between the two Asturcón populations is basically due to the coat colour phenotype. Both, the morphological and genetic information don't allow us to dearly differentiate the Asturcón population into two sub-populations. All these results were presented to the breeders associations as well as the Regional Government of Asturias.

Recently, the Asturcón breeders association has formalised the inclusion of individuals belonging to Garcia-Dory association (bay Asturcón breeders), into its breeding scheme and stud-book. At this moment Asturcón pony association has the possibility of including the coat colour genotype of all the reproductive animals under its control (both Asturcón pony populations) for mating strategies, as well as the advice of preferential matings for inbreeding to remain at low levels, based on genealogical and molecular data.
Acknowledgments.

This work was partially funded by a grant from the Instituto Nacional de Investigación y Tecnología Agraria y Agroalimentaria (INIA), no. RZ03-011. The authors would like to thank the members of Asociación García-Dory and Asociación de Criadores de Poni de Raza Asturcón (A.C.P.R.A) (http://www.asturcones.com/) for their kind help and support.

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DYSTOCIA IN DONKEYS CARRYING MULE FOALS IN MOROCCO: AN EVALUATION OF 32 CASES

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2Anogyra, Limassol, Cyprus

Introduction

Equine fetal growth is governed by maternal size and thus fetal oversize is unusual in horses despite the possibility of having a sire that is much larger than the dam. It has been shown that maternal size interacts with both maternal and fetal genotypes to influence the development of the allantochorion and the morphology of the microcotyledons and thereby control the extent of fetal growth; (Allen 2002).

Our experience of donkey dystocias at the SPANA clinics in Morocco suggests that donkeys carrying mule foals are more predisposed to complicated births than those carrying donkey foals, this may suggest that the ability of the maternal genotype to regulate fetal growth in donkeys is less developed than in horses. Most cases of dystocia are presented to the SPANA clinics belatedly, with an interval of 24-72hrs from onset of labor being not uncommon. Many of these dystocias would normally proceed to caesarian section but as the females are often endotoxic and in shock at presentation, they make poor candidates for general anesthesia. Over 50% of jennies undergoing caesarian section under general anesthetic at the SPANA clinics over the last two years, died in the peri-operative or post operative period. Anesthetic protocol in the resource limited settings of private or non governmental organizations in the developing world usually involves a xylazine/ketamine combination for induction and maintenance. In an attempt to improve this survival rate we decided to evaluate the efficacy of carrying out the surgery on the standing animal via a left lateral flank approach under local anesthesia and sedation as necessary.

The objective of the retrospective study presented in this paper is to: determine the incidence and etiology of these dystocia; to evaluate the treatment options that have been employed in their resolution; and to assess whether standing caesarian section in the jenny donkey improves the outcome for either dam and foal.

Materials and methods

Hospital records for all dystocias presented to any of the SPANA clinics in Morocco between 2002 and 2005 were evaluated. Dam and foal species were noted as well as clinical condition at presentation, time from onset of labor, reason for dystocia, intervention received and final outcome for jenny and foal. Fetal oversize was diagnosed when foal was in correct presentation but delivery of shoulders was impossible even with gentle traction.

Results

Of the 32 cases of dystocia admitted, all were jenny donkeys and 29 of these were carrying mule foals. Seven dystocias were successfully managed by manual correction with or without elevation of the hindquarters. Sixteen underwent midline section under intravenous anesthesia, of which 8 (50%) jennies and 2 foals survived. Three underwent lateral flank section in a standing position. Of these 2 (66%) survived and 1 foal survived. Three jennies died or were euthanized at presentation due to poor prognosis related to late presentation.

Of the 32 dystocias presented 22 (68.7%) of these related to fetal oversize. The results of this survey are tabulated below:
<table>
<thead>
<tr>
<th>Case number</th>
<th>Species</th>
<th>Clinical condition</th>
<th>Hrs since start of labor</th>
<th>Reason for dystocia</th>
<th>Obstetrical correction</th>
<th>Outcome Dam/Foal</th>
<th>Year</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>mule</td>
<td>poor</td>
<td>24 hrs</td>
<td>Fetal oversize</td>
<td>manual correction</td>
<td>Dam: live</td>
<td>2002</td>
</tr>
<tr>
<td>2</td>
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<td>48 hrs</td>
<td></td>
<td>died</td>
<td></td>
<td>2002</td>
</tr>
<tr>
<td>3</td>
<td>mule</td>
<td>Poor</td>
<td>16 hrs</td>
<td>Head blocked</td>
<td>manual correction</td>
<td>Dam: live</td>
<td>2002</td>
</tr>
<tr>
<td>4</td>
<td>anon</td>
<td>Medium</td>
<td>24 hrs</td>
<td>Head blocked</td>
<td>manual correction</td>
<td>Dam: live</td>
<td>2002</td>
</tr>
<tr>
<td>5</td>
<td>anon</td>
<td>Medium</td>
<td>74 hrs</td>
<td>Head blocked</td>
<td>manual correction</td>
<td>Dam: died</td>
<td>2002</td>
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<tr>
<td>6</td>
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<td>Dam: died</td>
<td>2002</td>
</tr>
<tr>
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<td></td>
<td>2002</td>
</tr>
<tr>
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<td>Dam: live</td>
<td>2003</td>
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<td>midline section</td>
<td>Dam: live</td>
<td>2003</td>
</tr>
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<td>24 hrs</td>
<td>Fetal oversize</td>
<td>midline section</td>
<td>Dam: live</td>
<td>2003</td>
</tr>
<tr>
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<td>24 hrs</td>
<td>Fetal oversize</td>
<td>midline section</td>
<td>Dam: live</td>
<td>2003</td>
</tr>
<tr>
<td>12</td>
<td>mule</td>
<td>Medium</td>
<td>74 hrs</td>
<td>Fetal oversize</td>
<td>midline section</td>
<td>Dam: live</td>
<td>2003</td>
</tr>
<tr>
<td>13</td>
<td>mule</td>
<td>Poor</td>
<td>48 hrs</td>
<td>Fetal oversize</td>
<td>midline section</td>
<td>Dam: live</td>
<td>2003</td>
</tr>
<tr>
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<td>Dam: live</td>
<td>2003</td>
</tr>
<tr>
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<td>24 hrs</td>
<td>Fetal oversize</td>
<td>midline section</td>
<td>Dam: live</td>
<td>2003</td>
</tr>
<tr>
<td>16</td>
<td>mule</td>
<td>Medium</td>
<td>24 hrs</td>
<td>Fetal oversize</td>
<td>midline section</td>
<td>Dam: live</td>
<td>2003</td>
</tr>
<tr>
<td>17</td>
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<td>24 hrs</td>
<td>Fetal oversize</td>
<td>midline section</td>
<td>Dam: live</td>
<td>2003</td>
</tr>
<tr>
<td>18</td>
<td>anon</td>
<td>Medium</td>
<td>24 hrs</td>
<td>Head blocked</td>
<td>midline section</td>
<td>Dam: live</td>
<td>2003</td>
</tr>
<tr>
<td>19</td>
<td>mule</td>
<td>Poor</td>
<td>48 hrs</td>
<td>Fetal oversize</td>
<td>midline section</td>
<td>Dam: live</td>
<td>2003</td>
</tr>
<tr>
<td>20</td>
<td>mule</td>
<td>Poor</td>
<td>48 hrs</td>
<td>Fetal oversize</td>
<td>midline section</td>
<td>Dam: live</td>
<td>2003</td>
</tr>
<tr>
<td>21</td>
<td>mule</td>
<td>Medium</td>
<td>12 hrs</td>
<td>Forelimb flexion</td>
<td>manual correction</td>
<td>Dam: live</td>
<td>2003</td>
</tr>
<tr>
<td>22</td>
<td>mule</td>
<td>Medium</td>
<td>12 hrs</td>
<td>Fetal oversize</td>
<td>midline section</td>
<td>Dam: live</td>
<td>2003</td>
</tr>
<tr>
<td>23</td>
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<td>Medium</td>
<td>24 hrs</td>
<td>Fetal oversize</td>
<td>midline section</td>
<td>Dam: live</td>
<td>2003</td>
</tr>
<tr>
<td>24</td>
<td>mule</td>
<td>Medium</td>
<td>12 hrs</td>
<td>Fetal oversize</td>
<td>lateral flank section</td>
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<tr>
<td>25</td>
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<td>24 hrs</td>
<td>Fetal oversize</td>
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<tr>
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<td>24 hrs</td>
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<td>lateral flank section</td>
<td>Dam: live</td>
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<tr>
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<td>mule</td>
<td>Medium</td>
<td>24 hrs</td>
<td>Fetal oversize</td>
<td>manual correction</td>
<td>Dam: live</td>
<td>2003</td>
</tr>
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<td>Fetal oversize</td>
<td>manual correction</td>
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<td>Fetal oversize</td>
<td>manual correction</td>
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<td>manual correction</td>
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<td>2003</td>
</tr>
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<td>2003</td>
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<tr>
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<td></td>
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<td>2003</td>
</tr>
</tbody>
</table>

Table 1. Results of 32 cases of dystocia presented to SPANA clinics 2002-2005
Discussion

In Morocco and other developing countries where mules are extensively bred and used, the loss of both dam and foal from a dystocia can represent an economic catastrophe for the owner. Fetal oversize of mule foals is one of the risks inherent in breeding mules. Establishing the optimal approach for treatment of these cases is important. Although we have only three cases of standing surgery to date, this approach appears to offer a viable alternative to the classic surgical approach. In developing countries where cost is an important consideration, the reduced anesthetic cost of this approach is an additional benefit of this technique. In the situation where the foal is dead a fetotomy might be an option to surgery. In resource limited settings the equipment for this procedure is often not available and given the small size of the reproductive tract the technique poses difficulties in the donkey. This study will be continued until December 2005 at which time it is hoped that a larger population of dystocia cases will allow a fuller evaluation of benefit.

References

EVALUATION OF A COMMERCIAL TEST TO DETECT ECF＜sup＞*＜/sup＞ (EARLY CONCEPTION FACTOR) IN SERUM OF MARES

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２Embryo Transfer Center, Keros, Passendale, Belgium

Introduction
For embryo transfer, embryos are recovered from the donor mares by uterine flushing performed 7 or 8 days after ovulation. Materials and medium used to collect the embryo and the time spent to search and collect the embryo are expensive. When, embryo collection is unsuccessful (in H 50% of cases), it is not possible to know if this is due to failure to conceive, early embryonic death or failure to collect the embryo that is present in the uterus. An accurate and reliable test for pregnancy at this stage of embryonic development would have great economic value. In addition, such test could be useful to study early embryonic death between fertilization and first pregnancy diagnosis by ultrasonography. An Early Pregnancy Factor (EPF) can be reliable if detected few hours or days after fertilization in serum of many mammalian species using a rosette inhibition test (RIT) (1). This test is very time consuming, and can only be performed in specialised laboratories. The aim of this study was to evaluate a commercial ELISA test that is easy to use and presumed to be able to detect ECF＜sup＞*＜/sup＞ (Early Conception Pregnancy) in serum of mares between the 3rd and the 30th days after fertilization.

Material and methods
In this experiment, blood samples were collected from broodmares inseminated 1 or 2 days before ovulation with fresh or frozen semen. A first blood sample was collected on Day 7 or 8 after ovulation. A pregnancy diagnosis was performed by ultrasound Day 14-16 and if the mare was pregnant another blood sample was collected. Within the hour after blood collection, the serum was tested using the commercially available Horse ECF Test＜sup>*</sup> following the manufacturer's instructions.

Results and discussion
Seventeen blood samples collected at Day 7 or 8 were tested. The test did not detect the presence of ECF in any of the samples although 6 mares were pregnant at Day 14 (one of them with twins). Samples were also collected on D14-16 from 16 mares confirmed pregnant by ultrasound. None of the serum samples tested with the ELISA test indicated the presence of a pregnancy.

In our study, the test gave no false positive result at day 7 or 8 in barren mares (11/11) but it did not detect any pregnancy both at day 7 or 8 (6/6) and at day 14-16 (16/16) in pregnant mares, even though the internal control of the test kit consistently confirmed the validity of the test. Our data suggest that the test is unable to detect ECF in pregnant mare serum.

A recent study (2) evaluated the same commercial test at day 7 or 8 of pregnancy. ECF was not detected in samples collected in 22 unbred mares. ECF was not detected in sera collected from 20 bred mares, although 12 mares were confirmed pregnant. ECF was detected in 26 sera collected from other bred mares, in only 14 of them an embryonic vesicle was observed by ultrasound at day 14-16.

The two studies indicate that the commercial test for ECF does not yet have the accuracy needed for commercial use in equine breeding industry.

References

Since national production of horsemeat is not sufficient to satisfy the great demand, in Italy are annually slaughtered 150,000 heads from the East of Europe. Nutritional and rheological properties of meat produced by imported horses do not always gratify consumer’s request, also for the impossibility of assuring traceability.

In the last years, equine breeding for meat production tended to increase in the South of Italy, thanks to the interest showed by farmers. Actually, this breeding typology represents a complementary economic activity, with great possibility of development in the future (Centoducati et al., 2003). For those reasons, we investigated rheological properties of meat from Italian Heavy Draught Horse (I.H.D.H.), with the aim of valorizing this local product.

Materials and methods

12 I.H.D.H. foals were used for the test. They were equally divided between the two sexes, naturally suckled and fed in the fattening phases with grazing and farm-made or commercial feedstuff. The foals were slaughtered at 11 months, according to the local tradition, at an EEC approved abattoir. The carcasses obtained were refrigerated at 4°C from slaughtering to selling (2-7d).

We recorded pH measurements after _h, 1 h, 24 h, 48 h and 72 h from the following muscles of the right half: Longissimus dorsi (LD), Semimembranosus (SM), Semitendinosus (ST), Quadriceps femoris (QF), Biceps femoris (BF). Using a portable colorimeter Minolta R300 we recorded colorimetric indexes L*, a* and b* according to HUNTER system and calculated color intensity or CHROMA (a*2+b*2)1/2 (Arcos-Garica et al., 2002). The same indexes have been recorded for renal and subcutaneous fat. From all the muscles above mentioned, we measured cooking weight loss, immersing a meat sample in a bain-marie until internal temperature amounted to 70°C.

All data were processed using the GLM from SAS (1990), according to the following linear model:

\[ y_{ijk} = \mu + a_i + b_j + e_{ijk}. \]

Where: \( y_{ijk} \) = dependent variable, \( \mu \) = overall mean, \( a_i \) = sex (i=1,2), \( b_j \) = muscle (j=1,…,5), \( e_{ijk} \) = error.

Results and discussion

Colour - L* values, indicating lightness, was always higher in males than females, particularly in BF and ST (differences respectively of 3 and 2). L* value, independently from sex, was higher in ST and QF muscles (about 31). Significant differences have been observed between LD and QF for lightness (P<0.05).

Also the redness index is higher in males, with differences of about 3 units for LD (P<0.05) and SM (P<0.01). The differences between males and females were less pronounced for the other muscles considered. The higher redness values have been recorded in SM, BF and QF muscles, with values superior to 14.

The b* index (yellowness) was always positive in females and negative in males. Chroma, that indicates color intensity and depends from redness, was always higher in males than females. Chroma value, that indicates the strenght or weakness of a chromatic color compared with neutral color (pure grey, C=0) was 15.48 in QF, superior to 14 for SM and BF and less than 13 for the other considered muscles. There weren't significant differences for color between sexes in subcutaneous and renal fats. L*, a*, and b* indexes showed almost similar values. Subcutaneous fat appeared bright, with L* equal to 63.21, but it also showed a light tendency to yellowish, with b* equal to 7.14.

The colour of renal fat in males is less bright ad yellowish than females. The yellowness is higher in renal fat, than in subcutaneous one, conferring a yellowish coloration, typical of equine fat. This phenomenon is earlier in renal fat and later in subcutaneous one.
<table>
<thead>
<tr>
<th></th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
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Subcutaneous fat

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Peri-renal fat

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Meat colour

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<td>SM</td>
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Chroma

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pH values

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<tbody>
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<tr>
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<td>QE</td>
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48h

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<tr>
<td>SM</td>
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<tr>
<td>QE</td>
<td>5.76±0.63</td>
<td>5.57±0.67</td>
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72h

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<td>ST</td>
<td>5.70±0.59</td>
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Different letters on the same line for each comparison show significant differences. A, B: P<0.01; a, b: P<0.05; different letters on the same column for each comparison show significant differences. X, Y, Z: P<0.01; x, y: P<0.05;
Cooking weight loss - Meat from males showed a higher cooking weight loss for all muscles except for SM. For BF muscle, the difference between sexes is more accentuated: (6.49%). In the general list we recorded in order: ST and QF, followed by SM with only about 39% of cooking weight loss.

pH - pH is a parameter that does not appear affected by sex. In fact, in all considered muscles, for both sexes, we recorded a sudden drop of pH values until 24 h. From 24h to 48 h, for QF e BF the trend is similar to the first 24 hours, although less drastic, while for LD, SM and ST pH values tended to increas. At 48 h pH is stabilized for all the muscles, with values ranging from 5.66 to 5.75. in the following 24 h, only LD, SM and ST showed a light decreasing of pH.

In conclusion, rheologic properties of meat from IHDH foals bred in the South of Italy satisfy the requests of both butchers and consumers. Considering the good results obtained, the interest of breeders for IHDH breed is justified. In the future it is necessary to investigate the ways for improving quantitative and qualitative meat production from IHDH horse, evaluating both feeding techniques and management.

References


EXERCICE INDUCED EPISTAXIS SYNDROM IN THE RACE HORSE: CONTRIBUTION TO STUDY ETIO-PATHOGENIC MECHANISM

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1. École Nationale de Médecine Vétérinaire, Sidi Thabet 2020, Tunisie.
2. Institut Pasteur de Tunis, Tunis 1006, Tunisie

Race horse's epistaxis syndrome induces bad performances and often restricts sportive career of athlete. Its etiopathogenic mechanism is not well elucidated. The immuno-allergic component and heart problems have been evoked. Our objective is to contribute to the study of these two components.

Material and methods

The authors have carried out electrocardiography recordings (ECG), according to Dubois's technique, on 26 horses suffering from exercise induced epistaxis (EIE), their average age was 7.7 ± 5.8 years and 42 witness healthy horses the average age was 4.4 ± 1.2 years. All are race horses. The diagnosis was realised on the basis of commemoratives and clinical examination associated, systematically to endoscopic examination. The witness healthy horses were horses that did not have any recent disease and have not received drugs or treatment since 3 weeks before examination and sampling and that endoscopic examination had confirmed that no EIE occurred in them.

In bipolar lead DII, the duration, the amplitude and the morphology of auricular depolarization wave (P-wave), ventricular depolarization wave (QRS complex) and ventricular repolarization wave (T-wave), also, the duration of PR-segment and RT interval have been investigated.

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

Moreover, the authors searched for the precipitant antibodies directed towards Aspergillus Fumigatus (AF) and mouldy hay total extract (Fo. Moi) by electroosyneresis on cellulose acetate membrane. The mouldy hay total extract were obtained according to the pepys technique, AF are commercial antigens (Diagnostics Pasteur). The diseased horses, at the number of 30, were racehorses that showed recurrent episodes of epistaxis after exertion. Their average age was 5.28 ± 1.79 years. The control horses, at the number of 49, are healthy horses taking part in the races at the same conditions than diseased horses. The average age was 4.95 ± 1.22 years. For each serum, a total score was attributed to each antigen, taking into account the quality, the intensity and the number of precipitation arcs.

This research was carried out in two periods, the first period was between 1996 and 1998 for ECG investigation and the second period was during 1999 and 2001.

Results and discussion

The Electrocardiograms reveal significant differences, between the 2 groups, in PR duration QRS duration, R wave and T wave amplitude have been observed. Horses suffering from EIE have PR longer, QRS shorter and R and T2 higher than that of healthy witness horses.

In horses suffering from EIE:

PR = 0.284 ± 0.068 s; QRS =0.131 ± 0.025; R = 0.454 ± 0.352 mv
T2 = 0.505 ± 1.95 mv ; in healthy witness horses: PR = 0.246 ± 0.005 s; QRS =0.152 ± 0.030; R = 0.224 ± 0.236 mv; T2 = 0.314 ± 0.126 mv

These ECG modifications are not pathologic, they are in the normal range for the species, but they are correlated with bad performances.

The electroosyneresis test shows nil and positive scores. The nil scores obtained toward the two antigens are comparable in healthy and diseased horses. Nevertheless the mean positive scores toward Fo. Moi show a significant difference between the 2 groups: horses with epistaxis show a mean level, 3.0 ± 1.6 statically higher than those of control horses, 1.6 ± 0.8. The distribution of Fo. Moi scores according to response intensity reveal that horses with epistaxis have rather greatly positive responses; in fact 86% of diseased horses have scores higher than a weakly positive type of responses. None of the horse with epistaxis, had reacted to AF. The type III hypersensitivity’s phenomenon, toward Fo Moi, could be one of the causes intervening in fitting out of epistaxis because we find more precipitin anti-Fo Moi with a greatly positive type of responses.

Horses suffering from exercise induced epistaxis have ECG parameters correlated with bad performances. Bronchopulmonary diseases caused by hypersensitivity to mouldy hay could be one of the causes intervening in fitting out of epistaxis.
CONTRIBUTION TO THE ETIOLOGICAL STUDY OF HORSE RESPIRATORY DISEASE EPIZOTIC OCCURRED IN 1998 IN TUNISIA

Chabchoub A*1, Dauphin G2, El Goulli A1, Landolsi F3, Ghram A4, Zientara S2

2. AFSSA, Service de virologie équine Alfort. 94704, France.
4. Institut Pasteur de Tunis 1006 Tunisie.

As a result of an outbreak of a contagious upper respiratory tract disease which affected horse population in the south of Tunisia in 1998, the authors carried out a serological and virological investigation in order to determine and identify the causes of this epizootic in horses.

Material and methods

Animals: 38 horses suffering from acute respiratory disease were sampled. Thirty one blood samples and 7 nasal swabs were taken.

In a first time serological investigations were carried out by inhibition of hemagglutination test (IHA) and fixation of the complement tests (FC) for, respectively, equine influenza and equine herpes virus infections (EHV).

In a second time, the authors have carried out a virological search in order to isolate and identify the circulating influenza virus type. Samples taken from diseased horses are submitted to RT-PCR after passing on SPF embryonated eggs. Four other influenza virus types have been included to compare them with the Tunisian strain. These types are A/eq/Grobois/1/93 (or Grobois/93), A/eq/Grobois/1/98 (or Grobois/98), A/eq/Miami/63 (Miami/63) and A/eq/Maroc/1/98 (Maroc/98). The RT-PCR products were revealed by electrophoresis. Two couples of amorces have been used to underline the H3 hemagglutinine sequence. The first couple of amorce, eq/H3/9/+ et eq/H3/1741/-, allows to underline an amplified DNA strip at the size of 1732 pb. The 2nd couple of amorce, eq/H3/9/+ et eq/H3/1170/-, allows to underline an amplified DNA strip at the size of 1161 pb. These strips are the product of the amplification of H3 hemagglutinine. They were identified in the 3 isolates Grobois/93, Grobois/98 et Miami/63. The presence of these strips confirms the appurtenance of these 3 isolates to H3 sub type. The same samples have been analysed again by RT-PCR after other passing on SPF embryonated eggs in order to characterize the influenza subtype responsible for this epizootic. This RT-PCR was carried out to detect H3 and N8 gene. These two surface-antigens define the subtype A Equi 2. Two couples of amorces have been used to underline the sequences of H3 hemagglutinin and that of neuraminidase N8. The 1st couple of amorce eq/H3/9/+ et eq/H3/1170/-, allows to underline an amplified ADN strip at the size of 1161 pb which is the gene amplicon coding for hemagglutinin; the second couple of amorce, eq/N8/2/+ et eq/N8/1460/-, allows to underline an amplified DNA strip at the size of 1458 pb which represents Neuraminidase amplicon. The detection and the amplification of these two regions allow confirming with certitude that it is a H3N8 subtype of Equine influenza virus.

Results and discussion

All the blood samples were negative for the EHV FC test. 17 serums from the 31 serums were positive to IHA test. These serological tests confirm that is the equine influenza disease but not EHV infection.

Within the IHA positive horses, 11 horses are not vaccinated of which 9 horses have high IHA titre ranged from 80 to 1280. They are IHA positive to Miami 63 (1 serum), Morocco 1998 (9) and Grobois 1998 (1) equine influenza subtypes. All of these subtypes are H3N8 subtypes. But no one of serum has reacted to A equi 1/ Prague 56 (H7N7).

Seven horses are vaccinated at list for one time before. They show a similar reaction toward the greater part of used antigen (table 1)
The detection and the amplification of DNA allow confirming with certitude that it is a H3N8 subtype of Equine influenza virus. The last influenza epizootic, in 1978-1979, was caused by H7N7 subtype.

Serological and virological investigations indicate that the H3N8 influenza virus subtype is responsible for the respiratory disease outbreak which occurred in 1998 in Tunisia.

<table>
<thead>
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<th>Subtype</th>
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</tr>
<tr>
<td>Miami 63</td>
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</tr>
<tr>
<td>Morocco98</td>
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<tr>
<td>Newmarket/1/77</td>
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</tr>
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<td>Grohois 98</td>
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<tr>
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</tr>
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<td>Miami 63</td>
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<tr>
<td>Morocco98</td>
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</tr>
<tr>
<td>Newmarket/1/77</td>
<td>1</td>
</tr>
<tr>
<td>Grohois 98</td>
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</table>

Table 1: Repartition of IHA positive serum according to influenza virus subtype
The existence of physiological rhythm at rest must be taken into consideration in the athletic horse. Measurement at resting state should be daily controlled to avoid potential misinterpretation later and the time of day may influence the preparedness of the subject for submaximal and maximal exercise performance.

Although the detailed study of rhythmic properties of individual variables can provide significant advances in the understanding of individual functions, the simultaneous study of many variables is a necessary step in the path to the understanding of the multiple temporal relationships of physiological processes. Thus, in the present study, we monitored simultaneously 21 different rhythms at rest in the jumper horse.

Materials and methods

The animals used in this study were 5 jumper horses (6 to 8 years old). Training lasted 2h each day and included walking, trotting, galloping and obstacle jumping. Horses were housed in individual indoor stalls under natural summer conditions: sunrise at 05:00, sunset at 20:00 with an ambient temperature of 19-26°C and relative humidity of 40-50%. Food (hay, oats, corn, and barley) was provided twice a day at 08:00 and 16:00. Water was available ad libitum.

Locomotor activity of individual animals was monitored by an activity data-logger (Actiwatch, Mini Mitter Co., Bend, OR) strapped to the animal's neck. Activity counts were recorded in 1-minute intervals. All other variables were recorded every 2 hours for 48 consecutive hours. Rectal temperature was measured with a digital thermometer with resolution of 0.1 °C. Heart rate, diastolic blood pressure, and systolic blood pressure were determined with an oscillometric apparatus using a cuff positioned at the previously-shaved base of the tail. The accuracy of the apparatus was 1 mmHg. The volume of water ingested by each animal in each 2-hour interval was recorded. Urination and defecation were recorded as binary events (yes or no in each 2-hour time interval). Blood samples were collected through jugular intravenous catheters every 2 hours and were later processed in the laboratory for assessment of the concentrations of melatonin, cholesterol, urea, albumin, creatinine, total protein, glucose, calcium, chloride, magnesium, phosphate, potassium, and sodium.

For consistency with the other 20 variables, the temporal resolution of the locomotor activity data was reduced to 2-hour bins by averaging of all 120 data points within each 2-hour bin. Thus, all data sets (for all 21 variables for the 5 horse) consisted of 24 data points spaced at 2-hour intervals over 2 days.

Four rhythmic parameters were assessed for each of the 21 variables: mean level, amplitude, acrophase (time of peak), and strength of rhythmicity. For each animal, the mean level of each rhythm was computed as the arithmetic mean of all values in the data set. The amplitude of a rhythm was calculated as half the range of oscillation, which on its turn was computed as the difference between peak and trough. The acrophase of a rhythm was determined by an iterative curve-fitting procedure based on the single cosine function. For each variable for each animal, a cosine wave was fitted to the data points according to the function \( Y_t = M + A \cdot \cos(\pi t + \phi) \), where \( Y_t \) denotes each data point in the time series, \( M \) is the mean level of the rhythm, \( A \) is the amplitude, \( \pi t \) is the trigonometric angle (in degrees) corresponding to time \( t \), and \( \phi \) is the angle displacement for the acrophase. The value of \( \phi \) was determined by iteration: the true value of \( \phi \) was considered to be the one that produced the smallest sum of squares of the deviations between iterated cosine functions and the raw data.

The significance of differences between means of acrophases and between means of strength of rhythmicity was tested by analysis of variance (ANOVA) followed by post-hoc pair wise comparisons by Tukey's HSD test. The level of significance (± = 0.05) was maintained at each ANOVA.

Results

Locomotor activity, rectal temperature, and plasma melatonin concentration exhibited robust daily rhythmicity. Whereas the rhythm of locomotor activity peaked in the middle of the light phase, the
temperature rhythm peaked early in the dark phase, and melatonin peaked in the middle of the dark phase. Heart rate and plasma glucose concentration also exhibited daily rhythmicity, albeit with lesser robustness. Although one might expect glucose concentration to be directly affected by the feeding schedule, the records do not suggest the presence of a strong effect. To evaluate the extent of inter-individual variability, a second analysis of variance was conducted in which the individuals (rather than the variables) were considered as one of the factors.

Because determination of the acrophase of a rhythm is predicated on the presence of rhythmicity in the data set, the precision of computed acrophases is expected to be greater in variables that exhibit greater rhythmicity. For the horses, the correlation was $r = -0.62$ ($p = 0.0028$); thus, strong rhythmicity was associated with narrow confidence intervals (that is, high precision of acrophase).

**Discussions**

The results indicated that, in horses, different physiological variables exhibit different degrees of daily rhythmicity and reach their daily peaks at different times of the day (fig. 1). Most of the recorded variables (13 out of 21 variables) peaked during the light phase of the light-dark cycle. Whether this is a characteristic feature of the physiology of diurnal animals cannot be ascertained without the availability of comparable data from nocturnal animals. The finding that different variables exhibit different degrees of rhythmicity is not surprising. In a previous study in eight species of small mammals, we founded that the rhythm of body temperature was consistently more robust than the rhythm of locomotor activity. Conceptually, a rhythm with low robustness cannot be the cause of a rhythm with high robustness. Thus, the rhythms of activity, rectal temperature, melatonin concentration, and glucose concentration must not be caused by any of the other 17 rhythms that we investigated. Whether any of these four rhythms is the cause of the other three rhythms (or of the remaining 17 rhythms) cannot be determined from the data on rhythm robustness. Of course, the rhythm of locomotor activity could be the cause of the temperature rhythm, but studies of human subjects in constant bed rest and correlational studies in animals have clearly shown that the temperature rhythm is independent of the activity rhythm.

The results from this study also elucidate the temporal relationship between the temperature and activity rhythms in horses. In previous studies on horses, we noticed that, in these diurnal animals, the body temperature rhythm peaked at the beginning of the night. Because we did not record locomotor activity in these studies, we were unable to confirm the phase disparity between the temperature and activity rhythms. In the present study, we clearly established that, in horse, the activity rhythm reaches its peak in the middle of the day, whereas the temperature rhythm reaches its peak at the beginning of the night. In summary, our data provide the evidence for existence of circadian changes in these resting parameters might mediate parallel changes in both performance and physiological responses to exercise over 24-hour period.

![Fig. 1:](image-url)
THE INFLUENCE OF OXYTOCIN INJECTION WITH OR WITHOUT LUNGING IN FOAL HEAT ON CONCEPTION RATE IN MARES WITH INTRAUTERINE FLUID ACCUMULATION

Ghasemzadeh-Nava, H.*, Bokaee, S., Gharagozloo, F.¹ and Ghasemi, F.²
¹Faculty of Veterinary Medicine, University of Tehran - Tehran - Iran
²Veterinary Organization, Asad-Abadi St. Tehran, Iran

Introduction

Post-breeding endometritis is a major cause of subfertility in the mare. Many factors have been suspected of contributing to this condition, although recent findings suggest that inadequate lymphatic drainage and inability to evacuate fluids from the uterus via the cervix may be important in the pathophysiology (5). It is believed that injection of ecbolic agents such as oxytocin stimulates myometrial contractions, resulting in drainage of uterine fluid (3). Our previous experience showed that lunging the mare after oxytocin injection may promote intra-uterine fluid (IUF) ejection to the outside of the reproductive tract (unpublished data).

The objective of this study was to examine the influence of oxytocin injection and also oxytocin plus lunging on IUF reduction and conception rate in mares with IUF accumulation.

Materials and methods

Mares in foal heat, (n=45, 8-14 years old) with more than 1 cm² fluid in their uterus (determined by ultrasonography) were selected and categorized randomly in 3 groups as follows:

- Group 1: Without treatment (control). n=17
- Group 2: Oxytocin injection (15 iu, IV), once daily, up to the end of foal heat (first injection on the 2nd day of foal heat). n=14
- Group 3: Treatment as group 2 plus lunging for 20-30 minutes, initiated 60 minutes after oxytocin injection. n=14

The interval from foaling to beginning of the heat and interval from foaling to ovulation in the mares in each group were on day 5 and 15 respectively. The mean of heat duration in group 1, 2 and 3 was 3.06, 2.93 and 3.07 and also the SE was 0.183, 0.195 and 0.164 respectively. The 1st day of foal heat was determined or suspected by teasing but it was confirmed by ultrasonography of reproductive tract and continued until the day of ovulation. Treatments stopped when ovulation was determined. Daily ultrasound examination of the uterus was done to determine fluid build up or elimination. All mares in 3 groups were artificially inseminated every other day with 500 million progressively motile spermatozoa once a follicle ≥ 40 mm was detected. The semen in all 3 groups used was just from a fertile stallion. Pregnancy was detected with ultrasonography 12 to 14 days after ovulation and re-examination was done on day 30 to 32 after ovulation.

Results

The results of this study showed that percentage of mare without any fluid in the uterus was 71.4% and 98.9% in group 2 and 3 respectively compared with 17.6% in control group (P<0.05). Furthermore, the amount of fluid after about 24 h following breeding in control group was more than that of other two groups. As shown in figure 1, the total conception rate in 3 groups with uterine fluid less than 1 cm² (61.5%) was significantly more than the total one in 3 groups with uterine fluid ≥ 1 cm² (26.3%) (P<0.05).

Figure 1: Conception rate in the 2nd heat after parturition on the basis of IUF accumulation in 3 groups
A: The mares with normal or involuted uterus (< 1cm² IUF) among 3 groups
B: The mares with abnormal uterus (≥1cm² IUF) among 3 groups a, b are significantly different (P<0.05).
Discussion

IUF accumulation often seen during foal heat has a negative effect on the pregnancy rate of mares bred during the heat (2). We showed that oxytocin injection during foal heat resulted in drainage of uterine fluid via its effect on myometrial contractions. We used 15 iu oxytocin in our study because it was previously reported that pregnancy rates decrease when mares are treated with 25 iu compared with 15 iu oxytocin after breeding (5). It is assumed that oxytocin treatment with doses higher than 15 iu can cause uterine spasm and result in a prolonged refractory period of the muscle cell (4). It was reported that effect of oxytocin was even more obvious in mares older than 8 years of age (4). That is why we studied the mares in this group of age (middle-age). The mechanical uterine clearance of older mares is not as effective in young mares; therefore, oxytocin treatment of older mares should be more successful. The significance of intrauterine free fluid in young mares is not clear and needs further investigation. The result of our study on the effect of oxytocin in middle-age group mares is in agreement with Rasch et al. However, our findings are in contrast with Malschitzky et al report. They reported that the presence of IUF during foal heat does not affect pregnancy rate. They considered mares aged 3-23 years old and did not study the effect of oxytocin on pregnancy rate in different age groups. Lunging the mares after oxytocin injection in foal heat to remove IUF is done by some practitioner in Iran. Although it was reported that 60 min after the administration of oxytocin the uterine fluid was seen in the cranial vagina (1), the result of present study suggests that additive effect of lunging (initiated 60 min after oxytocin injection) on IUF evacuation and pregnancy rate was low and was not significant as we expected. This may be due to the low sample size in each group.

Conclusion

Oxytocin injection in mares with IUF accumulation in foal heat can improve conception rate in next estrus.

References:

OXIDATIVE STRESS IN THOROUGHBRED  
DURING OFFICIAL RACES OF 1800 METERS  

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Free radicals are active chemical structures that are in constant production in the organism. It are unstable molecular fragments or molecules with an unpaired electron in their outer orbital. They may be formed in the course of physiological and pathological processes in aerobic organism, and the combination of a free radical with a cellular component may result in cellular dysfunction. Physical exercise is associated with a dramatic increase in oxygen uptake both by the whole body and particularly by the skeletal muscle. The production of reactive oxygen species (ROS) is believed to be the underlying mechanism for a series of biochemical and physiological changes that occur during exercise and are indicative of oxidative stress. In thoroughbred race horses to physical activity, gives rise to an imbalance between free radical production and antioxidant agents leading to oxidative stress. This stress can produce damage in several biomolecules creating metabolic alterations affecting physical performance. The increased free radical production, identified as semiquinone, coincided with a series of cellular disorders, such as lipid peroxidation, loss of sarcoplasmatic reticulum latency, and mitochondrial uncoupling.

The aim of this study was to find possible relationships between physical exercise and oxidative stress in thoroughbred during an official race of 1800 meters.

Materials and methods

Six healthy thoroughbred, 3 gelding and 3 mares, aged between 3 and 5 years, with a body weight of 400±30 kg, feeded with oats and hay, were used. All horses were trained and competed regularly in “Mediterraneo” racetrack (Siracusa - Sicily- Italy). Thoroughbred took part to an official race consist of 1800 meters course. On all the horses blood samples were collected at rest, immediately after the race, 30 and 180 min. the race. On each animal blood samples, collected through jugular venipuncture using vacutainer tubes (Terumo Corporation, Japan) with no additive, were centrifuged at 3000 x g for 10 min. On the obtained serum, using a spectrophotometer (SEAC Slim), the following parameters were assessed: lactate dehydrogenase (LDH), creatine kinase (CK), reactive oxygen species (ROS), thiol antioxidant barrier (SHp) and antioxidant barrier (Oxy-adsorbent). Creatine kinase (CK) concentration. Since the intra-group variance was not significant, the statistical elaboration of data was carried out on mean values of studied parameters. On mean values we applied the analysis of variance (one-way and repeated measures ANOVA), as to evaluate the statistical significant differences obtained by comparing the different experimental conditions (at rest vs immediately after the race, at rest vs 30 min. after the race and immediately after the race vs 30 and 180 min. after the race).

Results

The application of linear regression model (y = a+bx), showed a low correlation between individual values of reactive oxygen species (ROS), and of creatine kinase (CK) in thoroughbred. On individual values of reactive oxygen species (ROS), and of creatine kinase (CK), a linear regression model (y = a+bx) was applied in order to determine the correlation degree between the studied parameters in the thoroughbred and the correlation coefficient (r) was determined. The ANOVA for repeated measures showed a highly significant effect of exercise on some of the studied parameters: LDH, F(3,15)=49.85, p<0.0001; ROMs, F(3,15)=60.71, p<0.0001; Oxy-adsorbent, F(3,15)=393.70, p<0.0001. No statistical significant differences were observed for CK and SHP (Table 1).
Discussions

In thoroughbred, the oxidative stress associated to physical exercise, would not seem to be responsible of muscular lesions. In fact, the significant increase of lactic dehydrogenates (LDH) after the race vs. rest, may be due to an increase of the mitochondrial membrane permeability instead of from muscular lesions; the increase in permeability, could be associated to the maximal physical exercise done. On the other hand, the lack of statistically significant variations of creatine kinase (CK), seems to exclude the insurgence of muscular lesions caused by oxidative stress exercise-induced. During the physical exercise in horses there is an increase of creatine kinase (CK), this increase is not necessarily an index of insufficient fitness or muscular fatigue. The anti-oxidant power of the plasmatic barrier would not seem to be adapted to prevent the reactive production of the reactive oxidation species (ROS), considering that together with the statistically significant decrease of Oxy-adsorbent concentration after the race vs. at rest there is a statistically significant increase of the ROS. The lack of correlation between ROS and CK after the race would indicate that although the physical exercise causes an increase of the production of the reactive oxidation species (ROS), these are not responsible of damages at muscular level. Heavy physical exercise characterized by a remarkable increase in oxygen consumption present challenge to the antioxidant system because of the increase production of ROS. The enzymatic (superoxide dismutase (SOD), glutathione peroxidase (GSH), etc) and non-enzymatic anti-oxidants (e.g. vit. E) usually does not protect tissues from the oxidative damage, but the depletion of some of these anti-oxidants systems could increase the vulnerability of tissues and of cellular components reactive oxidation species; however tissues seem to increase theirs anti-oxidants defence when exposed to a chronic activation.

Chronic exercise training seems to have dual effects: it induces antioxidant enzyme and perhaps stimulates GSH synthesis, thus theoretically facilitating the removal of ROS produced during exercise. So, a comparison between anti-oxidants effects and the modifications of the parameters of the athletic performance (e.g. lactic acid, heart rate, respiratory rate and O2 consumption), both at rest and after work increasing loads, is necessary to show that oxidative processes inhibition during physical exercise may improve the physical performance.

Table 1 - Mean values together with the relative standard deviations and statistical significance obtained on the different experimental conditions in 6 thoroughbred horses.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Experimental conditions</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rest</td>
<td>After race</td>
<td>After 30'</td>
<td>After 180'</td>
<td></td>
</tr>
<tr>
<td>LDH (U/l)</td>
<td>206.30±13.95</td>
<td>298.30±12.91*</td>
<td>220.50±14.61[1]</td>
<td>223.00±18.97[1]</td>
<td></td>
</tr>
<tr>
<td>CK (U/l)</td>
<td>136.00±8.51</td>
<td>141.70±6.95</td>
<td>138.20±7.39</td>
<td>137.00±9.96</td>
<td></td>
</tr>
<tr>
<td>ROS (U/carr)</td>
<td>162.30±5.32</td>
<td>192.30±5.68 *</td>
<td>154.30±6.41[1]</td>
<td>157.32±6.44[1]</td>
<td></td>
</tr>
<tr>
<td>SHp (µmol/l)</td>
<td>499.20±21.08</td>
<td>482.50±25.04</td>
<td>515.50±21.30</td>
<td>515.80±22.89</td>
<td></td>
</tr>
</tbody>
</table>

Significance: * vs rest (p<0.001); _ vs after race (p<0.001).
EXPERIMENTAL ATTEMPTS TO MODULATE AND STIMULATE THE IMMUNE SYSTEM TO HORSES WITH EQUINE INFECTIOUS ANEMIA

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The study describes the results obtained in experimental attempts to modulate and stimulate the immunological potential in seropositive horses. In order to achieve this issue, two products, “Polidin” (bacterial product) and “Imunostim” (viral product) were used.

Materials and methods

“Imunostim” is an immunostimulating product which has viral inactivated components, some of them being strongly interferon inducers, suspended in oily adjuvant. This product is registered for patents propose. “Polidin” is a pharmaceutical immunomodulating product, having polibacterial origin, which contains 13 bacterial isolates heat inactivated and partially lysed with bile. The product was administered via intramuscular, to 9 horses with equine infectious anemia, in 2 ml dose, two by two days, during 10 days. Blood samples were harvested before and 21 days after inoculation. Also, hematological, biochemical and serological exams were performed.

Results

It was observed an increased number of leucocytes and platelet count, as well as for MCV and Hct. Among the serum proteins, the values of serum albumin, _2 and _-globulin were lightly increased.

Ten horses with equine infectious anemia were inoculated subcutaneous twice in a period of 14 days, in doses of 2 ml. Blood samples were harvested on the day 14 and 28 days after the first administration. Fourteen days after the first inoculation, the results showed the increase of some hematological (leucocytes number, hemoglobin, hematocrit, MCV, lymphocytes, monocytes) and biochemical parameters (serum albumin, _2 and _-globulin), which has presented same values 14 days after the second inoculation.

Horses that received “Polidin” as those that received “Imunostim” showed positive results at serological tests (Coggins test and ELISA).

Conclusion

Regarding our results, we could affirm that even the viral antigens effect was not stopped, both produces stimulated some blood cells proliferation, as well as a more active syntheses of hemoglobin molecule.

As we propose is a theoretical explanation about the involvement of immunomodulators in cytotoxic effectors activation on intra and extracellular level.

Concerning the product “Polidin”, our research started from the observation that this pharmaceutical product is inducing for a short time an increase activity in cytosome and phagolysosome of O2, H2O2, as well as a rearrangement of some lymphocyte and monocyte antigenic markers (TCR, Fc_R, CD2, CD46, CD32, CD64).

It may be possible that “Imunostim” provide a benefic effect in cellular cooperation between lymphocyte-TCR-CD3/antigen presenting cells, as well as in relation T CD8 lymphocyte-viral infected macrophages. This product seems to act as an “antigen translocation protein” with effects on T CD8 lymphocytes activation, the final effect being lyses of target cells and control of viral replication for a while.
THE EFFECT OF HYPERTHERMIA ON GELATINASES AND PRO-INFLAMMATORY CYTOKINES SYNTHESIS IN EQUINE TENDINOCYTES

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2 Department of Pathobiology, School of Veterinary Medicine, Rakuno Gakuen University, Hokkaido, Japan

Tendon injury, especially in superficial digital flexor tendon (SDFT), has proved to be a major problem for racehorses. In Japan as well as Western countries, tendonitis is a frustrating problem for the horse racing industry. Recent studies have shown that about 10-30% of Japanese racehorses suffer from tendonitis. In the degeneration of tendons, pro-inflammatory cytokines and gelatinase in matrix metalloproteinases (MMPs) are deeply involved. Pro-inflammatory cytokines, such as tumor necrosis factor (TNF) _ and interleukine (IL)-1__, can initiate the synthesis and release some types of cytokines. Gelatinase, particularly MMP-2 and MMP-9, appear to play an important role in tendinopathy, especially in the degeneration of extracellular matrix, collagen fibers and glycosaminoglycans, such as decorin and biglycan. The mechanism behind tendonitis has not been fully clarified; however, exercise-induced heat is highly plausible factor. During exercise, heat is generated in the tendon as it extends and contracts repeatedly. The temperature in the core region of the tendon reaches 40 to 45 oC when a horse is allowed to gallop. Such a high temperature may not only elicit the tendon degeneration but may also prompt the onset of tendonitis. The aim of this study was to clarify whether MMPs (gelatinases) and pro-inflammatory cytokines are induced by heat in tendon tissue in vitro, and to test the hypothesis that hyperthermia in the tendon causes the tendinocytes to synthesize pro-inflammatory cytokines, and that these cytokines, in turn, lead to the up-regulation of gelatinases.

Materials and methods

SDFT was collected from 2 healthy female racehorses (1 and 2 years of age), euthanized for reasons unrelated to the tendon or to musculoskeletal reasons. Tendinocytes were isolated by the outgrowth method. Isolated tendinocytes were cultured and all experiments were performed on cells passaged 3 or 4 times. At each passage, the cells were seeded at a density of 5 x 104/ml on 35-mm plastic plate for gelatin zymogram and Western blotting analysis. Cells were incubated at 37 oC with 5% CO2 and were cultured on medium with 5% fetal bovine serum and antibiotics (growth medium). For the counting of viable cells, cells were washed with PBS (-) and suspended in trypsin-EDTA. Cell suspension were adjusted to contain 1 x 105 cells/ml and the suspended cells were warmed in a water bath for 5, 10, 20, 30 and 60 min at 37, 40, 42 and 45 oC. After heat exposure, the cells were incubated 20 min at 37 oC and transferred to 96-well plates. Plates were incubated for 24 h and viable cells were counted with MIT assay kit. For the MMP assay, cells were incubated in 35-mm plates with pro-inflammatory cytokines, purified horse TNF_ (10 ng/ ml) and IL-1_ (10 ng/ ml), at 37 oC for 6-to-72 h, and another batch of cells was incubated at 40 oC for 5-to-60 min (heat-exposed cells). Additionally, some heat-exposed cells were incubated for 20 min at 5, 20 and 37 oC after incubation at 40 oC. Supernatant was collected and analyzed by gelatin zymogram to determine the amount of pro and activated MMP-2 and -9; heat-exposed cells were collected and analyzed by Western blot for the presence of TNF_ and IL-1_. Tendinocytes were also incubated for 60 min at various temperatures and examined by scanning electron microscopy.

Results

In the cells exposed to heat, the survival rate decreased sharply in a temperature and time dependent manner, especially at 43 and 45 oC. Electron microscope disclosed that the cellular structure collapsed in most high-temperature exposed cells, with many holes on the cellular membrane. Cells exposed at 40 oC, however, showed no change in survival rate, and multiple cellular projections were found, as if the cell had been activated by heat.

Gelatin zymogram revealed that proMMP-9 was the sole MMP remaining in the supernatant of the cultured tendinocytes, including that of non-treated cells. Addition of TNF_ and IL-1_ to the cultured tendinocytes accelerated proMMP-9 synthesis considerably. Heating the tendinocytes led to the
induction of proMMP-9 synthesis in a short time. The band density at 60 min was three-fold stronger compared to the density of the control cells (0 min). TNFα and IL-1β were detected in tendinocytes after heat exposure. TNFα appeared immediately after heating, and faded out by 60 min. In contrast, IL-1β increased with heating time passed.

Cooling treatment on heat-exposed cells from 40°C to 37°C considerably delayed up-regulation of cellular proMMP-9 synthesis. Furthermore, proMMP-9 level was strongly suppressed in the cells treated at lower temperatures, 20°C and 5°C.

In summary: (1) Heat influences the survival rate of cells and the cellular morphology. (2) ProMMP-9 synthesis in tendinocytes is induced by pro-inflammatory cytokines and by heating, and heated cells can produce pro-inflammatory cytokines in a short time. (3) Cooling treatments on heat-exposed tendinocytes reduces the proMMP-9 level effectively. Together, these findings support our hypothesis that hyperthermia in the horse tendon induces the tendinocytes to synthesize pro-inflammatory cytokines, and that the cytokines consequently bring on the up-regulation of gelatinases. Results of this study are particular clinical importance in the prevention of tendon degeneration possibly by control of the tendon temperature in the animal. It is conceivable that cooling the legs of the horses after training would be a simple but effective means of preventing tendinopathy.
EFFECT OF THE SUPERFICIAL DIGITAL FLEXOR TENOTOMY AND SUPERIOR CHECK LIGAMENT DESMOTOMY ON RADIO-METACARPAL, METACARPOPHALANGEAL AND INTERPHALANGEAL DORSAL ANGLES AND ON THE HOOF IN HORSES


UNESP - São Paulo State University, Faculty of Veterinary Medicine and Animal Science, Botucatu, Brazil; UNESP - São Paulo State University, Institut of Biosciences, Brazil.

The purpose of this study was to investigate the effects of the superficial digital flexor and the superior check ligament desmotomy on the radio-metacarpal, metacarpophalangeal, proximal and distal interphalangeal joint angles and on hoof parameters in horses. Under general anaesthesia the superficial digital flexor tenotomy and superior check ligament desmotomy were performed, on the right and left forelimbs of nine horses. Before surgery and on the 15th, 30th, and 60th postoperative days, the radio-metacarpal (RMA), metacarpophalangeal (MPA), and proximal (PIA) and distal interphalangeal joints (DIA) dorsal angles were measured by radiographic examination. Hoof parameters of both forelimbs such as toe (T), lateral heel (H), hoof length (L), width (W), and hoof angle (A) were also measured at the same time. Data were analyzed using a multivariate profile analysis (P< 0.05). Tenotomy significantly decreased the metacarpophalangeal joint angle (Decrease from a mean value of 140.7° at baseline to 126.9°, 126.6°, and 128.2° at 15, 30 and 60 days after surgery, respectively) and increased the distal interphalangeal joint angle (Increase from a mean value of 180.2° at baseline to 199.3°, 196.4°, and 197.3° at 15, 30 and 60 days after surgery, respectively). Desmotomy decreased the proximal interphalangeal angle (Decrease from a mean value of 172.6° at baseline to 170.3°, 169.6° and 167.6° at 15, 30 and 60 days after surgery, respectively). The surgical procedure did not change the radio-metacarpal joint angle. Both procedures produced hoof deformation (shorter and wider hoofs with taller heels). After tenotomy, the hoof angle was even higher (Increase from a mean value of 48.4° at baseline to 49.4°, 50.7° and 51.8° at 15, 30 and 60 days after surgery, respectively). Details of the results are on table 1. We concluded that superficial digital flexor tenotomy decreases the metacarpophalangeal joint angle and increases the distal interphalangeal joint and the hoof angle. The superior check ligament desmotomy decreases the proximal interphalangeal joint angle. Both surgeries do not influence the radio-metacarpal joint angle and results in wider, shorter hoofs with taller heels.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Pre-operative</th>
<th>15 days</th>
<th>30 days</th>
<th>60 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>RMA - right</td>
<td>172.5 ± 2.56°</td>
<td>168.9 ± 3.53°</td>
<td>168.8 ± 3.48°</td>
<td>169.8 ± 3.50°</td>
</tr>
<tr>
<td>RMA - left</td>
<td>172.1 ± 2.67°</td>
<td>171.4 ± 3.48°</td>
<td>172.2 ± 3.58°</td>
<td>174.1 ± 3.52°</td>
</tr>
<tr>
<td>MPA - right</td>
<td>140.7 ± 6.85°</td>
<td>126.9 ± 5.45°</td>
<td>126.6 ± 5.50°</td>
<td>126.2 ± 5.38°</td>
</tr>
<tr>
<td>MPA - left</td>
<td>140.2 ± 6.75°</td>
<td>136.8 ± 5.72°</td>
<td>137.5 ± 5.46°</td>
<td>140.0 ± 5.49°</td>
</tr>
<tr>
<td>PIA - right</td>
<td>172.8 ± 5.70°</td>
<td>168.3 ± 4.92°</td>
<td>169.4 ± 3.78°</td>
<td>169.1 ± 3.85°</td>
</tr>
<tr>
<td>PIA - left</td>
<td>172.6 ± 5.87°</td>
<td>170.4 ± 4.14°</td>
<td>170.6 ± 3.79°</td>
<td>167.6 ± 3.54°</td>
</tr>
<tr>
<td>DIA - right</td>
<td>188.2 ± 2.90°</td>
<td>189.2 ± 3.78°</td>
<td>189.4 ± 3.65°</td>
<td>187.4 ± 3.46°</td>
</tr>
<tr>
<td>DIA - left</td>
<td>182.9 ± 3.81°</td>
<td>183.8 ± 3.76°</td>
<td>186.3 ± 3.78°</td>
<td>185.2 ± 3.57°</td>
</tr>
</tbody>
</table>

Table 1: Means of the different parameters on the moments, with the standard deviation and statistical analysis results. (RMA - radio-metacarpal angle; MPA - metacarpophalangeal angle; PIA - proximal interphalangeal angle; DIA - distal interphalangeal angle; T - toe; H - heel; L - hoof length; W - width; A - hoof angle).

For each limb, means followed by the same capital letter do not differ from each other. For different limbs (right or left), means followed by the same small letter do not differ from each other (p<0.05).
EVALUATION OF STRESS IN HORSES IN TRAINING FOR TO PARTICIPATE IN ENDURANCE

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1 Faculty of Veterinary Medicine, University of Concepción, P.O.Box 537, Chillán
2 Equitation School, Quillota. Chile

The horses participate in different sports, being necessary that they have a good training that increases the muscular capacity the physiological structures and increase of the oxidative metabolism, specially when participate in competitions of endurance and do not have risks for your healthy.

The effects of exercise on the heart, blood lactate and other blood variables were reported in different studies, but current knowledge concerning the hormonal responses to exercise performed by horses is not numerous, and the determination of cortisol is one of them. The plasma cortisol levels show diurnal variations, the highest levels being measured in the morning between 6.0-9.0 am, and the lowest between 6.0-9.0 pm and hours of obtaining the sample is important.

The objective of this work was to evaluate the effect of exercise on the hemogram and the cortisol in mixed thoroughbred horses selected for to participate in endurance competition.

Materials and methods
Nine horses between 6 and 9 years old, clinically healthy were selected for this investigation.

The horses were training during three months (M1, M2, and M3) and they had the first evaluation after 3 months of training in field conditions.

The first and second evaluations were performed after galloping at 6 m/seg., during 15 minutes. The third were performed to galloping 75 minutes at 6 m/seg. During 70 minutes, all the evaluations were made in field conditions.

Samples blood of jugular vein to determine hemogramme and serum cortisol concentration were obtained at basal conditions and five and fifteen minutes after exercise.

All the evaluations were made between 9.0-12.0 am for standardized the samples for to determine the cortisol.

The hemogramme was made in a hematological counter and serum cortisol was determined by radioimmune assay.

Average, standard deviation and variance analysis were obtained for to determine significant variations of changes between each test and between months.

Results and discussion
The results show (Table 1) that the exercise produce significant variations (p < 0.05) of red blood cells, hematocrit and hemoglobin, during the test. The increase of them is product of adrenergic stimuli in the spleen; increase the red blood cells in circulation in about 30%. This increase of the red cells is beneficial because the capacity oxidative of the tissues is better and the utilizations of substrates by the metabolic pathways of the skeletal increase during the exercise. Furthermore others works reported that training result in a rapid increase of plasma volume.

<table>
<thead>
<tr>
<th></th>
<th>E x 10^6</th>
<th>Hto (%)</th>
<th>Hb (g/dl)</th>
<th>Le. x 10^7</th>
<th>Cortisol (µg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>M1-T0</td>
<td>8.21±0.66</td>
<td>36.4±3.17</td>
<td>14.26±1.2</td>
<td>7.02±1.65</td>
<td>2.68±1.35</td>
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<tr>
<td>M1-T5</td>
<td>10.98±2.4</td>
<td>50.5±11.5</td>
<td>18.90±3.6</td>
<td>8.76±1.64</td>
<td>5.5±1.67</td>
</tr>
<tr>
<td>M1-T15</td>
<td>8.02±2.7</td>
<td>41.2±2.43</td>
<td>15.8±1.07</td>
<td>8.29±1.47</td>
<td>6.25±1.79</td>
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<tr>
<td>M2-T0</td>
<td>9.20±1.16</td>
<td>39.8±5.51</td>
<td>14.6±1.66</td>
<td>7.93±1.03</td>
<td>3.05±1.14</td>
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<tr>
<td>M2-T5</td>
<td>11.2±0.89</td>
<td>49.2±2.30</td>
<td>19.1±0.74</td>
<td>9.12±0.86</td>
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<td>M2-T15</td>
<td>8.82±0.72</td>
<td>42.7±2.87</td>
<td>15.6±0.83</td>
<td>8.88±1.09</td>
<td>6.22±1.56</td>
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<td>M3-T0</td>
<td>7.87±0.72</td>
<td>33.4±2.6</td>
<td>13.6±3.1</td>
<td>6.23±1.61</td>
<td>3.44±0.61</td>
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<td>M3-T5</td>
<td>9.64±0.76</td>
<td>41.6±4.54</td>
<td>18.9±11.5</td>
<td>6.71±1.7</td>
<td>8.09±1.41</td>
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<td>M3-T15</td>
<td>8.25±0.7</td>
<td>35.3±4.05</td>
<td>14.0±4.34</td>
<td>6.28±0.81</td>
<td>6.8±1.16</td>
</tr>
</tbody>
</table>

Table 1.- Values of red blood cells (E), hemoglobin (g/dl), hematocrit (%), total leukocytes (Le), and cortisol (ºg/dl) in horses in training to participate in endurance competitions. (X±DE)
Fifteen minutes post exercise there are a decrease of red blood cells, however, are not similar at normal values. After exercise the red cells return al normal values between 30-60 minutes post exercise.

Leukocytes increase at 5 minutes post exercise and at 15 minutes return at the normal values, these increase it is relation with neutrophyles blood, the increase of leucocytes neutrophyles could be relation with the increase of neutrophyles.

Cortisol increase significant at the five minutes to finish the exercise and increase at fifteen minute post exercise. This increase is more significant than the horses galloping during 75 minutes and they is relation with the stress by the mayor intensity in this test. These results are similar at obtained in jumping horses, which present an increase of cortisol after exercise.

The results showed that the exercise produce significant changes in the hemograme and cortisol in the horses.

Acknowledgement

Grant FONDECYT 1040 415
HOW USEFUL IS TETANUS ANTITOXIN IN THE TREATMENT OF EQUIDAE WITH TETANUS?

A COMPARISON OF THREE TREATMENT PROTOCOLS USED IN THE MANAGEMENT OF 56 CASES OF EQUINE TETANUS PRESENTED TO THE SPANA CLINICS IN MOROCCO IN 2003/2004

Kay G
Technical Director of SPANA Society for the Protection of Animals Abroad Morocco 1999-2005

Introduction

Tetanus is a distressing and often fatal disease caused by a protein exotoxin with three components liberated by the bacteria Clostridium tetani of the family Bacillaceae. Equidae are particularly susceptible to the tetanus exotoxin (Radostits O, Blood D, Gay C 1994). It generally gains entry to the body via wounds. In Morocco, where all working equines are hobbled, pastern lesions from ill fitting or ill designed hobbles, are one of the major sites of entry.

Tetanus is considered enzootic in many countries in the developing world where vaccination programs for both man and equidae are not yet established. In Morocco tetanus is a major cause of death amongst horses, donkeys and mules. In countries where equidae play a key role in the rural economy and where the welfare of many families is intimately linked to the welfare of their draught animal, the prevention and treatment of this disease is an important issue. During an 18 month period between 2003 and 2004 SPANA (Society for the Protection of Animals Abroad) hospitalised 56 cases of equine tetanus and successfully treated 26 (approx 46%). The rest died or were euthanased on humane grounds.

The aims of treatment focus on the elimination of the source of the toxin, the neutralisation of any unbound toxin, the establishment of antitoxin immunity and the control of neuromuscular derangements. The use of tetanus antitoxin in the neutralisation of unbound exotoxin forms an important part of the treatment protocol but published dosages and routes vary widely and evidence on which to base therapy is scarce. Recommended doses range from a single administration of 5000 iu/animal to 2.5 million iu/animal followed by lower doses over five days. The economic implications of using high doses of antitoxin without evidence to indicate the increased chance of a more favourable outcome are important.

In an attempt both to establish a cost/benefit ratio to the use of high dose tetanus antitoxin, and to evaluate any prognostic indicators, SPANA conducted a clinical trial over an 18 month period in 2003 and 2004.

Equidae hospitalised for tetanus in this period were treated with one of three different dose rates of tetanus antitoxin, ranging from the first group who received none at all (14 animals), the second group who received anything from 1000IU to 39000IU IV over 1-3 days (17 animals) to the third group, which each received 50,000IU IV over two consecutive days (25 animals). There was no association between treatment group and outcome (P>0.8). This suggests that tetanus antitoxin may not be beneficial or economically justifiable in the treatment of tetanus in working animals in the developing world.

Materials and methods

Most of the equidae that presented to SPANA clinics in Morocco with Tetanus during the 18 months from Jan 2003 to June 2004 were included in this case series. Only those presenting in a terminal state were excluded. The diagnosis was made on clinical signs only. Other common differential diagnosis that had to be ruled out in each case included rabies, equine exertional rhabdomyolysis, West Nile Fever, and musculoskeletal trauma.

The clinical presentation was assessed for each case and details of age, sex, species, wound location, duration and severity of clinical signs at presentation, treatment protocol, number of hospitalised days, the outcome and any long term effects were recorded for each. Each case was allocated a 'clinical score' based on the following criteria as assessed at the time of presentation:

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1. Mild clinical signs (ie. slightly stiff gait but still walking and eating without difficulty).

2. Moderate clinical signs (ie. limbs stiff and walking with difficulty, trismus and generalised muscle spasm, animal still capable of eating and drinking).

3. Severe clinical signs (ie. Capable of maintaining an upright posture but incapable of walking, difficulty eating)

4. Terminal clinical signs (ie animal recumbent, incapable of eating). Animals presenting in this situation are considered candidates for immediate euthanasia and were not included in this series of cases.

Cases were assigned to one of three categories depending on the dose of tetanus antitoxin they received.

1. No dose: 14 cases
2. Low dose: 1000 - 39000IU - 17 cases
3. High dose: 50,000 IU - 25 cases

All animals were treated with a standard protocol consistent with those described in the literature. They all received 15000UI/kg procaine penicillin IM BID or TID (benzyl penicillin is not available for use in equidae in Morocco) for a minimum of 7 days. All cases received acepromazine 0.05mg/kg - 0.08mg/kg IV. Cases with symptoms that could not be controlled by this regime were treated with Diazepam. Wounds were systematically cleaned, meticulously debrided and subjected to copious lavage at 50psi.

None of the animals in this series had been previously vaccinated against tetanus.

Results

Of the 56 equidae in this series, 26 (46%) survived and 30 (54%) were euthanased or died.

A logistic regression model looked at survival as the outcome and age, wound position, clinical grade 1,2 vs 3, species, and dosage of tetanus antitoxin (TAT) by group. The results confirmed a strong association between survival and clinical grade at presentation (P=0.001) whilst the effect of species, age, wound position and dosage were not significant (P>0.4).

The survival rate by TAT dosage group is presented below.

<table>
<thead>
<tr>
<th>No TAT</th>
<th>Low dose</th>
<th>High dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of animals</td>
<td>14</td>
<td>17</td>
</tr>
<tr>
<td>Survival rate</td>
<td>50%</td>
<td>42%</td>
</tr>
</tbody>
</table>

Table 1 Survival rate associated with different doses of TAT

Discussion.

The mortality rate in this series was 54%. This compares favourably with rates from previous case series, (75% reported by Green et al in a series of 20 cases and 66% reported by Steinman et al in a series of 3 cases) The prognosis for survival is reported to depend on several factors; the immune and vaccination status of the host, the dose of clostridial inoculation, and the duration and availability of aggressive treatment and supportive care (Green et al). Green reported an association between survival and previous prophylactic vaccination with tetanus toxoid (P=.03) and stated that none of the nonvaccinated horses in their series of cases had survived.

We would have expected a higher mortality rate in this series given
- the negative vaccination status of all the animals.
- the poor immune status of most of the animals, (the majority of the animals in Morocco are in poor to very poor body condition and parasite control is nonexistent)
- that most patients were admitted more than 48 hours after onset of clinical signs and 12 were admitted 4 days or more after onset of clinical signs. No previous treatment had been administered in any of theses cases.
This relatively low mortality rate is puzzling given the poor vaccination and immune status of the animals in this series of all the clinical factors that were recorded for this series of cases the only one which has a clear association with prognosis is the severity of clinical signs (Grade 3) where the survival rate was 6%. (P<0.001). The apparent lack of association between dosage of tetanus antitoxin and outcome is important for clinicians in resource limited situations, and has dictated the protocol used by SPANA veterinarians in the treatment of equidae with tetanus.

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SEVERE OUTBREAK OF INFECTIOUS EQUINE ABORTION IN MOROCCO: EPIDEMIOLOGICAL, PATHOLOGICAL AND VIROLOGICAL FINDINGS

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Introduction

Infectious equine abortion or viral equine abortion is a worldwide distributed and naturally occurring late-time abortion of horses. It is due to equine herpesvirus type 1 (EHV1), a member of Varicellovirus genus in the Alphaherpesvirinae family, the causative agent of equine rhinopneumonitis. Beside abortion and rhinopneumonitis, EHV 1 may result in a severe fatal encephalomyelitis and constitutes a continuous threat to equine production. In this paper we present the epidemiological, pathological and virological findings of a severe outbreak of infectious equine abortion that occurred in the area of Rabat-Morocco during the spring of 2005.

Material and methods

Among affected equine herds, one herd with 6 abortion cases was subjected to further investigations. They all occurred at late gestation between the 8th and the 11th month of gestation without any complication for the mares. In this herd, which was regularly vaccinated against influenza and rhinopneumonitis, three aborted foals were necropsied and tissue samples including liver, lung, intestine, myocardium and placenta were submitted for histopathological, bacteriological and virological examination. Tissues samples were cultured into Mc Conkey Agar plates and incubated at 37°C. Isolated bacteria were identified according to standard bacteriological procedures. Pieces of organs were homogenized with MEM supplemented in antibiotics and centrifuged at 1200g during 10 minutes. The supernatants were stored at -20°C before virus isolation. The virus was isolated from organs of aborted foal by inoculating the homogenized and filtered tissue supernatants to recently prepared RK13 cell monolayers, at the concentration of 2 x 105 cells/ml in MEM medium supplemented with 2 % fetal calf serum, streptomycin 100 µg/ml and penicillin 100 UI/ml. Flasks were incubated at 37°C for 7 days and examined daily for the appearance of a cytopathic effect (CPE), characteristic of a virus infection. Such an effect may generally occur after one blind passage on RK13 cells. The confirmation of the virus identity was performed by virus neutralization test (VNT) in flat-bottom 96-well microtitre plates, using a constant dose of specific EHV1 antiserum and a 10 fold dilutions of the isolated virus. Fixed tissue samples in 10% buffered formalin were processed according to standard method. The tissues were dehydrated in alcohol and embedded in paraffin wax. Five µm thick sections were stained with hematoxylin and eosin and examined by light microscopy. Specific antibodies anti-equine herpesvirus (EHV) were determined on collected sera from affected mares by virus neutralization test (OIE Manual of Standards for diagnostic tests and vaccines, 1996).

Results

Main gross lesions included, jaundice, congestion and edema of the lungs, multiple grayish pinpoint necrotic foci in a congested liver, and congestion of the intestinal mucosa and the placenta. Microscopic changes in the lungs consisted of a fibrino-leucocytic alveolitis and bronchiolitis associated with the presence of acidophilic intra-nuclear (IN) inclusion bodies within hyperplasic bronchiolar epithelial cells. In the liver these changes consisted of an acute necrotizing hepatitis and the presence of acidophilic IN inclusion bodies within hepatocytes. The severity and distribution of the above described gross and microscopic findings which, were consistent with the diagnosis of equine rhinopneumonitis, are depicted in table 1. Sera collected from affected mares showed high titers of antibodies anti-equine herpesvirus (EHV). Klebsiella was isolated from intestinal content of one aborted foal. EHV1 was isolated for the first time in the country, from filtered samples of liver and spleen on RK13 cells line after 2 passages. The virus showed a typical cytopathic effect on cells and serotyping has been performed by virus neutralization test using a reference EHV1 antiserum.
Discussion

Although they have never been reported in the literature, rhinopneumonitis-related sporadic abortions were previously diagnosed in Morocco but never occurred as the epizootic abortions described here in. Based on clinical and epidemiological data, histopathological examination, virological isolation and serology, the cause of this outbreak of abortion was attributed to EHV 1. Furthermore, this paper represents the first report of the abortive form of equine rhinopneumonitis linked to EHV1 in Morocco. There are two EHV types, EHV-1 and EHV-4, which are known to be responsible for "viral equine rhinopneumonitis". Although these viruses share several laboratory and clinical features, they differ in epidemiology and pathogenic potential. EHV-4 is considered to be primarily associated with clinical respiratory disease, whereas EHV-1 is more frequently associated with abortions and neurological disease in foals worldwide. The isolation of Klebsiella from the intestinal content of one aborted fetus may be considered as an associated abortive agent in that case since this bacteria is one the most known causes of bacterial abortion in mares. The occurrence of this outbreak of rhinopneumonitis despite vaccination suggests failure of the vaccine used or the performed vaccination program to protect against the disease. It has been shown that vaccination alone can not fully protect EHV-infected mares from abortion, the implementation of good management and hygiene practices are key elements in reducing the risk of equine viral abortion. Since not all occurring equine abortions in Morocco are notified and no laboratory investigations are systematically performed, the prevalence of the disease countrywide is not known. Moroccan veterinary equine practitioners should be encouraged to notify and investigate abortions in equines which will allow to better document the incidence of this pathology among equine herds in Morocco.

<table>
<thead>
<tr>
<th>Lesions</th>
<th>Aborted foals (# &amp; age)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 (9 months)</td>
</tr>
<tr>
<td>Jaundice</td>
<td>+</td>
</tr>
<tr>
<td>Edema of subcutis and body cavities</td>
<td>+</td>
</tr>
<tr>
<td>Hypoaeomia and oedema of the lungs</td>
<td>++</td>
</tr>
<tr>
<td>Multiple grayish pinpoint necrotic foci in a congested liver</td>
<td>-</td>
</tr>
<tr>
<td>Hypertrophy of spleen and subcapsular petechiations</td>
<td>±</td>
</tr>
<tr>
<td>Hypoaeomia of the intestinal mucosa</td>
<td>+</td>
</tr>
<tr>
<td>Hypoaeomia and hemorrhage of placenta</td>
<td>-</td>
</tr>
<tr>
<td>Microscopic</td>
<td></td>
</tr>
<tr>
<td>Lungs</td>
<td></td>
</tr>
<tr>
<td>Hyapaemia and edema of the interalveolar septa</td>
<td>++</td>
</tr>
<tr>
<td>Fibrino-leucocytic and necrotizing alveolitis and bronchoitis</td>
<td>++</td>
</tr>
<tr>
<td>Acidophilic IN inclusions bodies within bronchiolar epithelial cells</td>
<td>++</td>
</tr>
<tr>
<td>Infiltration of portal spaces with mixed inflammatory cells</td>
<td>+</td>
</tr>
<tr>
<td>Liver</td>
<td></td>
</tr>
<tr>
<td>Micro foci of hepatic necrosis</td>
<td>++</td>
</tr>
<tr>
<td>Acidophilic IN inclusions bodies within hepatoocytes</td>
<td>-</td>
</tr>
</tbody>
</table>

Tableau 1: severity and distribution of gross and microscopic lesions in necropsied aborted foals
- : absent, ±: discrete, + present, ++: severe, +++: very severe
**INFORMATION AND COMMUNICATION TECHNOLOGIES IN EDUCATION (ICTE) FOR TEACHING EQUINE MEDICINE & SURGERY**

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**Introduction**

Information and Communication Technologies in Education (ICTE) can be used for Teaching with the goal of training students through interactive concepts based on theoretical or practical situations. ICTE include technologies needed for PowerPoint presentation with or without interactive vote, QCM on line, virtual classes and web-conferencing. In these last two cases teachers and students meet together at a same time, in a same place, except that the place is on the Web. It means that participants can be at different locations anywhere in the world. ICTE also include a teaching platform which is a protected site accessible on the Web by a login and an identified password where the student will find constantly personalized knowledge. Integration of these technologies at the Equine Department of the Veterinary School of Lyon is under evaluation since 5 years in teaching equine surgery at four different level of specialisation.

**Material and methods**

New constructions in the Equine Clinic include the concept of new interactive materials (camera, microphone, computer...) placement in four locations (1) the place of action (e.g. surgery room), (2) an adjacent teaching gallery, (3) distanced teaching rooms or theatres, (4) office of the teachers and post-graduate students (Fig. 1)

The teaching project is based on a regional and international cooperation between Equine and ICTE

(Fig. 1 - Interactive Equine Clinic)

Units of the Veterinary School of Lyon and the Faculty of Veterinary Medicine of Montreal.

This cooperation permitted to incorporate ICTE at four different level of teaching:

**Level 1**: power point presentations with interactive vote in undergraduate student teaching of equine medicine and surgery ; Using interactive vote during lectures gives the teacher the possibility to adjust his teaching and the students a way of auto-evaluation. (Fig. 2 et 3)
Level 2: discussion of clinical cases between Interns of both institutions through virtual classes (Fig. 4),

Level 3: surgery journal club through virtual classes and QCM dedicated to residents,

Level 4: web-conferencing to follow residents or graduate MS or PHD students with their research projects

Results and conclusion

Limitation of teaching concepts with ICTE is a need for computer and Internet access and managing simultaneous connections, taking into account time shifts. Webconferencing save time and travelling expenses especially when students and research promoters works at 2 or more sites. These technologies potentiates investment in teaching by multiplying the usual audience and supporting national and international cooperation. ICTE are very promising tools for teaching equine medicine and surgery and they foster new perspectives between training centres and veterinarians in Europe or anywhere in the world. For example in 2005 long distance selection of SPANA interns was easily and successfully performed between Rabat (Morocco) and Lyon (France).
PHENOTYPIC AND MORPHOMETRIC CHARACTERISATION OF BARBS AND ARAB-BARBS HORSES IN MOROCCO
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Morocco constitutes with the Maghreb countries the cradle of the Barb horse. The latter, one of the oldest equine breed in the world, with exceptional qualities, is paradoxically very little represented within the equine population officially recognized in Morocco and therefore less known.

However, during the last five years, this breed attracted a renewed interest as well by the National Stud farms as well as research institutions.

Thus, actions of promotion and encouragement were structurally initiated by the National Stud Farms in partnership with the National Association of the Barb Horses Breeders.

Genetic studies, carried by the Veterinary Genetic Analyses Laboratory at the Hassan II Institute of Agronomy and Veterinary Medicine, contributed to a better knowledge of this population of horses.

This work was aimed:

1. to determine morphological elements characterizing the Barb and to compare them with the official standard established by the World Organization of the Barb Horse.
2. to contribute to set up a standard of the Arab-Barb horses.
3. to characterize morphologically the population of horses known as of RNC "non determined breed" and to assign them to the one of the known breeds in Morocco.

495 horses originating from various breeding areas (mountainous, coastal and eastern zones) were used according to the following breakdown:

<table>
<thead>
<tr>
<th>Breed</th>
<th>Sex</th>
<th>Barb</th>
<th>Arab-Barb</th>
<th>RNC</th>
<th>Arab</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td></td>
<td>16</td>
<td>118</td>
<td>89</td>
<td>20</td>
<td>243</td>
</tr>
<tr>
<td>Female</td>
<td></td>
<td>14</td>
<td>132</td>
<td>77</td>
<td>29</td>
<td>252</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>30</td>
<td>250</td>
<td>166</td>
<td>49</td>
<td>495</td>
</tr>
</tbody>
</table>

The study related to the morphological aspects of the horses dealt with a certain number of measurements, carried out using the metric stand and ribbon and indices which make it possible to draw up a morphological profile for these horses (figures 1 and 2).
"the size or height at the withers (HG), the height at the croup (HC), the overall length or scapulo-ischial length (LT), the canon's length (LC), the height to the elbow level (VSS), the length of the neck (LEnc), the length of the head (LTe), the distance between the interior angles of eyes (DAIY), the chest circumference (TP), the neck circumference (TEnc), the forearm circumference (TAB), the knee circumference (TG), the circumference of the frontleg canon (TCA) and the circumference of the fetlock (TB). The liveweight (P), the body index, the index of compactness, the index of stoutness, the pectoral height index and the thoracic typist index”.

The data obtained from the sampled horses (all breeds combined) were initially analyzed by ANOVA test in order to determine effects of age, sex and breeding area, then by discriminating factorial analysis in order to highlight the differences between breeds. Finally decisional factorial analysis was used to assign horses known as RNC to one of the studied breeds.

The results obtained showed that the Barb horse may be described as a medioline horse, of average format, square head and a rather thick neck. Its average size is 154.46 ± 4.6 cm. It has a swallowed croup, a slightly convex cephalic profile, a powerful canon bone and large joints. The dominant colors are the bay, the chestnut and the gray. This description fits perfectly in the official standard of the Barb recorded by the OMCB.

The Arab-Barb can be described as a medioline horse, of average format and a square head. Its average size is 153.43 ± 5.97 cm. The dominant cephalic profile is the straight one and the croup presents a swallowed form. The Arab-Barb breed presents powerful canon bone and bulky articulations. The dominant colors are the bay, the chestnut horse and the gray. The blood ratio from Arab origin influences markedly the morphology of this horse. The discriminating and decisional factorial analysis carried out allowed assignment of RNC horses as follows (figure 3):
16 horses were assigned as belonging to the Barb breed, 7 with to the Arab breed and 143 with to the Arab-Barb breed. The results obtained made it possible to check that Barb and Arab-Barbs horses studied fit in the official standard of the OMCB.

The statistical tests showed a certain similarity between the Barbs and Arabs Barbs on the one hand, and between the latter and RNC horses. Arabian horses, introduced into the study to be used as a basis of comparison, are distinguished easily from the horses of the other breeds. In addition, sex, breeding area, service and the blood ratio from Arab origin influence the morphology of the horses.

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RADIOGRAPHICAL MEASUREMENTS OF FRONT FEET OF THE SOUND AKHAL-TEKE HORSES WITH RELEVANCE TO LAMINITIS

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Introduction

The Akhal Teke is an ancient horse originating from Asia where it was used by nomadic tribes as a war horse. The Akhal-Teke is among the most elegant of the world's horses. The conformation of this horse breed can be favorably compared to the Persian Arab, another breed of ancient origin. Laminitis is failure of the attachment between the distal phalanx and the inner hoof wall because of inflammation of the laminae within the hoof. Laminitis poses a significant threat to all domestic equidae. The normal radiological anatomy and measurements of the feet of normal Thoroughbred, Hanoverian, Pony and Arab horse races with relevance to laminitis have been described, but no study was found about Akhal-Teke horses. The objective of this study was to determine the radiological measurements of the third phalanx and hoof of sound Akhal-Teke horses.

Materials and methods

10 clinically sound and certified Akhal-Teke horses from a stable in the east of Tehran-Iran including 5 males and 5 females were selected. Lateromedial radiographs of hoof region of the right and left forelimbs using fixed exposure factors were obtained. Thickness of hoof wall at three levels (A, B and C), PCL: palmar cortical lengths of third phalanx (P3), D founder: perpendicular distance between extensor process of P3 and coronary band, CF founder: perpendicular distance between extensor process of P3 and frog corium, S founder: perpendicular distance between solar border of P3 and deepest point of solar surface in front of frog, angle H: hoof axis (angle S) minus P3 axis (angle T), angle R: P3 axis minus second phalanx (P2) axis (angle U), angle P: the angle between the lines through the palmar cortical and dorsal surface of P3 and several other important characters were measured on each radiograph (figure 1).

Results

The mean ± SD of some of the measured characters is shown in table 1.
Discussion

This study introduced S founder, CF founder and P angle as important criteria in evaluating laminitis and sinking of P3. Increased hoof wall thickness is the first radiographic sign of acute laminitis. The normal hoof wall thickness of Akhal-Teke horses were similar to previous reported studies in some of other horse breeds (e.g. Thoroughbred and Hanoverian). The results of this study can be used as a reference for radiological evaluation of laminitis and founder in Akhal-Teke horses.

<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (mm)</td>
<td>18.59</td>
<td>1.23</td>
</tr>
<tr>
<td>B (mm)</td>
<td>18.37</td>
<td>1.22</td>
</tr>
<tr>
<td>C (mm)</td>
<td>18.01</td>
<td>1.04</td>
</tr>
<tr>
<td>PCL (mm)</td>
<td>64.32</td>
<td>3.37</td>
</tr>
<tr>
<td>D founder (mm)</td>
<td>6.24</td>
<td>2.93</td>
</tr>
<tr>
<td>CF founder (mm)</td>
<td>48.65</td>
<td>5.96</td>
</tr>
<tr>
<td>S founder (mm)</td>
<td>10.77</td>
<td>3.65</td>
</tr>
<tr>
<td>Angle H</td>
<td>0.42</td>
<td>2.58</td>
</tr>
<tr>
<td>Angle R</td>
<td>-0.2</td>
<td>5.36</td>
</tr>
<tr>
<td>Angle P</td>
<td>36.87</td>
<td>2.48</td>
</tr>
</tbody>
</table>

No statistical difference could be found between right and left front feet measurements.
DIAGNOSIS OF RETINAL DETACHMENT ASSOCIATED WITH CATARACTS BY USING ULTRASONOGRAPHY IN SEVERAL HORSES IN IRAN

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Introduction

Ocular ultrasonography is a valuable diagnostic tool because it allows evaluation of the interior of the eye which may be obscured from direct visualization by any disease that causes ocular opacity. Four major ocular acoustic echoes are noted within a normal eye: anterior cornea, anterior and posterior lens capsules, iris and ciliary body, and retina-choroid-sclera complex. The optic disc is seen as a hyperechoic area at the back of the globe, from where the hypoechoic optic nerve may be identified running to the back of the orbit. Anterior chamber, lens cortex, lens and vitreous are anechoic. Cataract is any opacity, of any size, involving the lens or its capsule. Cataract can be uni or bilateral. Retinal detachment can be congenital or secondary to trauma, inflammation, neoplasia or hypertension.

Case history

Three horses were referred to the Veterinary Teaching Hospital, Faculty of Veterinary Medicine, University of Tehran-Iran, with a history of trauma to the one eye. Ophthalmoscopic examination revealed cataracts but evaluation of posterior structures to anterior lens capsule was impossible. Ultrasonography was achieved with a 7.5 MHz linear-array transducer and B-Mode real-time technique. Transpalpebral method through upper eyelid and applying acoustic gel was used to examine the eyes. Transverse and longitudinal scan planes were taken.

Results

Ultrasonography showed echodense linear structures resulted in classic funnel appearance of vitreous in one case and convex hyperechoic linear density of the back of globe in two cases. Other findings were including decreased anterior-posterior diameter of the globe (37 mm compared to the 42 mm in contralateral normal eye) and ultrasonographic signs of cataracts. Ultrasonographic findings were characteristic of total retinal detachment in one case (Figure 1) and partial retinal detachment in two cases. There is no effective treatment for retinal detachment in horses.
Clinical evaluation of intravenous administration of sodium hyaluronate in experimentally-induced arthritis in horses

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Hyaluronate (HA) is a major component of synovial fluid in joints. This molecule is composed of highly polymerized nonsulfated glycosaminoglycans, including glucuronic acid and N-acetylglucosamine. Intrarticular administration of HA is commonly used for treatment of human osteoarthritis to relief clinical symptoms for several weeks or months, which is believed to be acting as lubricant. In equine practice, intravenously-injectable HA is available for clinical use, which is likely to be as effective as intrarticular HA. Effects of intravenous HA may not be as direct lubrication of osteoarthritic joints as those of intrarticular HA. The purpose of the present study is to evaluate the effects of intravenous HA to synovial HA concentration and synovial membrane.

Materials and methods

8 clinically normal horses, 4 to 8 year of age, were randomly assigned for two groups; one was the group (HA group) consisting of horses with intravenous HA administration and another was the control group (Control). Experimental arthritis was induced in all 8 horses by the intrarticular injection of chymopapain (15mg/joint) into unilateral carpal joints under local anesthesia and sedation. In HA group, HA (40 mg) was administrated intravenously on days 7, 14 and 21 after arthritis induction. Blood and synovial fluids were aseptically corrected from carotid artery and bilateral carpal joints, respectively, on days 0, 3, 7, 10, and 17. Blood examination included white blood cell count (WBC), serum total protein concentration (serum TP), albumin/globulin (A/G) ratio were done. Examination of synovial fluids consisted of total protein concentration (synovial TP) and nucleated cell count (NCC). HA concentration in both serum and synovial samples were measured by sandwich enzyme-linked immunosorbent assay. At each time of blood and synovial sample correction, physical conditions and lameness in horses were also assessed. In each group of horses, half of experimental animals were sacrificed on day 17, while the rest were done on day 24. Articular tissue specimens were then collected for histopathological evaluation. Entire experimental procedure of this study was done in accordance of the guidelines of the Animal Care Committee of Graduate School of Veterinary Medicine, Hokkaido University.

Results

Horses treated with sequential HA intravenously seemed to have lower scores of lameness (less lame than the control) ones on days 17 and 21. WBC, serum TP and A/G ratio in both groups were within normal limits in entire period of examination. No significant differences were found between two groups, while transient increases were noted immediately after intraarticular administration of chymopapain in all treated horses. Serum and Synovial HA concentrations in HA group were seemed to be less affected by chymopapain injection in comparison with Control group. Histopathological examination focused on inflammation in synovium, cell proliferation of synovial surface, and changes of capillary circulation and glycosaminoglycan contents in synovium. Histological scores of synovium in intravenous HA treated horses were likely to be better in morphology of synovium than those in untreated controls. Accumulation of endogenous HA was evident in synovium of horses treated exogenous HA, which was administrated intravenously.

Discussion and conclusion

These results suggested that intravenous administration of HA appeared to restore synovial HA concentration and to relief clinical symptoms related to experimental inflammatory arthropathies. No adverse reactions related to intravenous injection of HA were revealed in blood and histological analysis in the present study, while encouraged HA production from inflamed synovium was evident. Degradation of synovial HA induced by inflammatory reaction in joints related to chymopapain injection might be restored by endogenous HA production in synovium. Exogenous, intraarticular injected HA might act as a mediator interacting to synoviocytes, which are responsible for regulation of synovial HA concentration in arthritic horses.
THE PREVALENCE E/H OF HORSES DURING 2001-2004, IN ROMANIA

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Institute for Diagnosis and Animal Health, Bucharest, Romania

The paper work contains the investigations results made between 2001-2004 on 23,170 sacrificed horses in the slaughter-houses from 26 districts for detecting the animals infected with E/H.

There have been detected 3,042 horses (13,12%) infected with E/H at the national level, out of which 2,719 (89,38) were from Teleorman and all the rest were from districts: Covasna, Harghita and Timiș.

In other districts (22) was not recorded any E/H case. It is very important to mention that in Teleorman district the horses infection percentage with E/H has recorded increased in years 2001, 2002 (24% and 24,53%); in 2003 has been recorded a 6 % decrease and in 2004 the infection percentage decreased to 3,65.

In Covasna and Harghita districts the infection the infection percentages in 2004 somewhere around 13 % (Covasna), 9 % (Harghita) and Timiș district the infection with E/H was below 1% in 2002 and 2003 and in 2004 no case been recorded.

Material and method

The remarking and interpretation of the statistical data taken from the veterinary office in the districts.

Results and discussions

According to the data we have between 2001 and 2004, 23,170 horses were sacrificed at national level, from 26 districts. From this, 3,042 were infected with E/H (13,12%) and from this 2,719 horses (89,38 %) come from district Teleorman and the others from: Covasna, Harghita and Timis. In the others 22 districts we didn't register any cases of E/H (table 1).

In the annual analysis of the infection percentage with E/H of horses during 2001-2004 we found:

- In year 2001 there have been sacrificed horses only from Teleorman district and the infection percentage of horses E/H has been of 24%;

- In 2002 there have been sacrificed horses in 22 districts and the annual infection percentage with E/H has been minor in comparison with the year before; 19,48% (table 2). In this year (2002) all cases with E/H infection horses have been in Teleorman district (24,53%) and Timis (0,37%), we notice in fact that in Teleorman district the percentage has been a lightly higher in comparison with 2001 year.

- In 2003 there have been sacrificed horses in 10 districts and the annual percentage of infection has decreased with 10 percent in comparison with year 2002 (table 3). We notice in fact that E/H cases of horses infection were registered also in Teleorman (18,20%) and Timis (0,16%) but the percentage has been smaller in comparison with year 2002.

- In 2004 the E/H cases of horses infection have been registered in Teleorman district also (3,6%) where the percentage of infection has been a couple of times smaller than while in Covasna district the percentage was of 12,76% and in Harghita 8,78%. Although horses have been sacrificed in the previous years in these districts in years 2002-2003, no E/H infection cases for horses have been detected until 2004.

We present in figure 1 the geographical distribution of the E/H cases of horses in Romania.

The analysis of the geographical positioning of the districts shows that: Teleorman district is located in the south part of Romania, Timis district is located in the west part while Covasna and Harghita are located in the center part and these last two the only ones close together.

Although the infection percentage with E/H at horses in Teleorman districts has been in continous decrease during 2001-2004 it registered the highest annual percentage of infection in comparison with the others districts;

During 2002-2003 there horses have been sacrificed every year from districts: Covasna, Harghita, Salaj, Suceava Timis and Galati, but in Covasna, Harghita Salaj, Suceava and Galati we didn't register any cases of infection with E/H;
In 2004 the infection percentage with E/H at national level has been 5 times smaller than 2002 and 2 times a half smaller than 2003.

Although the infection percentage with E/H at horses registered a continous decrease during 2001-2004;

It is necessary to elaborate the National Control and Surveillance Programme of E/H.

<table>
<thead>
<tr>
<th>Nr. Crt.</th>
<th>District</th>
<th>Nr. Sacrificed horses</th>
<th>Nr. Horses with E/H</th>
<th>%</th>
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</thead>
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</tr>
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Table 1

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<th>Nr. Crt.</th>
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<th>Nr. of horses sacrificed</th>
<th>Nr. of horses with E/H</th>
<th>%</th>
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<td>Bihor</td>
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<td>Bistrita Nasaud</td>
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<td>Galati</td>
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The districts distribution of the horses E/H in 2002, in Romania

Table 2
The districts distribution of the horses E/H in 2003, in Romania

Table 3

<table>
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<tr>
<th>Nr. Crt.</th>
<th>District</th>
<th>Nr. of horses sacrificed</th>
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<th>%</th>
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<td>Neamt</td>
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<td>Salaj</td>
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<td>Suceava</td>
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<td>8556</td>
<td>865</td>
<td>10.10</td>
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</table>

The geographical distribution of the E/H cases of horses, in Romania

Figure 1
THE PREVALENCE AND ASSOCIATION WITH MORTALITY OF WOUNDS IN THE ETHIOPIAN WORKING EQUINE POPULATION

Powell, R.K.*
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Introduction.
One of the largest problems Ethiopian equines face is wounds. Donkeys are considered stoical, however cleaning and dressing wounds, especially backsores, appears to cause great pain and distress. The majority of wounds are caused by abrasion and/or by pressure necrosis occurring as a direct result of a load. Pain experienced by animals carrying heavy loads is a major welfare concern. The effect of such pain on the productivity and health of these animals has not been investigated but the detrimental effects of pain have been well documented in other species.

Materials and methods
A representative cohort of 1080 animals from 18 different sites was randomly selected in Tigray and Amhara. Animals were examined at 3 monthly intervals for one year. On each visit each animal had a full clinical exam and wounds were graded on a simple scale of 0-4. 0 - No wound, 1 - Lesion in cutis, 2 - Lesion involving sub cutis, 3 - Lesion involving structures underlying sub cutis, 4 - Heavily infected lesion involving deeper structures/exuberant granulation tissue. Appropriate treatments were always given.

A database was established and subsequently analysed.

Results
Annual period prevalence of wounds was 33.63%. Point prevalence at each visit was around 15% with no significant seasonal variation. Nor was there any significant difference in wound severity with season.

Anatomical locations/frequency with which wounds were seen was recorded. The most common cause of wounds at the various locations was determined after discussion with the owners. 29.92% of the whole population or 84.58% of the wounded population were injured annually by either inadequate packing under the load, friction with the load or by the straps holding the load in place i.e. harness and husbandry problems. 6.67% of the population as a whole or 19.16% of the wounded population were injured annually by trauma. 2.36% (4.48% in mules, 2% in donkeys) have easily noticeable sarcoids. Other causes of injury were recorded.

Susceptible populations
Risk factors associated with wounds were identified to aid future intervention strategies
There was positive correlation between age and wound incidence, age and backsore incidence and negative correlation between condition score and wound incidence and condition score and backsore incidence. There was also a sex linked variation in wound incidence with 48.58% of males being wounded annually compared to 22.63% of females.

Wounds and environment.
Of importance to the effective targeting of treatment, delivery and education in the field was the identification of environments associated with high wound incidence. Such information would allow targeting of high risk areas so special dressing/wound clinics could be run. Wounds are far more common in urban areas than rural areas. 87.18% of urban animals are wounded in any one year, c.f. 26.63% of rural animals. The frequency of wounds was similar in the two regions, Amhara and Tigray.

Wounds and mortality
There was a strong and significant association between wounds and death. The mortality rate in animals with backsores was 12.3%.
The mortality rate in animals without backsores was 4.59%.
Thinner animals were more likely to have backsores. However the physiological and behavioural changes
a wound such as a backsore places upon an animal strongly suggest, poor condition can be at least partly attributable to the wound. In this study the poorer the condition an animal was in, the stronger the association between premature death and wound incidence, a donkey of condition score (CS) 2 or less was 3 times more likely to die if it had a back sore.

“Other” includes use of long acting antibiotics, excision of exuberant granulation tissue, flushing and/or drainage of deep pockets of infection.

Interpretation of the effects of wound treatment in this study is difficult for several reasons.

1. No two wounds were the same. Size was not taken into account.
2. The animals were only seen once every three months.
3. Different treatment regimes were used for different grade wounds.
4. Advice was given to the owner regarding wound management, some owners would have been more diligent than others.
5. Wound environment varied from site to site and donkey to donkey.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Frequency</th>
<th>% healed after 3 months</th>
<th>Grade of wound</th>
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</thead>
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<td>Clean, debride + amicyn</td>
<td>74.15</td>
<td>72.48</td>
<td>1 &amp; 2</td>
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<tr>
<td>Clean, debride and zinc oxide + sulphoniamide</td>
<td>19.05</td>
<td>53.57</td>
<td>2 &amp; 3</td>
</tr>
<tr>
<td>Other</td>
<td>6.8</td>
<td>100</td>
<td>3 &amp; 4</td>
</tr>
</tbody>
</table>
Discussion

We have shown an association between wounds and increased mortality. To investigate further, wound incidence was analysed in parallel to other factors that the survey showed had a strong association with death. Showing that the presence of a backsore increased the risk of death threefold or more in thinner animals and doubled the death rate in older animals. In every other group with which a comparison was made the presence of a wound was the most significant factor associated with premature death.

This has led to the conclusion that the presence of a wound was a significant factor in mortality rates. The high prevalence of wounds (33.63%) is of major concern. Indeed if the 7.71 % difference between the mortality rates of wound ed and non wounded can be considered premature deaths attributable to wounds, then this equates to 17.7% of total annual mortalities or almost one in five deaths. Assuming wound incidence is similar across the country backsores are strongly associated with approximately one hundred thousand premature deaths in Ethiopian donkeys annually.

It is unclear how a chronic wound can lead to death, possible mechanisms include the resources an animal uses whilst healing and the loss of resources through wound exudates and in addition the pain of these wounds. Pain and its consequences are difficult to measure, however observations in the clinics showed these lesions to be extremely painful, stressful and undoubtedly the most critical welfare issue facing the animals.

In spite of the rate of healing there is no decrease in the prevalence of wounds at population level. New wounds are being generated as fast as old ones heal. As at least three quarters of these wounds are caused by the load or the harnessing, the only way to decrease wound incidence is prevention. Owner education and training in husbandry, loading and harnessing technique and basic wound management is essential to combat the problem of wounds in Ethiopian donkeys.

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1 National population=5,500,000. 22.56% or 1,240,800 have backsores. The difference between the wounded and non wounded group mortality rate is 7.7 %. 7.7% of 1240800 equates to 95,665 premature deaths attributable to, or strongly associated with wounds.
HAEMATOCHEMICAL PARAMETERS IN SADDLE HORSES DURING A 7-DAYS TREKKING

Pugliese, A.*1; Di Pietro, S.; Bosco, V.R.F.; Giudice, E.
1 Department of Veterinary Medical Science, University of Messina, Italy

Various researches are performed on the horse to study the metabolic mechanisms of the various kinds of physical performances in the equine sportive activities. In the prolonged endurance tests a lot of researches show the effects of the exercise on the haematochemical, enzymatic and hormonal variations. Little data are available about the influence of the several day equestrian events on the athlete horse metabolism. Trekking is defined as a not competitive exercise performed under extreme conditions (prolonged and intense workload). Trekking represent a maximal exercise by the horse and the muscular-skeletal involvement gradually starts and ends. The biochemical variations include the metabolism of glucides, lipids and proteins, the serum enzymatic concentrations and the electrolytic balance. In order to better understand the metabolic variations in the horse submitted to an endurance exercise, we titled the plasmatic levels of creatinine, urea, total protein, uric acid, \( \geq \text{GT} \), cholesterol and glucose in a group of horses participating in a 7 days trekking excursion, with a total length of 184 Km, with different altitudes ranged between 200 to 1500 meters.

Materials and methods

The research was carried out on 6 saddle horses, clinically healthy, aged between 8 and 12 years. They were untrained for competition. Each exercise consisted in a long walk of about 25 Km for each trial. Horses were allowed a 2 hours recovery period (1:00-3:00 p.m.) to eat and drink freely in green pastures. Blood samples were collected from jugular vein two times in day: at the morning, at rest and after 12 hours without food, and at the evening, at the end of each trial. The obtained data about the effect of the exercise on the evaluated metabolic parameters were analysed with the Analysis of Variance for repeated measures (ANOVAÆ) (p<0.05). To compare post-trekking with basal values a paired t-test was applied. The correlation between the different parameters related to the length of ride has been carried out by means of analysis of linear regression (r).

Results

Results are shown on Table 1. At the end of each trial the mean serum levels of creatinine and uric acid showed a significant tendency to increase than the start (p<0.005). The total proteins significantly increase only at the end of the first and second trials (p<0.02). The mean values of urea are resulted significantly at the former trial (p<0.05) and at the latter three trials (p<0.005), while during the other trials the differences were not significant. The difference between the mean values of \( \geq \text{GT} \) resulted significant only at the latter trial (p<0.02), while the glycemia showed more significant differences between the start and the end of exercises at the former four trials (p<0.05). It was possible to evaluate detectable cholesterol serum levels only after the third trial: the t-test was significant (p<0.05) comparing only the mean values of the fourth trial.

Table 1: Mean serum levels (± SD) of the considered parameters before and after trekking in 7 different trials; r: linear regression; n. s.: not significant
Discussion

The increase of the serum levels of creatinine and uric acid can be related to the intensity of the physical exercise by the horses. The lower increases of uric acid registered at the latter three trials can represent a homeostatic adaptation of this metabolite during the exercise.

The increase of serum total proteins recorded only at former two trials underlines that the animals did not show alterations of the fluid homeostasis with haemo-concentration. It seems that the pattern of nitrogenous catabolites is influenced by the intensity of the exercise than that by the length of the trial. Serum levels not showed significant variations during the exercise, showing a normal hepatic activity during the physical exercise. The serum variations of the cholesterol serum levels are related to the covered kilometres: it is possible that the weariness of the first days determine a mobilisation of lipids to the energetic requirements. The variations of urea are too related to the covered kilometres and each horse produced 0,47 mg of ureic nitrogen at each kilometre. The variations of glycemia show that the equine organism adapts oneself after some days of exercise, reducing the energetic consumes.

In conclusion, during trekking the energetic metabolism is notable stimulate and the haematochemical ad enzymatic parameters must be controlled as indexes of the physical integrity of the athlete horse, sometimes altered by a non competitive sportive activity as trekking.

References


Reproductive equine practices in Algeria are still in traditional model, beginning by detection of oestrus every day, insemination every two days until refusal. A field study showed that for most of the breeders, a mare not in heat is considered to be pregnant. With this traditional model, fertility results vary from 50 to 75% (RAHAL 2003).

The question is: what is the part of traditional management in these varying results?

Knowing that ultrasonography is today a worldwide technique to resolve many reproductive problems, the objective of the study is to determinate how much this technique may improve equine reproduction results in our country.

This is the first study of ultrasonographic parameters in equine reproduction in Algeria.

**Material and methods**

- Ultrasonograph with linear probe 5 MHz
- Heat detecting bar
- Arabian and Standarbred mares bred in northern central region of Algeria

Mares in heat season are followed every day by ultrasonography, where we measure:

- size of follicles
- presence of corpus luteum
- presence and size of embryo
- endometrial and intraluminal uterine state

**Results**

<table>
<thead>
<tr>
<th>Breed</th>
<th>104</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arabian</td>
<td>58</td>
</tr>
<tr>
<td>Standarbred</td>
<td>42</td>
</tr>
<tr>
<td>AQPS*</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Age Périod</th>
<th>104</th>
</tr>
</thead>
<tbody>
<tr>
<td>4 to 12 years old</td>
<td>75</td>
</tr>
<tr>
<td>2003 et 2004</td>
<td>25</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Région</th>
<th>104</th>
</tr>
</thead>
<tbody>
<tr>
<td>Northern center of Algeria</td>
<td>75</td>
</tr>
<tr>
<td>Blida, Chebli, Zemmouri...</td>
<td>25</td>
</tr>
</tbody>
</table>

**Table I: characteristics of mares followed in reproduction**

<table>
<thead>
<tr>
<th>Reproductive problems</th>
<th>104</th>
</tr>
</thead>
<tbody>
<tr>
<td>47%</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Leading to a cycle lost</th>
<th>30%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early embryo mortality</td>
<td>12%</td>
</tr>
<tr>
<td>Post-oestrus ovulation</td>
<td>0%</td>
</tr>
<tr>
<td>Silent heat</td>
<td>14%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Leading to a season lost</th>
<th>17%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Persistent Corpus luteus</td>
<td>10%</td>
</tr>
<tr>
<td>twins</td>
<td>07%</td>
</tr>
<tr>
<td>Not counted endometritis</td>
<td>83%</td>
</tr>
</tbody>
</table>

**Table II: Reproductive problems detected by ultrasonography**
Discussion

This study showed that 47% of mares had reproductive problems detected by ultrasonography; these abnormalities were in unequal importance:

- 30% lost a sexual cycle in the season, because of early embryo mortality, post-oestrus ovulation and silent heat. There are two majors consequences for the breeder. The first one is to lose a cycle of 21 days or more. The second consequence is to have a later foaling date in the next season, which is prejudicial for foal's growth and its economic value.

- 17% lost at least a season of reproduction, in the case of corpus luteum, twins or endometritis.

Reproductive problems leading to a cycle lost (30%)

1. Early embryo mortality

We generally first examine for pregnancy at 12, 15 and 18 days after ovulation. In 12% of cases, the embryo vesicle degenerated between the second and the fourth week of gestation, rarely more. This can be physiological as shown by BRUYAS (1996).

2. Post-oestrus ovulation

In 4% of cases, while monitoring increasing of a primary follicle by ultrasound, ovulation was happened after the end of oestrus. The mare refused the stallion, reducing the chances of fecundation.

The lifetime of spermatozoids is less than 48 hours so the percentage of no conception in this case is high.

In order to minimize the risk of no conception, we should reduce inter-oestrus period by using a luteolytic injection, as prostaglandines.

3. Silent heat

14% of followed mares didn't exhibit any oestrus behaviour with the stallion, but presented endometrial oedema at ultrasonography, an open uterine cervix and follicles more than 30 mm size, all criteria showing an oestrus state (MCKINNON 1993).

Most of these mares (47%) were primipares, while other (32%) were followed by their foal. These cases were described in literature (MCDONNEL 1999).

The young mares need to be longly stimulated by a stallion and if possible followed with transrectal palpation or ultrasonography (MCDONNEL 1986).

Reproductive problems leading to a season lost (17%)

1. Persistence of corpus luteum

In 10% of cases, we didn't find any embryo vesicle 14 to 21 days after insemination while the mares didn't present any heat sign. The cervix was hardly shut. Ovarian ultrasonography showed follicles of 10 to 20 mm with a corpus luteum. Heat was seen 3 to 4 days after the use of PGF2a. These criteria suggest that we were in the case of persistence corpus luteum (LIU 1987, SHARP 1993).

2. Twin pregnancy

The identification of twin pregnancies has been a major benefit of performing ultrasound on mares. In 7% of cases, we diagnosed twins in uterine cavity at 15th day, mostly after a double ovulation. It has been reported in the literature that one or both vesicles will degenerate, depending on the position of one vesicle towards the other (NEWCOMBE 1995, MEADOWS 1995). We considered here these cases of twins would conduct to the lost of cycle or a whole reproduction season according to cases.

In practice, when bilateral twins were diagnosed, the more easily accessible was squeezed manually. When unilateral twins were diagnosed, we tried to separate it before 18 days after ovulation. If it was not possible, we induced abortion with prostaglandins.

3. Endometritis

Endometritis is a major cause of infecundity in the mare (PYCOCK 1999). In our study, 3 mares presented slight discharge through vulvae. Its uterine origin was confirmed by ultrasonography, where
it was abnormal accumulation of uterine fluid (KAHN 1993). We could not certify how many mares presented an endometritis only with clinical symptoms.

We have to complete these ultrasonography results by other exams such as biopsy, cytology, bacterial culture and sensitivity, to identify the degree of inflammation, the causative organism and the appropriate antibiotic therapy (REIMER 1998).

However, these cases were treated successfully by many lavages with 2 litres of saline once daily for 2 days. Oxytocin (20 IU) was administered intramuscularly twice daily.

**Conclusion**

The frequencies of the various reproductive problems found in this ultrasonographic study were similar to those in the literature.

It permitted a significative increase in fecundity (95% instead of 77%), and in time of pregnancy, which was earlier in season.

In our field, the use of ultrasonography in reproductive equine can improve the fertility of the mare. However, in order to be profitable, a minimal number of females should be followed in the season.
MEASUREMENT OF TOTAL PROTEIN AND ISOLATION OF DIFFERENT PROTEIN FRACTIONS IN BLOOD SERUM OF IRANIAN HORSES (ARAB & TURKAMAN)

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Introduction

The Arab horse, the purest and the most magnificent looking horse was most probably the result of the attempt of the Iranian peoples at artificial selection and improvement of the race of the existing horses on the Iranian plateau that were later transported to the Arabian peninsula via Mesopotamia. Research on the biochemical factors of the blood serum of the Iranian thoroughbred, that is the Arab and the Turkman, could be a small step towards the preservation and the improvement of these races.

The aim of the present study was to present a comparison range for total protein and protein fractions in blood serum of Iranian horses (Arab and Turkman).

Methods and methods

Samples were collected from 119 clinically normal Iranian (Arab; n=95 and Turkman; n=24) horses. These horses were selected from both sexes and different age groups. Jugular Blood samples were collected from the horses. The serums were harvested and then kept cool in ice and carried over to the Veterinary School at the Tehran University and tests were carried out on them on the same day. Protein concentration of the serum samples were determined by Biuret method, using Eppendorf autoanalyser and commercially available kits (Abu-Rayhan Co.). Protein fractionation was performed using cellulose acetate electrophoresis at 220 volts for 30 minutes.

Due to the uneven number of samples in different sex and racial groups the Least squares analysis was used to analyze the data.

Results

The results of the measurements are shown in table 1.

<table>
<thead>
<tr>
<th>Race</th>
<th>No.</th>
<th>Total protein g/dl</th>
<th>Albumin g/dl</th>
<th>Globulin g/dl</th>
<th>Alpha globulin g/dl</th>
<th>Beta globulin g/dl</th>
<th>Gamma globulin g/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arab</td>
<td>95</td>
<td>6.35 ± 0.05</td>
<td>3.06 ± 0.03</td>
<td>3.30 ± 0.03</td>
<td>0.83 ± 0.07</td>
<td>0.91 ± 0.01</td>
<td>1.55 ± 0.07</td>
</tr>
<tr>
<td>Turkaman</td>
<td>24</td>
<td>6.67 ± 0.11</td>
<td>3.12 ± 0.04</td>
<td>3.53 ± 0.12</td>
<td>0.69 ± 0.04</td>
<td>0.94 ± 0.04</td>
<td>1.70 ± 0.07</td>
</tr>
</tbody>
</table>

Discussion

There are some discrepancies between the studies of the other research workers on protein concentrations in blood serum of horses and the present research. There are few points in this regard which need to be mentioned. First, 119 animals were tested in the present study, which is an acceptable number and the results of other studies have seldom been based on as many numbers of animals. Secondly, to increase the precision of our study after every 10 samples, 3 known serums were tested as control serums. And finally, because of the variation in nutrition, geographical factors, race, the use to which animals had been put and other factors, all scientists believe that every region and in fact every laboratory must fix its own reference range for each blood parameter.

The results obtained from regression analysis of the protein fractionation in blood serum of the Iranian horses show that albumin concentrations reduces (P<0.001, r = - 0.264) and the globulin concentration increases (P<0.001, r = 0.278) with the age. The A/G ratio also increases with age (P<0.001, r = 0.313).

References

Proceedings of the 9th International Congress of World Equine Veterinary Association, 2006 - Marrakech, Morocco
Comparison of the studied parameters in the serum of the Iranian horses, based on sex showed that the level of albumin and A/G ratio are significantly higher in the stallions (P<0.50) but the globulin concentration is higher in the mares (P<0.50). Student's t test showed that only total protein is higher in the Turkaman than in the Arab (P<0.50).

References


The dimorphic fungus, Histoplasma capsulatum var. farciminosum is the causative agent of Epizootic Lymphangitis. The two morphologically distinct phases include a yeast phase that may be isolated from infected tissues, and the saprophytic mycelial phase. Both forms may be cultivated under laboratory conditions. In vitro studies of survival of the organism in both soil and water were conducted by Gabal and Hennager (1983). The organism is thought to be widespread in the environment in endemic countries, but soil analysis studies are yet to be carried out to determine this aspect of the fungus's biology. The OIE (Office International des Epizooties) classifies this as a category B disease and in many countries; the disease has been eradicated through a slaughter policy. This is not an option in places such as Ethiopia where the socio-economic effects of such a large scale loss of livestock would be immense.

This disease is currently affecting working equines in parts of Eastern and Northern Africa today. It usually infects solipeds and camels, but has also been reported in cattle.

Recent epidemiological studies are few; however, studies by Ameni and Siyoum (2002) suggested a prevalence of 26.2% in 2,907 carthorses studied in three towns in Central Ethiopia. The incidence of the disease has been linked with altitude, humidity, season and a rising fly population. Spread is achieved via direct contact with ocular/nodular discharge or discharge on fomites, mechanical vectors such as ticks and flies, and inhalation of spores. However, more extensive epidemiological studies, preferably long term cohort studies, are needed in order to develop further understanding of the pathogenesis of this disease in order to better target potential points of control.

There are four presentations of the disease, cutaneous, ocular, respiratory and asymptomatic carriers. The links between the different forms of the disease have been postulated, but are as yet unproven. The cutaneous form is the most commonly presented to the SPANA veterinary teams in Ethiopia. There is initially a single papule which increases in size over many weeks to a hazelnut sized intra-dermal nodule with a thick fibrous capsule and a core of viscid pus. Histopathology of the lesions show a chronic granulomatous inflammatory response packed with histiocytes and multinucleated giant cells in the dermis and subcutaneous tissues, the singular lesion resembling that seen in tuberculosis. Spread occurs gradually along the lymphatics to the regional lymph nodes creating a cording appearance. As the disease progresses there may be widespread dissemination around the body. Severely affected animals may develop lameness and ill-thrift, reducing their ability to work. This condition must be differentiated from Ulcerative Lymphangitis, sporotrichosis and cutaneous glanders. Identification is achieved via stained aspirates from unruptured nodules or fungal culture. Other diagnostic methods include intradermal skin test 'Histofarcin' test, fluorescent antibody technique and serology.

Treatment response is variable, and various protocols have been suggested. However the course of treatment is lengthy and labour intensive, prognosis is unpredictable and usually guarded unless treated very early in the course of the disease. Currently, vets in Debre Zeit, Ethiopia, are using a mixture of topical treatment (involving incision of all visible nodules and infusion with tincture of iodine) and in moderate-severe cases combining this with intravenous or oral sodium iodide therapy. In vitro trials showed sensitivity to amphotericin-B and nystatin (Gabal 1983), however, controlled in vivo trials have not yet been carried out. Any future treatment or vaccine development should be of practical use in the animals affected. Expensive drugs may not be widely available to animals in developing countries and the method and duration of treatment must also be considered. Factors reducing the risk to the animal in endemic areas should also be addressed in collaboration with treatment and/or vaccination.

This is a disease with not only far-reaching socio-economical implications, but also a detrimental affect on equine welfare.
SUMMARIZING THREE YEARS OF EQUINE GASTRIC ENDOSCOPY

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1 Veterinary Faculty, University of Sassari-Italy
2 Varese-Italy

Introduction

Gastroendoscopic examinations were performed on 273 horses, ranging from one to 27 years, of different sex (37 males, 77 geldings and 159 females) in a period of 3 years (2000-2003). 92 horses had clinical problems including chronic, recurrent colic for more days without particular signs at rectal examination; 82 diminished appetite and poor bodily condition; 58 with episodes of colic after coarse meal; 41 chronic diarrhoea.

Materials and methods

Animals, after sedation with detomidine, were examined with a 3.2-m-long equine gastroscope.

Results

Lesions observed in 158 horses were ulcers in squamous fundus, squamous mucosa adjacent to the margo plicatus along the greater curvature, glandular fundus; in 112 animals were observed lesions by Gasterophilus spp; in 4 horses gastric squamous cell carcinoma was recognized.

Discussion

Endoscopy is indispensable for making diagnoses or ruling out several possibilities of alimentary tract disorders.
A POSSIBLE SKIN HEREDITARY CONDITION DETECTED IN THREE CROSSBRED VENEZUELAN HORSES. CLINICO-PATHOLOGICAL EVALUATION AND SURGICAL TREATMENT

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Central University of Venezuela, Maracay, Estado Aragua, Venezuela
Private Practice

Introduction

The hyper extensible focal, fragile skin of three related crossbred horses was studied and compared with the normal skin of ten horses from the same farm. The understudied group consisted of three horses: one stallion and two sons (three and four years old) with large areas of extensible and fragile skin.

These horses were located in a farm at Falcon state at the central coast region of Venezuela and were used as work animals

The objectives of this study are to diagnose and treat this skin condition. This entity which had not been described in Venezuelan crossbred horses, will allow Venezuelan practitioners to use clinical and histological findings to obtain a presumptive diagnosis. On the other hand, the evolution after surgical treatment of the area was evaluated after one year of the procedure.

Materials and methods

Hyperelastosis dermis is detected dorsally at the back in three related horses clinically evaluated. These lesions were in areas of 5 to 10 sq. centimeters with no referred history of cutaneous evolution nor previous local inflammation or trauma.

Punch biopsies of skin of affected horses were performed. Samples were fixed in 10% neutral buffered formalin solution, processed in paraffin inclusion and, stained with Hematoxylin-Eosine, Trichromic, Orcein, Verhoff Van Giesson and Von Kossa stains.

One horse skin lesion (stallion) was treated by surgical removal of the affected area and, monthly clinical control for one year was done.

Results and discussion

Microscopically, the affected skin showed fragmented and disorganized collagen fibers. With Trichromic stain, many fibers had abnormal red-stained centers. The Elastic tissue stained with Orcein and Van Verhoff Van Giesson stains, showed agglutinated material.

No inflammatory process was seen. Control skins showed no evidences of collagen or elastic alterations; except that in punch biopsies of skin of affected horses were thinner than similar biopsies from the 10 control horses.

These results were consistent with Hyperelastosis Syndrome where focal lesions of elastic fibers were implicated; the pathogenesis is unknown. Some authors have postulated that this lesion may represent hyperplasia of elastic fibers in response to damage; while others have suggested that degenerative and regenerative processes in elastic fibers were associated with this syndrome.

The pedigree data available for these three horses suggest an autosomal recessive mutation, but are also consistent with autosomal dominant inheritance; therefore, the disorder resembles the lesions described by Minor et al (1978) in dogs, minks and cats; showing similar features with those observed in Ehrles Danlos Syndrome in man. In the present cases, articular disorders were not evident.

So far, no treatment for this related skin condition has been reported. Oral, parenteral and topical route of treatment are known to be ineffective.

Surgical treatment performed in one of these horses, showed no lesion in the area after one year of clinical control which is a clear indication of the genetic origin of this skin hyperelastosis condition found in Venezuelan horses.
Clinical, histological and surgical studies on skin hyperelastosis detected at back of three (3) related Venezuelan crossbred horses. One stallion and two sons of four (4) and three (3) years of age respectively, were in agreement with the opinion of several authors (Mc.Cracken.1978, Bounts et al. 2001, Rashmir-Raven et al. 2004) in which the absence of any previous external inflammatory process as well no histological evidences of such tissue reaction were found.

Additionally to the above mentioned findings, the surgical removal of the affected skin area and its subsequent clinical observation done for a entire year, has revealed the inherited nature of this particular skin hyperelastosis condition observed in three related Venezuelan horses belonging to the same horse farm located at the north-central part of Venezuela.

Although, no routes of treatment, e.g. oral, parenteral or topical seem to be effective, yet, surgical involvement may be the only available approach. On the other hand, the biopsy for histopathological study when performed correctly is a very important tool to diagnose this disease.

References
FIVE CASES OF OVARIOHISTERECTOMY IN THE DONKEY

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This abstract summarizes clinical findings, surgical procedure and outcomes of five cases where ovariohysterectomy was performed in female donkeys. Three cases of pyometra, one case of uterine tumour, and one of cystic ovaries with uterine hyperplasia.

All the donkeys were aged, weights 140-187 kg, with no prior reproductive history available. The cases are summarized in Table 1.

A common feature was intermittent dullness and depression with vague colic signs, vaginal discharge was present in the cases of pyometra and uterine tumour. Diagnosis was confirmed by manual and ultrasound examination per rectum, and vaginal examination with a speculum. In each case of pyometra, an unsuccessful attempt was made to catheterise the cervix and obtain drainage of pus prior to surgery. This was done prior to and after attempts to relax the cervix using 0.5ml/125 µg of cloprostenol.

Pre-operative work up included a full clinical examination, haematological and biochemical profile. All donkeys were considered healthy enough to undergo exploratory laparotomy despite their advanced age.

Surgery was performed under general anaesthesia in dorsal recumbency with a view to surgical removal of the affected ovary/ovaries and uterus if appropriate.

Ovariohysterectomy was carried out using a technique similar to that described by Santchi and others 1995. All donkeys were maintained on intravenous fluids throughout surgery and had arterial blood pressure monitored.

A midline skin incision 15-20cm long was made between the umbilicus and start of the udder and continued through the linea alba. In each case the uterus was exteriorised and packed off from the abdomen with saline soaked drapes. Ovario-hysterectomy was performed starting with one ovary and continuing down the broad ligament on each side before the cervix was approached. The ovarian artery and all vessels within the broad ligament were double ligated and transected with either 5 or 3 metric polygactin 910. In the three cases of pyometra 3-10 litres of pus was suctioned off via a small incision in the uterine wall which was subsequently closed with a purse string suture.

The uterus was clamped distally from right and left to prevent remaining pus spilling into the abdomen, closure was ensured using a double layer inverting suture pattern. Following ovariohysterectomy and checking for haemorrhage, the linea alba and skin were closed routinely.

In case 4, the uterine tumour, the mass was localised to the right uterine horn. In the case of the enlarged and cystic ovary the left ovary was found to measure approximately 70 by 60 mm, the uterus felt grossly thickened but did not contain pus.

Post operatively donkeys were monitored intensively to prevent hyperlipaemia and given non steroidal anti-inflammatory, and antibiotic cover with Gentamicin and Sodium penicillin.

Post-operative complications are summarized in table 1. Case 3 (pyometra), was euthanized due to anorexia and ileus after 5 days. At post-mortem there were no obvious surgical complications noted. Case 4 (uterine tumour) was euthanized 7 months after surgery due to colic, at post-mortem the tumour was found to have metastasized to the liver and internal iliac lymph node. The other cases recovered well from surgery with minor complications.
Histopathology was performed on samples removed at surgery and confirmed pyometra in cases 1-3, an endometrial adenocarcinoma in case 4, and uterine hyperplasia with an ovarian thecoma in case 5.

There are a number of case reports in the literature of ovariohysterectomy in the horse for treatment of chronic pyometra, tumours, uterine torsion and haematoma, e.g. Rotting and others 2003, Santchi and others 1995, but only one report in the donkey, (Bonfig and Ingenhorst), similar indications and complications were found in our cases, although the age range of the patients was much greater.

Donkeys with abdominal disorders are often presented late in the course of disease due to the relatively minor clinical signs demonstrated - behavioural changes, general dullness and poor appetite. In our experience it is not uncommon to find uterine and/or ovarian disorders in elderly female donkeys, these donkeys may present with vague signs of discomfort and/or vaginal discharge. The evaluation of a donkey with abdominal pain should include full examination of the reproductive systems in female donkeys.

The conformation of the donkey cervix may predispose to the development of pyometra. The donkey cervix is usually longer than that of the mare and has a protusion into the vagina (Vendrammi and others 1998, Pugh 2002). This protusion can make catheterisation of the cervix difficult and may also be associated with cervical adhesions following dystocia, this may explain why pre surgical drainage of the uterus was not possible in these cases.

<table>
<thead>
<tr>
<th>Diagnosis, Case number</th>
<th>Age (Approx. yrs.)</th>
<th>Weight (kg)</th>
<th>Clinical findings and history</th>
<th>Duration (yrs)</th>
<th>Post operative complications</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pyometra 2</td>
<td>23</td>
<td>183</td>
<td>Intermittent episodes of colic and dullness, thick vaginal discharge. Enlarged uterus.</td>
<td>Minimum 4 years</td>
<td>None</td>
<td>Alive and well (surgery June 2005)</td>
</tr>
<tr>
<td>Pyometra 3</td>
<td>28</td>
<td>187</td>
<td>Intermittent episodes of colic and dullness, thick vaginal discharge. Enlarged uterus.</td>
<td>Unknown</td>
<td>Ileus, unresponsive to treatment. Wound haemorrhage and oedema.</td>
<td>Fœthansised 5 days post-op.</td>
</tr>
<tr>
<td>Uterine Tumour 4</td>
<td>31</td>
<td>163</td>
<td>Intermittent colic, bloody vaginal discharge. Uterine mass.</td>
<td>2 years</td>
<td>Mild dullness 4 days, responded to increased pain relief.</td>
<td>Fœthansised 7 months post-op. perforation due to metastasis.</td>
</tr>
<tr>
<td>Cystic ovary uterine hyperplasia 5</td>
<td>22</td>
<td>150</td>
<td>Several episodes of dullness and recumbency, enlarged ovaries and uterus.</td>
<td>Minimum 2 years</td>
<td>Haemorrhage and transfusion reaction</td>
<td>Alive, no further problems 1 year post-op.</td>
</tr>
</tbody>
</table>

Table 1: Summary details of five jennies that had an ovariohysterectomy performed.
Many donkeys in the United Kingdom survive into their 30's and surgery may be considered in elderly animals after a full evaluation of the general clinical condition and understanding of the risks. A significant number of geriatric donkeys do suffer from abdominal neoplasia (unpublished data The Donkey Sanctuary), and it can be difficult to predict the outcome of tumour removal due to a lack of published data. In the case presented here the donkey was clinically bright between bouts of colic and rapidly deteriorated due to metastatic spread of the initial tumour.

In the case with fibrothecoma/thecoma of the ovary the exact relationship between this and the uterine hyperplasia is unclear. In other cases with grossly enlarged ovaries seen surgical removal has resulted in long term remission of symptoms of abdominal pain. More information and long term follow up is required on the nature and incidence of ovarian and uterine disease in the donkey.

References


A STUDY ON EXPERIMENTAL TRANSMISSION OF EQUINE SARCOID

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2 Department of clinical science, Veterinary Faculty, University of Tehran
3Department of clinical pathobiology, Veterinary Faculty, University of Tehran

Introduction

The possibility of the experimental transmission of The Equine Sarcoïd with hypothesis of infectious etiology of the tumor.

The equine Sarcoïd, the commonest equine skin tumor, is a benign, locally invasive, fibroblastic tumor. The tumor may appear in wounds during the proliferate stages of wound healing, or, more often, at the wound site several months after obvious termination of original wound healing. There is no consistently effective therapy. Its definitive etiology is unknown but it is widely accepted bovine papilloma virus (BPV) type 1 and 2 are associated with the pathogenesis of sarcoïd disease. More sarcoïd appear to contain detectable viral DNA and are also known to express the BPV type 1 and 2 major transforming protein (E5), but appear not produce infectious virions. The viral hypothesis is also supported by apparent outbreaks among horses and mules in certain areas and within stable as well as experimental transmission. While the mode of transmission of infection hasn't been elucidated, horizontal transmission probably occurs by natural means such as contaminated fomites, halters and contaminated surgical instruments.

Material and methods

An Arabian horse and a Caspian pony, which had lesions similar to the sarcoïd, were selected. In both of them these lesions surgically removed and diagnosis of Equine Sarcoïd was confirmed by histopathological examination. Sarcoïd lesions extracts were prepared from the pathologically confirmed removed lesions. Nine healthy another equidaes (six Caspian ponies and three horses) were selected as the recipient animals. Experimental transmission of the extracts on the recipient equidaes were tested by the way of different inoculation of the extracts such as: the rubbing of the extract on the normal skin, the rubbing of the extract on the scratched skin and the intradermal / subcutaneous and intravenous injections of the extracts on the different sites of the body. The extract, which obtained from the sarcoïd of the pony, was inoculated to the recipient ponies and the extract that obtained from the sarcoïd of the horse was inoculated to the recipient horses.

Results

Lesions such as hypotrichosis, erosion, crust, nodule, hyperkeratosis, and a mild form of the granulation tissues had developed in different areas of the body in some of recipient animals during 12 - 15 days after inoculation. The summary of the results is in the following table:

<table>
<thead>
<tr>
<th>RECIPIENT ANIMALS</th>
<th>Breed</th>
<th>Sex</th>
<th>Site and Route of the inoculation</th>
<th>New lesions development</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pony No: 1</td>
<td>Caspian</td>
<td>Male</td>
<td>A/B/C/D#</td>
<td>A (+)/B (-)/C (-)/D (+)</td>
</tr>
<tr>
<td>Pony No: 2</td>
<td>Caspian</td>
<td>Male</td>
<td>A/B/C/D</td>
<td>A (+)/B (-)/C (-)/D (+)</td>
</tr>
<tr>
<td>Pony No: 3</td>
<td>Caspian</td>
<td>Male</td>
<td>E</td>
<td>E (+)</td>
</tr>
<tr>
<td>Pony No: 4</td>
<td>Caspian</td>
<td>Female</td>
<td>A/B/C/D</td>
<td>A (-)/B (-)/C (-)/D (+)</td>
</tr>
<tr>
<td>Pony No: 5</td>
<td>Caspian</td>
<td>Female</td>
<td>A/B/C/D</td>
<td>A (-)/B (-)/C (-)/D (-)</td>
</tr>
<tr>
<td>Pony No: 6</td>
<td>Caspian</td>
<td>Female</td>
<td>E</td>
<td>E (-)</td>
</tr>
<tr>
<td>Horse No: 1</td>
<td>Arab</td>
<td>Female</td>
<td>A/B/C/D</td>
<td>A (-)/B (-)/C (-)/D (+)</td>
</tr>
<tr>
<td>Horse No: 2</td>
<td>Arab</td>
<td>Female</td>
<td>A/B/C/D</td>
<td>A (-)/B (-)/C (-)/D (-)</td>
</tr>
<tr>
<td>Horse No: 3</td>
<td>Turkmen</td>
<td>Male</td>
<td>E</td>
<td>E (-)</td>
</tr>
</tbody>
</table>

*: A = Subcutaneous injection in the lower neck.
#: E = Intravenous injections. + = New lesion developed. - = New lesion didn't developed.
Multiplying of dermal fibroblasts, eosinophilic filtration and hyperkeratosis were revealed from histopathological examination of some of these new lesions. All of the new lesions disappeared until 45 days after development.

Discussion

In this comparative study, the sarcoid lesions removed from a pony and a horse. The extract obtained from the sarcoid lesion of the pony was experimentally inoculated in a series of the ponies and the extract obtained from the sarcoid lesion of the horse was experimentally inoculated in the horses, respectively. Developments of the new lesions were more detectable and noticeable in ponies than horses. In this study only the whole extracts of the sarcoid lesion were used although the routes of the inoculations were different. The most world studies about the experimental transmission of equine sarcoid were on the horses and mules and there were any documents about the ponies. The all of the new transmitted lesions developed during 12-15 days after inoculation (The incubation period were few days) and disappeared until 45 days after developmental, which weren't equal with the true sarcoid lesions. And too, the histopathological features of them were different from the true sarcoid lesions, thus the new developed lesions may not be the true sarcoid. Of curse in the various world studies, the sarcoid-like lesions induced experimentally from intradermal or subcutaneous injections of whole or cell-free extracts of the sarcoid have developed during 3-4 weeks and disappeared after a short time, too.
PRELIMINARY ULTRASONOGRAPHICAL FETOMETRY OF CASPIAN MINIATURE HORSE

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Introduction

The Caspian Miniature horse is an ancient breed previously believed to have been extinct for over one thousand year. This small horse is extremely rare and is probably one direct ancestor of the oriental breeds and subsequently of all light horse breeds. Transcutaneous fetal monitoring from the ventral abdominal window is relatively new application of diagnostic ultrasonography in brood mares. It is useful in the management of high-risk pregnancies and, in particular, for evaluating fetal well-being at all stages of gestation. The Caspian horse is a small horse and the fetal measurements are different to those measurements reported on horses elsewhere, and therefore these measurements cannot be used as reference. The purpose of this study was to obtain normal fetal measurements of Caspian horses using ultrasonography.

Material and methods

Seven healthy pregnant Caspian horses with defined ovulation time were selected from Khodjir Research Center, Tehran-Iran. Transcutaneous ultrasonography of the mares was achieved using a 3.5 MHz convex array transducer. Uterus was scanned and the fetus imaged from the ventral abdomen. The largest diameter of the trunk (at the level of the stomach and liver), stomach (the largest inner diameter of the hypoechoic lumen), braincase (the widest distance between the outer borders of the cranium at an angle of 90° to the falx cerebri), eye (the largest diameter), one rib with one intercostals space (the mean of 3 ribs and intercostal spaces to reduce the error in measurements) of fetuses were measured at different gestational age between 90-190 days of pregnancy. Ultrasound images were recorded on videotape and were subsequently analyzed off-line by a single observer. Body measurements were taken from frozen-frame pictures using Scion Image software.

Results

All cases had only one fetus. The fetuses were very active during the scan and were rarely quiet. Measurements of different fetal structures are shown in the figure 1.

Discussion

As we expected, the fetal measurements Caspian horses were significantly smaller compared to other horse breeds. The diameter of braincase in Caspian horse and Thoroughbred fetuses were similar until 145 days of gestation. Due to transrectal ultrasonography difficulties in Caspian miniature horses, transcutaneous ultrasonography showed to be a useful and safe technique in these small horse breeds. It is important to consider that ultrasonic fetal biometry constitutes a valuable method for pregnancy diagnosis and the state of pregnancy, as well as fetal well being, growth and development, characteristics that can allow for improvement of the reproductive management. The results of this preliminary study can be used as a standard reference in evaluation of gestational age of Caspian horses, although, more cases should be studied for more accurate estimations.

References


Figure 1: Growth of the largest diameter of the trunk, braincase, stomach, eye and one rib cross section with one intercostal space in Caspian horse during pregnancy.
BONE MARROW ASPIRATION AND ISOLATION OF MONONUCLEAR CELLS IN ADULT HORSES - PRELIMINARY RESULTS -


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Introduction

In the late 90’s, the medical interest has been directed to the stem cells therapy of degenerative diseases and in cases of deficient healing process. These cells are involved in the growth, remodeling and healing process (Richardson, 2004). During human embryonic development until 5 to 7 days, cells of the inner mass of the blastocyst retain the capability to regenerate an entire individual, and are therefore totipotent. The stem cells of an older embryo, fetus or blood cord are called pluripotent, because of the compromise with selected groups of tissues (endoderm, ectoderm or mesoderm) (Muschler et al., 2003). In adults, the stem cells are found at bone marrow and their isolation could be accessed by aspiration technique. The sternum is the site of choice for bone marrow aspiration in horses (Freeman, 2000).

This study aims to evaluate a simple technique that enables adult horses stem cells collection, allowing further researches of cellular therapy in this species.

Materials & methods

Usually the animal is submitted to general anesthesia (Hertel, 2002; Thomas, 2003; Freeman, 2000) and positioned in dorsal recumbence. This experiment was developed with ten sedated standing horses, as described before (Smith et al., 2003). After sedation with xylazin 10% and clipping, the sternebrae were identified by ultrasonography. The marked sites were scrubbed and local anesthesia done. A Jamshidi biopsy needle (8 gauge and 15 cm) was introduced in the 5th sternebra by a firm pressure and back-and-forth rotations until penetrate to cortical bone. Once firmly seated within the bone, the stylet is removed and a 20 ml syringe, preloaded with 5000 UI of heparin, was attached for aspiration. Strong negative pressure was applied until few drops of material were visible. The first sample was expelled into a Petri dish to verify the presence of spicules, confirming bone marrow origin. After that, a 5 ml heparinized syringe is attached and five aliquots of approximately 3 ml were taken from each animal. The samples were conditioned in ice and immediately transfer to the laboratory. Bone marrow can be separated into its constituent parts by density centrifugation, usually over Ficoll Hypaque as describe by Smith et al, 2003, Perin et al., 2003 and Sato, 2003. The samples were initially filtered through a IV transfusion set (Embramed®) to remove cell aggregates and then diluted in PBS. The filtered was layed gently onto Ficoll Hypaque d=1,077 (Sigma Chemical Co.) and centrifugated at 500g for 30 minutes at room temperature, so that a buffy layer, rich in bone marrow mononuclear cells, is formed in between the plasma and Ficoll erythrocyte residue. This layer was removed and washed twice in PBS by centrifugation (at 500g for 10 min.). Smears were immediately prepared and submitted to Romanowsky stain to verify the presence of hematopoietic precursors cells, suggesting the presence of mesenchymal cells.

Results & discussion

The use of mesenchymal stem cells in musculoskeletal injury has provided some promising results (Young et al, 1998; Herthel, 2002; Smith et al, 2003). Cytochemical or immunologic markers can be applied for accurate identification of cell types. The identification of human bone marrow mesenchymal...
cells were found to be positive for the surface antigens STRO-1, SH2, SH3, CD29, CD44, CD71, CD90, CD120a and CD 120 among others, but negative for CD14, CD34 e CD45, some of the markers for the haematopoietic cell lineage (Richardson, 2004). However, as yet, no antigen markers have been identified for equine mesenchymal cells, suggesting the need of further studies in this species.

No accidental pleural or cardiac perforation was reported in the bone marrow aspiration, demonstrating that the technique is reliable, but collector needs to be trained to maximize results. As the knowledge is obtained, the stem cell harvest experiment and clinical approach can be implemented in this species.

References


RICHARDSON, L. Department of Veterinary Clinical Sciences, The Royal Veterinary College. Dissertação não publicada. 2005


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3 Statistical analysis system (SAS) software (release version 8.2), Cary, NC.