Research Article

Histopathological Examination of the Possibility of a Purrfect Neuro Inflammatory Storm Leading to Choroid Blindness and Death Following Traumatic Brain Injury in Red Kangaroo (*Osphranter rufus*)

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Abstract | The cause of mortality in macropods like kangaroos may not always be known, and in many cases, it is difficult to determine. According to necropsy results, a male red kangaroo with a history of chronic neuro-ocular toxoplasmosis had an extensive mixed fracture of the posterior and base of the skull. Direct deep and countercoup brain bruises, independent fractures of the top and roof of both orbits, subdural hemorrhage with edema and shifting of the mid-line to the left, frontal lobe with severe hemorrhagic discharge in the ventricles, diffuse subarachnoid hemorrhage and exclusion of berry aneurysm in vertebral arteries, traumatic tear due to intracranial and intraspinal courses, temporal were seen. Due to unilateral brain herniation, the tonsillar hernia was associated with hemorrhage and linear necrosis rather than only bulging. The corpus callosum and fornix had small areas of hemorrhage that indicated a sign of diffuse traumatic axonal injury. In histopathology, activated microglia and reactive astrogliosis were seen. The cause of death was TBI followed by eye blindness caused by toxoplasmosis, during which was the formation of a hematoma larger than 60 mL, a primary Glasgow coma scale less than 8, and basal nucleus ganglia dysfunction. A 40-ml intracerebral hemorrhage occurred followed by severe blood aspiration and asphyxia.

Keywords | Choroid blindness, Neuroinflammation, Neuro-ocular toxoplasmosis, Red Kangaroo, Traumatic Brain Injury, Necropsy, Histopathology

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INTRODUCTION

Marsupials are a major order of mammals whose ancestors radiated from stem mammals over 180 million years ago. The cause of mortality in macropods like kangaroos may not always be known, and in many cases, it is difficult to determine. However, various types of etiology are known or hypothesized, with the most common being stress or nutritional infection. Several factors (many synergistic effects) may be associated with mortality (Rodger, 2020). Given kangaroos' physiological traits and their demand for broad expanses for movement and activity, the confined population of kangaroos in zoos is constantly in danger of damage and impacts due to collisions with objects or fences, especially if they do not have enough room. The kangaroo's brain is the bodily portion that is most vulnerable to clash with objects and injury (Dubey et al., 2021). A head jolt, blow, or bump; a sudden and severe clash with an object; or when a body pierces the skull and enters the brain tissue (Vanderveen,

2021). Traumatic brain injury (TBI) is a disorder of the brain's normal functioning caused by a jolt, blow, or bump to the head; when the head strikes an object suddenly; or when a body pierces the skull and enters the brain tissue. TBI symptoms can be minor, moderate, or severe, depending on the amount of the brain injury. A short shift in mental state or awareness may occur in mild instances.

Severe instances might result in coma, death, or persistent anesthesia. Rapid bleeding into one or more regions of the skull, extra muscular, subdural, subarachnoid, intraventricular spaces, or the cerebral cortex can cause sudden death. The causes differ based on the age and location of the bleeding in the body.

The neuropathological findings might range from a brain that seems quite normal to one with many possible subarachnoid or subdural hemorrhages.

Annually, several cases of death-related disorders strongly suggest that trauma is the primary cause (Capizzi et al., 2020). As a result, there might be external and internal evidence of traumatic injuries to the chest and abdomen. However, the volume and severity of the evidence are insufficient to give a convincing explanation for the death or even proof of head trauma. Under these circumstances, there's a good chance the cause of death was in the skull, and a fixed brain examination might provide the solution (Benaroya, 2019). While there may be signs of trauma, such as a thin layer of subcutaneous bleeding and minor surface contusion, the brain incision frequently displays no signs of internal injuries, such as parenchymal or intraventricular hemorrhage. As a result, there is still some doubt concerning the specific process of death, which may continue even after thorough histological research (Roldan and Kyriacou, 2021). Several pathological cases may occur as a result of concussions. The following are the most important.

Hematoma

A hematoma is a common problem that occurs as a result of damage to one of the larger blood vessels in the body. A hematoma can look like a bruise, but bruises occur due to damage to small blood vessels rather than large ones. An epidural hematoma (EDH) is a collection of blood that forms between your skull and the dura mater, the outermost protective membrane covering your brain. A subdural haematoma is a serious condition where blood collects between the skull and the surface of the brain. It's usually caused by a head injury. The history of ectopic hematoma following injury before anesthesia, on the other hand, is a "lucid" or "latent" gap, which allows most patients to reach the veterinary zoo clinic and undergo surgery (Quatman-Yates et al., 2020). Necropsy findings include cranial fractures at the point where the parietotomoral region meets a localized dark red blood clot caused by a rupture in the middle meningeal artery or one of its branches. An extradural hematoma is a collection of blood in the 'potential' space between the skull and the outer protective lining that covers the dura mater. It usually occurs because of a head injury. An operation to remove the hematoma may be needed and must be distinguished from the dark, brittle heat hematoma reported in certain fire fatalities by the pathologist. animals with a pure subdural hematoma may perceive a distinct distance before anesthesia, similar to an extracutaneous hematoma. Subdural hematoma caused by a head injury, such as from a fall, motor vehicle collision, or an assault. so frequently linked with skull fractures. While a fracture and a blood clot are always present in an ectopic hematoma, the blood clot in subdural hematomas is typically widespread, unilateral, or bilateral, and does not always match the fracture line. This process demonstrates a distinct type of damage, a subdural hematoma caused by bridging veins in the subcutaneous region rupturing as a result of a rotating motion. Identifying the source of bleeding during a necropsy is not always possible. Acute subcutaneous hematomas, which include dark red fluid or blood clots, can develop quickly (Kim and Priefer, 2020).

Brain contusion

Cerebral contusions, a form of traumatic brain injury, refer to bruises of the brain tissue, typically at the surface. Contusions, therefore, occur at sites where the brain can graze against adjacent bony surfaces. Typical sites include the basic-frontal lobes and anterior tips of the temporal lobes. Para-sagittal contusions known as "gliding" contusions are also commonly seen. Coup or contrecoup injuries also result in cortical contusions. In coup injuries, the brain tissue directly underneath the site of trauma shows evidence of contusion. Such injuries are usually associated with overlying fractures. In contrecoup injuries, the brain tissue opposite the site of impact shows evidence of injury (Payne and William, 2021).

Subarachnoid hemorrhage

Subarachnoid haemorrhage is an uncommon and severe subtype of stroke affecting animals at a mean age, leading to loss of many years of productive life. The rupture of an intracranial aneurysm is the underlining cause in 85% of cases. The most common observation following a head injury is bleeding in the region under the arachnoid. Identifying the source of bleeding during a necropsy might be challenging, which raises the risk of an artificial rupture during the procedure. Several methods have been described to identify the site of a vascular rupture. These include removal of the posterior brain block and upper cervical spinal cord and blockage of the necropsy or placement under the neck area, along with impure or post-mortem

angiography. Intubation of the proximal vertebral arteries in the neck and washing with isotonic salt while checking the ventral surface of the brainstem for fluid retrieval, which has already been removed, are examples of positive findings (Ziu and Mesfin, 2021).

Traumatic intraventricular hemorrhage

Intraventricular hemorrhage (IVH) is defined as the eruption of blood in the cerebral ventricular system and is mostly secondary to spontaneous intracerebral hemorrhage and aneurysmal and arteriovenous malformation rupture. IVH is a proven risk factor for increased mortality and poor functional outcome. Its seriousness is correlated not only with the amount of blood but also with the involvement of the third and fourth ventricles. Four mechanisms explain the pathophysiology of this event: Acute obstructive hydrocephalus, the mass effect exerted by the blood clot, the toxicity of blood-breaking products on the adjacent brain parenchyma, and, lastly, the development of chronic hydrocephalus. It is thus obvious that the clearance of blood from the ventricles should be a therapeutic goal (Reardon et al., 2022).

Diffuse vascular injury

The pathological finding of multiple small hemorrhagic brain lesions of traumatic origin was first identified by Cassaca. Subsequently, cases of this pathology were reported only by Tomlinson, Strich, and Adams *et al.*, who named it diffuse vascular injury. A more thorough knowledge of DVI is appropriate, since this type of lesion is relatively frequent in victims of severe head trauma caused by Quarrel between animals (Reardon et al., 2022). A forensic veterinarian rarely performs a necropsy on an animal that has died quickly, usually with the characteristics of an external blow to the head, however, without obvious external abnormalities of the brain. Under these conditions, neuropathologic examination after a period of stabilization may reveal multiple petechial hemorrhages throughout the brain, especially in the brainstem and white matter of the anterior parts of the frontal and temporal lobes located next to the thalamus. Posterior cerebral hemorrhages were formerly referred to as primary cerebral hemorrhages. However, due to their widespread distribution in the cerebral hemispheres and brainstem, they are more commonly considered the primary type of brain injury. Although there is no doubt that animals with this pattern of multiple petechial hemorrhages in the brain die instantly or within a quarter of an hour, increasing experience has shown that some animals with this type of acute vascular pathology survive the initial injury only to die several hours later. This shows that there may be a traumatic damage spectrum defined by several tiny hemorrhages in the brain caused by a diffuse vascular injury that is very similar to diffuse axonal injury. Both of these, previously known as shearing injuries, are severe kinds of diffuse traumatic brain damage that occur most often, but not always, following physical incidents. This form of petechial hemorrhage is symptomatic of damage of a type and distribution that is harmful to animal life (Stewart et al., 2022). Despite countless research on these species' biology and behavior, there are few studies and reports on illness processes and reasons for mortality. The present study aimed to examine histopathologically the likelihood of a purrfect neuro inflammatory storm leading to choroid blindness and mortality in Red Kangaroo following traumatic brain damage.

MATERIALS AND METHODS

Initially, before the necropsy operation of a 7-years old male Red Kangaroo (Osphranter Rufus), the information and history of the animal were recorded and completed with the help of the file in the Eram Zoo, Tehran, Iran. The health history included clinical symptoms, death circumstances, clinical blood work, diet and environment, recent treatments, cage mates, mixed-species exhibit, and temperature/ humidity. Then, during the gross examination stage, all organs were examined, with a sample collected from each component required for the pathology slide and microscopic analysis. Physical and nutritional condition, pelage, subcutaneous fat stores, body orifices, and superficial lymph nodes Checked out. Musculoskeletal system such as (Bones, marrow, joints, muscle), Body Cavities (fat stores, pleura, thymus, lymph nodes), spleen, respiratory system (nasal passages, pharynx, larynx, trachea, bronchi, lungs, regional lymph nodes), cardiovascular system (heart, pericardial sac, great vessels, myocardium, valves, chambers), digestive system (mouth, teeth, tongue, esophagus, stomach, small and large intestine, anus, liver and gall bladder, pancreas, mesenteric lymph nodes), urinary system (kidneys, ureters, bladder, urethra), reproductive system (testes, penis, accessory sex organs, mammary gland, placenta), endocrine system (thyroids, parathyroids, adrenals, pituitary), central nervous system (brain, meninges, spinal cord) and sensory organs (eyes, ears) were examined. All organs suspected of having a lesion were inspected and sampled for histopathological investigation and slides were made and evaluated for expert review. Then, in little plastic bags filled with liquid nitrogen, we froze 3-5 cm chunks of tissue from organs (e.g., lung, liver, kidney, spleen) to be stored ultra-frozen at 70 degrees Celsius. The tissues were preserved in a 10% buffered formalin solution with a one to ten tissue to solution ratio. Tissues were just 0.5 to 1 cm thick. Post-mortem serum (from the heart), urine, and any abnormal fluid accumulations were frozen. Most fresh tissue is very delicate, easily distorted, and damaged. Thus, it is impossible to prepare thin sections (slices) from it unless

it is supported in some way whilst it is being cut. Usually, the specimen needs to be preserved or "fixed" before sections prepared. Broadly, two strategies can be employed to provide this support (Feldman and Wolfe, 2014) first: The tissue can be rapidly frozen and kept frozen. second: Alternatively, specimens can be infiltrated with a liquid agent that can subsequently be converted into a solid that has appropriate physical properties that will allow thin sections to be cut from it. These sections are prepared with a "rotary" microtome. After Specimen reception, Fixation was done which is a crucial step in preparing specimens for microscopic examination. Its objective is to prevent decay and preserve cells and tissues in a "life-like" state. It does this by stopping enzyme activity, killing microorganisms, and hardening the specimen while maintaining sufficient molecular structure to enable appropriate staining methods to be applied (including those involving antigen-antibody reactions and those depending on preserving DNA and RNA). The sooner fixation is initiated following the separation of a specimen from its blood supply, the better the result will be. The most popular fixing agent is formaldehyde, usually in the form of a phosphate-buffered solution. specimens were fixed by immersion in formalin for six to twelve hours before they are processed. Then the Grossing step was done (Markovic et al., 2020). which, involves a careful examination and description of the specimen that will include the appearance, the number of pieces, and their dimensions. Larger specimens may require further dissection to produce representative pieces from appropriate areas. Then a device called a tissue processor is used. These instruments allow the specimens to be infiltrated with a sequence of different solvents finishing in molten paraffin wax. The specimens are in an aqueous environment to start with (water-based) and must be passed through multiple changes of dehydrating and clearing solvents (typically ethanol and xylene) before they can be placed in molten wax (which is hydrophobic and immiscible with water). The duration and step details of the "processing schedule" chosen for a particular batch of specimens will depend on the nature and size of the specimens. After processing, the specimens are placed in an embedding center and removed from their cassettes and placed in wax-filled molds. At this stage, specimens are orientated because this will determine the plane through which the section will be cut and ultimately may decide whether an abnormal area will be visible under the microscope. The cassette in which the tissue has been processed carries the specimen identification details, and it is now placed on top of the mold and is attached by adding further wax. The specimen "block" is now allowed to solidify on a cold surface, and when set, the mold is removed. The cassette, now filled with wax and forming part of the block, provides a stable base for clamping in the microtome. The block containing the specimen is now ready for section cutting. Sections are cut on a precision instrument called a "microtome" using extremely fine steel blades. Paraffin sections are usually cut at a thickness of 3 -5µm, ensuring that only a single layer of cells makes up the section. Sections are now "floated out" on the surface of warm water in a flotation bath to flatten them and then picked up onto microscope slides. After thorough drying, they are ready for staining. Then the slides were stained with the standard hematoxylin and eosin method and pathologically evaluated with a light microscope (Feldman and Wolfe, 2014).

RESULTS AND DISCUSSION

The necropsy findings of a male Red Kangaroo with a history of chronic neuro-ocular toxoplasmosis indicated an extensively comminuted fracture of the posterior and the base of the skull. Direct deep and countercoup brain bruises; independent fractures of the top and roof of both orbits; subdural hemorrhage with edema and shifting of the mid-line towards the left; frontal lobe with severe hemorrhagic discharge in the ventricles; Diffuse Subarachnoid hemorrhage and the absence of berry aneurysm in vertebral arteries, the traumatic tear due to intracranial and intraspinal courses, Contusions in the temporal, frontal, other sites, and coup and counter-coup injuries were seen. Due to unilateral brain herniation, the tonsillar hernia is associated with hemorrhage and linear necrosis rather than only bulging. The corpus callosum and fornix had small areas of hemorrhage that indicate a sign of diffuse traumatic axonal injury. Following the supra callosal herniation in the midline, multifocal infarction, ischemia, arterial territory, and intracerebral hemorrhage were seen. Other symptoms observed included nasal fracture and tenderness, floating chest and hemothorax, as well as internal eyelid rupture, corneal rupture, orbital fracture. The lungs were quite bloody and heavy due to the spiraling and widespread infiltration of blood. Other internal organs such as the kidneys, spleen, liver, and digestive tract were intact. All internal organs were sampled for microscopic examination. The cause of death was due to hematoma greater than 60 ml, primary GCS less than 8, and basal nucleus ganglia ICH volume of 40 ml, followed by severe blood aspiration and asphyxia.

Eye

Neuro Ocular toxoplasmosis is a parasitic infection of the eye caused by Toxoplasma gondii. The disease usually affects the posterior pole of the eye, and the lesions can be single or multiple and can be classified as active or frightening. Active lesions are gray-white and accompanied by choroiditis, vasculitis, and exudative discharge. The vitreous is normally clear and transparent, and it may become hazy or cloudy due to the infiltration of inflammatory immune cells. The scarring tissue starts from the peripheral parts

and progresses toward the center with clear pigmentation changes. Ocular lesions are asymptomatic and may progress years after infection. In this case, in the history of kangaroos kept in this collection, there was a history of toxoplasmosis years ago that was untreated and the disease was chronic in the herd. After the necessary examinations and according to lesions that threatened the optic nerve and large retinal vessels and macula that induced a large hemorrhage and inflammation. The treatment program started twenty days ago with a combination of pyrimethamine, folinic acid, and sulfadiazine. Neuro Ocular toxoplasmosis manifests as granulomatous inflammatory infiltration of the areas of necrosis in Bruch's membrane necrotic regions as well as in the choroid. In the present study, the main histopathological lesions observed were degeneration and atrophy of the retina, severe acute choroid inflammation, and necrosis (Figure 1).

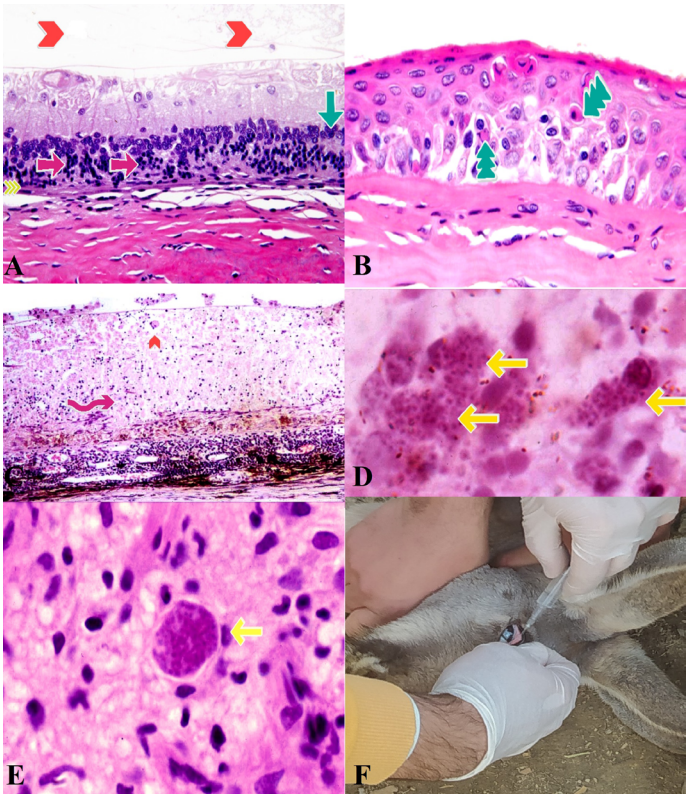

Figure 1: Eye, change in Traumatic Brain Injury. **A:** Mild degeneration of Retina, including loss of cone and rod photoreceptor processes, (yellow arrowheads), unicellular necrosis (single-cell) in the outer part of nuclear layer photoreceptor cells, (Pink Arrows), Disorganization and hypo cellularity of the outer and inner parts of the nuclear layers, (Green Arrow) and narrowing and absence of the plexiform layers. (redhead arrow). (H & E, x800). **B:** Cornea ,Necrosis. Hypereosinophilic and shrunken necrotic cells, (Green Arrow) (H & E, x800). **C:** overlying necrosis of retina, (Pink Arrow) and presence of a large Toxoplasma gondii tissue pseudocyst on the surface. (redhead arrow) (H & E, x600). **D, E:** Toxoplasma gondii cyst-containing discrete granule-type bradyzoites (yellow arrows) (H & E, x800). **F:** Intraocular injection in another patient kangaroo.

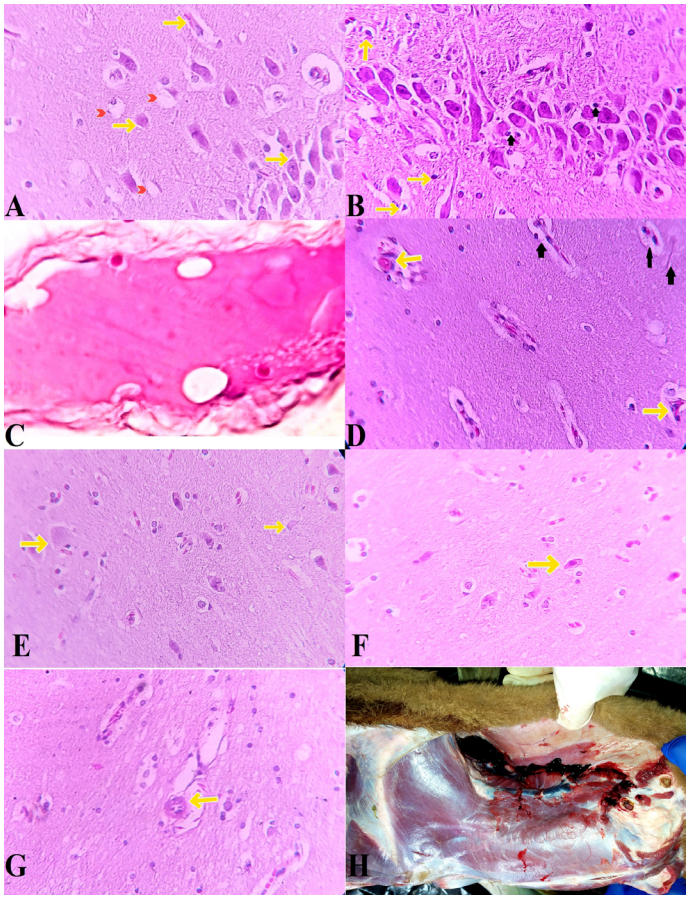

Figure 2: Brain changes in Traumatic Brain Injury. The early changes reflect alterations in ion gradients accompanying shifts in the perikaryon (Soma) and cause cloudy swelling. **A:** Swelling of the endoplasmic reticulum appears as a gap (cleft) in the periphery of the soma (Yellow arrows). This is different from crystalline inclusions and should not be mistaken. Astrocyte takes up Potassium + and the cytoplasm becomes pale due to intracellular fluid accumulation (red arrowhead) (H&E, x600). **B:** In response to brain-contusions injuries, neurons appear dark and pyknotic. (yellow arrows) (H&E, x200). astrocytes as well as Surveillant microglia activated to produce extra inflammatory mediators (Black Arrows). **C:** due to cellular edema and sufficient concentration of swollen neurons, some regions appear pale. (H&E, x12.5). **D:** necrotic neurons have a uniform eosinophilic cytoplasm. (yellow arrows). highe proportion of activated microglia and astrogliosis (Black Arrows) (H&E, x600). **E:** Some neurons become ghost cells without nucleus staining and a barely visible cell architecture (yellow arrows). (H&E, x600). **F:** rare eosinophilic neurons (yellow arrows) (H&E, x600). **G:** Toxoplasma gondii tissue cysts in the cerebral cortex (yellow arrows). (H&E, x600). **H:** Traumatic myositis in Cleidomastoid, Sternomastoid, Sternothyroid, Sternohyoid and Trapezius Muscles and sever damage to jugular vein, linguofacial vein, maxillary vein and caudal auricular vein, great auricular nerve lead to ruptures and hemorrhage.

Brain

The brain's histology revealed diffuse and basal subarachnoid hemorrhage, with aneurysms and vertebral arteries ruled out. The intracranial and intraspinal courses caused the traumatic tear. Contusions have also been found in the temporal, frontal, and other areas, as well as coup and counter-coup injuries. Tonsillar herniation was related to bleeding and necrosis rather than only bulging due to unilateral brain herniation. Brain swelling-gyri flattening in one cerebral hemisphere, and tiny pockets of bleeding in the corpus callosum and fornix, indicate diffuse traumatic axonal damage. In the midline due to supracallosal herniation, there were many foci of infarction, ischemia, arterial territory, and intracerebral hemorrhage (Figure 2).

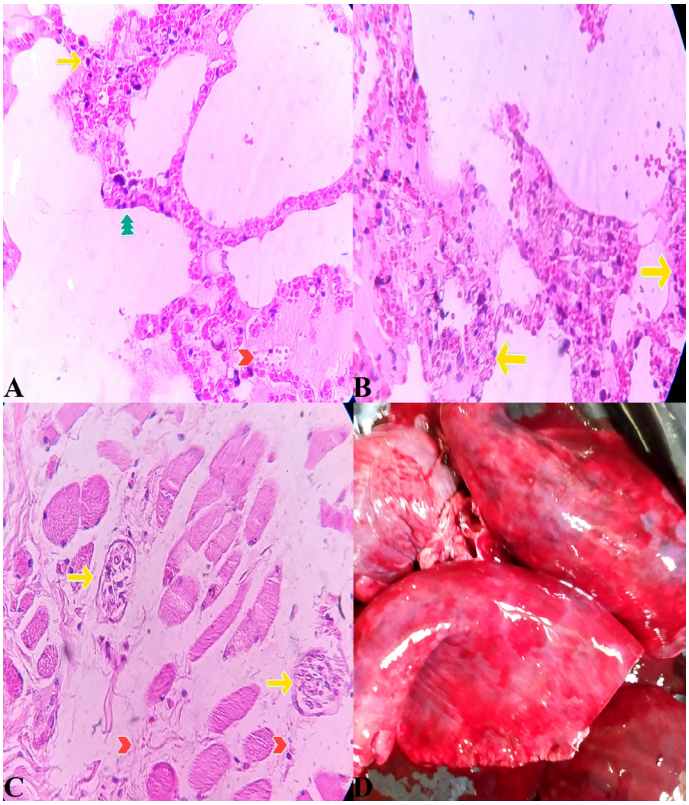

Figure 3: Lung changes in Traumatic Brain Injury. **A:** neutrophil infiltration (yellow arrow) and proliferation in the alveolar and interstitial space, thickening of the alveolar septum, (green arrow) as well as edema and alveolar hemorrhage (redhead arrow). (H&E, x600). **B:** destruction of type II alveolar cells, which leads to disruption of normal fluid transport. (yellow arrow). **D:** Toxoplasma gondii cyst-containing discrete granule-type bradyzoites (yellow arrows) and free tachyzoites (red arrowheads) (H&E, x600). **C:** Macroscopic view of Lung tissue.

Lung

TBI causes pyroptosis and an increase in the expression of inflammatory proteins in type II alveolar epithelial cells in lung tissue. These alterations include neutrophil infiltration and proliferation in the alveolar and interstitial spaces, thickening of the alveolar septum, edema, and alveolar bleeding, as seen in (Figure 3). In the acute phase of this injury, these are the most prevalent characteristics of histological diagnosis. In the present study, morphological abnormalities in lung tissue were more prominent 4 hours after damage and after necropsy and were linked to increased production of inflammatory proteins.

Within hours to days after the injury, microscopic lesions demonstrate diffuse damage to cells and structures of the alveolar-capillary membrane in the lungs. The loss of type II alveolar cells, which disrupts normal fluid transport, was also found in the investigation of lung slides in this lesion. Inflammation in the CNS is a crucial factor in the innate immune inflammatory response's initiation. The CNS's innate immune system has a role in the pathophysiology of pulmonary dysfunction following a TBI. Pyroptotic cell death may result from inflammatory activation and IL-1 release. A systemic inflammatory response has recently been discovered to have a significant role in TBI-induced lung impairment. The blood-brain barrier is permeable for up to 3 hours after a TBI, allowing protein and fluid to flow through the protective barrier between the brain and the intravascular portion. After a TBI, brain obstruction, and other alternative channels, such as lymphatic or glymphatic transport systems, inflammatory mediators are secreted, which can increase inflammation in the brain and harm other distant organs (Figure 3).

Tongue

Reduced range or control of tongue movements, alone or in combination with impairments such as delayed or missing pharyngeal swallow, are regarded to be the most common post-TBI oropharyngeal motor deficits. Aspiration is a common occurrence. Reduced laryngeal elevation, the reduced base of tongue retraction, reduced pharyngeal peristalsis, prolonged pharyngeal transit time, prolonged oral transit time, unilateral pharyngeal paralysis, absent or weak reflexive or voluntary cough, cricopharyngeal dysfunction, and primitive oral reflexes are all symptoms of pharyngeal paralysis (biting, pursing and rooting). Inconsistency or delay in the process of oral preparatory and chewing of food and early swallowing of food like plants and fruits can indicate poor tongue control. Facial asymmetry in these rodents is caused by aberrant facial muscle tone, which can be hypo or hypertonic and accompanied by abnormal oppositional muscular contraction. Cells seemed to be enlarged in this circumstance and due to hypoxia damage after TBI, and hypoxic death, cell, and nuclear membrane were differentiated. Because of the diminished efficacy of the plasma membrane's energy pump owing to energy depletion, potassium is released from the cell and sodium accumulates intracellularly. The oxidative phosphorylation pathway ends and the oxygen supply to the cell is reduced, and the cell switches to an anaerobic cycle (Figure 4).

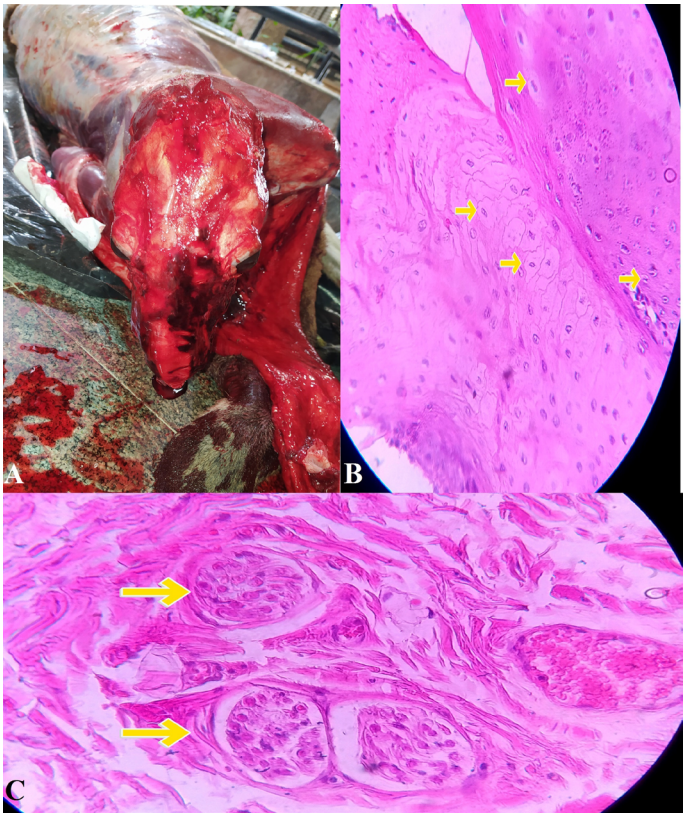

Figure 4: Tunge Muscle changes in Traumatic Brain Injury. **A:** Rupture of Levator nasolabialis, Levator labii maxiltaris, Temporalis, Masseter and Buccinator Muscles due to severe physical trauma that resulted in severe injury to Communicating branch of auriculotemporal nerve, transverse facial vein one facial artery and facial vein that lead to severe hemorrhage. **B:** hypoxic death cells appeared to be swollen and the cell membrane, as well as the nuclear membrane, was discernible. (yellow arrow) (H&E, x600). **C:** Toxoplasma gondii cyst-containing discrete granule-type bradyzoites (yellow arrows) (H&E, x600).

Muscle

Muscle atrophy is a key phenotype in neuromuscular injury that causes loss of functional contractile tissue and may result from any number of changes in neuromuscular connectivity and activity, including denervation, disuse, and inhibition of muscular activity. To alter pain or motor drive. Muscle degeneration such as cytoplasm and membranous disruption, phagocytosis, and centralization of myonuclei (subsequent regeneration) are processes that are different from atrophy and have been observed in degenerative musculoskeletal diseases that present with functional impairment. Muscle biopsy data shows that the vast majority of muscle regions, even in the absence of atrophy, show signs of fiber degeneration. However, atrophy-inducing stimuli versus degenerative or regenerative phenotypes remain vague. In particular, it is unclear whether these degenerative phenotypes are the result of local or peripheral denervation, inflammation, or impaired motor drive. Traumatic brain injury is a central nervous system injury in which external mechanical forces on the brain cause focal or diffuse nerve damage. Like other models of central nervous system injury, traumatic brain injury eventually leads to long-term muscle disorders, which are often accompanied by pain and lead to poor performance. Our findings provide the first morphological evidence for skeletal muscle degeneration and regeneration after CNS injury, in addition to motor cortex-induced muscle degeneration and regeneration but not atrophy in TBI. Given in this case, death occurred immediately after the brain injury. So, naturally, except for a few cases, the findings of pathology in the muscle cannot be considered completely related to the cause of death. Some are accidental findings, some are very rapid muscle responses to injury, and a few are related to the cause of death (Figure 5).

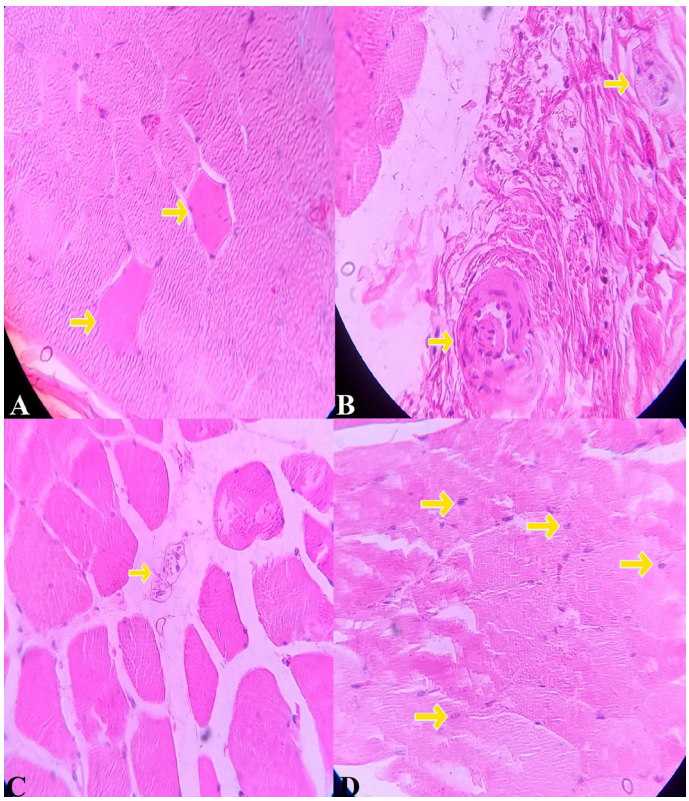

Figure 5: Muscle spino-acromio-clavodeltoideus. changes in Traumatic Brain Injury. **A)** Degenerated fibers, (yellow arrow) (H&E, x600). **B, C:** Toxoplasma gondii cyst-containing discrete granule-type bradyzoites (yellow arrows) (H&E, x600). **D:** Central nucleation can be seen in the injured M. infraspinatus (H&E, x600).

Spleen

Many types of immune cells, both within the central and peripheral nervous system, are activated soon after traumatic brain injury onset and significantly contribute to brain damage or repair. The spleen is associated with the brain by soluble mediators and autonomic innervation, and cross-communication between the brain and the spleen may be important to establish a systemic inflammatory

response to traumatic brain injury. Splenic responses after traumatic brain injury and their contribution to brain ischemic injury have attracted considerable attention. Preliminary animal studies have revealed that the spleen shrinks in the acute phase dramatically after middle cerebral artery occlusion. This morphological change is associated with the long-distance trafficking of splenocytes from the spleen into the ischemic region. It is noteworthy that the reduction in spleen size is induced by middle cerebral artery occlusion and is associated with the rate of ischemic injury. Present pathological findings suggest that spleen size is quite dynamic in traumatic brain injury animals, with early contraction followed by subsequent re-expansion. This change in spleen volume may be accompanied by the early release of circulating spleen cells, which in turn may contribute to the post-Traumatic Brain Injury inflammatory cascade. Despite existing awareness of the splenic alterations, the exact function of the spleen in ischemic brain injury is unclear. The spleen can establish long-term connections with the brain by mobilizing its cellular components during ischemic injury. The total number of cells inside the spleen decreases dramatically within a few days after traumatic brain injury. This decrease probably reflects the increase in cell death within the spleen as well as the increased efflux of immune cells from the spleen into the blood. Many of these immune cells penetrate the ischemic brain and contribute to brain lesions. Analysis of splenocytes showed an increase in various lymphocytes, including T and B cells, within 18 hours after traumatic brain injury, which was reversed in the CD74 deficiency, which resulted in smaller red neuron size and reduced nerve damage in neuronal Wallerian degeneration (Figure 6).

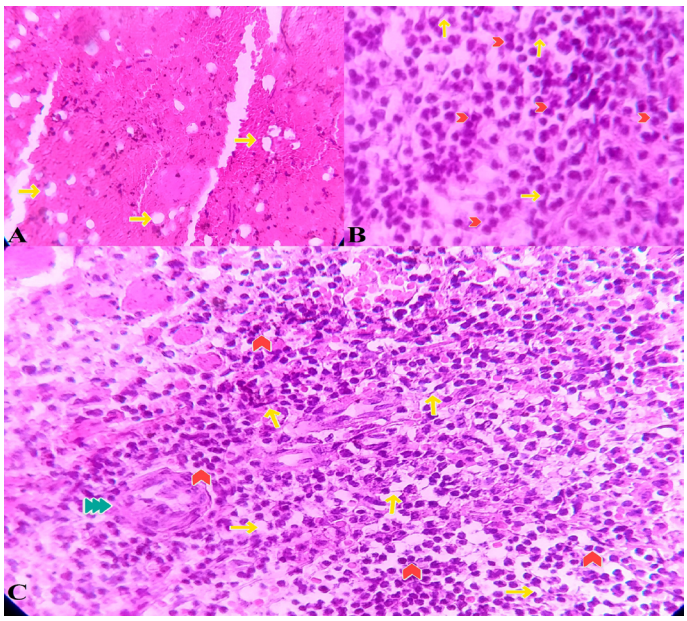

Figure 6: Spleen changes in Traumatic Brain Injury. **A:** internuclear bridges (yellow arrows) and binucleated erythroid precursors (redhead arrow) were readily recognized (H&E, x600). **B:** severe depletion of white pulp lymphatic tissue (yellow arrows) (H&E, x600). **C:** Toxoplasma gondii cyst-containing discrete granule-type bradyzoites (Green arrow) internuclear bridges (yellow arrows) and binucleated erythroid precursors (redhead arrow) (H&E, x600).

Heart

Following severe cerebrovascular diseases such as traumatic brain injury, severe cardiovascular lesions such as myocardial damage have been observed. Traumatic brain injury raises the heart rate, blood pressure, intracranial pressure, plasma norepinephrine, and cardiac troponin I, according to clinical studies. In addition, traumatic brain damage alters the ultrastructure of cardiac myocytes. The sarcoplasm has increased edematous areas and an expanded sarcoplasmic reticulum. Other observations included enlarged mitochondria and enhanced heterochromatin accumulation. Necrosis, hemorrhage, congestion, hypereosinophilia bundle, and leukocyte infiltration were among the histopathological findings of cardiac myocytes in this investigation (Figure 7).

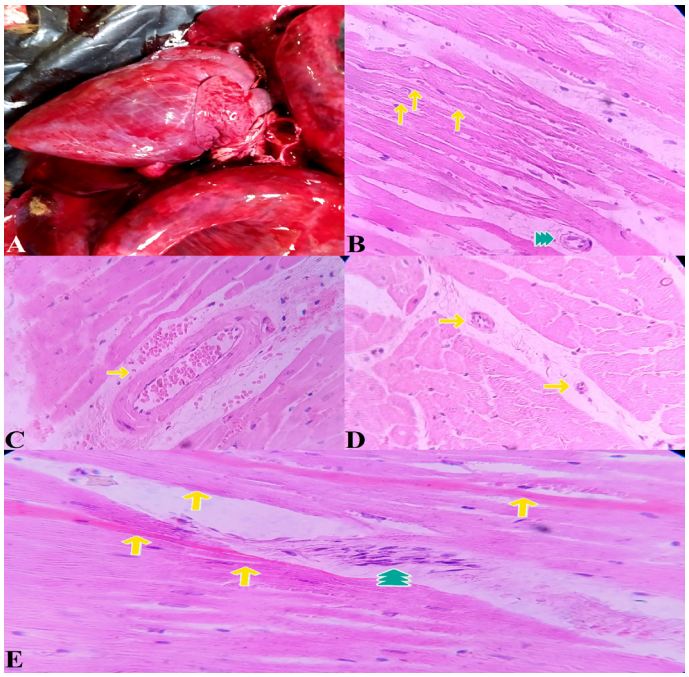

Figure 7: Heart changes in Traumatic Brain Injury. **A:** Macroscopic view of heart tissue. **B:** Degeneration and Atrophy of myocardial fibers (yellow arrows) Toxoplasma gondii cyst-containing discrete granule-type bradyzoites (Green arrow) (H&E,x600). **C:** Congestion and hemorrhage of cardiac tissue(H&E, x600). **D:** Toxoplasma gondii cyst-containing discrete granule-type bradyzoites (yellow arrow) (H&E,x600). **E:** Hypereosinophilic bundles(yellow arrows) and leucocyte infiltration(Green arrow) (H&E, x600).

Liver

After traumatic brain injury, swift microglial activation and release of pro-inflammatory cytokines occurred in the pericontusional areas around the brain necrotic tissue.

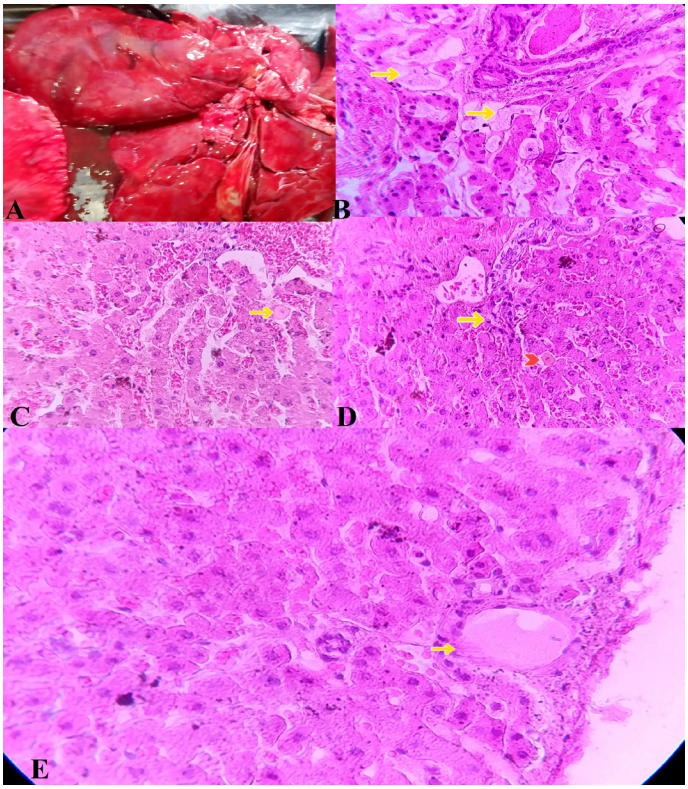

Figure 8: Liver changes in Traumatic Brain Injury. apoptosis, edema, and inflammation in hepatic tissue were increased. **A:** Macroscopic view of Liver tissue. **B:** Focal points of edematous fluid accumulation in the space between hepatocytes (yellow arrow) (H&E, x600). **C:** Rare apoptotic cell (yellow arrow) (H&E, x600). **D:** Infiltration of inflammatory cells (yellow arrow) Rare apoptotic cell (Red Head Arrow) (H&E, x600). **E:** Focal accumulation of edema (yellow arrows) (H&E, x600).

Other inflammatory cells, such as macrophages and neutrophils, are sources of toxic metabolites that can release a matrix of metalloproteinases, leading to the formation of axonal bulbs and red neuron lesions. Activation of these inflammatory cells is not limited to the CNS parenchyma. Further uptake of leukocytes and a further increase in neutrophils occurs in the PNS. However, these signals may not be emitted from the CNS after injury, and to activate leukocyte mobilization and priming They are released from damaged peripheral organs. If the process is sufficiently strong, this high level of circulating leukocytes is associated with fever and changes in serum levels. In addition, inflammatory cells from the bloodstream accumulate in organs such as the liver, leading to acute inflammation in the organ. These changes are referred to as the acute systemic phase response, and the liver is the major organ involved in coordinating this response. The liver contains the resident macrophages and is a major factor in increasing contributory serum chemokine levels after brain injury. Thus, it has been shown that traumatic brain injury is directly related to acute systemic phase response. After traumatic brain injury, the expression of a chemokine by the liver leads to the recruitment of neutrophils and hepatic lesions, which contributes to multi-organ dysfunction. It is accepted that pro-inflammatory cytokines and chemokines are important critical mediators of the acute systemic phase response after traumatic brain injury. Systemic inhibition shows that induction of chemokines and cytokines in the liver regulates the focal brain inflammatory response. The release of hepatic chemokine enhances the local injury response by increasing circulating immune cells' migration to the injured parts of the brain. However, the recruitment of leukocytes into the cerebellum may also depend on hepatic chemokine and acute systemic phase production, making their inhibition a useful therapeutic plan for this injury (Figure 8).

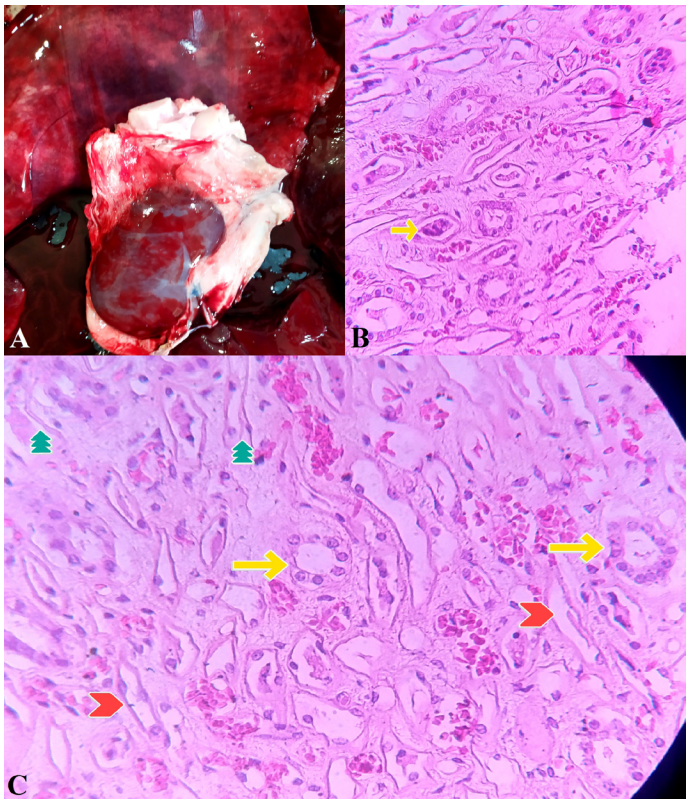

Figure 9: Kidney changes in Traumatic Brain Injury. **A:** Macroscopic view of Kidney tissue. **B:** Toxoplasma gondii cyst-containing discrete granule-type bradyzoites (yellow arrow) (H&E, x600). **C:** Damaged proximal cell tubules demonstrating epithelial thinning (Green arrows), epithelial blebs (Red Head arrows), and proximal tubular dilation (yellow arrows) (H&E, x600).

Kidney

Renal disorders can occur in traumatic brain injuries such as subarachnoid hemorrhage, cerebral ischaemic stroke, white matter lesions, head injury, and intracerebral hemorrhage. These traumatic lesions can influence renal function by three main mechanisms: Neuroinflammation, increased neurosympathetic activity, and the hypothalamic-pituitary axis. These different mechanisms are suppression of the immune system and depression of cell-mediated immunity.

Like in sepsis, the systemic inflammatory response triggered by cytokines plays a key role in the pathogenesis of kidney dysfunction and failure. Traumatic brain injury often activates the sympathetic nervous system and this activation results in reduced renal glomerular perfusion with increased renal sodium reabsorption. Sustained severe hypertension can lead to RBC hemolysis, fractured acute kidney injury, and acute tubular necrosis secondary to RBC thrombosis in the glomeruli. In the meantime, traumatic brain injury due both to changes in cerebral sodium wasting and vasopressin secretion can lead to acute changes in renal sodium handling. In traumatic brain-damaged animals, electrolytes and fluid imbalances can cause renal failure and increase morbidity and death. Other variables that can influence renal function include the administration of mannitol as a fluid, antibiotics, and radiocontrast solutions, as well as hemodynamic instability following traumatic brain injury. All of these lesions provide a characteristic microscopic appearance in the kidneys, which includes typical pathological findings in acute kidney damage in the analysis of slides, as shown in (Figure 9).

This case report has highlighted numerous areas of pathological overlap between *T. gondii* and TBI, which in theory could exacerbate the functional consequences of these conditions. According to what was reported in the pathological slides, to the best of our knowledge, no literature examining the overlap between *T. gondii* and TBI exists, and a great deal of future research is still required. First and foremost, studies must be done to comprehensively characterize if and how *T. gondii* infection modifies the aftermath of TBI. marsupial models will be invaluable to assist in characterizing the hypothesized synergism between *T. gondii* and TBI and validated Marsupial Mammals models of TBI and *T. gondii* infection exist already (Wang et al., 2019). captive wildlife models additionally allow for highly controlled study designs to assist the initial delineation of mechanisms for a given sex or age. Large studies of Free-range wildlife TBI populations should also be conducted in parallel to complement preclinical data. For example, *T. gondii* infection could be screened when TBI captive zoo marsupials present to veterinary clinics, thus allowing for analyses of pathophysiological and outcome differences between TBI patients with and without *T. gondii*. Indeed, it is still not known how many latent *T. gondii* parasites are found in any tissue type in kangaroos l*et al*one the brain. Current tests can only determine exposure by immunoglobulin G antibody response and are not informative about where latent forms reside in the kangaroo's body. This is an important consideration and something that needs to be determined as we would only expect synergy of *T. gondii* and TBI if there were sufficient levels of parasites in the brain to exacerbate inflammation. Taken together, these studies are imperative in our understanding of how TBI and *T. gondii* interact, and provide a foundation to develop and optimize appropriate treatments for TBI kangaroos with and without *T. gondii* to improve outcomes. The α2-adrenergic agonist Guanabenz may be another suitable drug candidate in the context of TBI combined with *T. gondii*, given its ability to downregulate inflammatory responses via elevation of eukaryotic initiation factor 2 alpha subunit (eIF2α) phosphorylation (Ziu and Mesfin, 2021). Moreover, Guanabenz is effective in reducing inflammation and cyst burden during the chronic stage of *T. gondii* (Zhao and Hu, 2021), as well as reducing endoplasmic reticulum (ER) stress and hence reducing neuronal loss post-TBI (Weiss et al., 2019). Guanabenz may also be beneficial by decreasing sympathetic hyperactivity (Wang et al., 2019). The eIF2α dephosphorylation inhibitor, Salubrinal, has additionally shown benefit in both *T. gondii* and TBI studies independently (Wilking et al., 2016). It should be mentioned that Guanabenz has been used by the author in Tehran's Eram Zoo and the treatment of wallabies suffering from toxoplasmosis and traumatic brain injury with great success and Guanabenz was able to control the inflammation process well.

In chronic *T. gondii* infection, Salubrinal has been demonstrated to inhibit the reactivation of bradyzoites (Zhao and Hu, 2021); and through TBI studies, it was established to suppress ER stress, as well as autophagy and apoptosis pathways, thereby reducing morphological and functional deficits post-injury (Zhang et al., 2014). Though Salubrinal does not target the overlapping neuroinflammatory pathways directly, it may still prove beneficial in reducing cell death acutely after injury, and bradyzoite reactivation if immunosuppression were to occur. However, it is important to consider the possibility that these treatments may prove detrimental in animals with a *T. gondii* infection, given that certain inflammatory processes are also necessary to control parasite replication. In other words, if an animal with a chronic *T. gondii* infection were to receive a neuroinflammatory-based drug candidate post-TBI, *T. gondii* tachyzoite replication may reactivate, resulting in uncontrollable parasite proliferation, exacerbated cell death, and clinical symptoms of toxoplasmosis. This therefore would have the opposite effect to what was intended. Hence, it would be beneficial to additionally investigate neuroinflammatory drug candidates with standard treatment strategies for reactivated toxoplasmosis, such as pyrimethamine-sulfadiazine therapy (Silva et al., 2022). This particularly highlights the importance of future research investigating TBI coupled with *T. gondii* infection, in both the preclinical and clinical settings. It would also be of interest to examine the incidence of TBI in animals both with and

without a pre-existing *T. gondii* infection. With growing evidence that *T. gondii* infection in and of itself may result in subtle behavioral abnormalities, it could be the case that these behaviors result in either increased or decreased risk of sustaining a future TBI. For example, in this case, contracting the ocular form of toxoplasmosis caused severe behavioral abnormalities in the kangaroo. So, he started running at a high speed and hit the fence around the animal's enclosure in completely abnormal conditions and causing a traumatic brain injury. *T. gondii* seropositivity has also been associated with increased aggression and impulsivity in Adult healthy kangaroos (Wickwire et al., 2018). These adults may engage in more risk-taking behavior than *T. gondii* seronegative individuals and additionally be at an increased risk of sustaining a TBI. As *T. gondii* seroprevalence increases with age, and free-range kangaroos above 6 years and captive kangaroos above 15 years (Average lifespan in kangaroos is 8 years in the wild; up to 25 years under human care) 15years of age account for a significant proportion of TBI-related deaths, it would also be important to consider the incidence of TBI individuals with or without infection for this age group (Zhao and Hu, 2021). Importantly, as aging in marsupials is associated with immune system dysregulation as well as increased levels of basal inflammation, future studies investigating the effect of age on the hypothesized synergism of chronic *T. gondii* infection with TBI are essential (Kalogeropoulos et al., 2022). Although the focus of this paper has been in the context of a kangaroo with chronic *T. gondii* experiencing a TBI, it should also be considered that a history of TBI may predispose kangaroos to worse outcomes upon a later *T. gondii* infection. For example, TBI can increase the proportion of activated microglia and neuroinflammation chronically after post-injury (Zhang et al., 2014). *T. gondii* would therefore be met upon migration to the brain parenchyma with a more robust neuroinflammatory response, potentially leading to excessive cell death. Moreover, if immunosuppression were to result from a TBI (Xu et al., 2018), and kangaroos were to be later infected with *T. gondii*, uncontrolled proliferation of tachyzoites may occur in enterocytes and once migrate into the brain parenchyma, exacerbated activation of apoptotic pathways may occur due to cell stress via tachyzoite proliferation, and necrotic tissue may result (Wilking et al., 2016). More broadly, it would also be of significance to determine whether peripheral parasitic infections, such as enteric parasites, can alter TBI outcomes. Much like with a chronic *T. gondii* infection, a significant proportion of the captive kangaroo TBI population would be likely to encounter an intestinal parasite at some point either before or after injury. Amplified microglial activation, pro-inflammatory mediators, and functional deficits have previously been demonstrated through peripheral lipopolysaccharide challenge post-TBI (Carlstrom et al., 2020). Additionally, as peripheral immunosuppression and brain-gut axis dysregulation can eventuate in the aftermath of a TBI, increased susceptibility to peripheral infection and increased mortality rate can occur (Fabiani et al., 2022). Therefore, it is reasonable to predict a similar pattern of exacerbation would occur as a result of peripheral parasitic immunological stressors, among other types of peripheral infections. However, to date, this has been an understudied topic, and this field of research requires greater attention in future studies. In general, due to the very high sensitivity of the kangaroo species to parasitic diseases, especially parasites transmitted through vermin such as mice and cats, as well as the irreparable damages caused by these diseases, and on the other hand, due to the wild nature of kangaroos and The impossibility of periodic check-ups and the need for anesthesia to check, which is a very high-risk and dangerous process, seems to be the best recommendations to prevent the entry of vermin into the place of keeping such species in closed environments and captive populations. and also, in closed conditions and captivity, it is possible to have a clinical check-up every 6 months, and it is better to do this for sure in the wild population, the policy should be to eradicate such diseases.

CONCLUSIONS AND RECOMMENDATIONS

In closing, Traumatic Brain Injury is a key contributor to the global burden of disease Among the captive and wild populations of kangaroos. but despite promising preclinical trials, to date, no effective treatments exist. This reflects the heterogeneity of Traumatic Brain Injury pathophysiology and presentation, such as the presence of infection. *T. gondii* chronically infects approximately one-fifth of the world's captive kangaroo population in zoos, which equates to a significant proportion of individuals who sustain a Traumatic Brain Injury being chronically infected with *T. gondii* at the time of insult. As there is a myriad of neuroinflammatory processes that are common to both conditions, exacerbated neuroinflammation, amplified functional deficits, and accelerated neurodegenerative processes may occur in Traumatic Brain Injury kangaroos who are confirmed with chronic *T. gondii* infection. The interplay between *T. gondii* and Traumatic Brain Injury however remains speculative and as such, further investigations should be conducted to assist Traumatic Brain Injury treatment development and future clinical practice. There are several existing drug candidates that could target overlapping mechanisms in *T. gondii* and TBI to minimize secondary injury. For example, the NLRP3 inhibitor MCC950, which has been demonstrated in several preclinical TBI studies to decrease neuroinflammation and improve cognitive and motor deficits, may prove efficacious when the conditions

appear concomitantly (Zhao and Hu, 2021), MCC950 has additionally been demonstrated to decrease IL-1β secretion upon monocyte infection with *T. gondii* (Weiss et al., 2019), however, there is an absence of research pertaining to this inhibitor in *in vivo* chronic infection models. The IL-1 receptor antagonist, Anakinra, is another potential drug intervention in this context, and has already demonstrated safety and tolerability in marsupial TBI trials (Zhang et al., 2014). Experimental studies have reported that Anakinra is beneficial in wallabies with a combined TBI and tibial fracture (Reinink et al., 2021). This injury combination results in exacerbated neuroinflammation compared to an isolated TBI (Wickwire et al., 2018), and may bear similarities to what would occur in a combined TBI and *T. gondii* setting.

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NOVELTY STATEMENT

This review has highlighted numerous areas of pathological overlap between T. gondii and TBI, which in theory could exacerbate the functional consequences of these conditions.In this paper it was shown for the first time that TBI can increase the proportion of activated microglia and neuroinflammation chronically after post-injury . T. gondii would therefore be met upon migration to the brain parenchyma with a more robust neuroinflammatory response, potentially leading to excessive cell death. Moreover, if immunosuppression were to result from a TBI , and an individual were to be later infected with T. gondii, uncontrolled proliferation of tachyzoites may occur in enterocytes and once migrated into the brain parenchyma, exacerbated activation of apoptotic pathways may occur due to cell stress via tachyzoite proliferation, and necrotic tissue may result.

AUTHOR'S CONTRİBUTİON

PM performed the practical procedure and analyzed histopathological lesions. PM and BN wrote the draft of the manuscript. PM and AR revised the draft of the manuscript and removed language errors. PM and SM checked and approved the final version of the article for publication in the present journal.

Availability of data and materials

All data and related findings of the present study are prepared for publishing in the present journal.

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Further considerations

The manuscript has been spell-checked and grammar checked by a professional language editor.

Ethical approval

The work described in this manuscript involved the use of non-experimental (owned) animals.

Established internationally recognised high standards('best practice') of veterinary clinical care for the individual patient were always followed and this work involved the use of cadavers. Ethical approval from a committee was therefore not specifically required for publication in AAVS.

Although not required, where ethicalapproval was still obtained it is stated in the manuscript.

Informed consent

Informed consent (written) was obtained from the legal custodian of animal and it cadaver described in this work for all procedures undertaken(prospective or retrospective studies).

Conflict of interest

The authors have declared no conflict of interest.

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