

The Donkey and Mule Necropsy a Update

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Received: August 07, 2021; **Published:** September 08, 2021

Abstract

Necropsy in donkeys and mules, is a post-mortem pathological diagnostic tool. Necropsy can be defined as a systematic study of an animal cadaver, in this case the donkey or mule, is a diagnostic procedure with the objective of determining the cause of death of the donkey or mule and sampling for etiological diagnosis. There are few reports in the literature of necropsy techniques and pathologies in donkeys and mules. The aim of this study was a review an update of necropsy in donkeys and mules. Regular performance of post-mortem necropsy examination in donkeys and mules will greatly increase the likelihood of accurate diagnoses and often provides a more thorough case understanding. This article reviews the most relevant and up-to-date aspects of necropsy technical in donkeys and mules, including some specific protocols in case of musculoskeletal injuries, sudden death and pathologies of the central nervous system, specials pathologies in donkeys and mule, etiological agents, and sampling, additionally, the necropsy of the donkey and mule in the field. In conclusion the donkey and mule necropsy can be performed by equine veterinarian in the field including taking samples and/or by an accredited veterinary pathologist in an official institution laboratory diagnostic center. The results of the necropsy identify the cause of death of the donkey or mule, its etiology and the associated risk factors, and establish preventive measures for the stable or farm. We propose an international database with official institutions and practicing veterinarians to collect the long-term death data in donkeys and mules and establish future prevention measures..

Keywords: *Equine; Donkey; Mule; Necropsy*

Introduction

Necropsy in donkeys and mules, is a post-mortem pathological diagnostic tool. Necropsy can be defined as a systematic study of an animal cadaver, in this case the donkey or mule, it is a diagnostic procedure with the objective of determining the cause of death of the donkey or mule and sampling for etiological diagnosis. The diagnosis of the cause of death in donkeys and mules has many connotations, the first one determining the cause of death, the diagnosis of an individual pathology, for example an intestinal torsion in case of colic, or a tumor process, as well as a pathology with potential infectious for example Leptospirosis (*Leptospira* sp, *Rodococcus equi*, *Streptococcus equi* subspecies zooepidemicus, infectious-contagious such as rabies virus, Venezuelan Equine Encephalitis, EEO, West Nile virus and even zoonotic diseases. In recent years we have seen news of animal abuse in donkeys and working mules worldwide, despite many efforts by institutions and associations that work for animal welfare, in these cases, the necropsy may be useful for its legal connotations

this situation demands trained veterinary pathologists with experience in donkeys and mules and forensic veterinary medicine. Unlike horses, the necropsy on the donkey and mule is not counted in the Jockey Club Injury Database of each country; the reports are mostly institutional, for example Donkey Sanctuary or some enthusiastic researchers and veterinary pathologists who publish their results every year. A multicentric study, post mortem examinations were performed on 349 donkeys over an 18 month period (between 2005 - 2006), the presence and extent of specified dental disorders was a high prevalence (93%) of disorders was noted in the population with a median age of 31 years. Specially, the dental diseases: cheek teeth diastemata (with 85% of prevalence), often associated with advanced periodontal disease [1]. In the same multicentric study other disorders observed included missing teeth (in 55.6% of donkeys), displaced teeth (43%), worn teeth (34%), local overgrowths (15%), focal sharp overgrowths (3%) and dental-related soft tissue injuries (8%) [1]. A more recently study of gastric ulcers, in total, 426 donkeys were examined at necropsy to determine the presence of gastric ulceration or Equine Gastric Ulcer Syndrome, 41% (n = 174) of the donkeys studied had evidence of gastric ulceration at necropsy, 18% had hyperlipaemia prior to or death or euthanasia and this was a risk factor for donkeys developing gastric ulceration; 62% of hyperlipaemia cases also displayed gastric ulceration ($P < 0.001$), kidney disease was a potential high risk factor ($P = 0.02$), with 74% of these donkeys having gastric ulceration, donkeys that died or were euthanized due to respiratory disease were at a decreased risk of developing ulceration ($P = 0.01$), this recently study has shown that gastric ulceration or Equine Gastric Ulcer Syndrome, is commonly observed in donkeys at necropsy and may be extensive [2], but no report in mules. A full records from 1,444 UK donkeys over a 7-year period were included in the analysis, sixty-one categories of post-mortem finding were identified from 9,744 observations, the four most prevalent conditions noted were dental disorder (78.7%), vascular disease other than aneurysm (60.9%), arthritis (55.4%) and foot disorder (44.8%), gastric ulceration was found in 42% of the donkeys and gastrointestinal impaction in 18.6% [3]. In the same study the most frequent combination of two post-mortem findings in the same donkey was arthritis and dental disorder, the most common disorders were associated with age, body weight and/or body condition post mortem and, for some disorders, gender, for many of the post-mortem findings [3]. In other report were study by necropsy and histopathology 38 donkeys (*Equus africanus asinus*) and 9 mules (*Equus asinus* × *Equus caballus*), in Southern Extremadura (include locations: Arroyo de Molinos, Bodonal de la Sierra, Fregenal de la Sierra, Fuentes de León, Higuera La Real, Jerez de los Caballeros and Segura de León) Spain, during 2012-2016, the death or euthanasia was attributed to disease of the digestive system 43% (20/47) (colic, dental disorder, gastric ulceration, liver and pancreatic diseases); locomotor system 28% (13/47) (laminitis, arthritis, foot disorder and catastrophic injury); respiratory system 6% (3/47) (Epistaxis and Fibrosing pneumonitis); endocrine system diseases 4% (2/47) (adrenal tumor neoplastic and pituitary adenoma); reproductive system 4% (2/47) (ovarian carcinoma and squamous cell carcinoma, germ cell tumor), lymphoid system 4% (2/47) (tumor spleen and thoracic lymphomas), urinary system 2% (1/47) (cyst renal, membrano-proliferative glomerulonephritis and abscess) and nervous system 9% (4/47) (acute poisoning) [4]. A multicentre retrospective study of the causes of mortality in donkeys and mules in Southern Extremadura, Spain and Aragua State Venezuela 2007 - 2017, were study 84 donkeys, 42 male (32 castrate), 29 female, and 13 mules, death or euthanasia was attributed to disease of the digestive system 48% (40/84) (colic, dental disorder, gastric ulceration, liver and pancreatic diseases); locomotor system 23% (20/84) (laminitis, arthritis, foot disorder and fractures); respiratory system 5% (4/84) (Epistaxis and fibrosing pneumonitis); endocrine system 4% (3/84) (adrenal tumor neoplastic and pituitary adenoma); reproductive system 5% (4/84) (ovarian carcinoma and squamous cell carcinoma, germ cell tumor), lymphoid system 6% (5/84) (tumor spleen and thoracic lymphomas), urinary system 2% (2/84) (cyst renal, membrano-proliferative glomerulo-nephritis and abscess) and nervous system 7% (6/84) (acute poisoning and Venezuelan equine encephalitis) [5]. The aim of this study was a review an update of necropsy in donkeys and mules.

The donkey and mule necropsy

Necropsy findings can also be helpful for elucidating the severity of disease to owners who need further validation of their decision to euthanize in donkeys and mules [6]. There are multiple procedures involved in a necropsy [7] in equine. 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 [5], this include the necropsy in the field. For a better understanding, we will describe the systematic necropsy in the donkey and mule step for step.

History: (Anamnesis)

Always should be considered the medical data, convalescence time, therapeutics (treatment) and evolution, presumptive clinical diagnosis and productive history of the farm or stable (reproductive data, abortions, perinatal death). Is very important check the feeding, water, management aspects and population, vaccine (types) and deworming plan, quarantine and environmental. In donkeys and mules subjected to intense work days, they can develop the rhabdomyolysis-myoglobinuria-azotemia syndrome with collapse and death. In other cases they may present Exercise Induced Pulmonary Hemorrhage (EIPH), with bilateral epistaxis which is a characteristic sign of death from over-training or exhausted donkey syndrome. In Almonte Huelva-Spain, a preliminary report, all 36 donkeys were male castrated 10 - 20 years old on average and 22 mules with aged 12 to 25 years average, presented bilateral epistaxis and over-training [9,10]. Experiences in tropical countries in many cases associated with winter and summer climate changes at the end or beginning of each period, the number of vectors increases and generates a higher incidence of vector-borne diseases in donkeys and mules, such as *Trypanosoma evansi* including EEV, EEO, WNV and rabies, we can find donkeys and mules in the field with undifferentiated neuromotor syndrome, ataxia, or dead. Other examples of mortality in grazing donkeys and mules may be associated with electric shock during the winter period. As well as it is necessary to consider during the summer period the donkey mortality associated with dehydration in the field.

Position

Donkey or Mule: Right lateral decubitus (in cases of colic). Orientation of necropsy depends on clinical history and collection of 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 [11]. Bloody nasal discharge (epistaxis) very important in over-training or exhausted donkey syndrome, clear nasal and/or oral fluid or froth discharge, or in cases of infectious diseases, rectal or vaginal prolapsed, injection sites is very important for diagnosis. Melanomas can be observed in mucocutaneous junctions, perineal region and/or in some skin region, some donkey breeds may have a greater predisposition to this type of tumors on donkeys and mules of advanced age. Equine sarcoid can occur in donkeys, in regions close to cattle, cutaneous fibroids have a high incidence and cutaneous fibrosarcomas can be present in cases of chronic cutaneous infestations by *Habronema muscae* in areas of the foreskin, pubic region, face, perineal region and in legs, in many cases complicated with *Pythium insidiosum*, it can even be observed in the stomach associated with chronic parasitic nodular and fungal gastritis.

The time of death

In the external examination we must consider post-mortem changes, it must also consider in cases of forensic veterinary pathology to determine the cause of death and evidence of animal abuse, on the other hand, these post-mortem changes are irreversible and may limit the taking of samples. for microbial studies and histopathology, limiting the diagnosis.

Rigor mortis: In donkeys and mules, the same in others animals, the stiffening after death, is best related to the body temperature and the metabolic activity at the time of death. If death occurs during a high exercises or high fever disease, rigor can occur almost simultaneously with death. Rigor mortis also occurs rapidly in donkeys and mules that are excited or severely stressed just before death. The opposite is true for donkeys and mules that are moribund or cachectic for a long period before death special in animal abuse; rigor may not occur for hours, if it occurs at all, and when it does, it may not be easily noticeable [11]. **Algor mortis:** the corpse of animals 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:** in donkeys and mules is produced by the activation of lysosomal enzymes at the cellular cytoplasm level, and it gives a purple or green appearance to the tissues and is a limitation for the taking of samples in the necropsy. **Livor mortis** is

due to the effect of gravity, the blood is concentrated in the most ventral regions of the corpse is very important as a crime scene in donkey and mule abuse and neglect. *Tympanization* corresponds to the exacerbated production of gas by the microbial flora in the cecum and colon of the donkey and mule. 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 in donkeys and mules.

Skinned

Consists in the separation of the skin from the subcutaneous tissue, the skin and subcutaneous tissue are indicators of a systemic disease such as endotoxic shock in donkeys and mules. Feeding high in beta-carotenes in forage can give a yellowish hue in donkeys but it has no correlation with any pathology. In field necropsies in donkeys and mules, ectoparasites on the skin are common, ticks, which are important as vectors of hemoparasites, especially when they are in direct contact with cows, sheep, goats, including camels, and deer. In donkeys, lipomatosis/steatosis of the subcutaneous tissue is frequent after 5 years, but it is related in some cases to obesity and the crest of the neck, including endocrine pathologies such as Cushing's syndrome, equine metabolic syndrome and hyperlipemia. Deposits of lipids similar to lipomas without malignant characteristics can be observed in the dorsal region of the neck, in the mesentery and even fatty liver in some cases. At low frequency, liposarcomas can be observed in donkeys and mules. 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, reflect mamma and testicle separately, and consider cutaneous wounds and cutaneous habronemosis in this region. Reflect skin on right side and completely abduct right limbs by cutting muscular attachments of scapula and freeing femoral head consider wounds and bruises.

Separation of limbs

This step consists in incise axillary region at scapula and freeing femoral head (*Round Ligament*). Five joints are routinely checked, in the order given, in the necropsy of donkeys and mules [11], but specially third phalanx in cases of laminitis. These are representative and easily accessible during necropsy including field necropsy. 4.1-Right hip. 4.2- Right and left stifle. 4.3- Right shoulder. 4.4- Atlanto-occipital. 4.5- Right and left hock in young animals. The recommendation is to check all the synovial joints to rule out pre-existing injuries in donkeys and mules. Degenerative joint disease or chronic osteoarthritis is a pathology that affects donkeys after 7 years, in necropsies, joint effusions, hemoarthros, degenerative changes of the articular cartilage and loss with exposure of the subchondral bone can be observed. In grazing animals with little management, cases of chronic fractures with bone remodeling and adaptation as an acquired deformation can be observed with chronic lameness, as well as complicated cases with septic arthritis. It is important to mention the very common congenital bone and joint deformations in donkeys and those acquired associated with chronic fractures and foot problems, mainly overgrowth of the hoof. Laminitis has a higher incidence in donkeys than in mules, laminitis is observed associated with cresty neck, obesity, hyperlipidemia, metabolic equine syndrome and Cushing's syndrome. Laminitis can be devastating in many cases due to rotation of the third phalanx, with perforation of the sole of the hoof and osteomyelitis, however in other cases it can behave like chronic laminitis [12], in donkeys this disease represents a great importance.

Opening of cavities

The opening of the cavities brings us closer to the inspection of the organs in-situ, remove the ribs by cutting with rib cutters or saw, first close to the sternum, then several inches from the vertebrae in this point check the presence and position of all organs. In Miniature donkeys and donkey and mule foal you can do it dorsal decubitus. Arrange the GI tract to display all of the parts before removal, in donkeys and mules, carefully place cecum dorsocranially, small colon on left thigh, large colon cranially, and small intestine over right flank [11], and check the presence and position of all organs in situ. The small intestine is pulled ventrally towards the prosector or veterinarian or vet pathologist while cutting the mesenteric attachments close to the bowel and properly inspecting the gut as it is cut free [11]. The large

bowel of the donkey 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. The next step 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 [11]. Carefully to check the GI tract, cut along the greater curvature of stomach, forestomach, and representative lengths of duodenum, jejunum, and ileum, open ileocecal orifice and cecum, large and small colon, and rectum, incise major vessels when exposed consider some tumors such as lipomas and liposarcoma. The Equine Gastric Ulcer Syndrome: are 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. Previous studies suggest a high incidence of gastric ulcers in donkeys [1-3], it is suggested to emphasize the revision of the stomach, as well as consider ulcerative nodular gastritis associated with *Habronema muscae* and consider *Gasterophilus* sp. [13], which shows in the last 10 years of the author's experience a high incidence in Asia (Middle East) and Europe (Spain and Portugal) [14]. A high prevalence (90%) of ulcers and gastritis was found in donkeys during our study, is important to consider that none of the donkeys had previously presented clinical signs of Gastric Ulcer Syndrome, the lesions were necropsy findings [14]. Of the total population (137 donkeys), stomach ulcers and associated gastritis were found in 100% of the samples, antral edema in 56.9%, parasites (*Trichostrongylus axei*) in 10.2%, and impaction in 5.8%. Furthermore, *Hemomelasma gastric* was found in one stomach (0.7%), thus, it evidenced a high incidence of gastric pathologies in donkeys in different livestock holdings in Córdoba, Colombia among which squamous ulcers are the most frequent ones, followed by acute gastritis and antral edema. For this reason, it is necessary to do more research and to raise awareness about the treatment of these pathologies in these donkeys [15].

Opening of cavities (Peritoneal cavity: liver, kidney, spleen)

Once the digestive tract is removed, must continue remove the liver, leaving the diaphragm in place, make multiple inspection slices into the liver and incise major vessels. Carefully remove the spleen, make several inspection slices into spleen, cut each kidney longitudinally to pelvis. Consider in history the euthanasia method of splenomegaly associated with barbiturates and phenothiazines. Donkeys and mules natural infected with the Equine Infectious Anemia Virus (EIA), may show spleen contraction and pale spleen as well as petechial to equimal hemorrhage. Splenic hemangiomas and hemangiosarcomas can be an incidental finding of necropsy in donkeys, in few they are associated with the cause of death. Leave each kidney attached to the bladder to allow the ureter to act as a third hand when handling the small slippery tissue, is common to observe kidney cysts and ovarian cysts in young donkeys but also in old donkeys. Primary ovarian tumors are frequent in old donkeys, we have reported some cases in the literature [16]. The adrenal gland is very important in the diagnosis of stress, intensity of exercise and animal abuse, do not hold the adrenal itself, incise the adrenal [11], note the cortex-medulla-cortex (CMC) ratio, adrenal glands are indicators of death associated with stress in donkeys and mules petechial hemorrhage is key in the diagnosis. Endocrine tumors are common in old donkeys and mules, in mules we have diagnosed cases of pituitary adenoma as well as pheochromocytomas [17]. Additionally, carcinomas of the uterus and kidneys are common, another tumor with a high incidence in mediastinal lymph nodes and thorax is lymphoma with a high degree of local invasion and metastasis, it can also be observed in mesenteric, gastro-splenic lymph nodes and in the spleen [18].

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. Remove the symphysis. 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. Check the umbilical arteries that lie alongside the bladder and the omphalo-mesenteric veins from the umbilicus to the liver in newborn animals [11]. Some catastrophic lesions of the base of the pelvis with rupture of the obturator artery in accidents due to paddock accidents is common in donkeys.

Opening of cavities (Oral, tongue, cervical and thoracic viscera)

We perform to remove the tongue, cervical and thoracic viscera in masse, cut on the medial side of both mandibles close to the bone [11]. The symphysis may be split, if necessary, for easier removal, especially in the horse. Free the tongue manually (this is difficult in the donkeys and mules), pull the tongue down and back, cut through the prominent (keratoepihyoid) joint of the hyoid bones on both sides, continue traction, removing the trachea, esophagus, and other soft tissues down the neck and examine the jugular veins. Pharyngeal Lymphoid Hyperplasia: this is a common finding in young and adult donkeys and recognized by the irregularity of the pharyngeal mucosa, caused by numerous 2 - 3 mm, smooth nodules on the caudal dorsal surface of the tongue and the pharynx around the epiglottis, these are increased in periods of environmental stress in donkeys. Observe and incise the thyroids and parathyroids is key in the diagnosis of secondary nutritional hyperparathyroidism common pathology in equine the Middle East (Kuwait, Oman, Qatar and UAE), as well as in some regions of South America such as Brazil, Colombia and Los Llanos (Apure, Barinas, Guárico and Portuguesa) Venezuela, additionally, mineral imbalance loss of the calcium-phosphorous ratio, magnesium deficiency among others that can be observed in desert soils. Exostosis of bone, maxillary, frontal, nasal, limb deformities, bone fragility, spontaneous fractures, weight loss and lameness can be seen in this point. Cut down the full length of the esophagus, free the esophagus and the aorta from the dorsal caudal mediastinum to allow access to both bronchi. 5.5.-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 [11]. Tracheal froth: certainly this may be a lesion in some cases, but marked tracheal froth production, even with froth flowing from the nostrils, may be seen in euthanized animals that don't have a history of dyspnea or respiratory distress, is common to observe in African Horse Sickness virus (AHS), as well as gastric dilatation and rupture. Palpate the lungs gently, cut down the trachea and major bronchi and observe the cut ends of the pulmonary arteries for emboli. Incise the tracheobronchial lymph nodes, to cut down and examine the pulmonary arteries, it is best to turn the lungs over and cut from their ventral surfaces. In cases of sudden death in training and work, 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 in horses. Sampling is key in the diagnosis of EIPH, a protocol has been established for taking samples from specific areas of the lung in equine. This protocol can be used in donkeys and mules with a history of sudden death. Equine lung 12 sites sampled from EIPH, 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) [19-23]. This sampling of each lung region is accompanied by a histological study, from grade 0 or normal to severe grade 3. Previous experiences in cases of sudden death in donkeys and mules during work or in long horseback riding in Spain, Portugal and Venezuela, necropsy results in some cases were severe massive hemothorax, foalwong rupture of the segmental bronchial arteries, with associated edema, pulmonary congestion and hemorrhage petechial to equimotic in many cases confluent and subserosal petechial hemorrhages were observed in the dorso-caudal lung lobes. 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 [19,20,22]. In cases of sudden death or also in cases of possible environmental poisoning, 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 and 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): 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 [7]. The heart should be weighed after examination, but before sections are taken. Carefully 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, all blood should be removed before weighing. The next step to open the right ventricle, hold the heart in your left hand, with the left side of heart towards the prosector, pathologist or vet, make the incision, starting at the pulmonary trunk, into the right ventricle, close to the interventricular septum. Carefully open the pulmonary trunk past bifurcation, check the semilunar valves, turn the heart over with its right side towards you. 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 [11]. We have observed some cases of left ventricular hypertrophy associated with EIPH and sudden death and fat infiltration. Open the left atrium and ventricle with a straight incision. Incise through the parietal cusp of the left atrioventricular valve, check the left atrioventricular valve and openings to the pulmonary veins [11]. Carefully to open the aorta, insert the knife under the septal cusp of the left atrioventricular valve [21]. Incise through the wall of the atrium, out and down the aorta [21,22]. Check the semilunar valves of the aorta, orifices, and right and left coronary arteries, orifice of the brachiocephalic trunk [21]. Open the abdominal aorta and its major branches (mesenteries, iliacs, etc.) [11]. This protocol can be used in donkeys and mules with a history of sudden death [7] 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 [7]. 5.6- Opening of cavities: Brain, Cerebrospinal Fluid CNS): Move the head to locate the atlanto-occipital joint, in this moment, collected CSF at this time in cases of infectious and potentially zoonotic diseases such as equine encephalitis and rabies virus, this procedure requires expertise and biosecurity, if required, from a dorsal or ventral approach. Carefully insert the knife into the joint and transect the spinal cord and ligaments of the joint dorsally and ventrally to remove the head. 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 [11]. Place the head on its right side, another cut is sagittal, just medial to left occipital condyle, to continued place the head on its left side for one cut, at this time cut the olfactory peduncles, internal carotid arteries, and cranial nerves as the brain is removed, is important to collect the trigeminal ganglion for the diagnosis of Herpesvirus. If the brain is not to be kept, cut 1 cm transverse sections for inspection in brain 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, should be done for systematic purposes in strict order. Carefully to remove the pituitary gland: pick up the dura from the basilar part of the occipital bone between the sawn condyles, peel it forward to include the pituitary, consider pituitary tumors in donkeys and mules old. Neurological diseases: Unlike horses, donkeys and mules are longer-lived, age, this factor can be considered as a predisposing factor for the development of some pathologies in donkeys and mules that are not frequently observed in horses, among which we can mention degenerative pathologies such as dental wear and loss, tumors of the central nervous system, tumors of the endocrine system, among others should be considered about the necropsy and the taking of samples for the diagnosis. The diagnosis of pathologies of the central nervous system (CNS) in donkeys and mules is difficult, one or several etiological agents associated with the cause of death may be present, as well as several risk factors many differential diagnoses must be considered at the time of an necropsy, as well as an exhaustive systematic study complemented with the taking of samples of central nervous tissue, peripheral nervous tissue, CSF, for confirmation of the etiology associated with the cause of death. In absence of exact diagnosis, it is more appropriate to call all these diseases Equine Neuromotor Syndrome (ENMS) [24]. Etiologies include viruses (Equine Herpesvirus Subtype 1/4, Equine Viral Encephalitis Venezuelan, Easter, Weastern 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, lasalosisid, nicarbazine, heavy metals, lead, mercury, organophosphates, organ chlorates, insecticides, herbicides and fungicides) [24,25]. 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., *Ehrlichia* 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). In many geographic locations there are specific toxic plants, these should be considered at the time of necropsy and sampling including the environment (food, water, grass, grass, hay and potentially toxic plants).

Donkey and mule field necropsy

In my experience, most necropsies on donkeys and mules are carried out in the field, we must always be prepared as practicing veterinarians and as veterinary pathologists. The most important thing is to develop skills in necropsy, anatomical identification and taking samples during the field necropsy, guaranteeing personal and environmental biosecurity and disposition of the corpse of donkey and mule. The basic field necropsy instruments are necessary: knives, ax, scissors, scalpel, tweezers, sample collection containers, personal clothing: gloves, glasses, mouth cap, panties, apron, plastic boots (the use of disposable clothing is common) to guarantee personal biosecurity considering some zoonotic diseases. Environmental biosecurity should be considered to perform the necropsy in an isolated place with limited access from other animals, not to contaminate tributaries of water, soils and vegetation. The remains of the corpse must be removed in its entirety in many countries there are official companies for the collection of the remains of animal carcasses with total biosecurity, in case of not having this service it is recommended to incinerate the remains of the corpse and bury at least 1½ meter deep, consider the regulations by country for the disposal of the corpse. In many stables and farms we recommend establishing an area for necropsy that is easy to disinfect and has wide access for the transportation of dead animals. This technique of equine necropsy has been described by AAEP for horses [26,27], and can be easily applied on donkeys and mules. The donkey or mule is placed in left lateral recumbency and examined for overall body condition, to considerer obesity and cachexia on external examination. Any wounds or external abnormalities should be identified and associated with possible causes of death. Begin the necropsy by making a curving paracostal incision through the paralumbar fossa and ending at the xyphoid, It is a practical, fast and safe field technique. Carefully enter the abdominal cavity but avoid puncturing any underlying bowel [26]. This can be particularly difficult when substantial gas distention is present, and careful incision through each separate layer of abdominal musculature can help prevent inadvertent puncture. Extend the incision cranially by coursing between the front limbs and up the ventral neck, 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 [26]. The resulting muscular flap can be reflected dorsally to expose the abdominal cavity and rib cage to examine the cardiopulmonary system, make a stab incision into the diaphragm near the sternum. Suction of air into the incision confirms negative pressure within the thorax. Cut away the diaphragm along its attachment to the thoracic wall, 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 [26]. The respiratory system, the lungs can be examined in situ or removed from the chest for evaluation, consider lung abscesses, lung adhesions and chronic pulmonary fibrosis in donkeys with a history of confinement in stables with little ventilation for long periods, carefully the heart may be removed and opened for inspection of all chambers and valves. Dissect through the ventral neck incision to expose the trachea in some infectious diseases, such as African horse sickness, severe tracheal edema is observed, but also in other cases such as acute stomach dilatation, gastric rupture, and sudden death associated with exercise-induced pulmonary hemorrhage and dissect esophagus consider esophageal obstructions and reflux esophagitis and esophageal parakeratosis. 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, the entire “pluck” (tongue, larynx, trachea, esophagus, heart, and lungs) may also be removed en masse for detailed evaluation and sampling. As mentioned above,

the main cause of death in donkeys and mules is gastro-intestinal accidents that are why we must consider an exhaustive examination of the digestive system that consists to evaluate abdominal viscera for abnormalities in location and appearance before removal from the abdomen [26]. Locate the pelvic flexure and exteriorize the large colon, laying it alongside the carcass, removal of the large colon from the abdomen makes evaluation easier and allows better visualization of the remaining abdominal organs, in this step it is possible to take samples of tissues and intestinal content for the identification of intestinal parasites. Lipomas and liposarcomas are frequent donkeys and mules, in many cases as an incidental finding of the necropsy not associated with a pathology but in other cases it may be related to a gastrointestinal accident, the entire length of the small intestine and small colon should be examined as well as the liver, spleen, both kidneys and urogenital track, and adrenals and collect the samples at this time. The CNS is evaluated as follows to extract the brain, 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. Lift and remove the section of calvarium to expose the brain, evaluate the fresh brain and proceed to sample collection. Remember that the brain has a high content of lipids and this predisposes to rapid autolysis, consider rapid sampling in extreme environmental conditions in the field to avoid autolytic changes. Post-mortem examinations on animals potentially affected by these diseases should be performed by a diagnostic laboratory [26]. The field necropsy: an added service in rural veterinary medicine, in relation to equine 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 (animal abuse and negligence), including insured animals, prices range between 500\$ and 2500\$ [28]. These prices may have a 15 to 20% increase in the field, taking into account travel to the examination site/ranch (distance kilometers), 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

The identification, characterization and description of the lesions is essential during the necropsy procedure, we must use the same terminology for a better understanding of the observed lesions that is reflected in the necropsy report. The terms to be used are: location, color, size, shape, consistency, texture, number or extent (%), content, odor, distribution and surface appearance [11]. 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 [11].

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). Always remembering the necropsy is a single moment, once the corpse and its remains are eliminated, we must systematically collect the samples with emphasis on the laboratory where they will be sent. Tissue samples should be collected from organs of interest and any identified lesions, remember in forensic medicine cases the collected samples have legal connotations. 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 [26]. The routine fixation medium for histological study is Formaldehyde at 10% for 24 - 48 hours and Bouin or Davidson. All sections for fixation should be less than 1/4" Inches (0.5 - 1.5 cm) thick [11]. 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, this for comparative purposes. As in any technique description, experience is needed to increase proficiency in donkeys and mules. Always take sections of all tissues with a sharp knife, never with a pair of scissors except for bowel samples that can be performed with the enterotome instrument. Routinely take tissue samples of liver, kidney, lung, and all lesions [11]. In veterinary pathology we use the term "the whole animal inside the collecting container" this in order to collect all the samples necessary for the etiological diagnosis. Brain and heart sections are indicated in a grossly negative necropsy

as they may harbor non-grossly visible fatal lesions, however by routine we recommend collecting it. Certainly, other tissues than those listed are to be examined if lesions are suspected in them clinically. 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

The necropsy report is a legal document, must contain the identification of the donkey or mule (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 donkey or mule 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. The described method permits a thorough evaluation of the most common disorders while maintaining a relatively intact carcass, making this a desirable procedure for both clinician and owner [26].

Conclusion

The donkey or mule necropsy represents a post-mortem diagnostic tool, it can be performed by a 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 donkey or mule, its etiology and the associated risk factors, and establish preventive measures. We propose an international database with official institutions and practicing veterinarians to collect the long-term death data in donkeys and mules and establish future prevention measures and multidisciplinary studies.

Summary

The equine necropsy findings can also be helpful for elucidating the severity of disease to owners who need further validation of their decision to euthanize. There are few reports in the literature of necropsy techniques and pathologies in donkeys and mules. The aim of this study was a review an update of necropsy in donkeys and mules. Regular performance of post-mortem necropsy examination will greatly increase the likelihood of accurate diagnoses and often provides a more thorough case understanding. This article reviews the most relevant and up-to-date aspects of necropsy in donkeys and mules, including some specific protocols in case of musculoskeletal injuries, sudden death and pathologies of the central nervous system, etiological agents, and sampling, additionally, the necropsy of the donkey and mule in the field. In conclusion the donkey and mule necropsy can be performed by 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 donkey or mule, its etiology and the associated risk factors, and establish preventive measures.

Bibliography

1. Du Toit N., *et al.* "Post Mortem Survey of Dental Disorders in 349 Donkeys From an Aged Population (2005-2006). Part 1: Prevalence of Specific Dental Disorders". *Equine Veterinary Journal* 40.3 (2008): 204-208.
2. Burden FA., *et al.* "Necropsy Survey of Gastric Ulcers in a Population of Aged Donkeys: Prevalence, Lesion Description and Risk Factors *Animal* 3.2 (2009): 287-293.

3. Morrow L., *et al.* "Retrospective Analysis of Post-Mortem Findings in 1,444 Aged Donkeys". *The Journal of Comparative Pathology* 144 (2011): 145-156.
4. Morales-Briceño A., *et al.* "A retrospective study of the causes of mortality in donkeys and mules in Southern Extremadura, Spain. 1st Donkey Science Worldwide Meeting (2016).
5. Morales-Briceño A., *et al.* "A multicentre retrospective study of the causes of mortality in donkeys and mules in Southern Extremadura, Spain and Aragua State Venezuela 2007-2017". In 6th Annual Donkey Welfare Symposium. UCdavis, California (2018).
6. Ness S. "How to Perform an Equine Field Necropsy Sally L. Ness, DVM". Proceedings of the Annual Convention of the AAEP - Las Vegas, NV, USA (2009): 313-316.
7. Johnson D. "Horse necropsy or Post-mortem Examination (PME)" (2014).
8. Diab S., *et al.* "Sudden death in race horses postmortem examination protocol". *Journal of Veterinary Diagnostic Investigation* 29.4 (2017): 442-449.
9. Morales-Briceño A., *et al.* "Epistaxis in unmedicated equine during intense exercise in Almonte-Huelva-Spain. A preliminary report". In 21st International Conference of Racing Analysts and veterinarians". Montevideo-Uruguay (2016).
10. Morales A., *et al.* "Estudio retrospectivo de la casuística de muerte súbita en caballos en Almonte-Huelva, Andalucía, España. Revista del Instituto de Higiene "Rafael Rangel" 48.1-2 (2017): 211-213.
11. King J., *et al.* "The Necropsy Book". The Internet-First University Press (2013): 259.
12. Morales A., *et al.* "Problemas comunes del casco en burros en el Sur de Extremadura, España. Proceeding del XVI Congreso Internacional de Medicina y Cirugía Equina. SICAB'15 (2015).
13. Lamprea-Garrido A., *et al.* "Parasitismo intestinal en burros en Bodonal de la Sierra, Extremadura-España. Estudio preliminar". RE-MEVET (2016): 18-22.
14. Morales A., *et al.* "Gastric ulcers syndrome in donkeys". *Red Veterinary Medicine* 30 (2015): 27-32.
15. Cardona-Álvarez JA., *et al.* "Frecuencia de patologías gástricas en burros (*Equus africanus asinus*) en Córdoba, Colombia". *Revista de Medicina Veterinaria* 31 (2016): 23-34.
16. Morales Briceño A., *et al.* "Tumor ovárico en una burra. Reporte de un caso". *Revista Equinus* 47 (2017): 38-45.
17. Morales-Briceño A., *et al.* "Pheochromocytoma in a donkey. Report of case". *Revista Científica FVC-LUZ* 4 (2018): 270-273.
18. Morales-Briceño A., *et al.* "Patologías en équidos adultos. Serie de casos". In XVIII Reunión de la Sociedad Española de Anatomía Patológica Veterinaria. Córdoba Junio 26 (2016): 102.
19. Morales-Briceño A., *et al.* "Hemorragia pulmonar inducida por el ejercicio. Patrones de lesiones patológicas en PSI de carreras". *Equinus: Medicina y Cirugía Equina* 39 (2014): 62-74.
20. Morales A., *et al.* "Muerte súbita en caballos de alta competencia. Aspectos patológicos y serie de casos clínicos de revision". *Equinus: Medicina y Cirugía Equina* 41 (2015): 48-62.
21. Morales A., *et al.* "Sudden death, aortic rupture in horses, literature review, case studies reported and risk factors". *Brazilian Journal of Veterinary Research* 52.4 (2015): 298-309.

22. Morales Briceño, A., *et al.* "A retrospective study of causes for mortality of Thoroughbred horses in Caracas, Venezuela (2008-2012)". *Pesquisa Veterinária Brasileira* 38.5 (2018): 817-822.
23. Williams K., *et al.* "Regional Pulmonary Veno-occlusion: A Newly Identified Lesion of Equine Exercise-induced Pulmonary Hemorrhage". *Veterinary Pathology* 45.3 (2008): 316-326.
24. Bermúdez V., *et al.* "Aspectos anatomopatológicos y toma de muestras para el diagnóstico de Encefalitis Equina y Síndrome Neuromotor en caballos Pura Sangre de Carrera y otras razas equinas en Venezuela". Curso Internacional de Encefalitis Equina y Virus del Oeste del Nilo (WNV), Venezuela 2005. USDA-APHIS (2005): 1-4.
25. Bermúdez V., *et al.* "Equine Neuromotor Syndrome in Thoroughbreds and other Breeds in Venezuela A multidisciplinary Study". 9th Congress of the World Equine Veterinary Association. Proceedings Edited by Bakkoury M. and Dakkak A. Marrakech (2006): 62-62.
26. Necropsy of Racehorses. General Guidelines. AAEP (2009).
27. Ness S. "How to Perform an Equine Field Necropsy. Proceedings of the Annual Convention of the AAEP - Las Vegas, NV, USA (2009): 313-316.
28. Morales-Briceño A., *et al.* "La necropsia en campo: un servicio agregado en la medicina veterinaria rural". *Revista de Medicina Veterinaria* 34 (2017): 167-180.

Volume 6 Issue 10 October 2021

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