Hyperlipaemia and the asinine patient

The role of the veterinary nurse in the ongoing blood analysis of an asinine patient with hyperlipaemia is outlined by Owen Levins, veterinary nurse, Dundalk Institute of Technology

Hyperlipaemia is defined as an excess of lipids in the blood (Black's Veterinary Dictionary, 2005). It is a condition prevalent in donkeys (Barton, 2004), which is experienced as a result of negative energy balance (Grove, 2008). Free fatty acids (FFAs) are mobilised from the bodies fat reserves in response to the release of hormone-sensitive lipase. These are re-esterified by the liver to form triglycerides, which are re-released into the plasma (Grove, 2008).

AETIOLOGY

Hyperlipaemia may be either a primary or secondary condition. Primary hyperlipaemia is usually seen as a result of insulin resistance and is most commonly observed in overweight (Barton, 2004) or laminitic animals (Grove, 2008). The insulin resistance disrupts the normal metabolic pathways by not reducing lipase levels, leading to a higher level of FFAs in the blood.

Secondary hyperlipaemia can arise from many states, including stress, inappetance, lactation or concurrent disease and is the most common type seen in practice. This type of hyperlipaemia is due to the body intentionally releasing FFAs to correct a negative energy balance.

DIAGNOSIS

Early diagnosis of hyperlipaemia is crucial due to the high-mortality rates associated with untreated cases. Diagnosis of hyperlipaemia may be difficult, especially in donkeys, as clinical signs can be vague and non-specific (Grove, 2008). The clinical signs and risk factors can be interpreted together to indicate a preliminary diagnosis of the condition. This preliminary diagnosis can be validated on observation of grossly lipaemic plasma (Foreman, 2016). Definitive diagnosis can be made through biochemical analysis of a blood sample as outlined under the blood tests section below.

Clinical signs of hyperlipaemia are most often noticed as behavioural changes of the donkey by its handler (Grove, 2008). Table 1 shows the most common clinical signs associated with the condition.

Risk factors for hyperlipaemia can be either predisposing or precipitating (Bergero & Nery, 2008; Hammond, 2004), as shown in Table 2. When working with donkeys, it is important to keep these factors in mind and any change in behaviour in any patient with even one of these factors should warrant further investigation (Hammond, 2004). In addition to those outlined below in Table 2, Grove (2008) also mentions laminitis, Cushing's syndrome and surgery as possible risk factors for hyperlipaemia.

Stress is one of the main precipitating factors of hyperlipaemia in donkeys (Bergero & Nero 2008; Hammond 2004), so any donkey believed to be under stress should be closely observed and have blood tests carried out to allow for early intervention. Table 3 outlines the most common causes of stress induced hyperlipaemia (Grove, 2008).

Diagnostic testing is crucial to determining the extent of the condition and formulating a comprehensive treatment plan, as the level of triglyceride concentration in the blood

1. Treat any underlying/ concurrent disease
2. Fluid therapy
   a. Maintain normovolaemia
   b. Correct electrolyte imbalances
   c. Restore acid/ base balance
3. Symptomatic therapy
   a. NSAIDs, analgesics, anti-ulcer medication
   b. Multivitamins, anabolics
   c. Antibiotics
4. Nutritional support to maintain positive energy balance
5. Normalise lipid metabolism
   a. Reduce mobilisation
   b. Increase clearance

Figure 1: Five-point treatment plan for donkeys with hyperlipidaemia (Grove, 2008).

Dullness	Depression
Inappetence	Anorexia
Gut stasis with mucus-covered, dry faecal balls	Congested mucous membranes with delayed capillary refill time (CRT)
Halitosis	Pyrexia
Ataxia	Tachycardia
Tachypnoea	Ventral oedema
Hepatic encephalopathy	Abdominal pain

Table 1: Common clinical signs associated with hyperlipaemia (Grove, 2008).

Predisposing	Precipitating
Breed	Anorexia
Sex (Female)	Malnutrition
Obesity	Stress
Pregnancy/ Lactation	Disease

Table 2: Predisposing/precipitating factors of hyperlipaemia, adapted from Watson (1998) in Bergero & Nery (2008).

Relocation	The relocation of a donkey from one farm to another
Separation from herd/partner	Movement of a donkey from one herd to another or the movement of their partner
Death/illness of partner	The death of a donkey's partner or if the partner has been diagnosed with hyperlipaemia

Table 3: Most common causes of stress-induced hyperlipaemia (Grove, 2008).

Plasma triglyceride	Treatment approach	Prognosis
5-8mmol/L	Encourage voluntary feeding using tempting, succulent foods and grazing	Values should return quickly to normal if feeding continues
8-10mmol/L	Nasogastric intubation	Good
10-15mmol/L	Nasogastric intubation +/- IV fluids	Fair if results are reduced promptly. Treat seriously
15-20mmol/L	Nasogastric intubation and IV fluids	Guarded
>20mmol/L	Intensive IV fluids	Poor

Table 4: A rough guide to triglyceride concentration (Grove, 2008).

can indicate the prognosis and treatment approach (Grove 2008), as seen in Table 4.

TREATMENT

Treatment of hyperlipaemia can be quite intensive. Early diagnosis and appropriate treatment greatly improves the patient's prognosis. Grove (2008) outlines a five-point treatment plan for treating hyperlipaemia in donkeys, shown below in Figure 1.

This five-point plan is only an outline, and each patient must be assessed on an individual basis to determine the specific treatment required under these headings.

The veterinary nurse has an important role to play in the ongoing treatment and management of this condition, as most aspects of it can be carried out by a registered veterinary nurse. Fluid therapy, once prescribed by a veterinary practitioner, can be administered and monitored by a veterinary nurse. Veterinary nurses can administer most drugs under the direction of a veterinary practitioner and are also well equipped to monitor the patients' demeanour in a hospital setting and can easily gauge the patient's response to treatment and report back to the veterinary practitioner.

OBTAINING A BLOOD SAMPLE

PREPARATION FOR VENIPUNCTURE

Before attempting venipuncture, the patient identification should be checked. This ensures that the sample is taken from the correct animal. Mistaken identity can be prevented by "checking breed, sex, colour and markings, brands, tattoos, computer chips, photographs, and age by registration papers, if possible" (Vaughn, 2008).

EQUIPMENT

When gathering the necessary equipment, it is important to collect everything you could possibly need. It is also easier to ensure you have spares in the event of any unforeseen circumstances.

SITE PREPARATION

The chosen site of venipuncture should be clipped, ensuring the haircoat is removed directly over the vein. Although most literature states that it may suffice to simply clean the area for venepuncture in equines (Corley, 2008; Hanie ,2006), in this authors experience and on advice from professionals with experience in dealing with donkeys on a regular basis, it is necessary to clip the coat in these patients due to the thickness of their haircoat. Middlecote (2014) states that it is preferable to clip the site of venipuncture in horses with a thick haircoat and prepare the skin aseptically, to prevent septicaemia.

Gloves should be worn when preparing the site aseptically. If there is gross dirt present at the proposed site, a scrub should be performed using gauze swabs soaked in a 4% chlorhexidine solution. This should be repeated until the swabs come away free from dirt and debris. Following the scrub, the site should be rinsed with 70% isopropyl alcohol. The alcohol should be left to air-dry. This may take up to 15 minutes. Once the site is dry, venipuncture may be attempted. If the site is free from gross contamination, the scrub may be skipped and the site soaked in 70% isopropyl alcohol as outlined above.

Plasma triglyceride	Treatment approach	Prognosis
Clippers	Chlorhexidine 4% scrub	1.5in 18g needles
Spare clipper battery	Isopropyl alcohol	Gauze swabs
Spare 40 blade for clipper	20ml syringes	Vacutainers

Table 5: Equipment to be gathered for blood collection.

RESTRAINT

If a donkey is suspected of suffering from hyperlipaemia, they will usually be quite dull and depressed, therefore will not usually resent handling or manual restraint. Chemical restraint is less than ideal when collecting a blood sample, and is not usually necessary in these patients.

For manual restraint, a headcollar and lead rope should be applied. The head should be controlled by the person holding the lead rope. The head should be turned contralaterally to the proposed site of venipuncture to extent the neck and allow for easier visualisation and palpation of the jugular vein. The use of a stocks or chute is preferable for more aggressive patients. Matthews (2008) believes that nose twitches are not very effective on most donkeys.

VENIPUNCTURE

SUITABLE SITES

The site of choice for collection of a blood sample in the donkey is the jugular vein as it is large and easily accessible (Corley 2008). If the jugular vein is unavailable, other sites are the cephalic, lateral thoracic, saphenous or coccygeal veins (Hanie, 2006; Barr et al, 2009).

PROCEDURE

In this author's experience, it is more practical to collect the sample using a syringe rather than directly into a vacutainer, however both methods are well documented (Hanie, 2006; Barr et al, 2009). Pressure should be applied to the vein distally to the proposed site and the vein allowed to fill.

Hanie (2006) and Barr et al (2009) recommend a 1-1.5 inch, 20g needle be used in horses, but this author has seen 18g needles used successfully in donkeys. The needle (attached to the 20ml syringe) is inserted through the skin directly over the distended vein at approximately 45-60° to the skin. This angle is slightly larger than would be used in a horse, as donkeys have much thicker skin (Matthews, 2008). The vein can be steadied by using the thumb of the opposite hand. Once a flash of blood is observed in the hub of the needle, the plunger is withdrawn with slow even pressure. It is advisable to withdraw approximately 8-10ml of blood for each vacutainer to be filled. Once the desired amount of blood has been collected, the needle is withdrawn from the vein and digital pressure applied to the site for minutes using a clean, dry gauze swab.

COLLECTION AND STORAGE OF SAMPLE

Once the correct amount of blood has been placed in the required tube, the tube should be gently inverted eight times to ensure proper mixing of the sample with the anticoagulant.

For serum biochemistry, Malikides et al (2000) indicates that blood should be collected into a Lithium Heparin anticoagulant vacutainer tube (green top). Malikides et al (2000) suggest samples should be submitted for analysis as soon as possible after collection, but if this is not possible, the serum should be separated from the red cells within one hour of collection and stored in a clean, plastic vial. This can preserve the sample for 12-24 hours if refrigerated (Malikides et al, 2000). Holtgrew-Bohling (2016) states that analysis should take place within four to six hours of the samples collection, or else the sample separated and refrigerated.

Middlecote (2014) suggests that blood being collected for haematological analysis be collected in an EDTA Ethylenediaminetetraacetic acid (EDTA)-anticoagulant vacutainer tube (purple top). This sample can also be used to test the patients PCV. A plain tube should also be collected to allow for visual appraisal of the patients' blood. This is extremely useful when hyperlipaemia is suspected.

BLOOD TESTS

After collection of the blood sample, the plain tube should be left to stand for three to 10 minutes (Grove, 2008). This will provide an early indication of hyperlipaemia, as the plasma will be saturated with lipids causing it to appear milky. The difference between a normal sample and a sample with lipaemic plasma is shown in Figure 2.

Grove (2008) suggests that both serum biochemistry and routine haematology should be carried out to assess hydration, liver and kidney function, white blood-cell ratios and electrolytes, all of which may be affected in cases of hyperlipaemia and may indicate the severity of the case.

Step 1:	Place a small drop of blood near one end of a clean glass microscope slide
Step 2:	Place the narrow edge of a second slide against the surface of the first slide at a 30° angle
Step 3:	Draw the spreader slide back into the drop of blood
Step 4:	Allow the blood to spread along most of the width of the edge of the spreader slide
Step 5:	Push the spreader slide forward with a steady, even, rapid motion
Step 6:	Wave the slide gently to allow it to air dry

Table 6: Steps to prepare a wedge-film blood smear (Anthony & Sirois 2002).

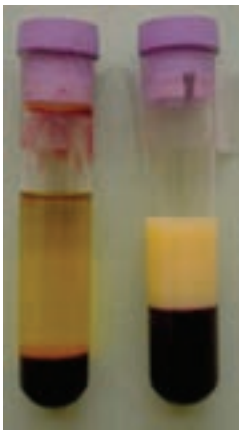

Figure 2.

BIOCHEMISTRY

Figure 3 shows suggestions for the biochemical analysis that should be carried out for the diagnosis and monitoring of hyperlipaemia. When assessing these results, it Is important to evaluate liver function, kidney function and electrolytes (Grove, 2008; Hammond, 2004).

HAEMATOLOGY

When undertaking haematological analysis, it is possible to use either automated or manual methods. If a haematology analyser is to be used, the manufacturer's instructions should be adhered to. The following test should be completed using the sample mixed with EDTA anticoagulant. Manual evaluation of a blood sample comprises of three parts: blood smear; packed cell volume (PCV); and total protein.

BLOOD SMEAR

Blood smears allow for the differential counting of white blood cells WBCs) and morphological assessment of red blood cells (RBCs). Anthony & Sirois (2002) outline the procedure to prepare a blood smear for a differential cell count. Although a haematology analyser may perform this function, it is important to carry out a manual assessment (Shoemaker, 2009; Anthony & Sirois, 2002).

To prepare a wedge-film blood smear, Anthony & Sirois (2002) suggest the method outlined in Table 6.

The prepared blood smear should then be stained with a Romanowsky type stain, as these afford the best overall morphological assessment (Anthony and Sirois 2002; Shoemaker, 2009).

PCV

PCV allows for the assessment of dehydration, which may occur secondary to hyperlipaemia.

Holtgrew-Bohling (2016) outlines the technique to carry out a PCV test as follows:

Fill a capillary tube ¾ of the way full, and seal the end with clay. Place the tube in a centrifuge and balance. Spin for three to five minutes. Record the colour and transparency of the plasma and record the percentage using a PCV card.

TOTAL PROTEIN

The assessment of the total protein uses a refractometer

Analyte	Normal range	Analyte	Normal range
TRIG (Triglycerides)	0.6-2.8mmol/L	Glob (Globulin)	32-48g/L
CPK (Creatinine phosphokinase)	128-525iu/L	Creat (Creatinine)	53-118µmol/L
AST (Aspartate aminotransferase)	238-536 iu/L	Urea	1.5-5.2mmol/L
GGT (Gamma-glutamyl transpeptidase)	14-69iu/L	Amy (Amylase)	1-10.6iu/L
GLDH (Glutamate dehydrogenase)	1.2-8.2iu/L	Lip (Lipase)	7.8-27.3IU/L
ALP (Alkaline phosphatase)	98-252iu/L	Ca (Calcium)	2.2-3.4mmol/L
Bile acids	2.6-18.6µmol/L	Na (Sodium)	128-138mmol/L
TBil (Total bilirubin)	0.1-3.7µmol/L	K (Potassium)	3.2-5.1mmol/L
TP (Total protein)	58-76g/L	Cl (Chloride)	96-106mmol/L
ALB (Albumin)	21.5-31.6g/L	Chol (Cholesterol)	1.4-2.9mmol/L

Table 7: Normal biochemical values (adapted from Burden et al, 2016).

and the plasma obtained during the PCV test. Holtgrew-Bohling (2016) details the procedure as follows:
After completing the PCV, break the capillary tube just above the buffy coat. Place a drop of plasma on the lens of a refractometer and look through it to record the value. When using a refractometer, it is important to ensure it is correctly calibrated and to read the correct scale through the eyepiece.

VALUES INDICATIVE OF HYPERLIPAEMIA

BIOCHEMICAL

- Triglyceride: The main biochemical marker of hyperlipaemia is serum triglyceride levels. Any elevation above the normal range is cause for concern and should be treated (see Table 4).
- Bile acids: An increase in bile acids may be seen in some cases of hyperlipaemia (Svendsen, 2008).
- Cholesterol: Increased cholesterol levels may be present in cases of hyperlipaemia (Svendsen 2008).

HAEMATOLOGICAL

- PCV: PCV may be increased due to dehydration, secondary to hyperlipaemia (Grove, 2008). It is important to note that unlike horses, donkeys do not haemoconcentrate until they are seriously dehydrated and so an increase in PCV will not be seen with mild to moderate dehydration (Matthews, 2008).
- NEU: Neutropenia with left shift may be apparent in patients with hyperlipaemia (Foreman, 2016). This can be detected through a differential WBC count.

SUGGESTED BIOCHEMISTRY PANEL

- Triglyceride and glucose
- Hydration status
  - » Packed cell volume
  - » Plasma protein
- Renal function
  - » Creatinine
  - » Blood urea nitrogen
  - » Electrolytes (Na^+, K^+, Cl^-, $Ca2^+$)
- Blood gas analysis
  - » Partial pressue of carbon dioxide
  - » Partial pressue of bicarbonate
  - » pH
- Liver damage
  - » Sorbitol dehydrogenase
  - » Gamma glutamyl transferase
  - » Alkaline phosphatase
  - » Alkaline aminotransferase
- Liver function
  - » Bile acids
  - » Ammonia

Figure 3.

CLINICAL RELEVANCE

Ongoing blood tests are indicated in cases of hyperlipaemia to assess the success of the treatment. These blood tests allow for the treatment plan to be updated and made more effective. The initial intensive treatment

Parameter	Normal range	Parameter	Normal range
RBC (Red blood cell count)	4-7.3 X1012/L	EOS % (% eosinophils)	1-10%
PCV (Packed cell volume)	25-38%	EOS T (Total eosinophils)	0.09-1.15 X109/L
Hb (Haemoglobin)	9-15.3g/dL	BAS % (% basophils)	0-0.8 %
MCH (Mean corpuscular haematology)	18.9-28.6pg	BAS T (Total basophils)	0-0.5 X109/L
MCHC (Mean corpuscular haematological concentration)	31.4-39.1g/dL	LYM % (% lymphocytes)	17-65%
MCV (Mean corpuscular volume)	57-79fl	LYM T (Total lymphocytes)	1.8-7.8 X109/L
WBC (White blood cell count)	6.1-16.1 X109/L	MON % (% monocytes)	0-5 %
NEU% (% Neutrophils)	28-78 %	MON T (Total monocytes)	0-0.8 X109/L
NEU T (Total neutrophils)	2.2-13.3 X109/L	Platelets	4-7.3 X109/L

Table 8: Normal haematological values (Grove, 2008).

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can be discontinued when the plasma triglyceride levels have been reduced sufficiently to allow for fluid therapy to be discontinued (see Table 4). For this reason, ongoing monitoring is important to guide the level of care required by the patient. The monitoring should be continued until the triglyceride levels are back within normal ranges, at which stage treatment can be discontinued and the patient simply monitored for any outward clinical signs of relapse. Depending on how early this condition is noticed, prognosis can vary widely, as seen in Table 4. As this condition is quite prevalent in donkeys, it is important that owners are made aware of the subtle signs they should be watching out for. Donkeys are stoic animals, and will do their best to avoid showing weakness or pain until it is often too late to restore them to full health. Donkeys are immensely affectionate and social animals, and a little 'tender, loving, care' can go a long way to making them feel more comfortable and responsive to treatment. This is a role that veterinary nurses excel at, and can make all the difference to these patients.

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