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The Equine Necropsy a Update

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Abstract

Necropsy can be defined as a systematic study of an animal's corpse, in this case the horse, is a diagnostic procedure in order to determine the cause of death. The aim of this study was to review an update of necropsy in horses. Necropsy in horses is very important for forensic medicine (in case of sudden death in the breed), population medicine (in the case of some diseases that affect the horse population, for example, the Equine Influenza virus, Equine Herpesvirus 1/4), public health (very important equine encephalitis, such as VEE, West Nile virus and rabies), research (in the case of catastrophic musculoskeletal injuries, in the case of study of some drugs for neoplasms in the equine melanoma model), legal necropsy (in case of animal abuse and very frequent use in recent years, as well as recent legal cases of drugs prohibited in horse racing) and horse insurance. The regular performance of the post-mortem necropsy exam will greatly increase the likelihood of accurate diagnoses and often provide a more complete understanding of cases. This article analyzes the most relevant and updated aspects of necropsy in horses, including some specific protocols in case of musculoskeletal injuries, sudden death and pathologies of the central nervous system, etiological agents and sampling. In addition, the horse's autopsy in the field. In conclusion, equine necropsy represents a post-mortem diagnostic tool in the horse, which can be performed by an equine veterinarian in the field, including sample collection and/or by an accredited veterinary pathologist in an official laboratory of the institution.

Keywords: *Equine; Horses; Necropsy*

Introduction

Necropsy can be defined as a systematic study of an animal cadaver, in this case the horse, it is a diagnostic procedure with the objective of determining the cause of death of the horse. A necropsy is an autopsy performed on an animal [9]. Simply put, a necropsy or post-mortem examination (PME) gives an explanation for the horse´s death, It is often important to investigate a horse's death to identify the reason or reasons [7] and establish measures for the prevention of mortality risk factors. The necropsy in horses is very important by forensic medicine (in case of sudden death in race), population medicine (in the case of some diseases that affect horse populations, for example the equine Influenza virus, equine Herpesvirus 1/4, public health (very important equine encephalitis such as VEE, West Nile virus and Rabies), research (in case of catastrophic musculoskeletal injuries, for example in relation to the recent cases observed in Santa Anita Park or in case of study of some drugs for neoplasms in the equine melanoma model), legal necropsy (in case of animal abuse and very frequent in recent years, as well as recent legal cases of prohibited medication in horse racing), and horses insurance, is a market that has

grown in recent years given the costs and market that demands trained pathologists with experience in equines. A recently online survey with 33 participants (equine veterinarians 70% and students 30%) available at https://docs.google.com/forms/d/1QfVSlLZl_RC4SMgO-0SU4NbYwXFViM7chvedmauXI0U/edit#responses in March 2020 from 15 countries (Bahrain, Brazil, Chile, India, Indonesia, Iran, Italy, Jordan, Nicaragua, Pakistan, Palestine, Russia, Spain, United State of America and Venezuela). 49% of the participants answered that they have experience in horse necropsies while 51% have no experience in horse necropsies. One of the questions in the survey you motivate yourself to include in your vet services the equine necropsy service 94% affirmed that yes, 6% answered that they were not interested in a horse necropsy service. Being able to conduct an equine field necropsy in a safe and proficient manner is a helpful skill for the equine practitioner [6] and necropsy in the field: an added service in rural veterinary medicine [18]. Use of a systematic process enables the practitioner to develop a familiarity with normal anatomic positioning and tissue appearance such that abnormalities are quickly identified [6], additionally, they are usually useful in the veterinarian-trainer-owner relationship to quickly identify the cause of death of a horse at a given time. This interesting information about the need for training pathologists in the area of equine veterinary pathology and for practicing veterinarians in equines who can offer a field necropsy service in some countries. The aspects required to develop this service comprise: basic training of the rural veterinarian or field in terms of identification and description macroscopic lesions (macropathology), in order of being able to relate it to the clinical presentation of the disease [18]. Therefore, the following is necessary: 1. Continuous training for veterinarians already licensed in Comparative Applied Veterinary Pathology, Applied Veterinary Pathology, Supply Macropathology or Veterinary Pathology of the Reproduction, all of them offered subjects in some postgraduate courses (University of Córdoba, Central University of Venezuela), in which the author participate as Professor-Instructor. Other options are continuing education of the American Colleges and Europeans of Veterinary Pathology (ACVP: American College of Veterinary Pathology, ECVP: European College of Veterinary Pathology) [18]. 2. Diagnostic laboratories: it is essential to know the reference laboratories for sending field samples, both official and private, and the requirements for sending such samples. In some cases it is necessary to send samples to international laboratories; therefore, this aspect should be considered. It is necessary to manage and include information on the cost of processing the samples [18]. 3. Training and education of the client (livestock producer): this aspect is essential for the producer understands the need to carry out the necropsy, the costs that this service represents in the field and the analysis of samples in the laboratories, as well as its impact on the farm in terms of prophylactic measures, in addition to the control and prevention of diseases [18]. Recently during the SARS-CoV-2 pandemic, the Davis Thompson Foundation http://www.cldavis.org/ has developed a weekly program of specialized training courses in the area of comparative veterinary pathology that includes horses "Equine Diseases" 12 May 2020, with approved continuing education (CE) credits using the delivery method of seminar/lecture in jurisdictions which recognize AAVSB's RACE approval. In Brazil the Associação Brasileira de Patologia Veterinária-ABPV, develop a Virtual VideoClass Support Program https://abpv.vet.br/programa-de-apoio-virtual-proav/"Necropsy in Equine" dictated by the author of this article. Necropsy and sampling gives greater confidence to the breeder, trainer in terms of identification of the mortality problem in the stable and the taking of awareness of prevention measures. American Association of Equine Practitioners (AEEP) in 2009 [20] recommend that all horses that die or are euthanized at a licensed track or training facility undergo a complete necropsy as it is for clinician doing his own necropsies. Sometimes it is not available and to some pathologist It is important to do a necropsy, without being prejudiced by an erroneous, misleading or incomplete history. Necropsy findings should be entered into The Jockey Club Equine Injury Database. For appendicular injuries, the affected limb at the site of the injury should undergo gross dissection (+/- diagnostic imaging, toxicology, histopathology) and appropriate documentation of findings (written description and photography) [20]. National Regulation of Horse Racing in Venezuela in the article 306: establishes a necropsy to be carried out on each of the deceased horses by an official veterinary pathologist accredited in the Venezuelans National Institute of Racetracks. (http://www.coproca.org/FINAL/RNC.pdf). The necropsy report should include identification of the affected anatomical structure(s) including a description of gross lesions found in bones, joints, ligaments, tendons, skin and blood vessels [1]. For non-appendicular conditions, reasonable effort should be made to determine and document the cause of death. For sudden death occurring during or immediately after a race, the cardiovascular and respiratory systems warrant as comprehensive an examination as is possible. For race-related fatalities, a 'best practice' inquest protocol is recommended that incorporates ante-mortem information (examples include: interviews with personnel relevant to the horse and/or the incident, exercise history, race replay video, medical history) and post-mortem findings. Ante- or immediately

post-mortem blood samples (and urine, when available) should be collected, maintained under chain of custody protocols, and submitted to the official racing laboratory. In the last 10 years veterinary pathologists in the equine area have developed some specific necropsy protocols and sampling for some specific pathologies presented by the horse [20], should always consider the chain of custody and all the legal aspects that can lead to a lawsuit for the collected samples. The aim of this study was a review an update of necropsy in horses.

The equine necropsy: Regular performance of post-mortem necropsy examination will greatly increase the likelihood of accurate diagnoses and often provides a more thorough case understanding. Necropsy findings can also be helpful for elucidating the severity of disease to owners who need further validation of their decision to euthanize [21]. There are multiple procedures involved in a necropsy [7]. The autopsy technique will vary depending on personal and institutional preference but, in all instances, a systematic examination of all visceral organs, including the brain, must be performed [4]. For a better understanding, we will describe the systematic necropsy in the horse step for step.

History: (Anamnesis): medical data, convalescence time, therapeutics (treatment) and evolution. Presumptive clinical diagnosis. Productive history of the farm (Reproductive data, abortions, perinatal death), feeding, management aspects. Population, vaccine and deworming plan, quarantine and environmental and other relevant health aspects. Never perform an necropsy without a medical history is a risk for health, many diseases have zoonotic potential.

Position: All animals are placed on their left side with the dissector facing the animal's abdomen in horse: Right lateral decubitus (colic) [7,8], is recommended for anatomical observation during the necropsy and sampling process in horses. Orientation of necropsy depends on clinical history and taking samples.

External examination: Following external examination, including natural orifices, eyes, and limb and joint palpation, the lymph nodes, nerves, and most vessels are examined when exposed [9], is important to detail wounds, traumatic wounds and surgical wounds. Bloody nasal discharge (epistaxis), clear nasal and/or oral fluid or froth discharge, rectal or vaginal prolapse, pseudoicterus of horses, injection sites is very important in horse racing.

The time of death: Post-mortem changes are irreversible in a corpse and in many cases can limit necropsy and sampling.

Rigor mortis: The stiffening after death, is best related to the body temperature and the metabolic activity at the time of death [9]. In cases of death occurs during a high fever disease, rigor can occur almost simultaneously with death. Rigor mortis also occurs rapidly in animals that are excited or severely stressed just before death, occurs frequently in horses that present sudden death during training or competitions. The opposite is true for animals that are moribund or cachectic for a long period before death for example in cases of Equine Infectious Anemia and in cases of chronic laminitis; rigor may not occur for hours, if it occurs at all, and when it does, it may not be easily noticeable [9].

Algor mortis: The corpse takes the environment temperature, if we are in a northern country the temperature decreases and if we are in a country in the Middle East the temperature will increase. *Autolysis*: is produced by the activation of lysosomal enzymes at the cellular cytoplasm level, and it gives a purple or green appearance to the tissues. Autolysis is a limitation for the taking of samples.

Livor mortis: It is due to the effect of gravity, the blood is concentrated in the most ventral regions of the corpse.

Tympanization: Corresponds to the exacerbated production of gas by the microbial flora in the cecum and colon of the horse. The environment (temperature and humidity), for example in the Middle East and Siberia, can influence post-mortem changes by accelerating or retarding, as well as some conditions such as sudden death during exercise and endotoxemia.

Skinned: Separation of the skin from the subcutaneous tissue. After an initial stab incision into the right axilla, extend skin incision cranially, just to right of midline, to chin and caudally to perineum, just above the genitalia, this incision is made only at the skin level. Reflect skin on right side and completely abduct right limbs by cutting muscular attachments of scapula and freeing femoral head, reflect mamma and testicle separately [9]. The skin and subcutaneous tissue are indicators of a systemic disease such as endotoxic shock.

Separation of limbs: Scapula (Sinsarcosis type joint) and freeing femoral head *(*Round Ligament).

Five joints are routinely checked, in the order given, in the necropsy of horses [9], consider a greater emphasis on catastrophic musculoskeletal injuries in competition horses. These are representative and easily accessible during equine necropsy.

- Right hip.
- Right and left stifle.
- Right shoulder.
- Atlanto-occipital.
- Right and left hock in young animals.

The recommendation is to check all the synovial joints to rule out pre-existing injuries. In a diagnostic pathologist's guide to carpal disease in racehorses [5]: the equine carpus incorporates 3 joints from proximal to distal: the antebrachiocarpal (radiocarpal, middle carpal, and carpometacarpal joints. Before or during the necropsy we can use diagnostic tools such as X-rays, computed tomography and magnetic resonance imaging. Carpal bones comprise 7 - 9 cuboidal bones that include, from medial to lateral: the radial (Cr), intermediate (Ci), ulnar (Cu), and accessory (Ca) carpal bones forming the proximal row of carpal bones; and the second (C2), third (C3), and fourth (C4) carpal bones forming the distal row, with the first carpal bone reported in 25 - 50% of horses, and the fifth carpal bone rarely present (1 - 3%) [5]. C3 is the largest of the 3 bones in the distal row and is "L" shaped with 3 distinct regions: the palmar portion that is rarely injured, and the dorsal portion that is divided by a distinct sagittal ridge into radial (medial) and intermediate (lateral) facets, which are frequently injured in racehorses [5]. Despite their relatively small size, inspection of these intercarpal ligaments is imperative because lesions, including avulsion fractures, and degeneration or partial to complete ligamentous tears, are reported as significant sources of carpal lameness and instability, and are often concurrent with carpal osteochondral "chip" and bi-articular "slab" fractures. In our experience, the CMC joint is difficult to disarticulate for gross examination, and unless indicated in the history, is not often disarticulated during routine postmortem examination [5], consider arthrocentesis points and septic arthritis in some cases. In young foals (i.e. < 2 mo of age), cuboidal bones of the carpus are incompletely ossified and thus are inherently susceptible to injury [5]. Although the degree of ossification at birth varies among foals, neonatal carpal bones typically have a rounded appearance with thick radiolucent spaces that represent hyaline cartilage surrounding ossification centers of the carpal bones [5]. Ossification is often incomplete, is best seen on a dorsopalmar radiograph, and can be difficult to discern grossly or histologically in demineralized specimens. In addition to leading to disrupting growth plates or increasing loads on joints, incomplete ossification of the cuboidal bones is one cause of angular limb deformities [5]. Angular limb deformities are common, with one study of neonatal Thoroughbred foals reporting only 2 of 67 (3%) carpi having straight conformation and valgus angular deviations up to 4° considered normal [5], but we have also observed it in other breeds such as the Arab, American Quarter-Horse and especially in donkeys. Osteochondrosis is a well-documented equine developmental bone disease at multiple predisposed sites as a result of endochondral ossification [5]. As mentioned above, osteochondromata are cartilaginous exostoses characterized by a radiolucent hyaline cartilage cap overlying endochondral bone that communicates with the medullary cavity of the parent bone. Septic arthrosynovitis or tenosynovitis of the carpal region can arise from hematogenous spread or introduction of bacteria into the joint or synovial sheath through a penetrating wound or iatrogenic injections and surgical procedures [5]. Most common isolates comprised

gram-positive bacteria (e.g. nonhemolytic and hemolytic *Staphylococcus* spp., beta-hemolytic and non-beta-hemolytic *Streptococcus* spp.) and gram-negative bacteria (e.g. *Enterobacteriaceae* family, *Escherichia coli*, and *Pseudomonas* spp.) with no bacteria isolated in 27% of cultures [5]. Typical postmortem changes to intrasynovial structures include diffuse synovial hyperplasia and hyperemia, often with mats or strands of fibrin packed into synovial recesses, adhering to, or spanning the cartilage and synovial membranes [5]. Articular cartilage also shows focal-to-diffuse yellowing and thinning, representing degeneration and atrophy, with ulcers and osteomyelitis extending into the subchondral bone. A collaborative initiative developed a standardized grading scheme, based on modified Mankin and OARSI (Osteoarthritis Research Society International) scoring systems, for characterization of macroscopic and microscopic lesions associated with experimental or naturally occurring osteoarthritis in horses [5]. Recently schemes were designed to assess all components of the joint, including lesions identified in the synovium (inflammatory infiltrates; hyperplasia; edema; and fibrosis), cartilage (superficial, partial to full-thickness erosions; fibrillation and fissures; chondrocyte necrosis and loss; chondrone formation; and changes to the extracellular matrix), and subchondral bone (splitting of the osteochondral matrix; subchondral bone disruption and collapse; and subchondral bone remodeling) [5]. In conjunction with degenerative osteoarthritis, osteochondral fragmentation (i.e. fracture) of the equine carpus results from naturally occurring, repetitive impact trauma associated with racing and training [5].

Opening of cavities: Remove the ribs by cutting with rib cutters or saw, first close to the sternum, then several inches from the vertebrae and check the presence and position of all organs [9], the cut must avoid directly affecting the abdominal viscera. In foals you can do it dorsal decubitus. Arrange the GI tract to display all of the parts before removal, in the horse, place cecum dorsocranially, small colon on left thigh, large colon cranially, and small intestine over right flank [9]. Examine but leave the pancreas attached to the duodenum or the root of mesentery, always requires inspection in cases of equine metabolic syndrome, as well as some neoplasms. Before any visceral organs are removed from the peritoneal cavity, look for abnormal vessels (shunts) to or from the liver or intestine and especially leading to the caudal vena cava [9], can be seen in foals with congenital abnormalities and check the presence and position of all organs in the abdominal cavity. The small intestine is pulled ventrally towards the prosector while cutting the mesenteric attachments close to the bowel and properly inspecting the gut as it is cut free [9], at this point you can perform the sampling, also identify gastrointestinal accidents. The large bowel of the horse is removed by pulling it further over the back while using blunt finger dissection to release it from its dorsal attachments and by finger stripping the major mesenteric vessels free while doing so, at this point you can perform the sample collection, also identify gastrointestinal accidents and neoplasms such as lipomas and liposarcomas, also intestinal adhesions. Always consider lay GI tract in relative order esophagus, stomach, small and large intestine, to be opened later as the last major procedure of the necropsy to prevent fecal contamination of the tissues and instruments [9]. To check the GI tract, cut along the greater curvature of stomach, for stomach, and representative lengths of duodenum, jejunum, and ileum for review, open ileo-cecal orifice and cecum, large and small colon, and rectum, incise major vessels when exposed [9], can use the enterotome.

Equine gastric ulcer syndrome: Gastric lesions (inflammatory, erosive, and ulcerative) can be classified according to their evolution over time: acute gastritis, chronic gastritis; according to the severity of the chronic erosive gastritis lesion, chronic ulcerative gastritis. In 2003 Merritt [11] established a specific gastroendoscopic classification system for the evaluation of the gastric mucosa in the horse, which is described below: Grade 0: Intact epithelium, without signs of hyperemia, hyperkeratosis, Grade 1: Mucosa intact but with areas of hyperemia and/or hyperkeratosis, Grade 2: Small multifocal erosions or ulcers, Grade 3: Large multifocal ulcers, with extensive regions of erosion, Grade 4: Extensive ulcers with exposure areas of the submucosa. The European College of Equine Internal Medicine Consensus Statement-Equine Gastric Ulcer Syndrome in Adult Horses [22], the committee recognizes that the terminology for EGUS requires clarification and proposes that the nomenclature be: Equine Gastric Ulcer Syndrome (EGUS) as a general all encompassing term to describe erosive and ulcerative diseases of the stomach consistent with the use of the term PUD in man; Equine Squamous Gastric Disease (ESGD) and Equine Glandular Gastric Disease (EGGD) as terms that more specifically describe the affected region anatomically. Within ESGD, both primary and secondary disease is recognized. Primary ESGD, the more common of the 2 forms, occurs in animals with an otherwise normal gastrointestinal tract. In contrast, secondary ESGD occurs in animals with delayed gastric outflow secondary to an underlying

abnormality such as pyloric stenosis [22]. The pathophysiology of EGGD remains to be elucidated and as such further subclassification of lesion type is not possible at this time [22]. Instead, the committee recommends the use of descriptive terminology with a clear distinction of the anatomical region affected (cardia, fundus, antrum, or pylorus and the gross appearance of the lesion as well as taking samples from each of the stomach regions. The committee emphasizes that the affected region of the stomach should be clearly identified when communicating research and clinical findings. European College of Equine Internal Medicine Consensus Statement-Equine Gastric Ulcer Syndrome in Adult Horses [22]. However, in our experience, in some cases, the extension and depth of ulcers can affect the squamous region on the margo plicatus border confluent with the glandular region, it is difficult to apply this classification by affected area as we describe in some of our previous articles: Morales A, Bermudez V, De Vera M, Contreras M, Garcia M, Gueneau P. 2006. A multidisciplinary study of gastric ulcers in Thoroughbreds of Venezuela. Vet Pathol. 45:5, 2006.822; Morales A, Perdigón M, García F, Bermúdez V, Leal L. 2009. Síndrome ulceroso gástrico equino del Hipódromo "La Rinconada" Caracas, Venezuela. *Analecta Veterinaria.* 29, 2. 05-07; Morales A, García F, Bermúdez V. 2010. Detection of *Helicobacter* like`s organisms in Thorough bread horses from Venezuela. Brazilian Journal of Veterinary Pathology. Vol. 3 no. 1. 52-55; and Morales A, Méndez A. 2013. Histopathological detection of *Helicobacter* Like Organisms in gastric mucosa of Spanish horses. Intern J Appl Res Vet Med, 11:3. This classification has currently been complemented by our research group since 2005, with a histopathological study using gastric biopsy or in postmortem evaluation using the necropsy technique, as well as molecular for the detection of bacteria of the *Helicobacter* genus and its impact on gastric mucosa. The histological patterns are: Grade 0: Intact epithelium, without signs of hyperemia, hyperkeratosis. histopathology: gastric mucosa without apparent lesions; Grade 1: Intact mucosa but with areas of hyperemia and/or hyperkeratosis, histopathology: hyperemia, congestion, orthokeratotic hyperkeratosis, neutrophilic inflammatory infiltrate; Grade 2: Small multifocal erosions or ulcers, histopathology: focal erosion of the mucosa, mixed neutrophilic and lymphocytic infiltrate; Grade 3: Large multifocal ulcers, with extensive regions of erosion, histopathology: exposure of the submucosa (ulcers), lymphocytic infiltrate; Grade 4: Extensive ulcers with exposure areas of the submucosa, histopathology: exposure of the submucosa (ulcers), lymphocytic infiltrate [15].

Opening of cavities (Peritoneal cavity: liver, kidney, spleen): Remove the liver, leaving the diaphragm in place. observe the color and characterize the consistency and texture, make multiple inspection slices into the liver and incise major vessels, we have observed a high incidence of hepatic telangiectasia associated with medication for long periods of anabolic. Remove the spleen, make several inspection slices into spleen. Cut each kidney longitudinally to pelvis, remove kidney capsule, leave each kidney attached to the bladder to allow the ureter to act as a third hand when handling the small slippery tissue, consider papillary necrosis associated with NSAIDs in the horse. Incise the adrenal (9), do not hold the adrenal itself, and note the cortex-medulla-cortex (CMC) ratio, adrenal glands are indicators of death associated with stress in racehorses.

Opening of cavities (Pelvic cavity): Open the pelvic cavity by sawing (or using rib cutters) through the pubis to the obturator foramen, then through the ischium, both sides and remove the symphysis [9], some suggest a single cut through both the symphysis and ilial shaft. Cutting close to the bone, remove the pelvic viscera, kidneys with ureters, and bladder, genitalia, and rectum, some tumors may occur in this anatomical region. Check the umbilical arteries that lie alongside the bladder and the omphalo-mesenteric veins from the umbilicus to the liver in newborn animals [9]. Some catastrophic lesions of the base of the pelvis with rupture of the obturator artery in race.

Opening of cavities (Oral, tongue, cervical and thoracic viscera): To remove the tongue, cervical and thoracic viscera in masse, cut on the medial side of both mandibles close to the bone [9]. The symphysis may be split, if necessary, for easier removal, especially in the horse. Free the tongue manually (this is difficult in the horse), pull the tongue down and back and cut through the prominent (keratoepihyoid) joint of the hyoid bones on both sides [9]. Continue traction, removing the trachea, esophagus and other soft tissues down the neck, identify the key parathyroid gland in the diagnosis of developmental bone diseases trauma to the teeth, infection, calcium deficiency, deficiencies of vitamins A, C, or D and nutritional secondary hyperparathyroidism. Observe and incise the thyroids and parathyroids. Examine the jugular veins, very is important for being an intravenous injection point in race horses. Cut down the full length of the esophagus, this

step is important in cases of partial obstruction of the esophagus, free the esophagus and the aorta from the dorsal caudal mediastinum to allow access to both bronchi [9].

Opening of cavities (Thoracic cavity): Leave the heart attached to the lung for best evaluation of the vessels involved and leave the lungs attached to the diaphragm to act as another third hand in pulling the esophagus and trachea tight when cutting down each of them, and down the bronchi [9]. Palpate the lungs gently, can identify at this point chronic obstructive disease, chronic pulmonary fibrosis, foci of pneumonia and neoplasms, including areas of metastasis, cut down the trachea and major bronchi and observe the cut ends of the pulmonary arteries for emboli and incise the tracheobronchial lymph nodes [9]. To cut down and examine the pulmonary arteries, it is best to turn the lungs over and cut from their ventral surfaces, in special cases of sudden death in training and running, an exhaustive examination of the thoracic cavity, lungs and heart should be carried out. In recent years we have worked on some pathological injury patterns in EIPH. Sampling is key in the diagnosis of EIPH, a protocol has been established for taking samples from specific areas of the lung. Equine lung 12 sites sampled from EIPH horses, representing left (L), right (R), dorsal (D), and ventral (V) lung fields, are LV1: Left Ventral Lobe (Craneal), LV2: Left Ventral Lobe (Medium), LV3: Left Ventral Lobe (Caudal), LDL1: Left Dorsal Lobe (Craneal), LDL2: Left Dorsal Lobe (Medium), LDL3: Left Dorsal Lobe (Caudal), RVL1: Right Ventral Lobe (Craneal), RVL2: Right Ventral Lobe (Medium), RVL3: Right Ventral Lobe (Caudal), RDL1: Right Dorsal Lobe (Craneal), RDL2: Right Dorsal Lobe (Medium), and RDL3: Right Dorsal Lobe (Caudal) [10,14,16,17,19,23]. This sampling of each lung region is accompanied by a histological study, from grade 0 or normal to severe grade 3. Necropsy results in some cases were severe massive hemothorax, foolwong rupture of the segmental bronchial arteries, with associated edema, pulmonary congestion and hemorrhage petechial to equimotic in many cases confluent [10,14,16,19] and subserosal petechial hemorrhages were observed in the dorso-caudal lung lobes associated with EIPH. These histopathological lesions generally showed severe congestion, marked interstitial edema, and acute pulmonary hemorrhage due to rupture of focal bronchial arterioles, including red blood spilled, is possible to observe areas of pulmonary emphysema and EIPH [14,16,19], these findings represent the diagnostic key in cases of sudden death in racehorses. Toxicology testing in cases of SD is challenging because, in the majority of cases, there is no history of specific toxicant exposure prior to death [4]. The toxicological study in racehorses is expanded every year due to the high impact of environmental exposure through pasture contaminated, including natural plants in some regions observed in international competitions according to the reports of the FEI (Federation Equestrian International) and specialized toxicological laboratories in the world. In some cases, specific testing might be directed by anecdotal history of use of specific substances, presence of postmortem lesions compatible with specific chemical exposures, or an a priori hypothesis regarding the role of a particular chemical in causing SD, in such cases, the samples analyzed and the analytical methodology employed is driven by the kinetics or chemical properties of the toxicant of interest [4]. In cases of sudden death, is recommended to take blood and urine samples for toxicological studies immediately after death of the animal, we can take 60ml of blood, 120ml of urine, 50 grams of tissue. Keep refrigerated sample (ice/ice pack), not frozen, is very important to maintain the chain of custody of the sample for medically legal aspects.

Opening of cavities (Thoracic cavity): The approach to the gross examination and dissection of the heart in animals varies according to personal or institutional preference and the literature consulted [4]. Regardless of the heart dissection method chosen, all of the major components of the heart, including the pericardium, myocardium, mural and valvular endocardium, great vessels, right and left coronary arteries, and the regions where the main components of the conduction system are located must be carefully examined for gross abnormalities (e.g. changes in color, hemorrhage, fibrosis, valvular thickening, narrowing of coronary arteries, etc.), even when, in our experience, the vast majority of hearts from racehorses are grossly normal [4]. The heart should be weighed after examination [9], but before sections are taken of tissue. To evaluate the heart is necessary with a hand grasping the base of the heart, cut the pericardium and major vessels, the pulmonary artery, and aorta as they extend through the pericardium observe carefully [9]. All blood should be removed before weighing. To open the right ventricle, hold the heart in your left hand, with the left side of heart towards you. Make the incision, starting at the pulmonary trunk, into the right ventricle, close to the interventricular septum. Open the pulmonary trunk past bifurcation, check the semilunar valves observe carefully. Turn the heart over with its right side towards to prosector or pathologist, continue the incision,

following the interventricular septum, into the right atrium. Open the right ventricle and atrium, check the right atrioventricular valve, the orifices of the cranial vena cava, the caudal vena cava, the fossa ovalis, and the coronary sinus observe carefully. We have observed some cases of left ventricular hypertrophy associated with EIPH and sudden death. Open the left atrium and ventricle with a straight incision, incise through the parietal cusp of the left atrioventricular valve [9]. Check the left atrioventricular valve and openings to the pulmonary veins observe carefully. To open the aorta, insert the knife under the septal cusp of the left atrioventricular valve [17], noise through the wall of the atrium, out and down the aorta [17,19]. Check observe carefully the semilunar valves of the aorta, orifices, and right and left coronary arteries, orifice of the brachiocephalic trunk [17], observe carefully in obese and old horses it is possible to observe atheromatous changes (atheroma plaques) and aneurysms. Open the abdominal aorta and its major branches (mesenteries, iliacs, etc.) [9], consider equine verminous arteritis associated with parasite migrations in the horse, especially *Strongylus vulgaris*. Diab., *et al*. in 2017 [4] describe the key areas for heart sampling in cases of sudden death in horses, the histologic sampling protocol of the heart is composed of 11 routine samples plus any areas with gross abnormalities. H1- Right ventricular free wall. H2- Pulmonary artery semilunar valve. H3- Right atrial appendage. H4- Sinoatrial node region. H5- Left atrial appendage. H6- Left ventricular free wall. H7- Left ventricular papillary muscle 2 area. H9- Atrioventricular node region. H10- Interventricular septum. H11- Aortic semilunar valve, aorta [4].

Opening of cavities (Brain, CNS): Move the head to locate the atlanto-occipital joint, obtain CSF at this time, if required, from a dorsal or ventral approach [9]. Cut all of the soft tissues around the joint, insert the knife into the joint and transect the spinal cord and ligaments of the joint dorsally and ventrally, do not direct knife into brain proper. Remove the head in this point. To the right is a diagram showing the location of the brain in a dorsal view of the skull, dotted lines represent the lines of incision, remove the major muscle masses from inside the area of dotted lines [9]. Look into the foramen magnum to note the normal absence of the cerebellar vermis. One cut is transverse through the frontal bone, caudal to the zygomatic process of frontal bone. Place the head on its right side, another cut is sagittal, just medial to left occipital condyle. Place the head on its left side for one cut [9] and cut the olfactory peduncles, internal carotid arteries, and cranial nerves as the brain is removed, the trigeminal ganglion must be collected for the diagnosis of Herpesvirus type 1/4. In some cases if the brain is not to be kept, cut 1 cm transverse sections for inspection can be carefully evaluated fresh. The routine procedure of SNC is to make 7 transversal cuts: caudate n. (gl. Pallidus putamen), parietal cortex, hippocampus thalamus, cerebellum, mesencephalon, post. Colliculus and obex. To remove the pituitary gland: Pick up the dura from the basilar part of the occipital bone between the sawn condyles, pituitary adenomas are common in old horses from the age of 14, these tumors present the effect of mass occupying space producing neurological signs such as blindness, headache and ataxia, peel it forward to include the pituitary. Remember anatomical characteristics of the horse for the description of injuries.

Neurological diseases: In the horse can be difficult to assess in a field condition, there are several infectious and non-infectious causes of nervous system diseases (Bermudez., *et al*. 2005). In absence of exact diagnosis, it is more appropriate to call all these diseases Equine Neuromotor Syndrome (ENMS). Etiologies include viruses (Equine Herpesvirus Subtype 1/4, Equine Viral Encephalitis Venezuelan, Easter, Western and West Nile Virus), bacteria (*Enterobacteria*, *Streptococcus* spp.), parasites (*Sarcocystis neurona:* EPM, *Trypanosome evansi*), mycoses (*Fusarium moliniformes*, Mycotoxin *Fumonisine* responsible for Equine Leucoencephalomalacia, *Aspergillus* sp., *Phycomicetes* and *Dimorphic fungi*), degenerative diseases (Equine Motor Neurone Disease Syndrome), Immune-mediated/granulomatous (Caudal Equine Neuritis), intoxications (ionophores anticoccidiales, momensine, salinomycine, narazine, lasalosid, nicarbazine, heavy metals, lead, mercury, organophosphates, organ chlorates, insecticides, herbicides and fungicides) [2,3]. In neurological cases, a multidisciplinary study is recommended and the taking of samples is key in the morphological and etiological diagnosis of the cause of death of the horse. A detailed clinical neurological evaluation, hematology, viral serology, (EVR, EEV, EEE, WEE, WNV), parasitology (*Trypasonoma* sp., *Babesia* sp., *Erhlichia* sp.), CSF analysis (sediment, biochemistry, microbiology, EPM and immunology); necropsy (viral isolation from brain, cerebellum and cervical-thoracic-lumbar spinal cord), and general histological study and toxicological from liver, kidney, stomach content feed and pasture (mycotoxins: aflatoxins, fumonisines, ocratoxins, organophosphate, insecticides, fungicides, herbicides, ionophores, heavy metals, peroxides levels in feed), blood, urine and environmental samples (water, food, hay, natural hay, soil samples).

Equine field necropsy: Being able to conduct an equine field necropsy in a safe and proficient manner is a helpful skill for the equine practitioner, use of a systematic process enables the practitioner to develop a familiarity with normal anatomic positioning and tissue appearance such that abnormalities are quickly identified [6], most of the necropsies and sampling performed by practicing veterinarians are in the field, thus requiring systematic expertise. The equine field necropsy: This technique has been described by AAEP [20,21]. The horse is placed in left lateral recumbency and examined for overall body condition, any wounds or external abnormalities should be noted, begin the necropsy by making a curving paracostal incision through the paralumbar fossa and ending at the xyphoid. Enter the abdominal cavity but avoid puncturing any underlying bowel [21], this will allow easy opening of the abdominal and thoracic cavity for in situ evaluation and sampling. This can be particularly difficult when substantial gas distention is present (in most cases of acute abdominal crisis (colic), and careful incision through each separate layer of abdominal musculature can help prevent inadvertent puncture [21]. Extend the incision cranially by coursing between the front limbs and up the ventral neck, carefully avoid incising the jugular vein, ending at the mandibular symphysis, make a second vertical incision behind the shoulder, just caudal to the triceps, starting ventrally, sharply dissect the skin and Latissimus dorsi from the rib cage, and carefully avoid puncturing the diaphragm [21], this will allow us to know the thoracic pressure and rule out pneumothorax associated with traumatic events and/pneumonia and pleuropneumonia. The resulting muscular flap can be reflected dorsally to expose the abdominal cavity and rib cage to examine the cardiopulmonary system observe carefully in-situ, make a stab incision into the diaphragm near the sternum [21]. Suction of air into the incision or cut carefully confirms negative pressure within the thorax, cut away the diaphragm along its attachment to the thoracic wall observe carefully. Using rib cutters, transect each rib at its ventral and dorsal attachment and reflect the ribcage cranially, maintaining muscular attachment cranially will aid in the replacement of the rib cage back into position for later closure [21]. The lungs can be examined in situ or removed from the chest for evaluation detailed. The heart may be removed and opened for inspection of all chambers and valves, observe carefully, disseminated intravascular coagulation and subepicardial petechial hemorrhage are common in colic and septicemia. Dissect through the ventral neck incision to expose the trachea and esophagus, consider the thyroid and parathyroid glands. Reflection of the limb will provide a better view of the cranial thorax and thoracic inlet, but it may also make incision closure and replacement of limbs back into normal anatomic position after necropsy difficult [21]. The entire "pluck" (tongue, larynx, trachea, esophagus, heart, and lungs) may also be removed en masse for complete evaluation in detail and sampling. Gastrointestinal tract, must be carefully evaluated, remembering that the first cause of death in horses is gastrointestinal accidents (colic), evaluate abdominal viscera for abnormalities in location and appearance before removal from the abdomen. To examine locate the pelvic flexure and exteriorize the large colon, laying it alongside the carcass and continue removal of the large colon from the abdomen makes evaluation easier and allows better visualization of the remaining abdominal organs. The entire length of the small intestine and small colon rule out torsion, incarceration, intussusceptions and volvulus, should be examined as well as the liver, spleen, both kidneys, adrenals and urogenital tract. To extract the brain, should consider personal biosecurity there are many potentially zoonotic pathogens such as equine encephalitis, rabies among other pathogens, first remove the skin and muscles from the dorsal skull, use a hack saw to make a transverse cut through the frontal bone just dorsal to the eyes, and then form a triangle by connecting the first cut with points just medial to the occipital condyles [21], lift and remove the section of calvarium to expose the brain. Post-mortem examinations on animals potentially affected by these diseases should be performed by a diagnostic laboratory [21]. The field necropsy: an added service in rural veterinary medicine, in relation to necropsy costs, official and private services oscillate between 80 and 150$, with the animal or corpse sent to the necropsy room of a diagnostic center, in case of necropsies with medico-legal connotations, including insured animals, prices range between 500$ and 2500$ [18], the necropsy service can generate financial income each year for the equine veterinarian. These prices may have a 15 to 20% increase in the field, taking into account travel to the examination site/ranch (distance), and it may also vary according to the number of dead animals, which represents an added service for the field veterinarian/rural veterinarian, which somewhat increases the annual net economic gains for rural veterinary business.

Main features to include for each lesion: location, color, size., shape, consistency, texture, number or extent (%), content, odor, distribution and surface appearance [9].

Morphological descriptions and interpretations of lesions may include the following: Distribution: Organ: unilateral/bilateral, focal/multifocal, locally/extensive; time: peracute, acute, subacute, chronic, chronic active; severity: minimal, moderated, severed; cause: verminous, bacterial, viral, traumatic; type: hemorrhagic, purulent, fibrinous [9].

Collections of samples: Sampling is key to diagnosis and needs support in the auxiliary branches of veterinary pathology (Bacteriology, virology, serology, parasitology, mycology, molecular biology, histopathology, electronic microscopic, toxicology). Tissue samples should be collected from organs of interest and any identified lesions. Samples should be no thicker than 0.5 cm and should be immersed in 10% neutral-buffered formalin (10:1 formalin to tissue ratio) to fix for histological evaluation (Ness, 2009), all sections for fixation should be less than 1/4" Inches (0.5 - 1.5 cm) thick [9]. When taking sections for histological study from paired organs, make the left side pieces longer or larger (not thicker) for easier identification later when being trimmed or described to pathologists, another way to do it is to put the samples in separate containers but it takes more time. As in any technique description, experience is needed to increase proficiency. Always take sections of all tissues with a sharp knife, never with a pair of scissors, for the small and large intestine you can use the enterotome. Routinely take tissue samples of liver, kidney, lung, and all lesions [9] in the collecting container. Brain and heart sections are indicated in a grossly negative necropsy as they may harbor non-grossly visible fatal lesions as well as in cases of sudden death. Certainly, other tissues than those listed are to be examined if lesions are suspected in them clinically. The fixation medium for histological study is Formaldehyde (Formaldehyde) at 10% for 24 - 48 hours and Bouin or Davidson. Once the necropsy was performed, the correct sample was taken and sent to the laboratory and we fulfilled our objective of a morphological and etiological diagnosis: diagnosis of the cause of death, diagnosis of infectious diseases, identification of risk factors, animal health status and establish corrective and preventive measures.

Necropsy report: Must contain the identification of the horse (microchip), clinic history, description of injuries, examinations, samples and laboratory tests, diagnosis and observations. For the detailed description of the injuries, must take into account: size, shape, surface, coloration, consistency and surface. The report of necropsy must contain the presumptive diagnosis and definitive diagnosis, with its comments, and the appreciations framed in the epicrisis of the case. Always remember that the necropsy report has legal connotations. In summary we have a corpse, we review its medical history, and we perform the external examination, opening of cavities, on-site inspection, and collection of samples. Remember step by step to perform a systematic necropsy to continue taking samples. Necropsy is a valuable diagnostic tool that can be performed in the field with relative ease by the field veterinarian with the support of the equine veterinary pathologist. The described method permits a thorough evaluation of the most common disorders in horses while maintaining a relatively intact carcass, making this a desirable procedure for both clinician and owner [21].

Conclusion

In conclusion the equine necropsy represents a post-mortem diagnostic tool in the horse, it can be performed by an equine practicing veterinarian in the field including taking samples and/or by an accredited veterinary pathologist in an official institution laboratory. The results of the necropsy identify the cause of death of the horse, its etiology and the associated risk factors, and establish preventive measures.

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