

Diagnostic Tools for the Study of the Cresty Neck in Horses

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ABSTRACT

The aim of this study was to describe the tools for sampling and histopathological diagnosis of the cresty neck in horses. The morphological study considering the body condition and the score of adiposity is performed following the protocol. Neck length and circumference were measured, as well as clinical evaluations were performed. The samplings for the study of the deformation of the dorsal neck region (cresty neck) in horses are: Fine-Needle Aspiration (FNA), Conventional biopsy and Modified biopsy. Biopsy specimens were fixed in a 10% formalin solution and embedded in paraffin wax. Pathologic examinations included identification of cell type, overall cellularity, cytoplasmic features, nuclear atypia, mitotic index, immunohistochemical findings, morphometric study, and pattern recognition for histological evaluation of cresty neck deformation. The use of cytology and biopsies histology is invasive tools available

to assess in depth the muscles of the dorsal region of the neck, and the degree of fatty infiltration, and even the degree of lipomatosis. The dorsal neck deformation in horses is a condition that requires a comprehensive study using diagnostic tools available which area safe and effective method for tissue diagnosis of the cresty neck. In conclusion, we describe diagnostic tools for the study of the cresty neck deformation in horses. Further comparative prospective studies, with larger sample sizes, are required.

INTRODUCTION

The deformation of the dorsal neck (Cresty neck) in horses is a condition that affects the equestrian industry in Spain, particularly the Spanish Pure Breed horse. The deformation of the dorsal neck or cresty neck deformation (CN) is a condition that has a great clinical and economic impact (Ruiz Lopez, et al., 2010), presented in Spanish Purebred horses and other breeds like the Lusitano. It also has been described recently in Andalusian Breed donkeys (Morales, et al, 2014). Cresty neck is a common defect in

Figure 1.- Equine (Spanish Pure Breed Horses): with deformation of dorsal neck (Cresty neck Score 4). Crest grossly enlarged and thickened, and can no longer be cupped in one hand or easily bent from side to side. Crest may have wrinkles/creases perpendicular to top line



Figure 2.- Equine (Spanish Pure Breed Horses): with deformation of dorsal neck (Cresty neck Score 5). Left side view where the permanent fall of the neck is seen to the right side. Crest is so large it permanently droops to one side.



Figure 3. Equine (Spanish Pure Breed Horses): with deformation of dorsal neck (Cresty neck Score 5). Right side view where the permanent fall of the neck is seen to the right side.



Figure 4.- Donkey (Andalusian Breed): with deformation of dorsal neck (Cresty neck Score 5), left side view where the permanent fall of the neck is observed. Crest is so large it permanently droops to one side.



Figure 5.- Donkey (Andalusian Breed): with deformation of dorsal neck (Cresty neck Score 5), right side view where the permanent fall of the neck is seen to the left side.

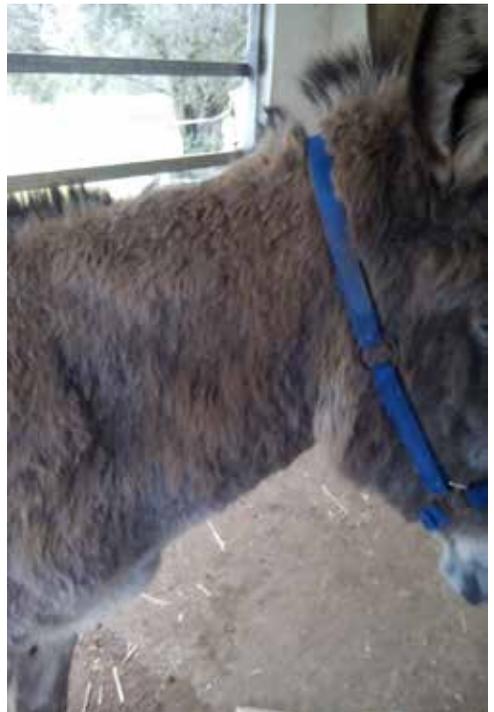


Figure 6.- Donkey (Andalusian Breed): with deformation of dorsal neck (Cresty neck Score 5), dorsal view where the permanent fall of the neck is seen to the left side.



Figure 7.- Equine (Spanish Pure Breed Horses): Fine-Needle Aspiration (FNA): After locating the dorsal neck (Cresty neck) for FNA, a special needle of very fine diameter is passed into the fat and muscle. The needle may be inserted and withdrawn several times (About five times).



Figure 8.- Equine (Spanish Pure Breed Horses): with deformation of dorsal neck (Cresty neck Score 2). Modified biopsy: basically sampling by biopsy technique system biopsy adjustable which a number of samples (punch) in the dorsal neck region are taken (cranial, medium and flow), the right and left sides, with a depth of 2-3cm, about 6 cm. long nuchal ligaments, as anatomical reference.



Figure 9.- Recognition patterns for histological evaluation of deformation of the cresty neck Grade 3.-Abundant fat vacuoles are showed in the space intermyofibrillar tendency to coalesce and low fat in muscle tissue infiltration (moderate lipomatosis) (H&E 10X).

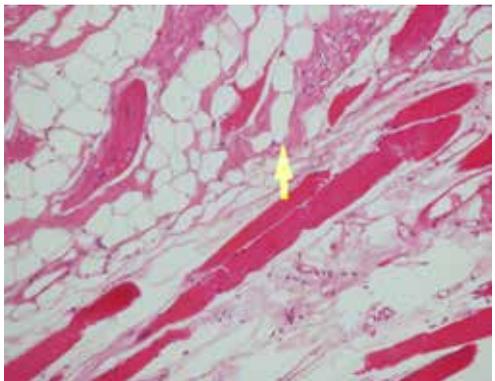
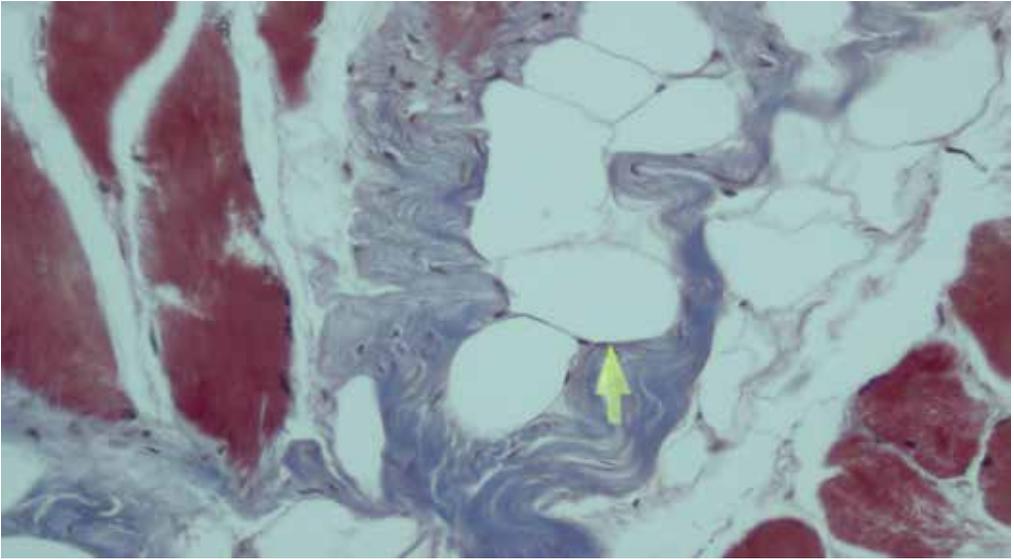


Figure 10.- Recognition patterns for histological evaluation of deformation of the cresty neck staining Masson's Trichrome positive (+++) (MT 20X).



Spanish Pure Breed horses, affecting 8.89% of the worldwide population (Sánchez, et al., 2016). Both environmental and genetic factors are associated with cresty neck (Sánchez, et al., 2016). This cresty neck condition has been widely described and has been associated with metabolic processes; however, it has limited its morphological study, given the limitations for sampling by biopsy or necropsy, and that affects the phenotype which can severely affect horse. Preliminary post-mortem studies have described the development of lipomatosis in the neck muscles with deformation as well as intestinal lipomatosis (Morales, et al., 2015). The aim of this study was to describe the tools for sampling and histopathological diagnosis of the cresty neck in horses.

MORPHOLOGICAL AND CLINICAL STUDY

Clinical: clinical evaluation of neck palpation is a detailed reference following the nuchal ligament and muscle region, considering the presence of pain, heat, swelling, and enlargement. Abscesses, trauma, scars, and tumors are common. Evaluate the horsehair, equine free grazing ticks can be observed in this region. The evaluation determines some kind of abnormality in the movement

of neck usually associated with lameness; it should not be discarded wobbler syndrome in foals as well as disorders such as cervical stenotic myelopathy which is common in Purebred Spanish horses.

Morphological study: morphological study considering the body condition (bone tips: spine of the scapula, ribs, coxal tuberosity and ischial tuberosity) and the specific weight, using a digital scale for horses or using the chest strap with the respective weight scale. The score of adiposity is performed following the protocol described by Carter, et al, 2009.

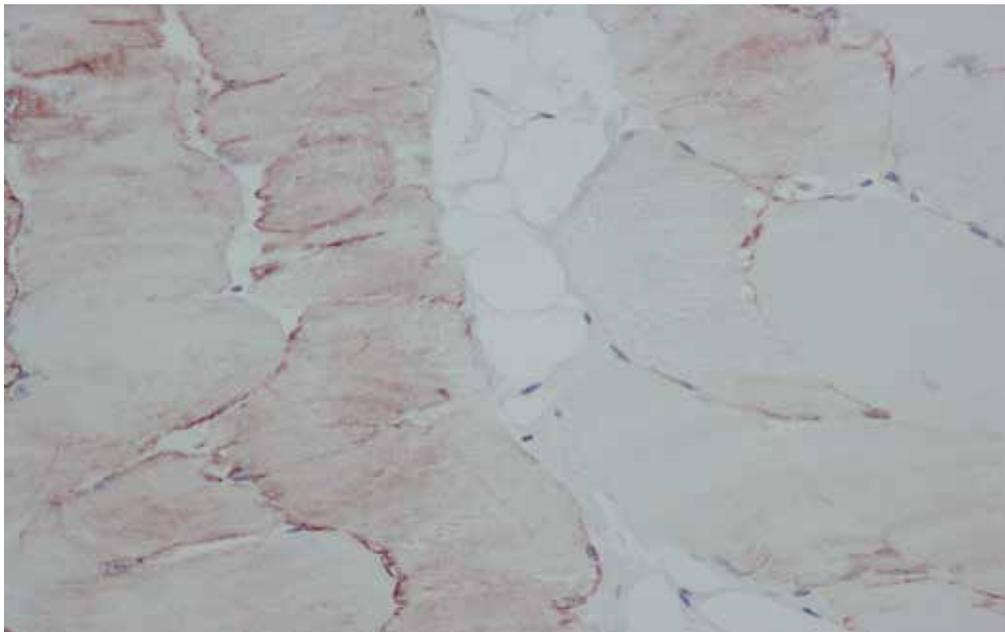
Score 0.-No visual appearance of a crest (apparent tissue above the ligamentum nuchae). No palpable crest.

Score 1.- No visual appearance of a crest, but slight filling felt with palpation.

Score 2.- Noticeable appearance of a crest, but fat fairly evenly deposited from poll to withers. Crest easily cupped in one hand and bent from side to side.

Score 3.-Crest enlarged and thickened, so fat is deposited more heavily in the middle of the neck than toward poll and withers, giving a mounded appearance. Crest fills a cupped hand and begins to

Figure 11.- Immunohistochemical study Desmin antibody (1:100), positive (++) (20X).



lose flexibility from side to side.

Score 4.-Crest grossly enlarged and thickened, and can no longer be cupped in one hand or easily bent from side to side. Crest may have wrinkles/creases perpendicular to top line.

Score 5.- Crest is so large it permanently droops to one side.

Neck measures: All neck measurements were taken while the neck was held in a relaxed position, at approximately a 45° angle. Neck length was measured from the poll to the highest point of the withers. Crest height was measured at 0.50 of neck length from the dorsal midline of the neck to estimated differentiation between the crest (tissue apparent above the ligamentum nuchae) and neck musculature, identified by palpation and visual assessment (Carter, et al., 2009). Neck circumference is measured perpendicular to this line 25, 50 and 75% of the distance between these two structures. The average neck circumference was calculated using the average of these three measures (Frank, et al., 2006 and Diez de Castro et al., 2012).

Sampling for the study of the deformation

dorsal neck edge (Cresty neck) in horses:

Fine-Needle Aspiration (FNA): The skin above the area to be Fine-Needle Aspiration is swabbed with an antiseptic solution and draped with sterile surgical towels. The skin, underlying fat, and muscle must be anesthetized with a local anesthetic. After locating the dorsal neck for FNA, a special needle of very fine diameter is passed into the fat and muscle. The needle may be inserted and withdrawn several times (about five times). It is recommended that the needle punctures on the right side of the dorsal and 6 on the left side of the dorsal neck. In cases of deformity, we take recommended samples in the margins of transition of the deformity.

Conventional Biopsy: The skin above the area to be biopsy is swabbed with an antiseptic solution and draped with sterile surgical towels. The skin, underlying fat, and muscle must be anesthetized with a local anesthetic. The biopsy is performed using sedation and local anesthesia directed into the subcutaneous, but not muscle, tissue. Following a vertical incision in the skin and muscle fascia, two parallel incisions two cm apart and one to three cm long are made

in the muscle. The muscle is grasped in one dorsal corner using forceps to avoid crushing other portions of the biopsy. A cross-sectioning incision is made dorsally, the muscle sample is excised in a ventral direction to a depth of one to two cm and the sample is excised ventrally. The samples obtained are fixed once a collector identified half of 10% formalin fixation and/or glutaraldehyde for microscopic electronic. Laboratories that use formalin fixation avoid the requirement for more intensive preparation required for frozen samples; however, formalin fixation has the disadvantage of creating a number of artifacts including cracking, sedimentation, and leaching of glycogen. These samples can also be used for chromatographic studies.

Modified Biopsy: The skin above the area to be biopsy is swabbed with an antiseptic solution and draped with sterile surgical towels. The skin, underlying fat, and muscle must be anesthetized with a local anesthetic. The biopsy is performed using sedation and local anesthesia directed into the subcutaneous, but not muscle, tissue. The development of this biopsy technique (System for Biopsy Adjustable), in horses is basically sampling by biopsy technique system biopsy adjustable which a number of samples (punch) in the dorsal neck region are taken (cranial, medium and flow), the right and left sides, with a depth of 2-3cm, about 6cm long nuchal ligament, as anatomical reference. The samples obtained are fixed once a collector identified half of 10% formalin fixation and/or glutaraldehyde for microscopic electronic.

Histopathology: The biopsy specimens were fixed in a 10% formalin solution and embedded in paraffin wax. The pathologic examinations included identification of cell type, overall cellularity, cytoplasmic features, nuclear atypia, mitotic index, immunohistochemical findings and morphometric study. Recognition patterns for histological evaluation of deformation of the cresty neck (Morales, et al., 2015), Grade 1.-Scarce and plentiful adipose deposit was observed. An unaltered muscle tissue is observed. Grade

2.-Revealed fat vacuoles, are evident in muscle tissue intermyofibrillar space and prone to coalescence. Grade 3.-Abundant fat vacuoles are showed in the space intermyofibrillar tendency to coalesce and low fat in muscle tissue infiltration (moderate lipomatosis). Grade 4.-degreeabundantfatvacuole-sin the space intermyofibrillar tendency to coalesceand fatty infiltration in muscle tissue (marked lipomatosis). Grade 5.-Showed vacuoles are observed only fat, not muscle tissue (severe lipomatosis) is evident.

Morphometric study: were performed Image Tools program with digitized picture of muscle biopsies (diameter sections muscle), in their employment grades 0-5. The procedure involves drawing a line on the graticule, the measurement unit (microns) is selected and the line length record. Once calibrated the program, the image of interest is maximized and the icon is selected, to measure variables (area and depth). In each measurement data corresponding morphometric variables were recorded on a score sheet and automatically program the mean and standard deviation of the measurements were obtained and statistical analysis were performed.

Special staining: Additionally staining was used Masson's Trichrome and other special stains can be used as Oil Red.

Immunohistochemistry: were performed the antibody (Desmin, Vimentin, ALT/WDLS, MDM2M, CDK4) for immunohistochemical (IHC) study (Garcia-Miralles et al, 2002; Rodriguez, et al., 2004; Al-Daraji, et al., 2009).

Ultrastructural study: An ultrastructural study of muscle fiber biopsies from the cresty neck revealed the presence of lipid droplets within the muscle fibers. This technique can be done for research purposes.

DISCUSSION

Currently the tools available for the study of the deformation dorsal neck (Cresty neck) in horses are the clinical evaluation, the morphological study which is comprised of the degree of adiposity and the morphomet-

ric measurements of the neck. The use of cytology and biopsies histology are invasive tools available to assess in depth but the muscles of the dorsal region of the neck, and the degree of fatty infiltration, and even the degree of lipomatosis. In relation to the cytology (FNA), small samples of tissue have been obtained using a needle to diagnose lesions in many anatomical locations. FNA cytology is generally less traumatic than core biopsy and may be more appropriate for equine high competition without affecting their phenotype and is the technique most accepted by the owners. The sampling procedure for FNA cytology is quicker to perform than core biopsy and is associated with a low complication rate, with results is available relatively quickly and relatively inexpensive to perform. The relative disadvantages of FNA cytology, compared with core biopsy, include: requires training in the preparation of quality smears and considerable cytology expertise is required to interpret FNA cytology, is generally inappropriate for the assessment of microcalcifications that may occur in the dorsal region of the neck. The definitive diagnosis of some lesions can be difficult to make on the basis of FNA cytology. The biopsy of the dorsal region of the neck (cresty neck), is the tool with greater reliability, specificity and sensitivity for the diagnosis of this condition (cresty neck) in horses, confirms the definitive diagnosis. Biopsy allows the study of muscle tissue and fat covers as well as enables morphologically characterize the status of the dorsal neck deformation and the potential risk of deformity. However the biopsy has severe limitations the main one is the low acceptance by the owners, because it can affect the phenotypic condition of the horse, being an invasive procedure. The conventional biopsy could only be applied in horses going to slaughter, however modified biopsy technique represents an important alternative for the study of the deformation of the dorsal neck, with acceptance in the owners. The sample size for histological study represents a limitation because the technique allows of biopsy modified

samples of approximately 5mm x 3mm, unlike conventional biopsy specimens may have a 1cm-3cm size, allowing studies more comprehensive and complementary histological and immunohistochemistry, electron microscopy and chromatographic studies. These diagnostic techniques may have applications in the study of endocrine diseases in horses as Equine Metabolic Syndrome, Cushing's syndrome, insulin resistance, obesity, laminitis, hyperlipemia (Johnson, 2002, Treiber, et al., 2006), possibly related to the deformation of the dorsal neck deformation. Cresty neck score has a moderate heritability, indicating that the prevalence of this defect could be diminished by genetic selection (Sánchez, et al., 2016). Cresty neck is not associated with girth-to-height ratio, but it is associated with systematic factors such as age, sex, coat colour and the stud location (Sánchez, et al., 2016). Finally you can have application in the pre-purchase exams in horse breeds at risk of deformation of the dorsal neck. The deformation of the dorsal neck deformation the horse is a condition that requires a comprehensive study using diagnostic tools available they are safe and effective method for the tissue diagnosis of cresty neck. However, we recognize the limitations of this study. In conclusion, we describe diagnostic tools for the study of the deformation of the cresty neck in horses. Further comparative, prospective studies with larger sample sizes are required.

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