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HAEMATOLOGY AND BIOCHEMISTRY OF
THE EQUINE ATHLETE AT REST

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Introduction

Haematological and blood biochemical testing of the equine athlete at rest is an integral part of equine
clinical practice. Specific organ system evaluations are as important within this discipline as they are in
any other discipline that involves internal medicine. The high commercial and sentimental value of elite
athletes means that they are seldom allowed to develop the diseases of neglect. However, they remain as
vulnerable to sudden onset disease and to all of the other conditions that inevitably arise within horse
populations, as any of their non-athletic, non-elite equine counterparts. The essential difference
between the elite performers and other horse populations is the importance in the athletic group, of sub-
clinical, potentially performance-limiting conditions. Many, but not all, of these conditions are
infectious diseases. The elite equine athlete is uniquely predisposed to these conditions because transport
to racing and competition venues and varying degrees of exposure to other horses, coupled with the
stresses inherent in competition and racing are essential components of all national and international
horse sport.

Screening for sub-clinical entities, differential diagnosis, evaluation of response (or the lack of it) to
treatment and decisions on when a return to full athletic demand can be permitted are therefore the
issues that confront the equine clinician and the laboratory.

It is important to realise that haematology and blood biochemistry screening is not a measure of athletic
fitness i.e. more correctly, the degree of exercise tolerance present in healthy individuals. These clinical
aids can however help to identify individuals that may not perform to the utmost limits of their ability,
because of a clinical or sub-clinical entity. Great care needs to be exercised when owners or trainers
request guidance on whether a horse with a sub-clinically entity should be allowed to compete, or be
withdrawn from competition. No one should ever compromise equine welfare, but it has to be
recognised that in the lower orders of horse sport, as with any other sport, those with sub-clinical
entities may significantly outnumber the so called normal individuals. Numerous clinicians have been
accused of giving poor quality advice when individuals for whom they have unsuccessfully advocated
withdrawal, have then won the contest in question.

Sample collection

The clinician's responsibilities begin with taking and critically evaluating the history and performing a
thorough clinical examination. Blood samples must be collected into the appropriate containers.
Collection into evacuated containers has become the norm. Potassium EDTA is the anti-coagulant for
haematology. Lithium heparin is not suitable for this purpose, because it does not permit differential
white cell counting. Samples for blood biochemistry testing can be collected into heparinised or plain
tubes for either plasma or serum biochemistry respectively. Coagulation studies and the modified Clauss
method of measurement the acute phase protein fibrinogen can only be carried out using sodium citrate
containers.

Sampling technique

Samples must be collected in an aseptic manner. There is no excuse for inducing sepsis, local or worse
still, general into an otherwise health horse. Collection must be sympathetic. There are few things worse,
in either the clients, the handlers or a colleagues eyes, than to see a Veterinarian lunging thoughtlessly
at a jugular vein with in total disregard of the hapless animal. There are at least two significant penalties
for failure of a quiet kind approach. They include loss of professional respect and the risks of creating
the potentially misleading artefacts that result from adrenal induced neutrophilia and splenic
contraction.
Timing of sampling

The timing of elective sampling should reflect the questions that are being asked. There is little point in taking post exercise samples or sampling within two or three hours of exercise, if the issue is whether muscle enzymes are within the normal resting range. Samples for elective testing are best collected pre-exercise, early in the morning or alternatively, late in the afternoon when exercise has been completed in the morning.

Sample handling

Misleading artefacts can also arise from poor sampling handling in the period between collection and evaluation in the laboratory. Samples should not be left lying on car seats or on the dashboard throughout the clinician's inevitably busy day. The minor investment involved in purchasing an insulated lightproof container, a laboratory blood sample rack and in hot climates, an ordinary household cold pack can yield rich dividends in terms of the accuracy and value of the information generated from the sampling. Labelling has to be legible. Thought has to be given to timing of transfer for analysis. There is little point in collecting samples late in the week and entrusting them to the post or courier, without addressing the issue of likely time of arrival in a distant laboratory and whether it can or will by prior notice, provide weekend service.

Laboratory selection and quality control

There are many sources of laboratory testing. They include generalised laboratories, highly specialised referral laboratories and the practice laboratory. Although they all have some common features, they are not synonymous entities and there are very important differences between the results that can be reasonably expected from them. Results generated by one laboratory can seldom be meaningfully compared to those emanating from another. Differences between haematology and biochemistry analysers and the reagents that they use will inevitably result in differing values, even between identical samples and especially between sequential samples. All too often, proper regular and detailed investment in accredited quality control systems is a low priority for practice laboratories, which often use relatively low initial cost bench-top dry chemistries. The subsequent high cost of the reagents for some of these systems is easily missed at the time of purchase. General multi-species laboratories can seldom supply the specialist interpretation and advice. High quality equine specialist laboratories, with rigid adherence to documented standard operating procedures and certified quality control accreditation are inevitably more expensive. Quite simply, in this area, as in so many others, you get what you pay for. Do equine clinicians need access to high quality haematology and biochemistry system? Sometimes yes, sometimes no - but they are always the only way of ensuring that minor changes, which may be critical in subclinical monitoring and case management are real and not just a reflection of variation in processing.

Reference ranges

The ranges published in the general texts are too wide to permit the identification of sub-clinical entities in the elite equine athlete. Differences in laboratory equipment and reagents result in differences in reference ranges. Reference ranges can be normally distributed (e.g. in Haematology) or have a more skewed distribution (e.g. Muscle enzymes). In both instances there is overlap between the 95% of the range that is considered normal and the 5% of individuals that also fall within this range, but are actually abnormal. This is why it is much more useful to try to compare the individual against itself, by serial sampling (where possible in both health and disease) than to compare it to a much more general range. In the authors laboratory the last three samples for the individual are wherever possible, tabulated on a single report sheet to facilitate the identification of meaningful trends.

Haematology

Red blood cells (RIB)

Every undergraduate and many laypersons understand that Packed Cell Volume (PCV 1/l or %) rises in dehydration and shock and falls in (extreme) haemorrhage. PCV is however, probably the least useful RBC measure in the elite athlete. As noted previously, it is a labile parameter influenced by splenic contraction following exercise or excitement and blood biochemistry is a better means of evaluating
hydration status. Identification of the anaemia that is associated with chronic infection is the entity that is of most importance in evaluating RBC criteria in the elite equine athlete. From a practical pint of view, anaemia can be suspected where Haemoglobin (Hb g/l) values are < 12 for Thoroughbreds and 11 for sport horses. Care must be exercised here however, as older Thoroughbreds e.g. mature National Hunt / Jump racing horses can be so relaxed on sampling that their Hb values can be misleadingly low. Equine erythrocytes do not follow the patterns associated with regeneration in other species. Anisocytosis and reticulocytosis are only seen in the authors experience in the most extreme and long-standing anaemia's. Serial evaluation of RBC, Hb and total protein are probably the best way to follow a post-anaemic erythropoietic response.

White blood cells (WBC)

In elite horses leucocytosis and leucopaenia can be defined as WBC >10x10⁹/l and <6.0 x10⁹/l respectively. Values lying close to these values should be interpreted with caution given the potentially confusing effects of catecholamine induced mobilisation of cells in this series and the need for appropriate filling of EDTA tubes. Failure to fill correctly can result in an incorrect coagulant to blood ratio with consequently misleading WBC values. Evaluation of the individual components of the WBC series is usually much more informative than reliance on WBC counts in isolation, hence the need for EDTA samples, rather than lithium heparin for Haematology as referred to previously.

Neutrophils / Polymorphonuclear cells (PMNL)

PMNL's are marginated in numbers on the endothelium of the peripheral blood vessels and are released into the circulation in response to stress, infection and inflammation. PMNL values are often reported as the percentage of Neutrophils (N %) in a differential cell count. When reported in this way, the Neutrophil: Lymphocyte ratio in mature adult elite horses is somewhere around a 60%: 40% ratio respectively. Neutrophilia and neutropaenia are better identified by recourse to absolute PMNL counts. Neutrophilia, when accompanied by hyperfibrinogenaemia, is usually noteworthy when PMNL's are > 7.0 x 10⁹/l. It is impossible to meaningfully differentiate between the neutrophilia of stress and that of infection and inflammation, without concurrent measure of acute phase proteins such as fibrinogen. Neutropaenia can be defined as PMNL's < 2.0 x 10⁹/l, when they can be a very disturbing indicator of impending catastrophes e.g. colitis / laminitis / peritonitis etc., etc. Immature neutrophils whether toxic or band form are associated with the presence of pus-forming infections and are best evaluated using manual differential counts derived from fresh blood smears.

Lymphocytes

Lymphocytosis (Lymphs >5.0 x 10⁹/l) is a feature of chronic bacterial infections and the secondary phase of bacterial infection that often follows from primary viral infections. Lymphopaenia (Lymphs <1.0 x 10⁹/l) may be seen in septic shock and severe viral infections and although reported as such, is seldom of much use in the authors experience in the identification of lymphosarcoma.

Monocytes

Monocytes are macrophage precursors. Monocytosis (Monocytes <0.5 x 10⁹/l) can reflect cellular responses to chronic bacterial infections.

Eosinophils

Eosinophilia (Monocytes <0.5 x 10⁹/l) can be seen in parasitism and allergy. It may also be seen in chronic eosinophilic granulomatous enteritis.

Blood biochemistry

General screening for sub-clinical and some clinical entities in elite equine athletes is often undertaken using a battery or package of tests that are chosen by the laboratory or by the individual clinician. Low-grade abnormalities or significant clinical entities can then be further investigated, by study of specific organ systems e.g. liver, kidney, GIT etc. This approach has much to recommend it, but it is important to remember that the statistically probability of having one or more values outside a reference range increases can increase with the number of tests carried out, regardless of the presence of disease or...
other compromise. Examples of some of the biochemical tests that are included in screening profiles are reproduced below.

**Total protein**

Hyperproteinaemia can occur in dehydration, extreme hyperfibrinogenaemia and the hypergammaglobulinaemia of chronic infection. Measurement of total protein is therefore too non-specific to be useful in the evaluation of the elite equine athlete. Hypoproteinaemia, when identified, similarly provides better information which broken down into its sub-components. Hypoproteinaemia is always disturbing.

**Albumin**

Hyperalbuminaemia (Alb >35.0 g/l) can be a reflection of dehydration. Clinicians have to recognise that this may be a transient and self-correcting entity in otherwise healthy and well-managed horses sampled at rest and may not merit recourse to fluid therapy. Consistent minor hyperalbuminaemia and electrolyte elevations may be used to remind management of the need for sufficient provision of water. Frank dehydration obviously necessitates correction. Hypoalbuminaemia often occurs as a result of preferential production of globulins in chronic infection, in severe gastrointestinal disease and in rarer conditions such as renal disease. Albumin may also be lost into body cavities e.g. in pleuritis and similar entities.

**Globulin**

Globulins can be divided into their alpha, beta and gamma components using protein electrophoresis. Alpha-2 globulins contain the acute phase proteins. Beta-1 elevations can be seen in parasitism and gamma elevations are seen in chronic infections, usually those that are bacterial in origin.

**Plasma fibrinogen**

Plasma fibrinogen can be measured in a variety of different ways. The modified Clauss method measure reference range is 0.9 to 1.7 g/l. Elevation above these ranges are very seldom seen in elite racehorses consistently performing at the highest levels. Heat precipitation method reference ranges are usually from 2 to 4 g/l. Hyperfibrinogenaemia reflects inflammation. The elevation response usually lags the causal insult by about 24hrs. Serial plasma fibrinogen evaluations are of great value in monitoring response to treatment and in decisions on when normal athletic activity can be resumed. It is important to remember that this is a non-specific indicator of inflammation and even minor entities such as sore heels or girth sores can result in misleading elevations, emphasising once again, the ever present need for clinical examination.

**Creatine kinase (CK)**

Rapid elevations occur in myopathy and other causes of soft tissue damage, which peak within 6-12 hours of the insult. This primarily muscle enzyme has a short half life and even very high elevations can return to normal within 3-4 days. Over-interpretation of minor CK elevations should be avoided, except as a warning in cases of recurrent rhabdomyolysis.

Aspartame Aminotransferase (AST) and Lactate Dehydrogenase (LDH)

These are also muscle enzymes but the latter is less muscle specific than the former. AST has a longer half-life than CK. Because AST levels peak more slowly (24 to 48 hrs post insult) but do not revert to normal (in the absence of further insult) until 10-21 days they are more useful in monitoring severity and the progress of recovery than CK. LDH can be fractionated into its iso-enzymes. LD1 elevations can be seen in haemolysis and LD2 in cardiomyopathy.

**Gamma-glutamyl transferase (GGT)**

Consistent elevations can be seen in consistently successful racehorses. Elevation can also be a non-specific stress indicator, in association with introduction to fast exercise. Hepatic cirrhosis, pancreatitis and renal pathology can also be reflected in GGT elevations. Reliance on GGT as a sole indicator or monitor of hepatic pathology should be avoided.
Bilirubin (indirect)

Elevations can occur in inappetance and when samples are taken prior to feeding, especially morning feeding. Elevation can be a useful indicator of a need for further hepatic evaluation which may include measures of Bile acids, Glutamate Dehydrogenase (GLDH), perhaps followed by Bromosulphthalein (BSP) clearance and or hepatic biopsy.

Urea and creatinine

Elevations should be interpreted with caution in the presence of dehydration and in these circumstances; re-evaluation after correction of hydration status is always advisable. Elevations occur in the presence of renal disease and here, creatinine may be a more valuable diagnostic and prognostic indicator than urea. Renal disease is rare in elite racehorses and the inclusion of these renal parameters in standard screening profiles is therefore questionable.

Electrolytes

Most electrolyte values seen in screening profiles lie within or very close to, reference ranges in healthy horses. They are elevated in tandem with the elevated albumin levels seen in dehydration. Significant deviations from these norms are usually seen in profound illness such as systemic illness, GIT or urinary tract disease.

Conclusion

Any screening profile can be extended to include any of the many blood biochemical tests that are now available. Inclusion of parameters other than those listed above, is largely a matter of personal choice. Further testing and serial investigation is always appropriate where disease entities are identified.

Bibliography/Further reading

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