UNIVERSITI PUTRA MALAYSIA

FLUID THERAPY RESPONSE IN ENDURANCE HORSES WITH METABOLIC AILMENTS

KUAN KIT LENG

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A project paper submitted to the

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It is hereby certified that we have read this project paper entitled "Fluid Therapy Response in Endurance Horses with Metabolic Ailments" by Kuan Kit Leng and in our opinion it is satisfactory in terms of scope, quality, presentation as partial fulfilment of the requirement for the course VPD 4901 Project.

DR. NORANIZA MOHD ADZAHAN
DVM, MVM, PHD (UPM)
LECTURER,
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Supervisor)

ASSOC. PROF. DR. GOH YONG MENG
RLATG, DVM (UPM), PHD (UPM)
LECTURER,
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Co-supervisor)

Dedication

To my Loving Mummy & Daddy, Big Brother,

Awesome Horses,

Caring Family, Fantastic Friends,

& in Loving Memory of Poh Poh & Ah Mah

You All will be Loved Dearly as

Happiness is Real Only when Shared

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LIST OF ABBREVIATIONS

AST	aspartate aminotransferase
beats min^{-1}	beats per minute
BUN	blood urea nitrogen
Ca^{2+}	calcium ions
$[Cl^{-}]$	chloride ion concentration
CK	creatine kinase
CRI	cardiac recovery index
FEI	Fédération Équestre Internationale
G	gauge
GGT	gamma glutamyl transferase
Hb	haemoglobin
HCO^{3-}	bicarbonate ions
hr	hour
HR_0	heart rate at beginning of inspection
HR_1	heart rate after 40m trot within 1 minute
$[K^{+}]$	potassium ion concentration
kg	kilogram
km	kilometer
L hr^{-1}	litres per hour
LDH	lactate dehydrogenase
MCHC	mean corpuscular haemoglobin concentration

MCV	mean corpuscular volume
MD	metabolically-disordered
Mg^{2+}	magnesium ion
mins	minutes
$ml\ kg^{-1}$	millilitres per kilogram
N	total population
n	number of samples
$[Na^+]$	sodium ion concentration
p-value	significance level
PCV	packed cell volume
R	coefficient of correlations
r^2	coefficient of determination
RBC	red blood cells
RTES/UPM	Royal Terengganu Endurance Stable/ Universiti Putra Malaysia
SC	successfully completed
secs	seconds
WBC	white blood cells

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ABSTRAK

Abstrak kertas projek yang dikemukakan kepada Fakulti Perubatan Veterinar dalam memenuhi sebahagian daripada kursus VPD 4999 - Projek Tahun Akhir.

Respon Terapi Cecair dalam Kuda Lasak yang mengalami Masalah Metabolik

oleh :

Kuan Kit Leng

2015

Penyelia : Dr. Noraniza Mohd Adzahan

Penyelia bersama : Prof. Madya Dr. Goh Yong Meng

Aktiviti kuda lasak adalah disiplin FEI yang berkembang pesat di seluruh dunia. Kuda lasak yang menghadapi penyingkiran metabolik umumnya memerlukan terapi cecair. Kajian ini menilai perubahan fizikal, hematologi dan biokimia dalam kuda lasak yang mengalami penyingkiran metabolik sebagai respon terhadap terapi cecair. Kajian ini juga mengkaji korelasi kelajuan tunggangan dan jarak perjalanan dengan keabnormalan fizikal, hematologi dan biokimia parameter yang mengakibatkan penyingkiran metabolik. Sembilan daripada enam puluh satu ekor kuda lasak yang menyertai aktiviti kuda lasak telah disingkirkan akibat masalah metabolik dan dihantar ke Hospital Ekuin RTES / UPM. Selepas pemeriksaan, terapi cecair intravena telah diperuntukkan dengan 0.9 % larutan garam pada kadar 5-10L jam^{-1}. Sampel darah diambil melalui vena leher sebelum dan selepas terapi cecair. Kadar denyutan jantung, tahap dehidrasi, kapilari isi semula, warna membran mukosa dan bunyi gastrousus didapati bertambah baik dengan ketara berikutan terapi cecair. Parameter hematologi dan biokimia adalah konsisten dengan pencairan darah tetapi sebahagian daripadanya kembali kepada nilai rujukan biasa. Kesimpulannya terdapat perbezaan yang ketara dalam parameter fizikal, hematologi dan biokimia berikutan terapi cecair intravena. Prognosis kuda lasak yang mengalami masalah metabolik dalam kajian ini adalah baik kerana mereka bertindak balas dengan baik terhadap terapi cecair intravena (0.9% larutan garam). Walau bagaimanapun, tiada hubungan yang ketara antara kelajuan tunggangan dengan keabnormalan parameter fizikal, hematologi dan biokimia yang mengakibatkan penyingkiran metabolik kecuali jarak perjalanan dengan indeks ikterus, bilirubin dan urea.

Kata kunci: *kuda lasak, metabolik, terapi cecair, dehidrasi*

ABSTRACT

An abstract of the project paper presented to the Faculty of Veterinary Medicine in partial fulfilment of the course VPD 4999 - Final Year Project.

Title: Fluid Therapy Response in Endurance Horses with Metabolic Ailments

By:

Kuan Kit Leng

2015

Supervisor: Dr. Noraniza Mohd Adzahan

Co-supervisor: Assoc. Prof. Dr. Goh Yong Meng

Endurance riding is a fast-growing FEI discipline worldwide. Metabolically-eliminated endurance horses generally necessitate provision of fluid therapy. This study evaluates the physical, haematological and biochemical changes of metabolically eliminated endurance horses in response to fluid therapy. This study also determines the correlation of riding speed and distance travelled with abnormalities in physical, haematological and biochemical parameters that constitute metabolic elimination. Nine out of sixty-one endurance horses participating in an endurance ride were metabolically eliminated and sent to RTES/UPM Equine Hospital. These horses were examined by veterinarians and intravenous fluid therapy was indicated with 0.9% saline solution at rate of 5-10L hr^{-1}. Blood sampling via jugular venipuncture was performed before and after fluid therapy. Heart rate, skin tenting duration, colour of mucous membrane, capillary refill time and gastrointestinal sounds improved significantly following fluid therapy. Haematological and biochemical parameters were consistent with haemodilution but several were restored back to normal reference values. In conclusion, there was significant difference in physical, hematological and biochemical parameters following intravenous fluid therapy. Since endurance horses with metabolic ailments in this study responded well to intravenous fluid therapy (0.9% saline solution), their prognosis was good. However, there was no significant correlation between riding speed with physical, haematological and biochemical parameters except for distance travelled with icterus index, bilirubin and urea.

Keywords: *endurance horses, metabolic, fluid therapy, dehydration*

1.0 INTRODUCTION

Endurance is one of the most thriving FEI disciplines to date. Endurance riding is a long-distance competition to challenge the athlete’s skill to safely manage the stamina and fitness of the horse over an endurance course crossing all kinds of track, distance, climate, terrain and not forgetting a race against the clock (Fédération Equestre Internationale, 2014). However, endurance riding poses greater emphasis on finishing in good condition rather than finishing first.

Equine welfare is of paramount importance, thus veterinary examinations are conducted at the end of each phase to ensure that the endurance horses are fit to continue the race. In fact, endurance horses may get disqualified from the race due to lameness or metabolic reasons. According to Robert (2014), although musculoskeletal injuries may arise at any point along an endurance ride, metabolic disorders generally pose an emergency situation. Besides that, endurance horses also face elimination if they fail to be present at the vet-gate after cooling within the stipulated time of 20 mins recovery period.

The most common reasons for metabolic elimination in endurance horses includes poor cardiovascular recovery, synchronous diaphragmatic flutter, reduced or absent borborygmi, exertional myopathy, colic and exhausted horse syndrome. In practise today, horses with metabolic elimination are submitted to the nearest Equine Hospital and receive fluid therapy as needed. However, question remains, “Is fluid therapy justifiable or able to improve the alarming metabolic status at hand?”

The purpose of this study was to find out if fluid therapy is justifiable on the grounds of metabolic elimination and to determine the response of fluid therapy based on changes in physical, haematological and biochemical parameters in metabolically eliminated horses. According to Jose-Cunilleras (2014), no wild horse will run over a protracted period of time at high speeds, as we force endurance horses to do so over long distances. Thus, the objective of this study was to determine the correlation of riding speed and distance travelled with abnormalities in physical, haematological and biochemical parameters that constitutes metabolic elimination of endurance horse.

2.0 LITERATURE REVIEW

2.1 Composition of equine sweat

Equine sweat, unlike human sweat, is a hypertonic fluid consisting of $[Na^+]$= 150mmol/L, $[K^+]$=50mmol/L, $[Cl^-]$=200mmol/L (Mcconaghy *et al.*, 1995). Horses losing sweat will lead to a hypotonic and interstitial fluid (Kronfeld, 2001). As mentioned by Schott & Hinchcliff (1998), with respect to equine sweat composition, since increase in plasma osmolality is a more potent stimulus for thirst compared to hypovolemia, thirst in these athletic horses is not triggered until a significant loss of body fluid takes place. Thus, horses performing strenuous exercise or athletic events of several hours duration are at risk of losing significant amounts of water and electrolyte.

In fact, the most important route of dissipating the heat load generated by working skeletal muscles during exercise is the evaporation of sweat which produces a cooling effect which is critical in preventing hyperthermia during exercise (Hodgson *et al.*, 1994). Horses have a thick, waterproof hairy coat that would normally slow down rapid translocation of sweat from skin to surface of hair for evaporative cooling. Thus, to solve this, horses produce a detergent-like protein called latherin which helps to wet the hairs to enhance spread and evaporation of sweat (McDonald *et al.*, 2009).

2.2 Exercise-associated dehydration/ exhausted horse syndrome

Exercise-associated dehydration or exhausted horse syndrome is most commonly seen in horses competing in athletic events such as endurance rides or exercising for protracted periods in hot and humid environments (Jose-Cunilleras, 2014). This multisystemic disorder occurs as a result of compound effects of dehydration, hypovolaemia, acid base and electrolyte disturbances, energy substrate depletion and hyperthermia (Robert, 2014). Essentially, it is a condition that necessitates medical intervention which focuses on correction of fluid and electrolyte deficits and aggressive cooling of overheated horse (McGowan and Geor, 2014).

2.2.1 Recognition of exercise-associated dehydration/ exhausted horse syndrome

Dehydrated horse typically exhibit clinical signs such as anorexia, lack of thirst despite obvious dehydration, persistently elevated heart rate, respiratory rate and rectal temperature. Besides that, these horses may have delayed jugular and capillary refill time and skin tenting duration, pale or congested, tacky or dry mucous membrane, produce highly concentrated urine and/or dry faeces, poor gastrointestinal motility, poor anal tone, ileus and even colic (Whiting, 2009). Cardiac arrhythmias may be an uncommon finding (Jose-Cunilleras, 2014). However, in severe cases, cardiovascular collapse and hypovolemic shock may ultimately be fatal (Whiting, 2009).

Meanwhile, initial signs of an exhausted horse include mild alterations in attitude and alertness and may develop gait abnormalities due to muscle soreness. Unfortunately, more severe clinical signs may manifest as development of mental alterations, for example, obtundation and lethargy which may progress to marked neurologic signs such as ataxia, circling, seizures or head pressing. These exhausted horses become reluctant to continue exercising and display muscle stiffness, cramps extending to fulminant rhabdomyolysis and eventually recumbency. Metabolic disturbances may contribute to the onset of synchronous diaphragmatic flutter or also known as 'thumps'(Whiting, 2009).

2.2.2 Clinicopathological findings in metabolically disordered endurance horse

According to Muñoz *et al.* (2010), metabolically disordered (MD) endurance horses had higher packed cell volume (PCV), total serum protein, albumin, serum urea nitrogen and creatinine than successfully completed (SC) horses which may signify severe dehydration. Adamu *et al.* (2012), illustrated that MD endurance horses exhibited lactate concentration ranging around transitional level of aerobic to anaerobic threshold while SC endurance horses had a concentration essential for aerobic performance in fit horses during a 120 km race. Teixeira-Neto *et al*. (2008) reported that there was no significant alteration in plasma creatine kinase (CK), aspartate aminotransferase (AST) and lactate dehydrogenase (LDH) between eliminated endurance horses and SC endurance horses throughout the race.

Fielding *et al.* (2009) reported that MD endurance horses had lower plasma chloride (Cl^-) and potassium (K^+), and higher total protein that SC endurance horses in a 160 km race. Exhausted horses usually show decline in serum electrolyte such as K^+, Cl^-, calcium (Ca^{2+}), magnesium (Mg^{2+}), and occasionally Na^+ (Robert, 2014). Whiting (2009) states that plasma [Na^+] can vary in exhausted horses, as significant amount of sodium lost via sweat is masked by accompanying losses of total body water. In addition, Al-Qudah and Al-Majali (2008) reported that 9 horses eliminated for synchronous diaphragmatic flutter in a 120 km race had a significant decrease in plasma Ca^{2+} level.

2.3 Metabolic indicators monitored during endurance race

Competing horses are subjected to a series of examinations and inspections complying with the FEI Veterinary Regulations to protect their welfare, safety and health throughout the race. Physical parameters that reflect on the metabolic status of a horse and will be assessed by veterinarians include, however not entirely exclusive to, cardiac recovery index (CRI), demeanour, mucous membrane, capillary refill time, hydration and intestinal activity. CRI, which was developed by Kerry Ridgway, is evaluated by recording the recording the heart rate at the beginning of inspection (HR_0) and 1 minute later after 40m trot out and back for gait assessment (HR_1) (Fédération Equestre Internationale, 2014). Robert *et al.* (2002) reported that heart rate ($HR_0 \geq 60$ beats min^{-1}) and CRI (HR_1-$HR_0 \geq 4$) were significantly higher in eliminated horses compared to those successfully completed.

2.4 Fluid resuscitation for dehydrated or exhausted horse

2.4.1 Fluid rates and administration of intravenous fluid therapy

Fortunately, exercise-induced dehydration and exhausted horse syndrome is responsive to medical therapy if fluid therapy is aggressive. Generally, fluid administration focuses on current fluid deficits; however, maintenance requirements and ongoing losses can be forgone in exhausted or dehydrated horses unless the horse develops diarrhoea, ileus with abundant nasogastric reflux or polyuric renal failure. In practice, administration 5 to 10L hr^{-1} is carried out via a fully open 14 G intravenous catheter inserted in one or both jugular veins. However, exhausted horses with hypovolemic shock may require 10 to 12 G intravenous catheter to all rapid administration of up to 30 to 40 L within 1 to 2 hrs for resuscitation (Jose-Cunilleras, 2014). According to Bergero *et al.* (2005), replacement fluid therapy should be based on sweat losses occurring per hour of exercise, typically about 2 to 5 L, but can be much greater losses of 10 to 15 L if exercising under hot and humid conditions.

2.4.2 Comparison between fluid types

Isotonic crystalloids may be sufficient to restore fluid and electrolyte homeostasis in horses suffering from mild to moderate dehydration of short duration in the absence of sustained gastrointestinal dysfunction and on-going losses (Seahorn and Seahorn, 2003). Furthermore, crystalloids are preferred in cases of isotonic dehydration as colloids do not restore fluid lost from the interstitial fluid space (Cook and Bain, 2003). Hypertonic saline is indicated in conditions involving hypovolaemia or traumatic brain injuries (Fielding and Magdesian, 2010). Fielding and Magdesian (2011) reported that hypertonic saline (7.2 %) administered at 4 ml kg^{-1} promotes greater expansion of plasma volume compared to isotonic saline (0.9 %) and is a preferred fluid choice for rapid resuscitation during emergency medical treatment. However, it must be immediately followed by administration of large volumes of isotonic or hypotonic intravenous or oral fluids. Seahorn and Seahorn (2003) reported that electrolyte levels should be monitored prior and following hypertonic saline due to risk of iatrogenic hypernatremia and hypokalaemia. Dehydration associated with exhausted horse syndrome or hyperthermia in endurance race is a field situation and usually treated with rapid infusion of 10 to 20 L isotonic sodium chloride intravenously. In addition, sodium chloride is the fluid choice for patients alike as their acid base status is variable and commonly suffers from metabolic alkalosis. According to Jose-Cunilleras (2014), Ringer's solution, which is also an acidifying solution, is preferred over lactated Ringer's solution as the latter produces a mild alkalinizing effect and thus, contraindicated in the already alkalotic horse. However, volume of fluid administration is much more important compared to the acidifying or alkalinizing nature of fluid and veterinarians must not abandon fluid therapy although the only choice of fluids is alkalinizing.

2.4.3 Oral versus intravenous rehydration

Schott (2006) states that oral rehydration could help to stimulate of intestinal motility however is contraindicated if there is presence of nasogastric reflux or severe resistance by horse during nasogastric intubation. Oral fluids are ideal in mild to moderately dehydrated (5 to 7 %) horses but absorption may be compromised due to reduced blood flow to intestinal tract in more severely dehydrated (7 to 15 %) horses. According to Jose-Cunilleras (2014), it is generally recommended to administer isotonic electrolyte solutions at a rate of 6 to 8 L every 30 to 60 mins with the aid of nasogastric tube. If there is no improvement of clinical condition within the following 2 to 4hrs, intravenous fluids should be administered immediately.

2.5 Effect of riding speed and distance travelled

According to Adamu *et al.* (2012), SC endurance horses travelled at constant speeds throughout the 120 km race while MD endurance horses decreased gradually and subsequently eliminated from the race. In another 120 km endurance race, majority of horses stopped drinking in the second loop and subsequently in the third loop, most were eliminated due to reduced speeds (Adamu *et al.* 2014). Ecker and Lindinger (1995) reported that increasing speed contributed significantly to all ion losses excluding K^+ while increasing distance for Na^+, Cl^- and K^+ losses.

3.0 MATERIALS AND METHODS

3.1 Study design

A total of 61 horses participated in the endurance race for three categories, which are 40 km, 80 km and 120 km. Those of which consist of 14 horses for 40 km, 21 horses for 80 km, and 26 horses for 120 km. The age of horses in 40 km category were ≥ 5 years old while those in 80 and 120 km categories were ≥ 6 years old. Each of the categories consists of a few loops that range 20 to 30 km each. After each loop, veterinary inspection was done on all competing horses. Physical parameters evaluated were recorded in the National Federation Log Book which includes heart rate, cardiac recovery index, gait, skin tenting duration, jugular and capillary refill time, mucous membrane colour, gastrointestinal sounds, anal tone, muscle tone, girth, withers and back, leg injuries and overall grading. All races were conducted with strict compliance to FEI regulations. Each horse serves as its own control. Baseline data (before intervention-fluid therapy administration) was compared to the outcome (after intervention).

3.1.1 Criteria for metabolic elimination

The maximum heart rate acceptable for 40km is 56 beats min^{-1} whereas for 80 and 120km is 64 beats min^{-1}. Besides persistently elevated heart rate, poor cardiac recovery index can also cause metabolic elimination. Other parameters assessed which deem horse failure to qualify due to metabolic reason included delayed skin tenting duration and capillary refill time, poor mucous membrane colour, reduced or absent intestinal activity, and even poor demeanour.

3.2 Inclusion criteria

All horses that fail to qualify due to metabolic elimination, which came up to a total of 9 horses were chosen as study subjects.

3.3 Riding speed and distance travelled

Competing horses, N=61, were subjected to a defined course (loops or phases) and minimum or maximum speed limit (Table 1-3). After the endurance race, the speed and distance travelled by the horses with metabolic elimination, n=9, were recorded.

3.4 Fluid therapy

After the first blood collection, site for intravenous catheter was prepared aseptically. Jugular vein was inserted with BD Angiocath™ 14 G intravenous catheter. Intravenous fluid therapy was initiated with administration of 0.9 % saline solution at approximately 5 to 10 L hr^{-1}. Fluid therapy continued till physical body parameters improved, for example, heart rate was $\leq$ 60 beats min^{-1}, normal mucous membrane, skin tenting and capillary refill time. Total amount of fluid administered was recorded in the Treatment Card.

3.5 Physical parameter

Horses that fail to qualify due to metabolic elimination, n=9, were submitted to RTES/UPM Equine Hospital immediately and were evaluated by Treatment Veterinarian. Physical parameters were monitored and reassessed every 15 to 20 mins interval with regard to treatment response. All physical examination findings were recorded in the Treatment Card. In addition, physical parameter findings from

National Federation Log Book that caused horses voted out for metabolic reasons were recorded.

3.5.1 Heart rate

Heart rate was the first parameter to be assessed with the aid of a stethoscope. The heart rate was recorded for 1 min.

3.5.2 Skin tenting duration

Skin tenting was performed by pinching of the skin in a fold nipped between a thumb and forefinger at the eyelid or point of the shoulder region. Skin tenting duration is the holdup of the skin returning to its normal shape after skin tenting. Mildly dehydrated skin requires approximately 1sec while severely dehydrated skin may require >3 secs to return to its normal position (Adamu *et al*., 2014).

3.5.3 Colour of mucous membrane

Colour of mucous membrane of gingiva and conjunctiva was also assessed. Normal mucous membrane = A is pink, mildly congested mucous membrane = B appears pale, severely congested mucous membrane = C is obviously reddened, and cyanotic mucous membrane = D appears as bluish discolouration.

3.5.4 Capillary refill time

Normal capillary refill time is observed when blood refills instantly after slight pressure applied to gums is released. Mild capillary refill time is when it barely takes one second to refill while severe capillary time is observed when it takes >3 secs for the pinkness to resurface.

3.5.5 Gastrointestinal sounds

Gastrointestinal sounds are evaluated with the aid of stethoscope at the 4 quadrants which consist of left upper and lower quadrant and right upper and lower quadrant. Normal gut sounds can be heard as 2 long rolls, followed by several small gurgles within 1 min (Gore *et al*., 2008). Normal gut sounds = A, mild decrease in gut sounds = B, moderate decrease in gut sounds = C, marked decrease in gut sounds or absent = D.

3.6 Blood sampling

After the first physical examination done by Treatment Vets, blood sampling was done via jugular venipuncture using a Venoject® Tube Holder, 21 G × 1.5" (0.8 × 38 mm) BD Vacutainer® PrecisionGlide™ Multiple Sample Needle, BD Vacutainer® containing lithium heparin (75 USP Units). Approximately 4 ml of blood was sampled prior and immediately after intravenous fluid administration. Second blood collection was done via jugular vein on the opposite side of the intravenous catheter placement.

3.6.1 Haematology test

Erythrocyte, Hb concentration, leukocyte, and thrombocyte were analysed using scil ABC Vet™ Animal Blood Counter with a predetermined setting using a "Horse" Smart Card®. PCV was analysed using Hettich-Haematocrit 210 and Hawksley micro-haematocrit reader. MCV and MCHC were calculated using formulae. Plasma protein was analysed using Atago® hand-held refractometer. Icterus index was analysed visually using standardized coloured diagrams.

3.6.2 Serum biochemistry test

Plasma electrolyte levels and biochemical parameter- Na^{+}, K^{+}, Ca^{2+}, urea, creatinine, glucose, bilirubin, AST, CK, total protein, albumin, globulin and lactate were determined using chemical analyser (Abaxis VetScan VS2 with Equine Profile Plus and Hitachi 920).

3.7 Statistical analyses

The normally distributed data were analysed using Paired T-Test and Wilcoxon Signed-Rank Test while non-normally distributed data were analysed using Pearson and Spearman Correlation Coefficient Tests. Data are reported as mean S.E. for normally distributed data and median for non-normally distributed data. The statistical software package IBM SPSS Statistics 20 was used for the analysis. Analyses were considered as significant at $P<0.05$.

4.0 RESULTS AND DISCUSSION

4.1 Physical parameters

4.1.1 Heart rate

Table 4: Pre-treatment and post-treatment physical parameters (based on Treatment Card)

Parameter	Treatment		Reference Range	Percentage Difference %
	Pre-	Post-		
Heart rate (beats min^{-1})	$66^{a} \pm 5$	$48^{b} \pm 2$	40 km: ≤ 56 80 &120 km: ≤ 64	-27.27

Values are expressed as mean ± S.E [a,b]. Means within rows with different superscripts are significantly different at $p<0.05$.

In this study, heart rate measured upon arrival at Equine Hospital was $66^{a} \pm 5$ beats min^{-1}, which was above the maximum limits. However, following fluid therapy, heart rate reduced significantly by 27.27% to 48 ± 2 beats min^{-1}.

According to Adamu *et al.* (2012), eliminated horses have higher heart rates compared to good performance endurance horses. Heart rate is an important criterion to accurately assess the horse's recovery and fitness to continue. However, evaporative cooling becomes ineffective in hot and humid climate. The heart pumps diligently to remove excess heat from the body core to periphery. However as dehydration starts to take a toll, there is decrease in effective circulating blood volume and cardiac stroke volume as well. Thus these horses under compromise tend to have persistently elevated heart rates or poor cardiac recovery index. In the present study, intravenous fluid therapy administered manage to improve cardiovascular function by volume expansion and cooling thus bringing down the heart rate to normal range.

Table 5: Pre-treatment and post-treatment physical parameters (based on Treatment Card)

Parameter	Treatment		Reference Range	Percentage Difference %
	Pre-	Post-		
Skin tenting duration (sec)	2.00[a]	1.00[b]		-50.00
Mucous membrane colour	7.00[a]	1.00[b]		-85.71
Capillary refill time (sec)	2.00[a]	1.00[b]		-50.00
Gut sounds	7.00[a]	1.75[b]		-75.00

Values are expressed as median [a,b]. Means within rows with different superscripts are significantly different at $p<0.05$.

4.1.2 Skin tenting duration

Skin tenting duration reflects on part of the extracellular fluid compartment of the body. Extracellular fluid comprises of intravascular compartment, interstitial fluids and any part excluding the cells accounting for one-third of total body weight. On the other hand, intracellular fluid comprises of fluid within the cytoplasm of cells accounting for two-thirds of total body weight (Schott, 2006). As sweating occurs, fluid is lost progressively from extracellular fluid followed by intracellular fluid. Thus, delayed skin tenting duration is one of the indicators reflecting on dehydration status. In the present study, there was significant difference in skin tenting duration with 2 secs at pre-treatment and improved to 1 sec post-treatment. This improvement may be due to the fluid administered being gradually distributed in the extracellular compartment.

4.1.3 Colour of mucous membrane

Colour of mucous membrane is also an indicator of blood circulation. Mucous membrane pre-treatment was observed to be severely congested = Grade C indicating the body circulatory system is greatly compromised especially in dehydrated endurance horses and improved significantly to normal mucous membrane which is pink = Grade A at post-treatment indicating good blood circulation.

4.1.4 Capillary refill time

Capillary refill time reflects on tissue perfusion to capillary bed. Prolonged capillary refill time reflects also on reduced effective circulating volume in blood circulation. Similarly to skin tenting duration, capillary refill time improved significantly from 2secs at pre-treatment to 1 sec at post-treatment.

4.1.5 Gastrointestinal sounds

The gastrointestinal tract has most of the total body fluid reserves. Sweating increases losses of fluid from this fluid reserve. Reduction in fluidity in gastrointestinal tract also impedes gastrointestinal movement. These endurance horses may exhibit clinical signs of colic. In addition, during strenuous exercise, the sympathetic nervous system is activated, consequently reducing blood flow to the gastrointestinal organ and increasing blood supply to the muscles. Thus, gastrointestinal motility is impaired and upon auscultation typical findings of reduced or absent borborygmi are present. In the present study, gastrointestinal sounds was moderately decreased = grade C which improved significantly to mild decrease gut sounds = grade B. Most of the metabolically eliminated horses refused to eat or drink during presentation at hospital, but following administration of intravenous fluids and resumption of gastrointestinal motility, evidenced by return of audible sounds in the abdomen and passage of faeces, they were again keen to eat and voluntarily started drinking.

4.2 Haematological parameters

Table 6: Pre-treatment and post-treatment haematology parameters

Parameter	Treatment		Reference Range	Percentage Difference %
	Pre-	Post-		
RBC ($\times 10^{12}$ L^{-1})	$10.65^a \pm 0.27$	$7.81^b \pm 0.24$	6.00-12.00	-26.67
Hb (g L^{-1})	$165.44^a \pm 5.52$	$121.00^b \pm 4.19$	100.00-180.00	-26.86
PCV (L L^{-1})	$0.51^a \pm 0.02$	$0.36^b \pm 0.01$	0.32-0.52	-29.41
MCV (fL)	47.22 ± 0.92	46.22 ± 0.72	34.00-58.00	-2.12
MCHC (g L^{-1})	$326.33^a \pm 4.14$	$334.67^b \pm 4.85$	310.00-370.00	+2.56
WBC ($\times 10^9$ L^{-1})	$12.94^a \pm 0.54$	$11.08^b \pm 0.59$	5.50-12.50	-14.37
Thrombocyte ($\times 10^9$ L^{-1})	123.00 ± 10.61	137.33 ± 11.92	100.00-600.00	+11.65
Plasma protein (g L^{-1})	$85.78^a \pm 4.38$	$56.33^b \pm 1.86$	60.00-80.00	- 34.33
Icterus index (Unit)	$66.67^a \pm 5.89$	$29.44^b \pm 5.80$	<20	-55.84

Values are expressed as mean ± S.E [a,]b. Means within rows with different superscripts are significantly different at $p<0.05$.

There was significant changes seen red blood cells (RBC), haemoglobin (Hb), packed cell volume (PCV), mean corpuscular haemoglobin concentration (MCHC), white blood cells (WBC), plasma protein, icterus index which also consistent with haemodilution following fluid therapy. RBC, Hb, and PCV were at high normal with mild increase in plasma protein at pre-treatment which may reflect more on exercise-induced fluid shifts. Increased sweating in hot and humid conditions and dehydration could contribute to these alterations. According to

Lindinger (2014), the key haematological variables for diagnosis of dehydrated horse includes elevation of PCV and plasma protein concurrent with reduction of plasma chloride (Cl^-). Increase in RBC, Hb, PCV and plasma protein results in increased blood viscosity thus increasing cardiac workload as well. Post-treatment values for RBC, Hb, PCV and plasma protein were significantly reduced by approximately 30 % indicating that there was fluid replacement and redistribution.

No significant changes in mean corpuscular volume (MCV) were found following fluid therapy. Besides that, MCHC was 326.33 ± 4.14 g L^{-1} at pre-treatment but rose to 334.67 ± 4.85 g L^{-1} at post-treatment. According to Poole and Erickson (2014), circulating haemoglobin concentration increases approximately two-fold above normal values as red blood cells are released due to splenic contraction in response to increased sympathetic activity during exercise. However, MCHC in this present study still remained within normal range at pre-treatment.

The white blood cell (WBC) count in metabolically eliminated horses was mildly elevated ($12.94 \pm 0.54 \times 10^9$ L^{-1}) at pre-treatment which may be sequestered in the spleen together with the erythrocytes contributed by enduring protracted periods of strenuous exercise. According to Snow *et al.* (1983), increase in neutrophil:lymphocyte ratio occurred with respect to neutrophilia and lymphopaenia at 3 and 4 hrs post-exercise in Thoroughbred horses. This altered ratio will return to basal levels within 6hours following exercise. However, differential count was not performed in this study. WBC count in this study is of limited use without differential count to aid assessment between normal physiological process or disease mechanism. Similarly to erythrocytes and leukocytes, thrombocytes or also known as platelets are also subjected to exercise or excitement-induced splenic contraction. However, in this study, thrombocytes values showed no significant changes. Elevation in icterus index 66.67 ± 5.89 unit is associated with the increase in serum bilirubin as mentioned below. Furthermore, icterus index reduced significantly to 29.44 ± 5.80 unit in response to fluid therapy.

4.3 Biochemical parameters

Table 7: Pre- and post-treatment biochemical parameters

Parameter	Treatment		Reference range	Percentage difference (%)
	Pre-	Post-		
Na (mmol L^{-1})	132.89 ± 1.20	134.44 ± 1.04	132.00-144.00	+1.17
K (mmol L^{-1})	3.38 ± 0.32	3.44 ± 0.34	3.00-4.50	+1.78
Cl (mmol L^{-1})	80.25^{a} ± 3.68	96.75^{b} ± 2.27	90.00-100.00	+20.56
Ca (mmol L^{-1})	2.77 ± 0.38	2.81± 0.08	2.20-3.00	+1.44
Urea (mmol L^{-1})	8.86 ± 0.76	8.49 ± 0.74	3.60-8.90	-4.18
Creatinine (μmol L^{-1})	211.56^{a} ± 23.64	162.33^{b} ± 15.56	<176.00	-23.27
Glucose (mmol L^{-1})	6.53 ± 0.36	6.38 ± 0.26	3.30-5.50	-2.30
Bilirubin (μmol L^{-1})	61.00^{a} ± 6.51	44.33^{b} ± 5.94	8.50-34.00	-27.33
GGT (U L^{-1})	21.22^{a} ±2.29	14.78^{b} ± 1.44	10.00-30.00	-30.35
AST (U L^{-1})	770.89^{a} ± 360.09	584.33^{b} ± 284.81	120.00-160.00	-23.81
CK (U L^{-1})	3997.44^{a}±2580.13	1946.33^{b} ± 789.76	100.00-500.00	-51.31
Total protein (g L^{-1})	85.11^{a} ± 3.89	59.22^{b} ± 1.87	55.00-75.00	-30.42
Albumin (g L^{-1})	37.78^{a} ± 1.28	26.33^{b} ± 0.83	25.00-35.00	-30.31
Globulin(g L^{-1})	47.33^{a} ± 2.72	33.00^{b} ± 1.33	25.00-50.00	-30.28
Lactate (mmol L^{-1})	6.66^{a} ± 0.83	4.42^{b} ± 0.72	1.11-1.78	-33.63

Values are expressed as mean ± S.E [a,]b. Means within rows with different superscripts are significantly different at $p<0.05$.

4.3.1 Electrolytes

As mentioned earlier, equine sweat is a hypertonic fluid to plasma. According to Coenen (2005), plasma Na^+ and K^+ may be normal to mildly decreased but plasma Cl^- decreases markedly despite duration of exercise. In this present study, it was found that at pre-treatment Na^+ and K^+ were low normal while plasma Cl^- was obviously indicative of hypochloraemia. Thus, loss of Cl^- through sweat leads to an increased reabsorption of bicarbonate ions (HCO_3^-) to maintain the anion gap. Therefore, increasing the risk of hypochloraemic metabolic alkalosis, which is common in endurance horses. Metabolic alkalosis may manifest as gastrointestinal stasis, cardiac arrhythmias, muscle cramps, spasm including synchronous diaphragmatic flutter.

The use of 0.9% saline is a fluid choice especially for horses with hypochloraemic metabolic alkalosis. The normal equine plasma has Na^+ concentration of approximately 130 to 140 mEq/L and Cl^- about 90 to 102 mEq/L. However, the chloride content in 0.9 % saline is relatively greater than plasma Cl^- concentration. Therefore providing greater amounts of Cl^- than Na^+ and producing a net effect which creates a mild hyperchloraemia which increases risk of hyperchloraemic metabolic acidosis (Magdesian, 2015). Hyperchloraemia was not observed in this study. Thus metabolic status either acidosis/alkalosis still requires further study for example, blood gas analysis, anion gap or strong ion difference calculations.

Intravenous fluids given without supplementation of potassium for prolonged period could subsequently lead to hypokalaemia which was inconsistent finding with this study. According to (Kronfeld, 2001), both hypokalaemia and hyperkalaemia can result in muscle weakness. Prolonged hypokalaemia may also cause loss of gut motility and paralytic ileus.

In cases of hypocalcaemia, it may pose risk of decreased tone and motility of gastrointestinal tract and also associated with weakness of skeletal muscle (Kronfeld, 2001). Fortunately, the horses showed Ca^{+} levels within normal range. In fact, there were two horse in this study with clinical synchronous diaphragmatic flutter despite normal calcium levels, thus there could be other on-going acid-base imbalances that contribute to this phenomena such as hypochloraemic metabolic alkalosis.

4.3.2 Kidney parameters

According to Cook & Bain (2003), blood urea nitrogen and creatinine should reduce to normal values following rehydration. Typically, if creatinine concentration is abnormally raised on admission, then it should be measured every 24 hours until it falls to normal values. This is because persistent creatinine concentration may signify persistent hypovolaemia and reduced renal blood flow or even underlying kidney disease. In the present study, (blood urea nitrogen) BUN was high normal while creatinine level was elevated ($211.56^{a} \pm 23.6$ μmol L^{-1}) pre-treatment which is consistent with dehydration or pre-renal azotaemia. Fortunately, creatinine levels reduced significantly to normal values at post-treatment. In addition, if urine was sampled, it is expected to be highly concentrated due to severe dehydration. However, in cases of ischemic renal tubular damage, urine is poorly concentrated despite obvious dehydration (Jose-Cunilleras, 2014).

4.3.3 Glucose

Glucose levels at pre-treatment showed hyperglycaemia (6.53 ± 0.36 mmol L^{-1}), which reflect on the physiological response to stress to meet increased metabolic demand of muscles. Glucose is a substrate which is essential in metabolism and energy production. However, blood glucose level may be variable. As blood glucose eventually depletes, this may contribute to occurrence of exhausted horse syndrome in endurance horse.

4.3.4 Bilirubin

Increases in serum bilirubin 61.00 ± 6.51 μmol L^{-1} may be due to intravascular haemolysis due to increased red blood cell fragility consequently causing elevation in icterus index as well as mentioned below. Kristensen (2014) reported an elevation in haemoglobin levels and drop in haptoglobin and iron levels may be due to exercise-induced haemolysis which is similar to a case of athlete's anaemia in humans. For example, foot strike haemolysis, is common physiological process particularly in endurance sports. Mairbäurl (2013) illustrated that in spite of stimulated erythropoiesis, exercise can cause intravascular haemolysis typically affecting senescent red blood cells. When these red blood cells pass through capillaries in contracting muscles, compression of red blood cells can result in mechanical rupture. There are many other factors that influence the osmotic fragility of red blood cells such as frequent accumulation in the spleen, oxidative stress, anaerobic exercise causing drop in pH, increase in particle pressure of carbon dioxide and increase in blood lactate concentration (Schwarzwald, 2014). However, serum bilirubin post-treatment reduced significantly to $44.33^{b} \pm 5.94 \mu mol\ L^{-1}$ due to haemodilution.

4.3.5 Liver-derived enzymes

In this present study, Gamma-glutamyl transpeptidase (GGT) was still within normal range. There was minimal impact of prolonged submaximal exercise on liver derived enzymes as seen pre-treatment. However, GGT reduced significantly by approximately 30 % to $14.78 \pm 1.44\ U\ L^{-1}$

4.3.6 Muscle-derived enzymes

According to Teixeira-Neto *et al*. (2008), plasma enzymes of AST and CK are most useful in evaluating muscular leakage. In the present study, AST and CK were elevated above normal values. However, these muscle-derived enzymes were reduced significantly by 23.81 % and 51.31 % respectively at post-treatment. Muscle disease should be considered when significant increase in AST and CK is coupled with clinical signs of muscle stiffness, myoglobinuria and others. However, despite elevated AST and CK, the endurance horses may have clinically occult rhabdomyolysis (Kerr and Snow, 1983).

4.3.7 Total protein

Pre-treatment showed hyperproteinaemia with hyperalbuminaemia, which is consistent with dehydrated horse. Globulin levels were still within normal range which is normal in a healthy horse despite dehydration. However, post-treatment total protein and albumin reduced significantly to normal values by 30.42 % and

30.31 % respectively. This also means intravenous fluids administered restored the dehydrated status.

4.3.8 Lactate

Endurance racing is typically an aerobic event. Lactate values pre-treatment were elevated markedly (6.66 ± 0.83 mmol L^{-1}) which may be due to anaerobic performance of metabolically eliminated horses. According to Geor *et al.* (1999), decrease in anaerobic muscle metabolism occurred as a result of adaptation to endurance training. Holloszy and Coyle (1984) reported that, endurance training also increases mitochondria in skeletal muscles thus enhancing respiratory capacity. In addition, this low-to-moderate intensity exercise also increases capacity of utilization of fat thus slowing glycogen depletion and reduces lactate production (Putman *et al.*, 1998). Therefore, in this present study, markedly elevated lactate levels may indicate poor performance of metabolically eliminated endurance horses.

4.4 Correlation between riding speed and distance travelled with derangements of physical, haematological and biochemical parameters constituting metabolic elimination

Table 8 shows Pearson and Spearman correlation coefficient test done with respect to normally and not normally distributed data in this study. The results showed that there was large positive correlation for distance travelled with icterus index (p=0.014, r=0.839), bilirubin (p=0.018, r=0.756) and BUN (p=0.024, r=0.737). This is further supported by coefficient of determination, r^2, whereby 70.39 %, 57.15 %, 54.32 % of variability in icterus index, urea and bilirubin can be explained

Table 8: Correlation between riding speed, distance travelled, with physical (based on National Federation Log Book), haematological and biochemical parameters constituting metabolic elimination at vet gate (n=9)

Parameter	Riding speed r	Riding speed p-value	Distance r	Distance p-value
Heart rate	-0.113	0.772	0.226	0.559
Skin tenting duration	0.000	1.000	-0.187	0.630
Mucous membrane colour	-0.334	0.380	0.197	0.611
Capillary refill time	-0.446	0.228	-0.346	0.362
Gut sounds	0.079	0.841	-0.237	0.540
Muscle tone	0.548	0.127	0.420	0.261
Girth, withers and back soreness	0.411	0.272	0.280	0.466
RBC	0.058	0.882	0.110	0.779
Hb	-0.73	0.853	0.372	0.325
PCV	-0.47	0.904	0.200	0.605
MCV	-0.182	0.639	0.236	0.541
MCHC	-0.090	0.819	0.324	0.396
WBC	-0.161	0.679	0.376	0.318
Thrombocyte	-0.361	0.340	-0.346	0.362
Plasma protein	-0.111	0.777	-0.096	0.805
Icterus index	-0.137	0.725	0.839	0.014
Na	-0.502	0.169	-0.200	0.606
K	0.288	0.452	-0.296	0.440
Cl	0.019	0.964	-0.457	0.255
Ca	0.267	0.488	-0.179	0.645
Urea	-0.175	0.653	0.756	0.018
Creatinine	0.371	0.326	0.371	0.326
Glucose	0.264	0.493	-0.317	0.405
Bilirubin, Total	-0.096	0.806	0.737	0.024
GGT	0.499	0.172	0.079	0.840
AST	-0.102	0.794	-0.102	0.794
CK	0.111	0.777	0.111	0.777
Total protein	-0.152	0.696	-.098	0.801
Albumin	0.036	0.927	-0.142	0.716

Globulin	-0.074	0.850	-0.074	0.850
Lactate	-0.016	0.967	0.121	0.757
Total intravenous fluids	0.116	0.767	0.108	0.783

by variable distance travelled. Table 8 shows Pearson and Spearman correlation coefficient test done with respect to normally and not normally distributed data in this study.

On the other hand, distance travelled had positive significant correlation with blood urea nitrogen in blood. This may be due to exercise-induced dehydration linked to reduced renal perfusion and low renal urea clearance (Meyer, 1987).

The results also showed that there was positive significant correlation for distance travelled with icterus index (p=0.014, r=0.839), bilirubin (p=0.018, r=0.756) and urea (p=0.024, r=0.737). This is further supported by coefficient of determination, r^2, whereby 70.39 %, 57.15 %, 54.32 % of variability in icterus index, urea and bilirubin can be explained by variable distance travelled.

In this study, there was no significant correlation between riding speed with physical, haematological and biochemical parameters. In contrary, increasing speed contribute significantly to ion losses in a study involving 243 horses (Ecker and Lindinger, 1995). However, this was not seen in this study which may be due to small sample size (n=9).

Distance travelled showed significant positive correlation with bilirubin and icterus index. Increasing bilirubin coupled by increase in icterus index when travelling greater distance could possibly be due to exercise-induced haemolysis in endurance sports. Horses are amazing athletes with the unique ability of spleen to sequester approximately 50 % of total red blood cell volume, facilitate by sympathetic nervous system and contraction of smooth muscle within splenic capsule (Hanzawa andWatanabe, 2000). According to (Murakami, 1974), haemolysis occurs in strenuous exercise such as long distance endurance race evidenced by presence of hemoglobin in plasma

However as haematopoiesis is very high, possibility of exercise-induced haemolysis to cause anaemia is unlikely in regard to the athletic horse. As long as the rate of red blood cell destruction is less than rate of red blood cell production, detrimental effects on athletic performance should not occur (Hanzawa and Watanabe, 2000).

5.0 CONCLUSION

This study illustrates the changes in selected physical and laboratory variables of metabolically eliminated horses in response to fluid therapy. The most consistently recorded signs for the metabolically eliminated horses that clinically indicated dehydration were persistently elevated heart rate, severely congested mucous membrane, prolonged capillary refill time and skin tenting duration, and reduced to absent gastrointestinal sounds. Thorough physical examination is of paramount importance to protect the welfare of these endurance horses. Furthermore, following fluid therapy, clinical signs of metabolically eliminated horses improved remarkably. Haematological and biochemical parameters were consistent with haemodilution however most of the parameters that were beyond normal values were restored. Blood sampling is a simple and relatively fast diagnostic tool providing information regarding the function of a number of body systems that are routinely assessed. However, care must be taken during interpretation as the physiological state of the horse can influence several blood parameters. In conclusion, there was significant difference in physical, hematological and biochemical parameters following intravenous fluid therapy. Since endurance horses with metabolic ailments in this study responded well to intravenous fluid therapy (0.9 % saline solution), their prognosis was good. In addition, there was no significant correlation between riding speed with physical, haematological and biochemical parameters except for distance travelled with icterus index, bilirubin and urea.

6.0 RECOMMENDATION

The effects as seen in this study is more representative of the reality than those reproduced in experimental conditions, however, they should be confirmed with a larger sample size and providing longer study period to do so. It is recommended to take into account bodyweight of endurance horse pre- and post-ride to assess dehydration status more accurately and to perform additional blood sampling at pre-ride and 24-hour post-treatment as well. Study of comparison of responses between different types of fluids or different methods of fluid administration namely oral and intravenous route can be performed. A sweat sample collection is also possible for sweat analysis.

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TABLES

Table 1: 40km category

Loop	Distance (km)	Cumulative Distance (km)	Minimum speed
1	20	20	8km hr^{-1}
2	20	40	Maximum speed
			16km hr^{-1}

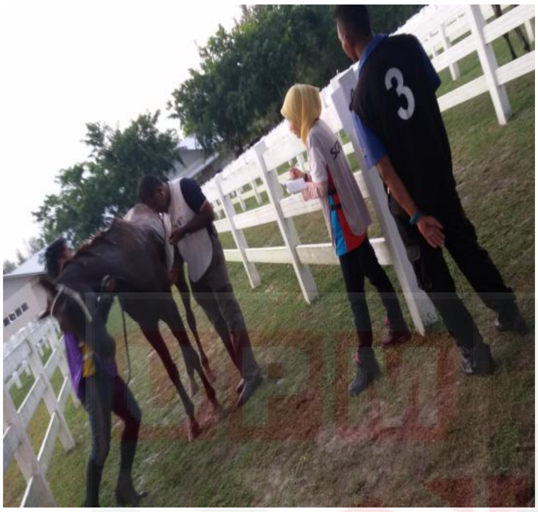
Table 2: 80km category

Loop	Distance (km)	Cumulative Distance (km)	Minimum speed
1	30	30	10km hr^{-1}
2	25	55	
3	25	80	

Table 3: 120km category

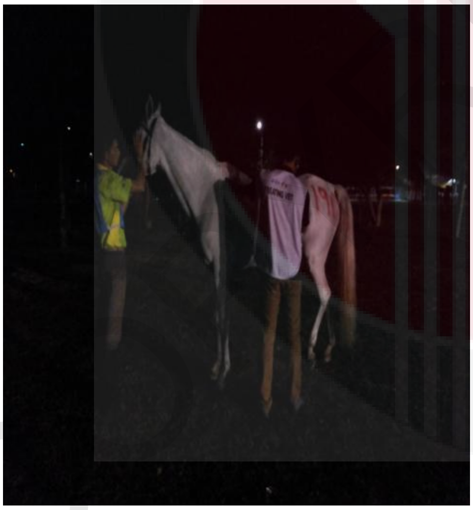
Loop	Distance (km)	Cumulative Distance (km)	Minimum speed
1	30	30	12km hr^{-1}
2	25	55	
3	20	75	
4	25	100	
5	20	120	

APPENDICES

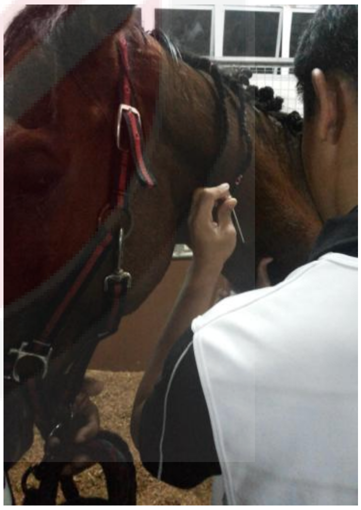


Appendix 1: Veterinary inspection at vet lane

Appendix 2: Example of exhausted horse

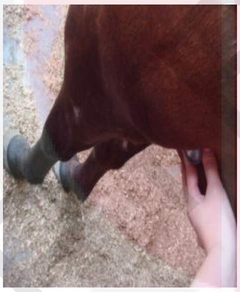


Appendix 3: Evaluation of endurance horse

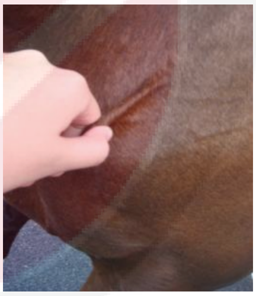


Appendix 4: Administration of 14G intravenous catheter by Treatment Vet at RTES/UPM Equine Hospital

Appendix 5: Intravenous fluids administration: 0.9% saline solution at 5-10L hr^{-1}



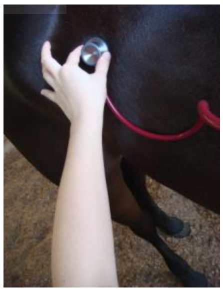
Appendix 6: Heart rate assessment



Appendix 7: Skin tenting duration

Appendix 8: Mucous membrane colour

Appendix 9: Capillary refill time



Appendix 10: Gastrointestinal sounds