UNIVERSITI PUTRA MALAYSIA

CLINICO-PATHOLOGICAL EVALUATION AND BIOMARKERS EXPRESSION ASSOCIATED WITH BACK PAIN IN HORSES

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By

MAYAKI ABUBAKAR MUSA

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Doctor of Philosophy

May 2021

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DEDICATION

It is my genuine gratefulness and warmest regard that I dedicate this work to my entire family "The Mayaki's"

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

CLINICO-PATHOLOGICAL EVALUATION AND BIOMARKERS EXPRESSION ASSOCIATED WITH BACK PAIN IN HORSES

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May 2021

Chairman : Associate Professor Intan Shameha binti Abdul Razak, PhD
Faculty : Veterinary Medicine

Back pain is among the most common causes of poor performance in athletic and riding horses. Currently, there is lack of suitable approach for the evaluation of back pain (BP) in horses, thus, there is need to develop quantifiable and objective means to diagnose the condition. Therefore, the main objective of this study was to evaluate clinico-pathological changes and grading, and biomarkers expression associated with BP in horses. The investigation comprised of a retrospective study on 181 cases of equine BP referred to the University Veterinary Hospital, Universiti Putra Malaysia between years 2002 and 2017, development of a grading system based on spinal abnormality-associated clinical features and determination of spinal pathological changes in horses with BP. Immunochemical staining method was used to determine the expression of glial fibrillary acidic protein (GFAP), and ionized calcium-binding adaptor molecule 1 (Iba-1) in the spinal cord to demonstrate gliosis, while ELISA was used to determine the serum concentrations of pNF-H, GFAP, and Iba-1 as potential biomarkers of equine BP.

Back pain in the horses were centred at the area under the saddle between T8 and L5 vertebrae. Most horse suffered from primary type (92.27%) of BP, with the main causes, in order of frequency, being soft-tissue lesions (57.48%), vertebral lesions (18.56%), tack-associated (16.77%), and neurological lesions (7.19%). Fourteen horses were graded for severity with BP using response to pain on palpation, muscular hypertonicity, joint stiffness, and physical dysfunction as differentiating parameters. The horses, in order of frequency, suffered from mild-moderate, mild, moderate, and severe BP. The common clinical features that differentiated between horses with and without BP were poor hindlimb impulsion (85.7%), longissimus dorsi spasm at palpation (78.6%), paravertebral muscle stiffness (64.3%), and resistance to lateral bending (64.3%).

Spinal cord gross and histopathological investigations on three horses with BP showed kissing spines involving 6 to 9 vertebrae, haemorrhagic malacic lesions with medullary disintegration, degenerative nerve fibres, dilated myelin sheaths, myelin macrophages, axonal swelling and/or loss, and satellitosis. The horses also showed reactive microgliosis and astrogliosis, suggesting that these changes play an important role in development and progression of equine BP. The most common active microglia in horses with BP were the elongated phenotype. This study also reported, for the first time, the presence of hypertrophied microglia phenotype in the spinal cords of horses with lameness.

Serum from horses with BP, concurrent BP and lameness, lameness only and healthy horses were used to determine serum pNF-H, GFAP, and Iba-1 concentrations as potential biomarkers in the diagnosis of equine BP. Based on the normal serum creatine kinase and aspartate aminotransferase concentrations, the BP in these horses was not due to muscle disorder. The high serum pNF-H concentration in horses with BP and its good discriminatory capacity in the detection of axonal loss and degeneration suggest that this analyte is a good biomarker for determination of BP in horses. Serum Iba-1 and GFAP concentrations vary considerably among groups of horses; however, the concentration of these serum biomarkers were higher in BP horses than either those with concurrent BP and lameness, lameness or healthy horses.

In conclusion, this study showed BP in horses is mainly caused by soft-tissue lesions and kissing spine syndrome, and can be diagnosed and graded using clinical abnormalities - pain response to plapation, muscle hypertonicity, joint stiffness and physical dysfunction. The typical pathological features of the vertebral and spinal cord in equine BP are kissing spines, haemorrhagic myelomalacia, axonal degeneration and reactive astrocytosis and microgliosis. Serum GFAP (cut-off: >0.690 ng/mL) and Iba-1 (cut-off: >26.99 pg/mL) have high diagnostic capacity to differentiate between equine BP due to spinal disorders and other causes.

Keywords: horse, back pain, spinal cord, astrogliosis, microgliosis, biomarkers, pNF-H, GFAP, Iba-1

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

PENILAIAN KLINIKOPATOLOGI DAN PENGUNGKAPAN YANG BERKAITAN DENGAN KESAKITAN BELAKANG PADA KUDA

Oleh

MAYAKI ABUBAKAR MUSA

Mei 2021

Pengerusi : Profesor Madya Intan Shameha binti Abdul Razak, PhD
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Kesakitan belakang (KB) adalah di antara penyebab prestasi buruk yang paling kerap pada kuda atlet dan tunggangan. Pada masa ini ada kekurangan dalam pendekatan yang sesuai untuk menilai KB pada kuda, justeru, adalah sangat perlu untuk ditubuhkan cara yang boleh ditentu kuantitinya dan lebih objektif untuk mengdiagnosis keadaan ini. Justeru, objektif utama kajian ini ialah untuk menentukan perubahan klinikopatologi dan penyataan biopenanda sel glial korda spina dan axon dalam KB ekuin. Penyelidikan ini terdiri daripada kajian retrospektif terhadap 181 kes KB ekuin yang dirujuk kepada Hospital Veterinar Universiti, Universiti Putra untuk tahun 2002 hingga 2017, pembangunan suatu sistem penggredan yang berasaskan ciri klinikal berkaitan keabnormal spina dan penentuan perubahan patologi spina pada kuda KB. Kaedah pewarnaan imunokimia digunakan untuk menentukan penyataan protein asidik fibrillari glia (GFAP), dan molekul terion-adaptor kalsium-pengikat 1 (Iba-1) dalam korda spina untuk menunjukkan gliosis, sambil kaedah ELISA pula diguna untuk menentukan kepekatannya dalam serum pNF-H, GFAP, and Iba-1 sebagai bakal biopenanda untuk KB ekuin.

Kesakitan belakang pada kuda tertumpu pada kawasan belakang di bawah pelana, iaitu di antara vertebra T8 dan L5. Sebilangan besar kuda mengidap KB jenis primer (92.27%), dengan penyebab utamanya, mengikut urutan, ialah lesi tisu lembut (57.48%), lesi vertebra (18.56%), peralatan tunggang kuda (16.77%), dan lesi neuron (7.19%). Empat-belas ekor kuda digredkan untuk menentu keterukan KB mengguna gerak balas kesakitan apabila dipalpat, kehipertonan otot, ketegangan sendi, dan disfungi fizikal sebagai parameter pembeza. Kuda ini, mengikut urutan kekerapan, mengidap KB pada kadar sedikit-sederhana, sedikit, sederhana, dan teruk. Ciri klinikal paling kerap yang membezakan di antara kuda mengidap KB dengan yang tidak ialah impulsi kaki belakang yang lemah (85.7%), kekejangan longissimus dorsi

apabila dipalpat (78.6%), kekejangan otot paravertebra (64.3%), enggan apabila badan cuba dipaksa bengkuk lateral (64.3%).

Penyelidikan kasar dan histopatologi korda spina pada tiga ekor kuda mengidap KB menunjukkan spina bersentuh yang melibatkan vertebra 6 hingga 9, lesi malasik hemoraj dengan disintegrasi medulla, gentian saraf ternyahjana, sarung mielin kembang, makrofaj mielin, bengkak dan/atau kekurangan axon, dan satelitosis. Kuda ini juga menunjukkan mikrogliosis dan astrogliosis, menyarankan yang perubahan ini memain peranan penting dalam perkembangan dan penambahburukan KB ekuin. Mikroglia yang paling kerap terdapat dalam kuda mengidap KB ialah fenotip panjang. Kajian in juga melaporkan, buat pertama kali, wujudnya fenotip mikroglia hipertrofi dalam korda spina kuda yang tempang.

Serum daripada kuda mengidap KB, KB dengan ketempangan, ketempangan sahaja, atau sihat diguna untuk menentukan kepekatan pNF-H, GFAP, dan Iba-1 sebagai bakal biopenanda dalam diagnosis KB ekuin. Berasaskan kepekatan kreatin kinase dan aspartate aminotransferase serum yang normal, KB pada kuda ini bukan disebabkan oleh gangguan otot. Kepekatan pNF-H tinggi tererti dalam kuda mengidap KB dan keupayaan diskriminasinya yank baik dalam pengesanan kekurangan dan penyahjanaan akson menyarankan analit ini adalah biopenanda yang baik untuk menentukan KB pada kuda. Kepekatan Iba-1 dan GFAP menunjukkan banyak kelainannya di antara kumpulan kuda; bagaimanapun, kepekatan biopenanda serum ini adalah lebih tinggi pada kuda pengidap KB daripada yang mengidap KB dengan ketempangan, ketempangan sahaja, atau sihat.

Kesimpulannya, kajian ini menunjukkan KB pada kuda adalah disebabkan oleh lesi tisu lembut dan sindrom spina bersentuh, dan boleh digred berasaskan ciri-ciri abnormalitiklinikal- tindakbalas kesakitan terhadap palpasi, hipertonositi otot, kekejangan sendi dan disfungsi fizikal Ciri utama patologi vertebra dan korda spina dalam KB ekuin adalah spina bersentuh, Gliosis reaktif, yang ternyata sebagai astrosit dan mikroglia teraktif, merupa mielomalasia hemoraj, degenerasi aksonal dan reaktif astrogliosis dan mikrogliosis.. GFAP (titik potongan: >0.690 ng/mL) dan Iba-1 (titik potongan: >26.99 pg/mL) di dalam serum mempunyai kapasiti diagnosis yang tinggi bagi membezakan KB yang disebabkan oleh kerosakan spina dan penyebab-penyebab lain.

Katakunci: kuda, kesakitan belakang, korda spina, astrogliosis, microgliosis, biopenanda, pNF-H, GFAP, Iba-1

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In the name of Allah, the Most Gracious, the Most Merciful

All praise is due to Allah, the lord of the universe, for His blessing in completing this thesis. May the peace and blessings of Allah be upon our noble prophet Muhammed, his family, his Companions and all those who follow them with righteousness until the Day of Reckoning.

"Indeed, after hardship, there is ease" (Quran 94:6)

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

LIST OF ABBREVIATIONS

AAEP	American Association of Equine Practitioners
AIF-1	Allograft inflammatory factor 1
AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
ANOVA	Analysis of variance
AST	Aspartate aminotransferase
AUC	Area under the curves
BEVA	British equine veterinary association
BP	Back pain
BPL	Back pain concurrent with lameness
C	Cervical vertebra
ºC	Degree Celsius
CD	Cluster of differentiation
CI	Confidence interval
CK	Creatine kinase
CNS	Central nervous system
cm	Centimetre
cm^2	Centimetre square
CR	Complement receptors
CSF	Cerebrospinal fluid
CT	Computerized tomography
CVM	Cervical vertebral stenotic myelopathy
Cy	Coccygeal vertebra
DNA	Deoxyribonucleic acid
DPX	Dibutylphthalate Polystyrene Xylene

DRG	Dorsal root ganglion
DSP	Dorsal spinous process
ECL	Electrochemiluminescence
EDM	Equine degenerative myeloencephalopathy
EGS	Equine grass sickness
EHV-1	Equine herpesvirus 1
ELISA	Enzyme-linked immunosorbent assay
EMND	Equine motor neuron disease
EPM	Equine protozoal myeloencephalitis
FP	False positive
G	Gelding
GBq	Gigabecquerel
GFAP	Glial fibrillary acidic protein
HC	Healthy control
H&E	Haematoxylin and eosin
IACUC	Institutional Animal Care and Use Committee
Iba-1	Ionized calcium-binding adaptor molecule 1
kDa	Kilodalton
L	Lumbar vertebra
lb	Pound
LFB	Luxol Fast Blue
LN	Lameness
LS	Lumbosacral
M	Mare
MAPK	Mitogen-activated protein kinase
mL	Millilitre

MRI	Magnetic resonance imaging
ng	Nanogram
NMDA	N-methyl-D-aspartate
NSAIDs	Nonsteroidal anti-inflammatory drugs
mCi	Millicurie
mHz	Megahertz
mm	Millimetre
OD	Optical density
OR	Odds ratio
PAS	Periodic acid-Schiff
PCR	Polymerase chain reaction
pg	Picogram
pNF-H	Phosphorylated Neurofilament H
PNS	Peripheral nervous system
r	Correlation coefficient
rpm	Revolutions per minute
RNA	Ribonucleic acid
ROC	Receiver operating characteristic curve
S	Sacral vertebra
SCI	Spinal cord injury
SD	Standard deviation
SDH	Spinal dorsal horns
SEM	Standard error of means
T	Thoracic vertebra
TB	Thoroughbred
^{99m}Tc-MDP	99m technetium-labelled methylene diphosphonate

TMB	3,3',5,5'-tetramethylbenzidine
TN	True negative
TNF-α	Tumour necrosis factor-alpha
TP	True positive
TPs	Transverse processes
U/L	Units per litre
UPM	University Putra Malaysia
UVH-UPM	University Veterinary Hospital, University Putra Malaysia
µL	Microlitre
µm	Micrometre
μm^2	Micrometre square
WB	Warmblood

CHAPTER 1

INTRODUCTION

1.1 Background of the Study

Quality performance of an athletic horse is subject to coordinated movements and the interrelationships among the body systems. If anyone of these systems becomes compromised, the horses' ability to perform at its best could be affected. The common clinical conditions responsible for loss of performance in riding horses include cardiorespiratory diseases (Davidson & Martin, 2003; Wilsher et al., 2010; McGurrin, 2015), back (Jeffcott, 1980a; Haussler, 1999a; Fonseca et al., 2006), muscular, neurological disorders (Rech & Barros, 2015; Cruz Villagrán et al., 2016), dental (Anthony et al., 2010; Salem et al., 2017; Pehkonen et al., 2019) and gastric disorders (Picavet, 2002; Mckeever et al., 2006; Banse & Andrews, 2019), lameness (Dyson, 2000; Faber et al., 2003; Wennerstrand et al., 2004; Sardari, 2008; Wennerstrand et al., 2009; Dyson, 2016; Dyson & Rasotto, 2016), and other systemic illness (Martin et al., 2000; Fraipont et al., 2011).

The spine comprises of multiple structures; vertebral bones (cervical, thoracic, lumbar, sacral and coccygeal), intervertebral joints, muscles, ligaments, nerves, and blood vessels, that provide support for coordinated and effective mobility of the spine. Abnormalities in these structures may manifest as back pain (BP). Back disorders or dysfunctions are among the most common and least understood equine afflictions causing poor performance (Jeffcott, 1980a). In back disorders, the clinical signs are often non-specific, thus, making the determination of underlying factors and diagnosis of back pain (BP) very difficult (Lesimple et al., 2013). This also led to variations in opinion among equine practitioners as to whether clinical signs are truly due to BP or lesions or abnormalities of the axial skeleton or other body systems.

Back pain can be classified as primary, secondary or presumed (Haussler & Jeffcott, 2014). Primary BP is associated with lesions in the spinal structures, while secondary BP is due to strain exerted as the result of axial skeleton lesions. Presumed BP is an alleged back disorder with either limited or no pathophysiological or anatomical evidence.

There has been an increase in the reported prevalence of equine BP from 0.9% into 100% particularly due to the difference in the area of clinical expertise employed and survey targets (Jeffcott, 1980b; Haussler, 1999b; Landman et al., 2004; Sardari, 2008). The diagnosis of BP mostly relies on case history, observable clinical signs, response to injection of local anaesthesia, radiography, scintigraphy, ultrasonography, and thermography (Jeffcott, 1980b; Erichsen et al., 2004; Fonseca et al., 2006; Girodroux et al., 2009; Meehan et al., 2009). The difficulty in evaluation of BP makes instituting appropriate therapies very challenging.

Pain is a complex system of undesirable sensory and emotional sense resulting in behavioural changes that represent the perception of an actual or perceived tissue injury (Viñuela-Fernández et al., 2007; Dubin & Patapoutian, 2010; Viñuela-Fernández et al., 2011; Verma et al., 2015). It is difficult to evaluate pain in horses because behavioural changes, particularly temperament may mask their expression of pain (Fureix et al., 2010; Manfredi et al., 2010; Fureix et al., 2012; Lesimple et al., 2012; Barstow & Dyson, 2015). Some horses are "cold-backs", that is they are highly sensitive to touch on the back. In normal horses, cold-back response is behavioural response and not associated with true BP. Thus, it is imperative that clinical evaluation of horses suspected of BP must be done thoroughly and accurately, and presence of conformational, behavioural, and structural abnormalities be ascertained before the signs can be positively associated with BP.

Physiologically, pain serves as a protective mechanism in the prevention of further insults on the tissues from noxious stimuli. The pain become a major medical issue only when it no longer serves to benefit the organism (Baron et al., 2010; Gao & Ji, 2010; Gosselin et al., 2010; Loggia et al., 2015). Pain can either acute or chronic (Chiang et al., 2012; von Hehn et al., 2012; Upp et al., 2013; Chapman & Vierck, 2017; Dorsey et al., 2019; Reckziegel et al., 2019; Wang et al., 2019). Acute pain is of short duration and gradually resolves as the injured tissues heal. Chronic pain is relatively sharper and more severe than acute pain and it can either be nociceptive, if it results from injury to somatic and visceral organs, or neuropathic, if it is due to nervous tissue injury. In pain disorders, acute pain can change to chronic pain due to central sensitization. Central sensitization is a condition in which regular inputs start to produce abnormal responses due to an increase in neuronal excitability in the central nervous system (CNS) (Graven-Nielsen & Arendt-Nielsen, 2002; Khasabov et al., 2002; Ji et al., 2003; D'Mello & Dickenson, 2008; Latremoliere & Woolf, 2010; Nijs et al., 2010; Wen et al., 2011; Nijs et al., 2014; West et al., 2015). An example of central sensitization is seen in chronic low BP in human (Nijs et al., 2015; Allegri et al., 2016). Currently, it is not known whether the mechanism of persistent or chronic BP disorder in horses is similar to that in humans (Baron et al., 2016).

Current researches focus on the use of biomarkers in the detection and diagnosis of neuronal and glial injuries. The estimation of these markers in the cerebrospinal fluid (CSF) and serum will improve the clinical assessment and monitoring of disorders causing prolonged or chronic pains. The biomarkers currently in used are either of the structural or functional components of nervous tissues, and their releases into CSF and general circulation reflects injury to the nervous tissue and/ or nerve-supporting glia cells (Alexander et al., 2007; Fukui et al., 2012a, 2012b; Sato et al., 2013; Intan-Shameha et al., 2017; Wu et al., 2019).

There is a paucity of information on the involvement of the spinal cord in pain modulation in the horse with BP. However, recent studies showed that persistent or chronic pain is associated with the role of glial cells in the spinal cord (Mika et al., 2013; Grace et al., 2014; Yang & Wang, 2015; Nasseri et al., 2016; Lambert et al., 2018). It is possible that spinal cord involvement in pain modulation in horses with BP occurs through development of reactive astrogliosis and microgliosis in the

dorsal horn of the spinal cord causing the synaptic plasticity, the underlying factor for persistent pain (Ikeda et al., 2009; Ruscheweyh et al., 2011; Sandkühler & Gruber-Schoffnegger, 2012; Zhou et al., 2019). It was also shown in humans that central and peripheral sensitization are linked with inflammatory changes in the spinal musculature, ligaments and intervertebral joints, as the result of postural changes in the lower back (Dubin & Patapoutian, 2010), while spinal cord injury and musculoskeletal pain are common symptoms.

Glial cells, particularly microglia and astrocytes, have been reported to be sensitive to activation and they play major roles in establishment and maintenance of chronic or persistent pain following nociceptive stimulation in the spinal cord or spinal injury (Ji et al., 2013; Mika et al., 2013; Nasseri et al., 2016). The activation of glial cells result in functional and morphological modifications that could cause release of pro-inflammatory mediators, contributing to the development of chronic pain (Gao & Ji, 2010; Gosselin et al., 2010; Hol & Pekny, 2015; Bogoslovsky et al., 2016; Takala et al., 2016; Ydens et al., 2017). Although, clinical determination of subtle spinal injuries in large animals like horses is often difficult, but with the biomarkers of microglia and astrocytes, particularly, ionized calcium-binding adaptor molecule 1 (Iba-1) and glial fibrillary acidic protein (GFAP), it is possible to associate spinal cord pathology with equine BP (Allison et al., 2016; Amaral et al., 2016; Bogoslovsky et al., 2016; Ozkunt et al., 2017; Ydens et al., 2017).

1.2 Statement of the Problems

Back pain is one of the commonest non-lameness musculoskeletal disorder in equestrian horses (Williams et al., 2001; Wylie et al., 2017). In Malaysia, BP is one of the main reasons for horse euthanasia, particularly those that show no response to more than six months of treatments (Noraniza, Mohd Adzahan, personal communication). The detection of pain and location of the exert point of pain in animals are difficult, particularly in horses. In equine BP, this is even more challenging because of multifactorial causes and multiple spinal structures involved (Denoix, 1999a; Cousty et al., 2010; Stubbs et al., 2010; Zimmerman et al., 2011; Clayton, 2012; Barstow & Dyson, 2015). The nonspecific signs, variable pathological features, history or complaint by the horse owners, and coupled with lack of good diagnostic imaging make accurate diagnosis of the condition problematic. Therefore, assessments by equine veterinarians generally rely on case history, physical examination, and response to anti-inflammatory medications (Johnston et al., 2004; Wennerstrand et al., 2004; Roethlisberger-Holm et al., 2006). When available, imaging techniques including radiography, ultrasonography, nuclear scintigraphy, and thermography are used in the assessment of possible lesion associated of BP in horses (Fonseca et al., 2006; Meehan et al., 2009; Stubbs et al., 2010; Zimmerman et al., 2011; Polidori et al., 2018). The possibility of neurological conditions like spinal compression, nerve pinching, and peripheral nerve afflictions causing BP in horses has been suggested; however, the commonly used diagnostic methods are either invasive (e.g., cerebrospinal centesis) or costly (e.g., magnetic resonance imaging) and not readily available to field equine veterinarians (Intan-Shameha et al., 2017) for the determination of neuron pathology.

1.3 Justification of the Research

In horses, BP is associated with various non-specific clinical abnormalities and manifestations (Cauvin, 1997; Martin & Klide, 1999; Riccio et al., 2018), and reduced or loss of performance. The main clinical signs in equine BP is pain response to palpation, muscle hypertonicity, and reduced vertebral flexibility (Jeffcott, 1980a; McGowan et al., 2007; Sardari, 2008; Dyson, 2016). Thus, these abnormalities are most appropriate to be used as parameters in a system for the quantification, grading, and categorization of back disorders in horses and to allow equine practitioners to assess, monitor, and manage horses with BP.

1.4 Research hypothesis

Hypothesis 1

Ho - BP in horses is not associated with specific clinico-pathological features

Ha - BP in horses is associated with specific clinico-pathological features

Hypothesis 2

Ho - The severity of BP in horses cannot be graded using spinal abnormalities and clinical features

Ha - The severity of BP in horses can be graded using spinal abnormalities and clinical features

Hypothesis 3

Ho - There is no spino-pathological changes and microglia and astrocyte activation in the spinal dorsal horn of horses with BP.

Ha - There is spino-pathological changes and microglia and astrocyte activation in the spinal dorsal horn of horses with BP.

Hypothesis 4

Ho - Serum biomarkers of microglia (Iba-1) and astrocyte (GFAP) activation cannot be use in the clinical diagnosis of BP in horses

Ha - Serum biomarkers of microglia (Iba-1) and astrocyte (GFAP) activation can be use in the clinical diagnosis of BP in horses.

1.5 Research Aim and Objectives

1.5.1 Main Objective

The main objective of the research is to evaluate clinico-pathological changes and grading and biomarkers expression associated with BP in horses.

1.5.2 Specific Objectives

The specific objectives of this research are to:

i. Evaluate the clinical characteristics, diagnosis and management of equine BP cases presented at the University Veterinary Hospital, Universiti Putra Malaysia.

ii. Develop a grading system for BP in horses based on thoracolumbar spinal abnormalities and clinical features.

iii. Evaluate thoracolumbar vertebrae and spinal cord pathology and immunochemical expression of microglia and astrocyte in the spinal dorsal horn of horses with BP.

iv. Evaluate the potential of serum markers of glia cell activation (GFAP and Iba-1) in diagnosis of BP in horses.

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