What's New in Cresty Neck Horses? Diagnosis in Mature and Aged Equids

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ABSTRACT

The aim of this study was to determine cresty neck (deformation of the dorsal neck) prevalence in mature or aged equids (15–25 years of age) and their associated factors. The study included 116 horses (Eqqus caballus) of Cordoba, Sevilla, and Southern Extremadura, Spain. There were 68 males and 48 females consisting of the following breeds:

- Spanish Pure Breed Horses
- Lusitano
- Spanish Arabian, and
- Crossed horses.

The equids were then grouped in the mature category (15–19 years) or the aged category (≥20 years). Additionally we included the variables: location, feeding, and exercise. A clinical evaluation of neck palpation was practiced. Morphological study considering the body condition and the score of adiposity following the protocol cresty neck score (CNS) was performed. Neck circumference, modified biopsy, histopathology, morphometric study, and immunohistochemistry were also recorded. Statistical analysis using Ordinal Logistic Regression Model for the response variable CNS was established. The cresty neck was observed in all breeds studied. Forty-four percent (51/116) of the horses had cresty neck and obesity (Grade 3, 4, and 5). All cases were confirmed by histopathology and immunohistochemistry. The results from this study show that 43% of mature horses (37/86) are cresty neck, overweight or obese. The results obtained from the multivariate model shows statistically significant effects of breed, gender, and exercise, expressed in Odds Ratio (OR) and the corresponding 95% confidence intervals.

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 (Morales, et al., 2017). The association of genetic and environmental factors are involved in the development of the cresty neck. However, preliminary studies suggest that metabolic changes in the muscles of the dorsal region of the neck characterized deposition of fat. Metabolic myopathies include a group of diseases that have the common feature of accumulating normal orabnormal products of metabolism such as glycogen and lipids (Gil, et al., 2006).

Several factors have been associated with the cresty neck in horses, but studies in mature horses have been limited. The recent American College of Veterinary InternalMedicine (ACVIM) consensus statement on EMS indicates that there is a scarcity of epidemiological data on the components of EMS, including obesity (Thatcher, et al., 2012). In 1998 and 2005, United States Department of Agriculture (USDA), National Animal Health Monitoring System (NAHMS) studies estimated that approximately 1.5% and 3.4%, respectively, of the U.S. horse population was overconditioned or obese (NAHMS, 1998, 2005) (Martinson, et al., 2014). Equids with a BCS (Body Condition Score) of ≥ 7 had a greater likelihood of being overweight, and the model suggested cutoffs at the 48th and 83rd percentiles for underweight and overweight individuals, respectively (Martinson, et al., 2014). Jensen, et al. (2016) observed 24% of obese horsesin mature Icelandic horses. The increase in the ages of the horses has been attributed to improved husbandry and owners' willingness to keep animals that no longer perform their intended function (Miller, et al., 2015).

In clinical practice, obesity is common in mature horses because they decrease their participation in equestrian activities. The observations made by equine veterinarians suggest that obesity is a growing problem in companion equid populations (Carter, et al., 2009). Equine obesity has been associated with conditions such as insulin resistance, and also with the increasing risk for laminitis (Quinn et al., 2006; Geor, 2008, Bruynsteen, et al., 2015). Because obesity and health problems in horses and ponies is related, it is important to measure the overall and regional adiposity (Carter, et al., 2009). While BCS is an accepted score for assessing the overall adiposity, CNS would standardize the assessment of regional fat distribution in the neck crest (Carter, et al., 2009). A study of seasonal prevalence and risk factors for nuchal crest adiposity in domestic horses and ponies using the Cresty Neck Score using a mixed effects logistic regression model. It concluded the prevalence of a CNS ≥3/5 was higher at the end of winter than at the end of summer, which was the opposite pattern seasonal variation to that observed for general obesity (Giles, et al., 2015). The aim of this study was to determine cresty neck prevalence in mature or aged equids (15–25 years old) and their associated risk factors.

MATERIALS AND METHODS

Sample

The study included 116 horses (Eqqus caballus), of Cordoba, Sevilla, and Southern Extremadura, Spain. The gender distribution was 68 males (51 castrated and 17 not castrated) and 48 females of the following breeds:

- 52/116 Spanish Pure Breed Horses
- 36/116 Lusitano
- 16/116 Spanish Arabian, and
- 12/116 Crossed horses.

The equids were grouped in the mature category (15–19 years) and the aged category (≥20 years). Additionally were considered the variables: feeding and exercise.

Clinical

A clinical evaluation of the neck by palpation, consideration of the presence of pain, heat, swelling, and enlargement was conducted. Abscesses, trauma, scars, and, tumors are common in the neck of horses.

Equine free grazing ticks can be observed in this region. Morphological study

A morphological study considered 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 Cresty Neck Score (CNS) 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 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 topline.
- 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).

Modified Biopsy

The development of this biopsy technique (System for Biopsy Adjustable) in horses is basically sampling a number of samples (punch) in the dorsal neck region. They are taken (cranial, medium, and flow) on the right and left sides. The samples obtained are fixed once a collector identified half of 10% formalin fixation for microscopic optic (Morales Briceño, et al., 2017).

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, etal., 2015, Morales, et al., 2017), were:

- Grade 0--Muscle fibers are observed, no fat vacuoles are observed.
- Grade1--Scarce adipose deposit was observed. An unaltered muscle tissue is observed.
- Grade 2--Fat vacuoles are evident in muscle tissue intermyofibrillar space and prone to coalescence.
- Grade 3--Abundant fat vacuoles in the intermyofibrillar space with tendency to coalesce, and low fat infiltration in muscle tissue (moderate lipomatosis).
- Grade 4-- Abundant fat vacuoles in the intermyofibrillar space with tendency to coalesce and fatty infiltration in muscle tissue (marked lipomatosis).
- Grade 5.- Only fat vacuoles are observed, without muscle tissue (severe lipomatosis).

Morphometric Study

Morphometric study was performed utilizing the Imagen Tools program with digitized pictures 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 that automatically programed the mean and standard deviation. The measurements were obtained, and statistical analysis were performed (Morales Briceño, et al., 2017).

Inmunohistochemistry

The immunohistochemistry (IHC) were performed the antibody (Desmin) 1:100 for (Garcia-Miralles et al, 2002; Morales, et al., 2017).

Statistical Analysis

A bivariate descriptive statistical analysis for all variables with respect to CNS was done. An Ordinal Logistic Regression Model (McCullagh & Nelder, 1989) was established for the response variable Cresty Neck Score (CNS). Firstly, there are the results of the bivariate models (taking a single variable and CNS) for breed, gender, location, food, exercise, and age. The other variables are closely related to the CNS so the models make no sense.

Finally, we present the results of a multivariate model including the covariates: breed, gender, exercise, and age. We have not included food and location because they are highly correlated with exercise and they presented problems of multicollinearity in the model.

All results have been obtained with the software SAS System 9.4 (SAS Institute Inc., Cary, NC, USA). The significant level was fixed at 0.05.

RESULTS

The cresty neck was observed in all studied breeds. Forty-four percent (51/116) of the horses presented cresty neck and obesity (Grade 3, 4 and 5). All cases were confirmed by histopathology and immunohistochemistry. An 8% (9/116) got a CNS=0; a 19% (22/116) showed CNS=1; 29% (34/116) got a CNS=2; 27% (31/116) present CNS=3; 12% (14/116) showed CNS=4; and 5% (6/116), present CNS=5. The results for Desmin antibody (1:100 dilution) were positive (+++) in grades 0-2 and negative (-) in grades 3-5. The distribution for cresty neck score and breeds are presented in table 1.

A high incidence of horses with cresty neck and obesity was observed in the Spanish Arabian breeds, while a low incidence in mature horses of Pure Breeds such as the Spanish Pure Breed and Lusitano. This occurred because the horses with this defect are immediately excluded from the genealogical record (ANCE), given their depreciation, are mostly sent to slaughterhouses, and do not reach maturity, except for some cases such as those observed in this study 6% SPB and 14% Lusitano.

The results of the bivariate ordinal logistic regression models are in seen in Table 2. We displayed the odds ratio (OR) and the corresponding 95% CI for the all bivariate models, one for each variable (breed, gender, exercise, and location). Statistically, significant variables are: breed, gender, exercise, and location. For the breed variable, we are going to comment on the results for the pairs with statistically significant differences. So, Crossed horses vs Spanish Pure Breed had an OR = 8.163, which indicates that the odds of large CNS

Table 1. *Cresty Neck Score (CNS) column profile for each breed*

Score/ Breed	Spanish Pure Breed	Lusitano	Spanish Arabian	Crossed horse
0	6%	5%	12%	17%
1	27%	17%	12%	0%
2	48%	17%	6%	17%
3	13%	39%	25%	25%
4	4%	22%	38%	8%
5	2%	0%	6%	0%

are 8.2 times higher in Crossed than Spanish Pure Breed.

In the comparison of Spanish Arabian horses vs Spanish Pure Breed, we obtained an OR = 5,595. These results indicate that the odds of large CNS are 5.6 times higher in Spanish Arabian than Spanish Pure Breed. In Lusitano horses vs Spanish Pure Breed, we obtained an OR = 2.772, which indicates that the odds of large CNS are 2.8 times higher in Lusitano than Spanish Pure Breed.

Gender was statistically significant. In the comparison between females and castrated males, we obtained an OR = 3,641, so the odds of high CNS are 3.6 times higher in females than castrated males. Non castrated males vs castrated males had an OR = 6.593, so the odds of large CNS are 6.6 times higher in non castrated males vs castrated males. The variable exercise was statistically significant.

For the comparison between horses in the high exercise group vs the medium exercise group, we obtained an OR = 32,866, so the odds of high CNS is 33 times higher in high vs medium exercise horses. Comparing high exercise vs low exercise we got an OR = 39,329, so the odds of large CNS is 39 times higher in high respect low exercise groups.

Finally, the location variable was statistically significant. Andalucia had an odds of large CNS 3.2 times higher than Extremadura. A multivariate ordinal logistic regression model was established with co-variates: breed, gender, exercise, and age. We note that age was included as a control covariate, even though it was not statistically significant. The variable age was not statistically significant. For variable Breed, Spanish Pure Breed had statistically significant differences with respect all the other breeds: Crossed horse vs Spanish Pure Breed (OR = 10.324); Spanish Arabian horses vs Spanish Pure Breed (OR = 3,602); and Lusitano horses vs

Table 2. *Results for bivariate ordinal logistic regression models. (*) means statistically significant differences*

Variable	Comparison	OR	$CI_{95\%}$(OR)
BREED	Crossed vs Spanish Arabian	1.459	[0.379, 5.616]
	Crossed vs Lusitano	2.944	[0.894, 9.702]
	Crossed vs Spanish Pure Breed	8.163*	[2.497, 26.684]
	Spanish Arabian vs Lusitano	2.018	[0.695, 5.861]
	Spanish Arabian vs Spanish Pure Breed	5.595*	[1.959, 15.976]
	Lusitano vs Spanish Pure Breed	2.772*	[1.267, 6.065]
GENDER	Female vs not castrated male	0.552	[0.204, 1.496]
	Female vs castrated male	3.641*	[1.736, 7.637]
	Not castrated male vs castrated male	6.593*	[2.344, 18.538]
EXERCISE	High vs Medium	32.866*	[5.820, 185.589]
	High vs Low	39.329*	[7.357, 210.241]
	Medium vs Low	1.197	[0.576, 2.485]
LOCATION	Andalucia vs Extremadura	3.165*	[1.594, 6.286]

Table 3. *Results for the multivariate ordinal logistic regression model with covariates: Breed, Sex, Exercise and Age (*) means statistically significant differences.*

	OR	$CI_{95\%}$(OR)	
Breed Cross vs Spanish Arabian.	2.866	0.669	12.281
Breed Cross vs Lusitano.	2.977	0.827	10.723
Breed Crossed vs Spanish Pure Breed.	10.324*1.039	2.829	37.675
Breed Spanish Arabian vs Lusitano.	3.602*	0.347	3.109
Breed Spanish Arabian vs Spanish Pure Breed.	3.468*	1.235	10.508
Breed Lusitano vs Spanish Pure Breed.	0.139*	1.520	7.911
Sex female vs not castrated male	4.555*	0.038	0.507
Sex female vs castrated male	32.830*	2.053	10.103
Sex not castrated male vs castrated male	46.838*	8.074	133.486
Exercise High vs Medium	32.045*	71.72	305.894
Exercise High vs Low	0.684	5.677	180.896
Exercise Medium vs Low	2.134	0.279	1.678
Age 15-19 vs >= 20		0.862	5.283

Spanish Pure Breed (OR = 3.468).

For variable gender, there are statistically significant differences among all groups: Female vs non castrated male (OR = 0.139); Female vs castrated male (OR = 4.555); and non castrated Male vs castrated male (OR = 32.83). For variable exercise high category there were statistically significant differences with respect to all of the other categories: High vs medium exercise (OR = 46.838), and high vs low exercise (OR = 32.045).

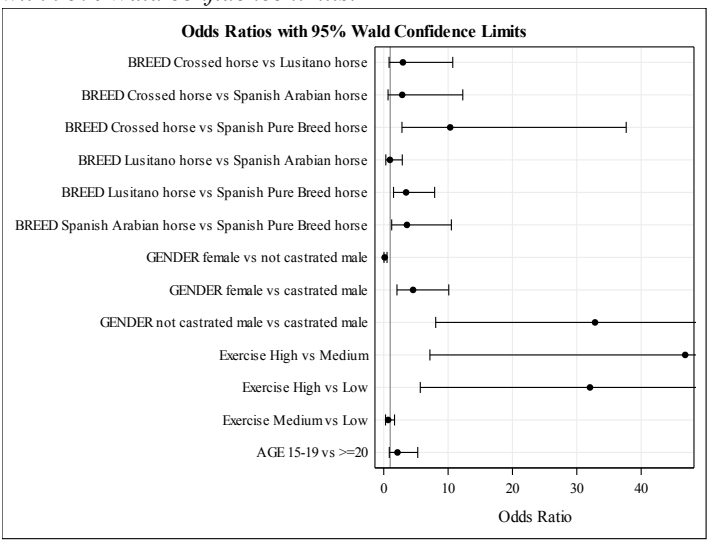
DISCUSSION

In 51 of the 116 mature horses (44%) in the current study, cresty neck and obesity were observed. Other studies in domestic horses and ponies show the prevalence was 45.83% (95% CI 36.76% - 54.90%) at the end of winter, falling to 33.33% (95% CI 26.76% - 39.90%) at the end of summer (Giles, et al., 2015) The prevalence seems to coincide with our results. However, our study did not consider winter or summer. The distribution among breed groups in descending order of frequency of cresty neck was: Spanish Arabian, Lusitano, Crossed, and Spanish Pure breed. Twenty six percent of the cases had more than ≥20 years old, while 74% of the cases presented an age of 15-19 years old. Other indices have been considered in other studies. Body condition scoring (BCS) is currently the most common management tool for assessing and monitoring body condition in horses (Silva, et al., 2016), since we use the weight. Body condition scores of the 254 horses varied from 3 to 9. There was no effect of age ($P > 0.10$) or sex ($P = 0.10$) on the BCS, but the farm had an effect ($P < 0.001$) (Jensen, et al., 2016).

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 themorphometric measurements of the neck (Morales Briceño, et al., 2017). Image analysis is an additional method that can be used for the determination of CNS in horses.

Cytological and biopsy techniques have been developed such as the tools available

Figure 1. *Results for the multivariate ordinal logistic regression model with covariates: Breed, Gender and Exercise Odds Ratios with 95% Wald confidence limits.*



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 (Morales Briceño, et al., 2017). However, they are invasive methods. Thus, in this study, we used non-invasive methods (Clinical, morphological study and neck measures) and invasive methods (modified biopsy, histopathology, morphometric study, and immunohistochemistry) for determining the cresty neck and obesity in horses. The association analysis between cresty neck scores and post-mortem nape fat measurements in horses, obtained after photographic image analysis, revealed the existence of a significant correlation between CNS and nape fat measurements in horses obtained after image analysis, in particular with nape fat thickness (Silva, et al., 2016).

The statistical analysis of this study was addressed using an Ordinal Logistic Regression Mode, the variable (CNS) had defined with six ordered categories. This model is an extension of the Logistic Regression Model for a binary response variable, which allows a response variable with more than two ordered values. The results obtained in the multivariate Ordinal Logistic Regression Model show a statistical significant effect on the CNS of breed (p=0.0007), gender (p<0.001), and exercise (p=0.0002), while the age was not statistically significant (p=0.1012). The location, Andalucia and Extremadura (p=0.0010) was only statistically significant in the bivariate model.

CONCLUSION

The results from this study show that 44% of mature Spanish Arabian, Crossed, Lusitano, and Spanish Pure Breed horses located in Andalucia and Extremadura, are cresty neck, overweight, or obese. Breed, gender and exercise are statistically significant risk factors in the presence of the cresty neck and obesity in mature horses.

REFERENCES

1. Bruynsteen L, Moons C.P.H, Janssens G.P.J, Harris P.A, Vandevelde K, Lefère L, Duchateau L, Hesta M. Level of Energy Restriction Alters Body Condition Score and Morphometric Profile in Obese Shetland Ponies. *Vet J* 206 (1), 61-66. 2015 Jun 11.
2. Carter, R.A., Geor, R.J., Staniar, W.B, et al. Apparent adiposity assesed by Standardised scoring systems and morphometric measurements in horses and ponies. *Vet J*: 179 (2):204-210. 2009.
3. Diez De Castro E, Pineda Martos C, Martin Cuervo M, Quintero Felices S, Mendez Vazquez N, Aguilera Tejero E. Valoración de obesidad y resistencia a la insulina en el Caballo de Pura Raza Española (PRE). *Equinus*: 34 Tercer Cuatrimestre: 58-75. 2012.
4. Frank N, Elliot Sb, Brant Le and KeislerDh. Physical characteristics, blood hormone concentration, and plasma lipid concentrations in obese horses with insulin resistance. *J Am Vet Med Assoc*: 228 (9): 1983-1390. 2006.
5. García-Miralles, T.; Gonzalbes-Garcia, J. Marcadores inmunohistoquimicos del desarrollo embriológico en el músculo estriado de las extremidades fetales. Available in: http://www.uninet.edu/conganat/conferencias/C006/. 2002.
6. Geor, R.J. 2008. Metabolic predispositions to laminitis in horses and ponies: obesity, insulin resistance and metabolic syndromes. *Journal of equine veterinary science* 28 (12), 753-759.
7. Gil F, Alemán M, Rivero M, Latorrre R, Carrión

M, Aguirre C, Ruiz I, Ayala I. Neuromuscular dosease associated with glycogen storage in a Spanish-breed filly. *The Veterinary Record*, April 15, 2006.

8. Giles S, Nicol C, Rands S, Harris P. Assessing the seasonal prevalence and risk factors for nuchal crest adiposity in domestic horses and ponies using the cresty neck score. BMC *Veterinary Research*. 2015. 11:13.2-9.
9. Javali S,Pandit P. A comparison of ordinal regression models in an analysis of factors associated with periodontal disease. *J Indian Soc Periodontol*. 2010 Jul-Sep; 14(3): 155–159.
10. Jensen R, Danielsen S, Tauson A.H.. Body condition score, morphometric measurements and estimation of body weight in mature Icelandic horses in Denmark. *Acta Vet Scand*2016, 58(Suppl 1):59.
11. McCullagh, P, Nelder, J.A. Generalized Linear Models. 1989. Chapman & Hall.
12. Martinson K.L, Coleman R.C, Rendahl A.K, Fang Z, McCue M.E. Estimation of body weight and development of a body weight score for adult equids using morphometric measurements. *J. Anim. Sci.* 2014.92:2230–2238.
13. Miller M.A, Moore G.E, Bertin R.R, Kritchevsky J.E. What's New in Old Horses? Postmortem Diagnoses in Mature and Aged Equids. *Veterinary Pathology* 2016, 53(2) 390-398.
14. Morales A. Mendez A, Perez Arevalo J. Infiltrative lipomatosis in the neck and colon of a Spanish Pure Breed Horse.Volumen: *Braz J Vet Pathol*, 8(1),170-173.2015.
15. Morales Briceño A, Méndez-Sanchez A, Perez-Arevalo J. Patrones de reconocimiento histopatológico de la deformación del borde dorsal del cuello en équidos. Estudio preliminar .ProceedingXXVII Reunión Anual SEAPV, Barcelona. P17-95. 2015.
16. Morales A, Escamilla Sánchez A, Méndez Sánchez A, Méndez J, Pérez Arévalo J. Histopathological pattern recognition of cresty neck in horses in Spain. *Braz J Vet Pathol*, 2017, 10(1).170-174.
17. Morales Briceño A, Méndez Sánchez A, Méndez-Angulo J, Escamilla-Sánchez A, Pérez-Arévalo J. Diagnostic tools for the study of the cresty neck in horses. *Intern J Appl Res Vet Med*. 15, 1, 52-60. 2017.
18. Quinn, R.W., Burk, A.O., Hartsock, T.G., Petersen, E.D., Whitley, N.C., Treiber, K.H., Boston, R.C. 2008. Insulin sensitivity in thoroughbred geldings: effect of weight gain, diet and exercise on insulin sensitivity in thoroughbred geldings. *Journal of equine veterinary science* 28 (12), 728-738.
19. Silva S, Payan-Carreira R, Guedes C, Coelho S, Santos A. Correlations between cresty neck scores and post-mortem nape fat measurements in horses, obtained after photographic image analysis. *Acta Vet Scand* 2016, 58 (Suppl 1):60.
20. Thatcher C.D, Pleasant R.S, Geor R.J, Elvinger F. Prevalence of over conditioning in mature horses in southwest Virginia during the summer. *J Vet Intern Med*. 2012 Nov-Dec;26(6):1413-8.