THE PATHOLOGY AND PATHOGENESIS OF CANINE CEREBRAL BABESIOSIS

By

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DEDICATION

For my husband:

Stephan

and my children:

Astrid and Theresa.

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SUMMARY

The pathology of canine cerebral babesiosis was examined at the gross, histological and ultrastructural levels. Gross lesions could be categorised as either global or regional. Congestive brain swelling, diffuse cerebral congestion and diffuse cerebral pallor were classified as global lesions. Multifocal haemorrhage and malacia were classified as regional lesions. Oedema was inconsistently present and could be either focal or diffuse.

The majority of histological changes were observed in both cerebral babesiosis and control cases. Regional lesions were unique to cerebral babesiosis and had specific histological features. Highly localised endothelial injury was the primary lesion. Early lesions were multifocal and strictly associated with the microvasculature. Intermediate lesions, with perivascular haemorrhage and neutrophil infiltration, were suggestive of reperfusion injury. Advanced lesions were locally extensive and similar in appearance to haemorrhagic infarction. It is likely that the pathogenesis of regional lesions is by a process of microvascular infarction, as venous thrombosis could not be demonstrated.

Ultrastructural evidence for adherent contact between erythrocytes and capillary endothelium was demonstrated. Endothelial cell necrosis occurred early in the development of lesions, before neuronal and glial injury. It is postulated that endothelial injury is the primary event in the development of regional lesions and secondary lesions develop as a consequence of microvascular infarction.

SAMEVATTING

Die patologie van die serebrale vorm van bosluiskoors in honde is ondersoek. Die letsels is makroskopies, histologies en elektronmikroskopies beskryf. Letsels kon makroskopies in twee groepe verdeel word: Globale letsels en gelokaliseerde letsels. Kongestiewe brein swelling, diffuse serebrale kongestie en serebrale anemie kom voor as globale letsels in serebrale babesiose. Multifokale bloeding en nekrose kom voor as gelokaliseerde letsels. Edeem was nie konsekwent teenwoordig nie, en was algemeen of verspreid.

Die meeste algemene histologiese veranderinge was in beide serebrale en kontrole gevalle teenwoordig. Gelokaliseerde letsels waarin spesifieke hisotpatologiese veranderinge voorgekom het, was kenmerkend van serebrale babesiose. Die primêre letsel is hoogs gelokaliseerde beskadiging van endoteelselle. Beskadiging van die kapillêre bloedvate ontstaan vroeg in die ontwikkeling van letsels. Verdere ontwikkeling van die letsel word gekenmerk deur peri-vaskulêre bloeding en neutrofiel infiltrasie wat aanduidend is van reperfusie beskadiging. Volontwikkelde letsels is plaaslik-ekstensief en het die voorkoms van hemoragiese infarkte. Dit is waarskynlik dat mikrovaskulêre infarksie 'n rol speel in die patogenese van die letsels, aangesien veneuse trombose nie ontstaan nie.

Noue kontak tussen rooibloedselle en kapillêre endoteel is elektronmikroskopies bevestig. Endoteelselnekrose ontstaan voordat tekens van beskadiging geidentifiseer kan word in neurone of gliaselle. Dit blyk dat kapillêre endoteelselbeskadiging die primêre letsel by die ontstaan van gelokaliseerde lesels is, en dat sekondêre lesels ontwikkel as gevolg van mikrovaskulêre infarksie.

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List of Abbreviations

ARDS	Acute respiratory distress syndrome
ARF	Acute renal failure
C_3	Complement 3
CNS	Central nervous system
DIC	Disseminated intravascular coagulation
F no.	File number
FDP	Fibrin degradation product
GIT	Gastro-intestinal tract
ICAM-1	Intercellular adhesion molecule – 1
ICP	Intracranial pressure
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IL-1	Interleukin 1
INF-gamma	Interferon gamma
iNOS	Inducible nitric oxide synthase
Mag.	Magnification
MODS	Multiple organ dysfunction syndrome
nm	Nanometre
NO	Nitric oxide
PAF	Platelet activating factor
pcv	Packed cell volume
PM no.	Post mortem number
pRBC	Parasitised red blood cells
RCC	Red cell changes
SIRS	Systemic inflammatory response syndrome
S. no.	Histopathology sample registration number
SPA	Soluble parasite antigen
TNF	Tumour necrosis factor
VAH	Veterinary Academic Hospital
VR-space	Virchow-Robbins space
µm	Micron

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Glossary of Terms

Adhesion: Flattening of parasitised erythrocytes along the vascular wall following margination.

Arterial supply territory: Volume of cerebral tissue supplied by an individual major cerebral artery.

Apoptosis: Individual cell death initiated by genetic mechanisms from within the affected cell.

Autoregulation: Compensatory vasoconstriction or dilatation of arteries and arterioles in response to physiological stimuli such as hypercapnia, hypoxia and intravascular pressure fluxes.

Border zones: Cerebral parenchyma situated on the periphery of adjacent arterial supply territories.

Cerebral flush: See congestive brain swelling.

Cerebral oedema: Increase in water content of brain tissue.

Cerebral vasomotor paralysis: Loss of cerebral vasomotor tone in arteries and arterioles. This phenomenon is a consequence of loss of autoregulation.

Compound granular corpuscles: Mononuclear phagocytic cells within the central nervous system actively engulfing necrotic debris and hence bulging with lipid vacuoles. These cells can be of microglial or adventitial origin.

Congestive brain swelling: An increase in intravascular fluid volume of the brain leading to raised intracranial pressure.

Definitive host: Host in which the parasite undergoes the sexual stage of the life cycle (in babesiosis this is the tick vector).

Delayed neuronal death: Neuronal injury that becomes morphologically appreciable by light microscopy 48 hours or more after a brief period (5 – 10 mins) of ischaemia.

Erythrocyte ghosts: Erythrocyte remnants consisting of the injured plasmalemma devoid of haemoglobin.

Fibrin degradation products: (FDP) Fragments of fibrin polymers following enzymatic breakdown of strands.

Gitter cells: See compound granular corpuscles.

Hypoxia: Oxygen deficiency as a result of various causes such as reduced concentration of oxygen in the blood (hypoxic hypoxia) or interrupted blood supply or reduced blood flow to an area (stagnant hypoxia).

Homogenizing cell change: Late stage of the ischaemic cell process in which neuronal cytoplasm stains homogeneously acidophilic with complete loss of nuclear definition.

Infarction: The process by which all cell bodies (neuronal and glial), blood vessels (arteries, veins and capillaries) and nerve fibres (myelinated and non-myelinated) in a given volume of tissue, undergo necrosis as a result of a reduction in blood flow. Ischaemic infarction occurs as a result of total obstruction of an end-arterial system. Haemorrhagic transformation of infarction occurs as a consequence of distal migration of a thrombus (embolisation deeper into area of infarction). Haemorrhagic infarcts develop as a consequence of venous obstruction.

Intermediate host: Host in which the parasite undergoes the asexual stage of the life cycle (in babesiosis this is the vertebrate host).

Ischaemic cell change: Middle stage of the ischaemic cell process in which the soma is shrunken with loss of Nissl substance and the nucleus is shrunken, dark-staining and often triangular. The cytoplasm is acidophilic, staining pink with eosin and mauve with Luxol fast blue. As neuronal injury progresses, basophilic incrustations become discernable as minute granular deposits on the plasmalemma.

Ischaemic cell process: Morphologically appreciable stages of neuronal injury commencing with microvacuolation and progressing through the stages of ischaemic cell change without incrustations, with incrustations, finally culminating in homogenising cell change after which there is disappearance of the neuron.

Karyorrhexis: Cellular necrosis characterised by nuclear fragmentation.

Karyopyknosis: Cellular necrosis characterised by nuclear shrinkage.

Margination of parasitised erythrocytes: Abnormal spatial positioning of parasitised erythrocytes against endothelium.

Microvacuolation: The earliest appreciable morphological change in neurons undergoing the ischaemic cell process (perfusion fixation). Basophilic neuronal cytoplasm contains numerous small vacuoles that cluster beneath the plasmalemma and around the nucleus.

Micro-vessel: Vessels less than 50 μm in diameter including capillaries and post-capillary venules.

Necrosis: Irreversible injury leading to cellular death within living tissue.

Perivascular space: The interstitial space around blood vessels. In normal brain, this is only a potential space, between astrocyte foot processes and the cells of the vessel wall. In microvasculature, only the basement membrane lies between the endothelium and astrocyte foot processes. Enlargement of this space may be an artifact in tissue sections, or alternatively, may represent oedema.

Pink brain: See congestive brain swelling.

Pyknosis: See karyopyknosis.

Reperfusion injury: The re-establishment of circulation after a critical period of anoxia-ischaemia which will allow a suboptimal degree of metabloic activity to occur, may be associated with a greater degree of morphologically visible tissue damage than if reperfusion did not occur.

Selective vulnerability: Site-specific neuronal response to hypoxia and other noxious stimuli. Cells showing the highest sensitivity are those of the cerebral cortex, particularly layers III,

V and VI, and large neurons such as the cerebellar Purkinje cells. Neurons in Sommer's sector of the hippocampus and in the border zones between arterial territories, are particularly sensitive to hypoxia.

Sequestration of parasites: Invasion of erythrocytes by parasites in order to escape detection by the immune system.

Sequestration of parasitised erythrocytes: Accumulation of parasitised erythrocytes in the microvasculature of organs in order to allow parasite proliferation by avoiding entrapment in the spleen. Unless otherwise specified, sequestration in the text refers to sequestration of parasitised erythrocytes.

Severe cell change: The cell body is swollen with loss of Nissl substance around a swollen nucleus. The cell margins are irregular with formation of ringlets and sometimes large vacuoles. The cell processes are stained.

Sludging (of erythrocytes): Intravascular haemagglutination of unparasitised erythrocytes in small caliber vessels. Individual erythrocytes are not necessarily discernible. Inflammatory cells and parasitised erythrocytes may be trapped in the sludge.

VR-space: Virchow-Robbins space (see perivascular space).

Wall shear stress: Force of friction acting on endothelium as a consequence of blood flow