Investigating whether the Caspian horse breed has primitive traits such as full Equine Nuchal Ligament Lamellae and interosseous muscle II and IV using ultrasound

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Abstract:

Through the thousands of years of domestication horses have undergone, they have undergone a myriad of changes. While certain atavistic traits have been seen to return in certain modern breeds like the Shetland and Welsh pony. In some older breeds, similar atavistic traits were never bred out. Through understanding the links between the extinct, primitive and modern breeds of horses relevant traits can better be preserved.

In previous research it has been shown that primitive breeds of *Equus caballus*, like the Dutch Konik and the Bosnian Mountain horse have what was described as primitive traits. In particular two traits have been found that are present in these breeds, but not in modern breeds. The first is the Nuchal ligament lamellae attachments from the second cervical vertebrae till the seventh (C2-C7), while in more modern breeds this trait is only present from the second cervical vertebrae till the fifth or at times till the sixth (C2-C5, C2-C6). The second is two additional interosseous muscles, on either side of the third interosseous muscle, otherwise known as the suspensory ligament. These muscles have been identified as the second and fourth interosseous muscle (IM2 and IM4, respectively), more commonly seen at the metacarpal and metatarsal bones in polydactyl species. These additional ligaments described are important in energy preservation and providing stability for the horse.

During this study we looked at whether the Caspian is potentially another breed that carry the aforementioned primitive traits. The Caspian horse is an old oriental breed, dating back to around 3000 B.C., these miniature horses hail originally from Iran and are a very rare breed. They are considered to be the ancestor of other oriental breeds such as the Turkoman and the Arabian horse. In the Netherlands there is but one place where they are bred, at Levende Landgoed NOVA, where there are a total number of around 27 horses. For this research 22 of the horses at this stable were scanned using ultrasound and the presence of the aforementioned primitive traits were recorded.

We found that 100% of the Caspian horses have a full Nuchal ligament lamellae attached from C2-C7. 77% of the horses had IM2 present, and 60% had IM4 present. Meaning that the Caspian horse is a third member of the *Equus cabballus* in which these primitive traits have been. Making it a primitive breed. Due to the small sample size no clear connection could be made between the presence of the interosseous muscles and the sex of the horses. However there was a trend visible where more of the mares had the IM2 present, while for the males it varied. For IM4 there was no clear difference to be seen between the mares and the stallions. A correlation could also not be made between the presence of IM2 and IM4 and the medical history, therefore an answer could not be given as to whether the health and stability of the horses are affected by the presence of these traits. Further research is important to better understand the implications of breeding choices made by breeders and how it can reflect on the health of the horses. Through better understanding the extent of breeds that have these primitive traits and the effect on their health and stability, it can be understood what not having these traits means to the modern breeds.

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Table of contents:

Table of Table and Figures:

Introduction

Understanding evolutionary links between extinct, primitive and modern breeds of horses is important in order to preserve certain traits. With increasing levels of domestication, certain primitive traits were bred out in favour of more preferential traits and some traits, this process however has also had consequences on traits deemed to be non-essential for the breeder's purposes, even though they might have long term modern day disadvantages to the animals. While the equine evolution has been well documented with regard to skeletal changes, the same cannot be said for soft tissues. However, recent studies have shown two examples of primitive traits that are not usually found in modern breeds, but are found in some primitive breeds of horses, donkeys and the Grant's Zebra. These are the Lamellae of the nuchal ligament fully attached from C2-C7, and functional interosseous muscle II and IV. *(May-Davis, Vermeulen and Brown, 2019)*

The two primitive traits under investigation, the attachment points of the nuchal ligament lamellae and the interosseous muscles II, III IV. Along with the measurement tools and the Caspian breed. Will all be further introduced in the following paragraphs.

Nuchal ligament lamellae

The nuchal ligament has been described as the most influential of the elastic structures in the neck. Its main function is to provide extra support during extension of the head and neck. It is made up of two parts, the funicular that runs dorsally from the occiput to the insertion on the withers and the lamellae, which connects the funicular to the cervical vertebrae. The nuchal ligament contains high-crimp collagen fibres in the lamellae which suggests that its purpose in the neck is energy preservation during locomotion. The less elastic ligamentous tissue in the funicular reduces the necessary muscular effort and stabilises the head and neck. *(Getty and Grossman, 1975)*

While considerable research has gone into the evolutionary development of the hoof and the limbs, information on the evolutionary changes of the vertebral column, especially the cervical part, is still lacking. *(May-Davis, Vermeulen and Brown, 2019*). Anatomy books depicting equine nuchal ligament lamellae (NLL), have used images that depicted the lamellae as being attached to all cervical vertebrae from C2-C7. This is shown below in the depiction created by equine anatomist; Snape in 1683 (fig. 1) *(May-Davis and Kleine, 2014).* Such Illustrations can also be found in more recent anatomy books used by veterinarian students, such as in "the horse anatomy workbook illustrated by Maggie Raynor, in 2004 (fig 2) *(Raynor and Goody, 2006)* based on the works of Goody. And in the "Anatomy of the Horse" by Klaus Diter Budras et al, illustrated by Gisela Jahrmärker, Diemut Starke, Renate Richter published in 2011 (fig. 3) *(Budras, Sack and Röck, 2011)*. In this book they also describe the lamellae as extending "cranioventrally from the spinous processes of T2 and T3 and attaches on the spinous processes of all cervical vertebrae except the first."

Fig 1: Illustration by Snape. (May-Davis and Kleine, 2014)

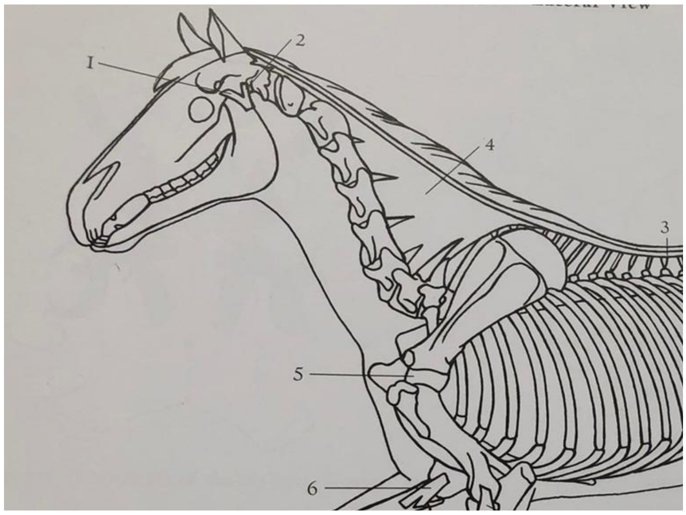


Fig 2: Illustration by Maggie Raynor (Raynor and Goody, 2006)

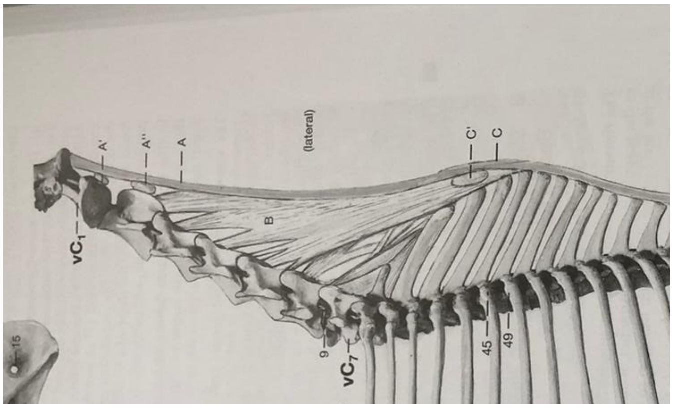


Fig 3: Illustration from “Anatomy of the horse” (Budras et al, 2011)

This is, however, something that has been shown in recent studies by Sharon May-Davis and her team to be missing in many breeds of domesticated horses. In one study of 35 unrelated horses of no specific breed, with mixed gender and age where dissected. It was noted that the Nuchal ligament lamellae (NLL) was not attached to the sixth and seventh cervical vertebrae. Furthermore, the attachment of the NLL on the fifth cervical vertebrae was quite thin and feeble in nine of those horses and that incomplete fibres were noted in the caudal attachments of the NLL in 15 horses. (*May-Davis and Kleine, 2014).* They have also done another, more recent study with 98 equids and 88 of the 98 had lamellae attached at C2-C5; 2 of 98 with attachments C2-C6; and 8 of 98 with attachments at C2-C7. *(May-Davis, Brown and Vermeulen, 2018)* Therefore the study concluded that many of the current breeds of equids have lamellae attached from C2-C5 unlike illustrated in the images above.

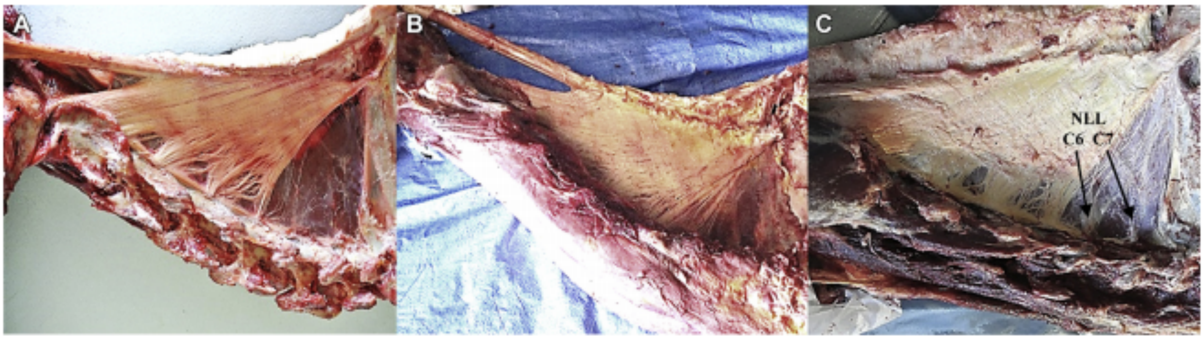


Fig 4: Attachment points of the NLL in A) shetland pony C2-C5, B) Welsh Cob C2-C6, C) Bosnian mountain horse C2-C7, C2-C5. (*May-Davis, Brown and Vermeulen, 2018)*

Interosseous muscle II and IV

Interosseous muscle II and IV in the distal limb have also been investigated, similarly to NLL as an indicator for primitive traits. Atavistic or vestigial soft tissue structures have been reported in modern horses, though it is considered rare. It can be stipulated that these soft tissue structures were once strong and functional in the polydactyl horse but now, the morphology is generally regarded as ineffective. However, two such structures, the interosseous muscle II (IM2) and interosseous muscle IV (IM4) have been found in two existing descendants of the Tarpan, namely the Bosnian mountain horse and the Dutch Konik. This finding is based on dissections that were performed on 575 distal limbs (DL) from 151 individual equids of the genus *Equus*; 488 DL were sourced from 122 *E. caballus* (domestic and primitive); 15 DL from 9 *E. przewaslkii* (Przewalski’s horse); 11 DL from 3 *E. asinus* (donkey) and 4 DL from 1 *E. quagga boehmi* (Grant’s zebra). The Bosnian mountain horse was the only domestic horse, and the dutch Konik in which the IM2 and IM4 were present. Furthermore it was shown in all distal limbs of the Przewalski horses, donkey and Grant’s zebra that were dissected.

These ligamentous structures showed longitudinal fibres with a skeletal origin and soft tissue insertion into the medial and lateral branches of the interosseous muscle III (suspensory ligament) dorsal to the sesamoids, similar in orientation to the inferior check ligament. This provided a functional medial and lateral stability to the fetlock joint, similar to the function of the medial and lateral digits in the Mesohippus and Merychippus. This is not generally observed in modern horses who only have one functional interosseous muscle, interosseous muscle III (IM3). This muscle has evolved to contain a large number of strong ligamentous fibres with elastic properties that functionally support the fetlock joint when the limb is either standing, or during locomotion. *(May-Davis, Vermeulen and Brown, 2019)*

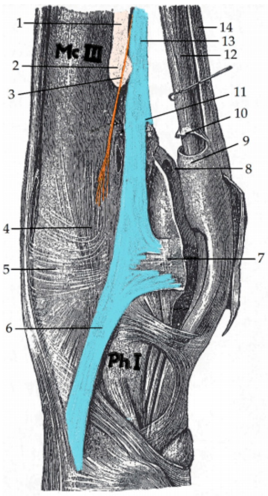


Fig 5: ligaments and muscles around the metacarpal; The metacarpal II and its distal node (2) and rudimentary tendon of the interosseous muscle II (3) are shown. The medial branch of the interosseous III muscle, and its branches are depicted (6). *(May-Davis, Vermeulen and Brown, 2019)*

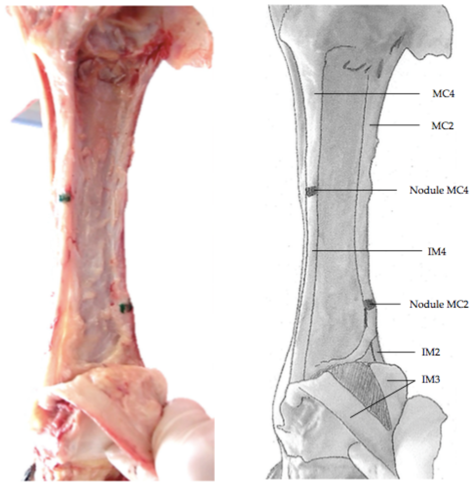


Fig 6: Interosseous muscle II and IV in a Przewalski's horse *(May-Davis, Vermeulen and Brown, 2019)*

<u>Using ultrasound as a non-invasive method to study the aforementioned traits:</u>

For this study, the horses were scanned using ultrasound. Other diagnostic tools exist such as magnetic resonance imagery (MRI) and computed tomography (CT), however, these methods are more stress inducing, less cost effective and more time consuming. Since ultrasound is lightweight and portable it is more suitable for field research. Ultrasound is often used for scanning the soft tissues in the distal limbs. The third interosseous muscle, also known as the suspensory ligament, is therefore scanned when necessary to locate injury *(Ross and Dyson, 2003)*. Hence it is possible to use such method to conclude the presence of IM2 an IM4, or the lack there of. By using the right type of transducer probe at megahertz frequencies it is also possible to study the nuchal ligament lamellae using ultrasounds. The suitability of this imaging method was verified by Sharon May-Davis's group. They first performed an ultrasound scan and then dissected the distal limbs, comparing the two results (Fig 7.) It can be seen that in both cases shown the attachment points of the NLL to C2 to C5, and the abscence of C6 and C7 attachments were already clearly visible in the ultrasound recording. *(May-Davis, et al 2020)*

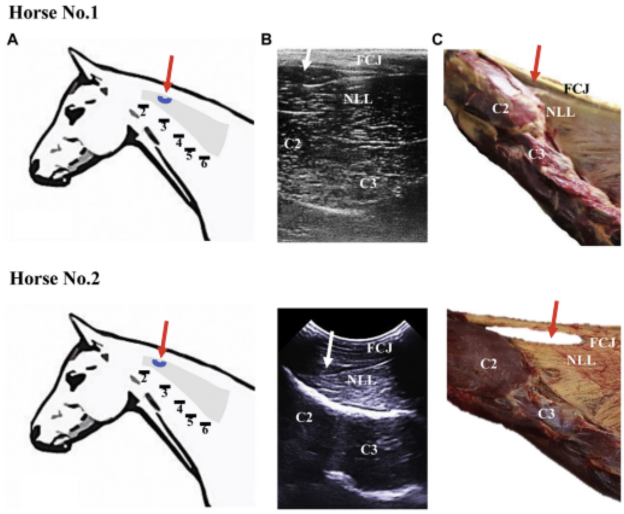


Fig 7: Comparison depicting the similarities with the ultrasound and the dissection. (May-Davis et al, 2020)

Caspian Horse:

The Caspian horse breed is an old breed of horse dating back to Persia as far as 3,000 B.C. Friezes, artefacts, ancient writings showed that small elegant horses were the favourites of Persia's kings. King Darius the Great used them to pull his chariot in lion hunts and honoured them on his Tri-Lingual Seal, ca. 500 BC. Wars between Islam and Mongolia destroyed most of the great libraries and museums, which meant that after the 7th century AD there is no further recorded trace of these horses. It was assumed that the breed had gone extinct (Caspian Horse, 2020)**.** Until Louise Firouz, the American-born wife of an Iranian aristocrat, re-discovered them while searching for a suitable riding pony for her riding school in 1965**.** Back then they were locally known as 'Mouleki' or 'Pouseki' ponies (little muzzle) used as pack or cart-horses *(Silvers, 2015).*

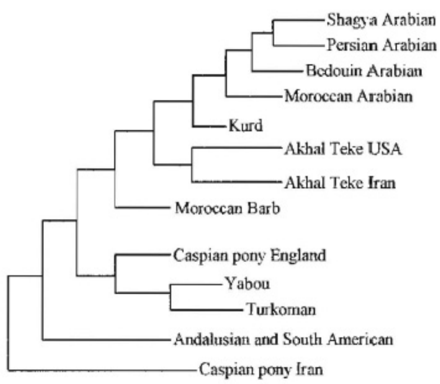


Fig 8: Family tree of the Caspian horse in regards to the other oriental and Iberian horse breeds.The Iranian Caspian horse holds the most primitive position. *(Firouz, 2020)*

Although not proven, some regard the Caspian as the descendants of the similarly structured Mesopotamian horses that disappeared during the 7th century AD." (*VioVet, 2020*) There is however evidence that a small breed of horse has existed in what is now known as Iran from at least the 4th millennium B.C. to the present. There have been genetic, osteological and graphical foundings that illustrated a small, *Equus caballus* whose influence on the development of the oriental horse has been instrumental in determining its unique characteristics *(Firouz, 2020).* Therefore the Caspian horse is considered the original horse type 4, known as the ancestral line of the Arabian and thus most hot-blooded breeds of horses and ponies that are known today. *(Caspian Horse, 2020)*

The skeleton of the Caspian horse was studied and compared with the Turkoman horse, Plateau Persian and Percheron. They have found four basic differences between the Caspian and the other breeds studied.

1) the Caspian's skull possess no Parietal crest
2) The scapula is wider, forming an isosceles triangle, more closely resembling that of a ruminant.
3) The metacarpal and metatarsal bones of the Caspian are much longer and slimmer
4) The first six thoracic vertebrae of the Caspian are longer, generating higher withers than their croup. *(Firouz, 1970)*

In the Netherlands, the Caspian horses are being breed at a stable called "Levende Landgoed NOVA" where the owner Nanda Smit-le Poole breeds them with the specific goal to keep the original bloodlines alive. Currently owning 27 Caspian mares, stallions, gelding and foals of varying ages.

Problem definition:

Since the first horses have been domesticated, over 500 different breeds of horses have been documented. Through the years their tasks and the breeding goal have varied in correlation with humanities needs for these animals. Today horses are used primarily for sporting and recreational purposes and are bred with the goal to be the very best in the specific sport they are used in. These breeding goals are based greatly on visible characteristics, such as size, character and visible genetic defects. However, not all genetic defects that have occurred due to breeding are visible, such as traits like the NLL and IM2 and IM4 described in the introductions. These changes can effect the horse's stability, and core strength in ways not fully explored. Through looking into the breeds that do and do not possess these traits and what it means in terms of health for these breeds, veterinarians and breeders can be better informed. If breeders are better informed they can be more aware of the effect their breeding goals have on their horses. Furthermore, medical professionals can potentially fine-tune their therapies in order to better help the horses by compensating for such changes. The Caspian horses have had a great part in the creation of many oriental horses and can potentially be part of the bloodline of many sport horses who also have Arabian blood, therefore studying the aforementioned traits in this breed is a step in learning how to help the modern breeds.

Research objective:

The objective of this study is to determine whether Caspian horses at "Levende Landgoed NOVA" have the following primitive traits; full attachment of the Nuchal ligament lamellae (C2-C7) and the interosseous muscles II and IV. In order to gain more insight into which breeds still have these traits and what this means for the modern horse breeds.

Main research question:

1. Do Caspian horses possess the primitive traits of having the lamellae and the interosseous muscle, and do these have an effect on their health and stability?

Sub research questions:

1. How many of the Caspian horses at the "Levende Landgoed NOVA" have a NLL attached from the second cervical vertebrae to the fifth/ sixth or sevenths vertebrae?
2. How many of the Caspian horses at the "Levende Landgoed NOVA" have IM2 and IM4 present in their distal limbs?
3. Is there a difference in the amount of Caspian horses that have full NLL and IM2 and IM4?
4. Is there a difference between the sex of the individual and the presence of IM2 and IM4?
5. Is there a difference between the type of trait they possess and the medical history?

<u>Literature review:</u>

History of the Caspian:

In 2017, a study performed by Fages and colleagues looked at the Y chromosomes in stallions to determine the main ancestor. In this study, they screened a region of the male-specific region of the Y chromosome (MSY) in 52 horses from 21 breeds. They state that most modern horse breeds come from centralised and organised horse breeding over the past few hundred years, and have been strongly affected by in-breeding and line breeding traditions. A trend, up until the 18th century, in central Europe was also to improve local herds by introducing "Oriental stallions". These oriental breeds were mainly Turkoman and Arabian stallions. With the exception of some northern European breeds, all modern breeds are clustered together in a group roughly 700 years-old, which came to Europe through the importation of these Oriental stallions. Their study also showed that especially the English thoroughbred's MSY was derived from Turkoman lineage. In turn, the English Thoroughbred had a great part in spreading this haplotype onto modern horse breeds. (*Wallner, et al, 2017)* It has also been suggested that the candidates for the genetic origin of modern domesticated breeds of equids are placed in the Pontic-Caspian steppes, Anatolia and Iberia. *(Fages et al, 2019)*

Archaeological sites found in the Pontic-Caspian steppes related to the early, to middle Holocene found bones of three species of equids, the Onager (*Equus hemionus),* wild ass *(Equus hydruntinus),* and the (domesticated horse) *Equus caballus*. The *Equus caballus* ranging across both Caspian Depression and the Black Sea steppes. *(Anthony, 2007)* Another study had assembled temporal and geographical information of 3070 horse occurrences across Eurasia, over 1120 archeological layers in Europe and based on this looked at the potential centres of domestication, not taking note of climate change being a potential driver for horse population dynamics. Three domestication centres were proposed, central Asia, eastern Anatolia, and Iberian Peninsula. The latter region was found to be very suitable for horses throughout the entire Holocene, while the Pontic-Caspian steppes showed high values. This meant that, while not considered suitable for European horses that existed around the time when horses were first domesticated, it can still be considered that a part of the region would have been suitable for the Asian horses, whereby the Caspian Sea presented the westernmost boundary. This could suggest that domestication started with a population with a background related to Asian horse breeds. This would have meant that the spread of the domesticated horses into Europe involved either adaptation, potentially through selective breeding or through domestication techniques used on local horse populations pre-adapted to these environmental conditions. *(Leonardi et al, 2018)*

The tree topology shown below in Figure 9, was created using 16.8 million transversion sites in 278 ancient equids, not taking into account potential migration. The name of each sample provides the archaeological site as a prefix, and shows how many years ago the specimen lived as a suffix. Suffixes (E) stands for European breeds, while (A) Asian breeds of ancient horses. The Caspian horses can be considered under ancient DOM2 remains found in Persia. *(Fages et al, 2019)*

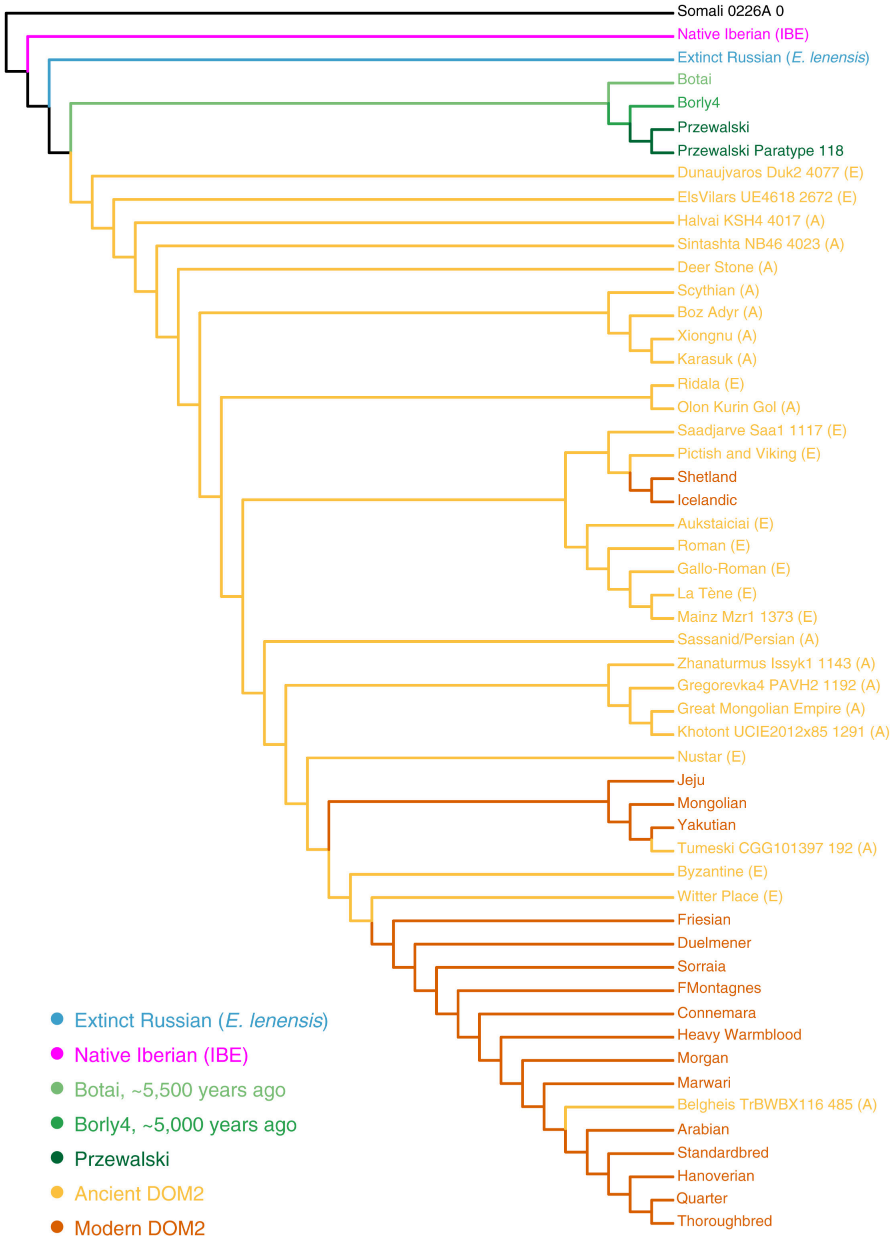


Fig. 9: Tree topology of horse breeds *(Fages et al, 2019)*

Certain modern European native breeds (for example the Friesian, Duelmener, Sorraia, and Connemara) were found to belong to a single group that was first seen to appear in Europe in the 9th century at Nustar, Croatia. At that time, it was not yet seen in northern Europe. This would suggest the introduction of new domestic lineages to the southern mainland of Europe between the 7th and the 9th century. It was at this time that eastern Europe saw the peak of Arab raids, affecting Croatia greatly. This would suggest the the oriental bloodlines began having a growing influence at this time in mainland Europe. This theory is further strengthened by the fact that the earliest identification of this group can be traced to two Sassanid Persian horses from Shahr-I-Qumis, Iran (4th till 5th century). *(Fages et al, 2019)*

Dr. Gus Cochran of Kentucky University found while researching international horse breeds concluded that the Caspian horse and the Turkoman existed from 3,000 BC, and he claimed with almost certainty that they are a direct ancestor of all oriental horses such as the Arabian. The Caspian is the base breed or breeds such as the Yabou (another breed native to Iran that inhabited the same area, and the Akhal Teke, which is closely related to the Turkoman. *(Dalton, 2000)*. A study looking at the genetic material of the Persian Arabian horse and has compared it to other

Iranian breeds among which the Turkoman and the Caspian. Their results also confirmed a distinct population structure between Persian Arabian, Turkoman and Caspian horse breeds. *(Sadeghi et al, 2019)* With the Caspians connection to the Arab, it can also consequently be said that it had more or less influence in British modern breeds such as the Welsh, the Connemara, the New Forest, and smaller influence on the Dartmoor and the Highland. Furthermore the Arab horse played a part in the creation of modern breeds in Rusiia, Eruope, Australia and America. The lipizanner horses can also be traced back to the Arabian stallion, Siglavy. *(Dalton, 1999)*

Fig 10: Painting from the tomb of Rekh-mi-Re circa 1450 BC. *(Littauer, 1971)*

Through studying stone carvings, in bas-reliefs, seals and rhythms throughout Iran's history, archaeologists have been made aware of the existence of a small equid with a head structure, not resembling the norm. However, it was widely accepted that the small size of the equids was merely due to lack of space. While, this was true when it was a way to make it possible to differentiate the status of two or more figures. Nonetheless this could not have been considered the case on statuettes or other pieces meant to tribute military animals of completely different sizes on the same relief. For example, the trilingual seal of Darius the Great (ca. 500 B.C.), which can now be found in the British Museum, shows a pair of tiny Equids with very slim legs, small ears and slightly convex faces pulling the royal chariot on a lion hunt. With the finding of the Caspian horse in 1965 by Louise Firouz, this breed could be considered living evidence that it was indeed a whole different breed and not an artistic choice. The miniature animal found on the aforementioned historical artefacts can be seen roaming from the Zagros Mountains in Iran long before the domestic horse was introduced by the Indo-Europeans. *(Firouz, 1969)*

It is generally accepted that domestic horse was an Indo-European import sometime between the latter part of the third millennium and the beginning of the second millennium B.C. Whilst this can be considered the case, it can now also be assumed that in Iran domesticated horses already roamed these areas. There is also evidence found for this by various archeologists such as those bones dug up in Iran by Carleton Coon and Bökönyi. Although, it cannot be said for certain which species of Equid the bones belonged to, as the bones were not sufficiently examined. The equid species were identified on the basis of the slenderness index. In this index archeologists compared the length and width of the bone. However, Bökönyi pointed out that "with certain metapodials it cannot be decided by mere measurements whether they (the bones) originate from horses or from asses." *(Bökönyi, 1968)*. It could especially have created complications in identifying the species in the Middle East and the Russian Steppes. This lead to controversy amongst zoologists for many decades. Leading to the possibility that the onager bones identified in sites from the Mesolithic through the Sassanian period in Iran did not all belong to onagers, but some of these may have been bones of horses with thinner metacarpal and metatarsal bones. Stone carvings, especially in Persepolis, show the horses that lived in the Achaemenian period possessing well developed foreheads. The powerful head of the Nisaeans show a pronounced swelling beginning at the occiput and extending through the parierals, frontal and nasal bones. Whilst there are certainly other breeds that resemble the structure of the head of the Nisaean, the "Lydian" ponies have been found to have the vaulted development only of the forehead, giving them a typical "Arab" look. In the present this head formation is only visible in the Caspian. No other breed has been found to possess this degree of similarity, not even the Arabian. There is evidence of continuity for the Caspian through the head formation, the dense slim bone, narrow hoof and size similar to that of the Nisaean. *(Firouz, 1972)*

Originally, the Caspian miniature horse was classified as a member of ponies, however, later on studies and comparisons have shown it to be a miniature horse. (Rezaian, 2006) While the distinction between a pony and a miniature horse can be vague, the classification is based on the conformation, gait and the character of the animal. The following characteristics place it under the title of a miniature horse. These horses stand from 100 to 120 cm at the withers. Their conformation resembles that of a horse. In terms of their character, Caspians generally have what can be considered a gentle disposition. They are intelligent and willing to work. Stud stallions have been regularly ridden in Iran by small children in groups and released afterwards in the same pasture without issues. In terms of their gate, Caspians are able to keep up with horse of larger proportion at all gaits except the rapid gallop. They have, what can be described as long walk, with the occasional natural single foot. During trot, their legs create a long swinging, far-reaching action. Their canter can be considered smooth, and they possess a rapid flat gallop. In short, the Caspian can be described as a small, yet well-proportioned horse. *(Firouz, 1972)* The Caspian horse originated from the Albourz mountains in Iran, therefore their movement can be considered almost deer-like as they had to scramble between rocks and browsing amongst spars shrubbery. *(Dalton, 1999)*

Other properties that are more specific for the Caspian are very important in order to later establish the importance of our findings. Such as, the development of the head is pronounced by the parietal bones, as well as the frontal bone, going from the occiput to the beginning of the nasal bones. This characteristic gives the appearance that the ears are set farther back on the head than they are. Some of the horses also have, what can be called an 'up-raised' nose which is caused by a marked development of the superior maxillary bones. *(Firouz, 1969)*. The ears do not exceed 11 centimetres. Their neck is slim, the shoulders sloping. Furthermore they have a straight back, the body has a thin frame, ending in a high-set tail. The legs are slim with dense bone structure. The average circumference of the foreleg, as can be measured just below the knee, is around 14 cm for stallions and 13 cm for mares. The hoofs have an oval-shape. While the Arabian was considered the original type 4 horse, in figure 11 it can be seen that this is actually the Caspian. *(Dalton, 2000)*

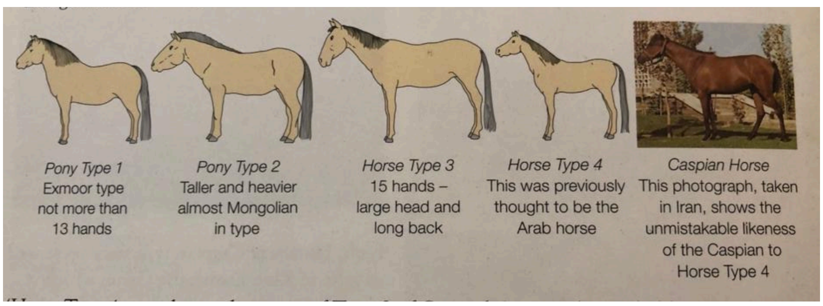


Fig 11: Depiction of the four horses with an image of a Caspian horse next to type, illustrating the likeness. *(Dalton, 2000)*

A three-year survey was conducted by Louise Firouz and her team, that begun in July, 1965. This survey covered a great stretch of Iran within sixty kilometers of the Russian border and the Turkoman Steppes. They discovered a rather small number of 54 horses. With the major concentration being about 30 in a 3,400 square km triangle. The individuals found during this survey were so scattered, that they concluded it impossible for any of the remaining Caspians to be considered the pure form of the breed. *(Firouz, 1972)* They observed that the Caspians rarely roamed together, it was therefore the question how they reproduced in the semi-wild. Louise concluded that the "Caspian was strong genetic recessive, which occasionally bonded amongst two phenotypic mongol-type horses to produce a phenotypic Caspian which, when bred with another Caspian produced genotypic Caspian." *(Dalton, 1999)* In April 1965 six Caspian mares and five stallions, were selected and have been used in a pilot project to preserve the breed, a project that extends till today to many parts of the world. *(Firouz, 1969)* Between 1965 and 1998 there close to 700 pure bred Caspians registered. *(Dalton, 2000)*

There are other traits observed in the Caspian that differentiate them from modern breeds. One such study looked at the Hyaline cartilage in the tongue of the Caspian, or rather the absence of this structure. They found that although the shapes of the papillae in Caspian horses were similar to modern horses, their distribution throughout the tongue were differing slightly. *(Rezaian, 2006)* Nickel et al, 1979 suggested that the root of the tongue in horses is free of filiform papilla, whereas in this study the findings showed filiform papilla in the Caspian, at the root of the tongue. The Caspian also seems to possess different types of salivary glands presented at the body and root. The difference of greatest interest for this study was the absence of hyaline cartilage in the special lingual structure of the tongue of the Caspian horse. This structure is observed in other breeds of horses mid-dorsally, under the body of the tongue. *(Rezaian, 2006)*

Another difference observed during breeding is in the growth rate of the Caspian foals. The greater part of their height will be reached within six months, after which growth in height is practically inconsequential. The development after six months mainly carries on in width. Sexual maturity is reached in foals, both in colts and in fillies, at around 18 months of age. *(Firouz, 1972)*

A preliminary comparative anatomy study compared the bones of the Caspian, Turkoman, Plateau Persian and one Percheron skeleton. They concluded that there were five basic skeletal differences between the Caspian and all other breeds of horses studied:

I. The Caspian's skull shows a pronounced elevation or bulging of the inter-parietal and parietal bones. Most distinctive of all, being that the Caspian possesses no parietal crest, the inter-parietal continuing unbroken to the nuchal crest of the occiput.
II. The scapula of the Caspian is wider than in other Iranian breeds, the neck of the scapula is narrower and the head much wider. It was noticed that that the scapula of the Caspian more closely resembled that of a ruminant than that of a horse.
III. The metacarpal and metatarsal bones of the Caspian are much longer and slimmer, in comparison with the height of the horse than those of other breeds.
IV. In the Caspian, the spinous processes of the first six thoracic vertebrae (N.1 to T.6) show a more pronounced elongation when comparing it with other Iranian breeds. Because of this difference the withers of the Caspian are much higher than the croup.
V. The hoof of the Caspian being narrow and oval-shaped, resembles the hoof of *Equus asillus* more than it does that of *Equus caballus*. *(Firouz, 1972)*

Another oddity has been found through analysis of the Caspian horse's DNA. This study revealed that a third of the investigated horses of this breed have 65 chromosomes. In Equids odd number of chromosomes can be found in hybrids such as mules and hinnies. These animals are, however, infertile due to an inability to create a homologous pairing during meiosis *(Makino, 1955)*. This is not the case in the Caspian horse, albeit it is considered an inefficient breeder. A number of animals in this study possess a modal number of 65 chromosomes, a number between that of the 64 chromosomes found in the *Equus caballus* and the 66 that can be seen in *Equus przewalskii*. Through looking at the form of the chromosomes and their G-bands, similarities have been found in the chromosomes of the Caspian horse to both the *Equus caballus and Equus przewalskii*. Therefore we can conclude based on the historical background and the genetic analysis of the Caspian horse that this breed is the outcome of natural hybridization between E. caballus and E. przewalskii. (Hatami-Monazah et al, 1979)

The history of the Caspian horse can be summarised in the chronological timetable in Figure 12 depicted below

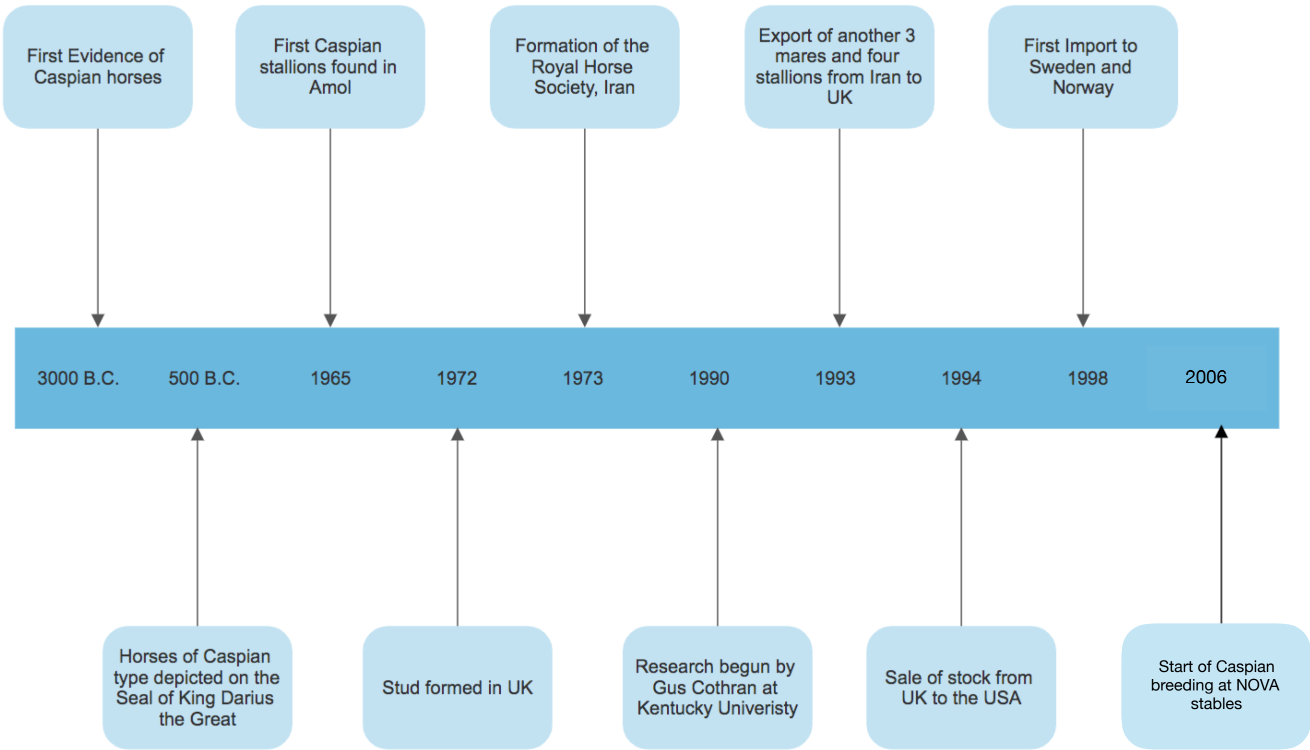


Fig 12: Chronological timetable depicting the history of the Caspian breed *(Dalton, 1999)*

Equine Nuchal ligament Lamellae:

The head and neck segments of the horse represent approximately 10% of the animal's total mass. *(Buchner et al, 1997)* An important contributor to motion in this segment is the nuchal ligament, a highly elastic ligament consisting of two parts. Dorsally located is the cord-like funicular nuchae, and ventrally the flat lamina nuchae. The funiculus extends from the edge of the occipital protuberance to the summit of the 3rd, 4th or 5th thoracic vertebrae where it continues through the supraspinous ligament till the sacrum. The part of the funicular at the base of the cranium is an oval cord that passes dorsally along the atlas and axis with no actual attachment. It then flattens in the mid-neck region and takes on a para-median position that carries on at the withers to the ends of the thoracic spines so that it almost makes contact with the scapular cartilages on either side. (*Budras et al. 2005)* The attachment of the NLL varies, originally depicted in veterinary anatomy books as having attachments over all cervical vertebrae with the exception of the first, while recent findings show it to be mainly found from the cervical vertebrae C2–C5 *(May-Davis and Kleine 2014)*. The

NLL is highly developed in many artiodactyls, but can be rudimentary or absent in birds, carnivores and primates. *(Gellman and Bertram, 2002a)*

The importance of this structure is realised for certain movements such as grazing, getting up or down from the ground, flexing, extending and rotating. *(Neck Issues — Burlington Equine Veterinary Services, 2015)* In ungulates, it is considered to assist grazing through raising the head after feeding. This occurs due to the stretching of the nuchal ligament, which allows the head to be lowered. When it is raised, the ligament returns to its previous length. (*Gellman and Bertram, 2002b*) However, if the grazing position was achieved simply by stretching the nuchal ligament, it would assumably be stretched beyond its range of elastic compliance. *(Gellman and Bertram, 2002a)* The equine neck contains various structures that play an important role in the musculoskeletal system. Its connection with the limbs is often overlooked. Nevertheless, a study by Brunsting et al, looking at acute instability of the nuchal ligament following cervical neuromuscular dysfunction in a dressage horse found that the occurrence of lameness in horses with a diagnosed back and neck problem was much higher than in animals solely with limb lameness *(Brunsting et al. 2017)*

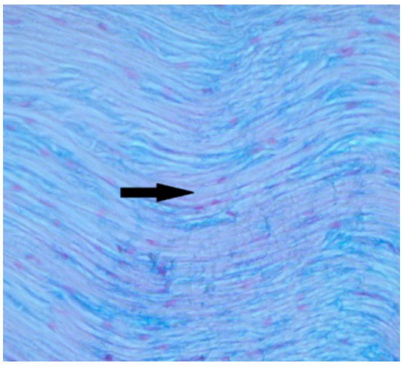


Fig 13: Histological image stained Alcian blue of the nuchal ligaments in horses. It contains regular bundles of collagen fibres as shown by the black arrow. (Lazarczyk et al, 2020)

Muscles actively elevate the head and neck, while the nuchal ligament does this passively. As a result of its elasticity it can passively relieve the dorsal extensors of the neck, thereby participating spinal movements on the median plane. The nuchal ligament aids in counterbalancing the cervical vertebrae in order to bring the forelimb forward in counterbalancing, working together with the hypaxial cervical muscles, the brachiocephalic and omotransversarius muscles *(Schmidt et al, 1982)*. Hence, this ligament helps alleviate the work of the muscles that are needed to hold the head, as well as create a plausible link between the motions of the limbs and that of the head and neck segment *(van Weeren, 2016)*. The stretch and recoil mechanism of the nuchal ligament can also be shown to assist the forward propulsion of the head and the neck during movement, particularly in the cervicothoracic region. *(Lazarczyk et al, 2020)*

The nuchal ligament is built up of fibrous connective tissue; collagen and elastin. These fibres are susceptible to distention. While the ligament is characterised by a high resistance to damage, with time, degeneration or fragmentation can occur thus losing its structure. *(Dowling et al, 2010)* The Nuchal ligament (NL) found in ungulates contains highly extensible biological polymer elastin, 80% elastin cogent by dry weight. It has a yellow colour and can extend long-range reversibly. However, when looking at the tissues found in NL, they differ from those of purified elastin. It has been shown in a study using electron microscopy and freeze-etch studies of the bovine NL that the elastic structures are surrounded by a matrix of interwoven collagen fibrils. This structure can be found lying parallel to the main ligament axis, along with periodical links to the elastic fibres. The authors conclude that the nuchal ligament may be considered a composite material composed of stiff fibres (collagen) connected by an elastin sturctures. *(Morocutti et al, 1991)* The movement of the NL is the result of the combined properties of these components. Elastic strain energy is stored in this ligament. The NL may therefore play a part in reducing the energy required to be exerted by the muscles that create the head movements as seen at varying gaits. The NL reduces the force needed to be exerted by the muscles potentially by a factor of 31% to 55% at the different gaits. If this was not the case, an animal of this size would not be able to deliver sufficient metabolic energy to their limbs in order to carry out the movement. In the extremely upright positions (< 60°), the NL was slack and unstrained. When lying down, the neutral position of the head and neck segment tended to be approximately 55°, the NL is then relaxed. When studying the movement of the head, the more caudal portions of the lamellar ligament underwent the least amount of strain. Nevertheless, it is plausible that should it be needed, these segments of the neck still play a role in increasing the energy storage of the NL, by a factor of as high as 20%. The NL is capable of keeping head and neck segment upright, against the forces of energy, and also aid in the correct head movements needed by senses in order to remain alert, or bend down to feed. *(Gellman and Bertram, 2002b)*

Although both elastin and collagen have visco-elastic properties, the strain rate induced by movement seen in the head and neck segment during locomotion is relatively slow. When placed under lower strain, its elastic fibre components are mainly in use. In contrast, when the NL is under high strain levels, its collagen components take a lead. At which point the structure resembles more the structure of a tendon. Such high levels of strain are, however, not likely to be seen. The possibility of high stiffness may still have its importance, in preventing that other structures found in this region would become overstrained. (*Gellman and Bertram, 2002a)*

What is known about the differences in attachments points of the NLL is fairly limited. A study done in 2018 by May-Davis et al, however worked towards giving as detailed an overview as possible of variances found in various breeds. They looked at 14 modern breeds of horses, out of which, all specimen had no attachment at C7 and two had attachments at C6, with the exception of the Bosnian mountain horse who had attachment from C2-C7. Furthermore, out of five "ancient breeds" of horses only 1 had a full nuchal ligament lamella from C2-C7, the rest had attachments only till C6. 2 of the 4 Dutch Konik horses that were considered a primitive breed had presented with the nuchal ligament lamella on C6 and C7. In *Equus przewalski*, *Equus asinus* and *Equus quagga boehmi* all showed attachments at C6 and C7 of the nuchal ligament lamella. In *Equus przewalski* and *Equus quagga boehmi* the attachments to C6 and C7 were clearly present, showing strong cord bands of bundled fibres. In *Equus asinus* the NLL appeared as a flat sheet of elastic fibres attached from C2-C7. The Spinalis dorsi attached to the fibres of the NLL, when the NLL was found attached to C6 and C7. The attachment between the NLL and Spinalis dorsi muscle could be seen as a very strong connective bond along the digitation of the NLL, going up until the dorsal edge of the Spinalis dorsi. The muscle had no other attachments to any other structures except the cervical vertebrae. *(May-Davis et al, 2018)*. The two breeds that were found to have a full NLL, Dutch Konik and Bosnian Mountain horses, have a common ancestor, the now extinct Tarpan (*Equus ferus gmelini*). *(Pasicka, 2015)* Dutch Koniks, were also found to have a unique splint bone asymmetry. This trait can be considered an adaption due to difference in soil found in the forest that they inhibited. *(May-Davis et al, 2018)*

A research from the Clayton group examined intersegmental vertebral motion during dynamic motion of the neck. Their findings showed that the largest difference in the motion of the cervical vertebrae during cervical flexion was observed at C1 and C6. *(Clayton et al, 2010)* In a subsequent study they showed further evidence that during later stages of the bending of the cervical region created the largest changes in the intersegmental angles at C6. Furthermore as the horse's chin moved further caudally, bending in this region increased. *(Clayton et al, 2012)* A potential reason for these cervical motions could be due to the lack of the nuchal lamellae at the C6 and C7, which compromises the stability of these cervical vertebrae during normal locomotion. While it has not been studied, a potential problem that this could result in is arthropathy in the caudal cervical articular process joints (APJs), as anatomical studies have reported APJs in horses of any breed older then 4-5 years in the caudal segment of the NL *(Ross and dyson, 2011)* Furthermore, the study by May-Davis et al. from 2018 showed that the Spinalis dorsi muscles attach to the nuchal ligament lamellae when it is present on C6 and C7. This could suggest that when connected to the nuchal ligament excessive hyper mobility is limited, due to the muscle having a better anchor point. *(May-Davis et al, 2018)* In humans, a link has been drawn between hyper mobility and premature development of degenerative joint disease. *(Kirk et al, 1967)* While it is speculation, this would correspond to the reported osteoarthritis in the caudal cervical APJs. The horses studied with this type of APJs were modern domestic breeds, that have been shown to lack attachment of the NLL at C6 and C7, which can lead to a correlation. *(May-Davis et al, 2018)* Nevertheless, better understanding of the anatomy and history of the nuchal ligament lamella, can lead to further understanding of issues in the neck region such as arthropathy in the caudal cervical vertebrae. *(May-Davis and Kleine, 2014)*

The NL of the camel has thick lamellae that attach it from the fourth to sixth cervical vertebrae, along with the more cranial cervical vertebrae. When they studied the NL in a camel during dissection, considerable force was needed to hold the carcass down, and the neck buckled sideways. This shows that the NL exerts great forces in order to support the neck while in a horizontal position, meaning the muscles are needed to pull the head down. In the neck of the camel the force seems sufficient in order to maintain balance, even when the neck is extended horizontally forwards. *(Mobarak and Fouad, 1977)* In deer and sheep the ligament does not exert enough force needed to balance the movements of the cervical/thoracic joint when the head and neck segment is in a downwards position. Hence, it can be assumed more musculature would be required in order to hold the head in this position should the NL be absent. The nuchal ligament lamellae in a deer has an attachment to the second, third and fourth cervical vertebrae. The sheep has a strong attachment to the second vertebra, and tenuous attachments to a few others. The figure below shows three different versions of the attachments of the Nuchal ligament lamellae. In the first depiction, should the NLL not be present at all, the muscles would be forced to use more metabolic energy, making it very difficult for the animal to hold its head upright. The difference in attachment points in the second two diagrams (b and c), allows for differences in how the head is held. Camels, much like horses are larger animals with a greater neck and head segment, which require more aid from such ligaments in order to be held upright. While in Sheep and deer, the fewer attachments have less consequence due to the size of the animals. *(Dimery et al, 2009)*

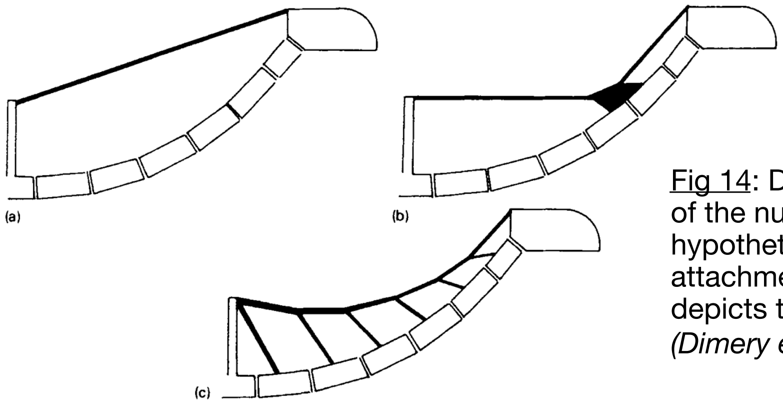


Fig 14: Diagram depicting the attachment points of the nuchal ligament lamella (a) shows a simple hypothetical arrangement, (b) shows the attachment points found in deer and sheep, (c) depicts the attachment points found in Camels *(Dimery et al, 2009)*

Interosseous muscle:

In modern day equids, the fetlock and phalangeal joints are supported by the third interosseous muscle (IM3) and the superficial and deep flexor tendons in the forelimb. The fetlock joint is somewhat overextended while standing still. Although the interosseous muscle is named a muscle, in the domesticated horse it is more a tendinous structure that arises from the proximal part of the third metacarpal, and attaches on the proximal sesamoid bones. *(Budras et al, 2011)* The proximal part of IM3 is partly separated into two main parts that originate from the third metacarpal bones, while in the hindlimb, the IM3 attaches to the third metatarsal bones. It then carries on in the metacarpal/metatarsal groove between the second and fourth metacarpal bones, and it is gradually separated from the palmar aspect of the third metacarpal bone. The point at which it gets separated into branches differs between horses, however, it generally occurs between mid-metacarpus/ metatarsus or the distal quarter of the metacarpal/ metatarsal bones. In the forelimbs, the IM3 is rectangular, strong, and ranging in length between 20 to 25 cm long. In the hindlimb it has a more circular formation, thinner and generally ranges from 25 to 30 cm in length. *(Denoix, 1994)*

When looking at the outside of the IM3, it appears to be like a ligament. Soffler et al found that about 10% of the IM3 is made up of muscle content. The remainder of the interosseous muscle mass is mostly composed of dense connective tissue, called collagen. The collagen allows the interosseous muscle to not get tired as it gives passive stability. It further consist mainly of short muscle fibres distributed throughout the proximal three-quarters of the IM3. Muscle fibres have been found to be surrounded by large bands of collagen. These regions of surrounding connective tissue are similar in appearance to what can be seen in the general mammalian tendon, while containing islands of muscle fibres. *(Soffler and Hermanson, 2006)*

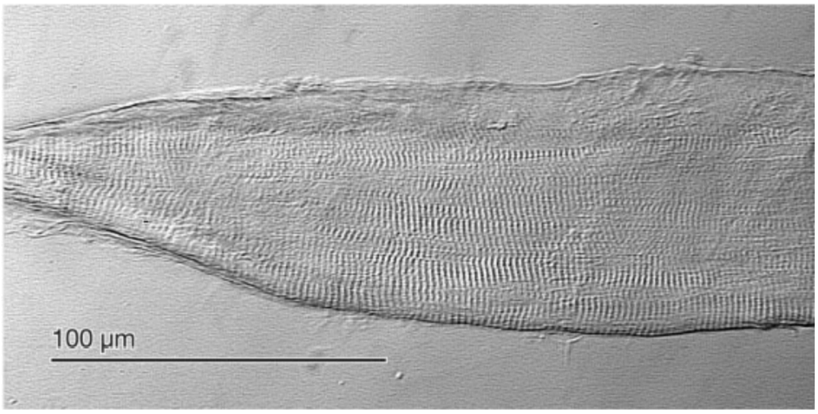


Fig 15: Single equine IM3, after treatment in Nitric acid and stored in Glycerol. When looked at with higher magnification, regular pattern of sarcomeres are visible in the tapered end of the fibre. *(Soffler and Hermanson, 2006)*

Another study by Wilson et al showed a difference in the quantity of muscle fibres in the IM3 in different breeds of horses. They found that Standardbreds have up to around 14% muscle, while Thoroughbreds have 10% of the dry weight. They also found that the amount of training makes a difference in the muscle fibre content. Thoroughbred horses in training were shown to have less muscle content in their IM3 than horses of the same age who where not in training. *(Wilson et al, 1991)* The muscle fibres in the IM3 were also shown to be 95% type I muscle fibres, presumed slow-twitch. Through pulling the distal tendon proximally, it can be seen that the interosseous muscle is able to generate large amounts of force yet it produces only little work. Even though during its evolution the muscle content in the IM3 in equids has been greatly reduced, the muscle content still has an important task. It likely contributes to the stability of the forelimb as well as to elastic storage of energy during locomotion. *(Soffler and Hermanson, 2006)*

The interosseous forms a sling around the fetlock joint that prevents collapse during quiet standing or weight bearing. The interosseous muscle might be greatly effective at preventing collapse, its task when exerting greater amount of force such as during galloping is not yet known. An in vivo study showed around 5% strain in walk and 9% in trot, in ponies. *(Jansen et al, 1998)* It was speculated that change the equine interosseous underwent in order to become predominantly a ligamentous structure started approximately 15 million years ago in the tridactyl Merychippus. This can be considered to be the case, due to the increase in body size and change in their environment to grasslands. *(Camp and Smith, 1942.)*

During the evolution of the horse, as the environment changed, ancestral polydactyl horse also had to adapt, since with the open environment came about the importance of speed and endurance. *(Matthew, 1926)* This then gave rise to the monodactyl horse that exist today. *(MacFadden, 2005)* Through evolution, in the modern horse the IM3 is now considered the only functional interosseous muscle. However atavistic traits sometimes reappear. Atavism is when a certain trait reappears that were seen at a previous point during the evolution of a species. For example, there have been certain reports of atavism in Shetland, Welsh ponies and miniature horses where ulnas and fibulas where found to be fused as well as horses with polydactylism. *(Tyson et al, 2004)*

Another atavistic trait recently found, relevant for this study, was the second and fourth interosseous muscle (IM2 and IM4, respectively) that have been found in certain Equids. To date it has been, shown in four species of Equus, two breeds of *Equus caballus* the Bosnian mountain horse and the Dutch Konik, *Equus asinus* (donkey), the Przewalski and *Equus quagga boehmi* (Grant's zebra).This structure has been described to be strong chord-like bands originating from the distal nodules of the metacarpals II and IV, and metatarsals II and IV. IM2 and IM4 was found to have an insertion point to the medial and lateral branch of the IM3, respectively. The thickness of these atavistic ligaments appears be constant from origin to insertion. Their structure appears to be in line with that of the IM3. The second and fourth interosseous muscle found in this study where shown to have similarities to the inferior check ligament, an accessory ligament of the deep digital flexor tendon. Both ligaments have proximal skeletal origins, insertion points to distal soft tissue, identical orientation and similar function. *(May-Davis et al, 2019)* Another study has also shown a similar structure in miniature donkey, the ligament was found on the palmar surface of metacarpal III with functional capabilities of stabilising the superficial digital flexor tendon (SDFT). They concluded this new found ligament to aid in running long distances, supporting more weight as well as provide additional stabilisation and support of SDFT. *(Nazem and Sajjadian, 2017)* What the Dutch Konik, Bosnian Mountain Horse, Przewalski's horse, *Equus asinus* and *Equus quagga boehmi* all have in common, is that throughout their evolution their main habitat consisted of mountainous terrain, soft pliable surfaces and more specifically, undulating environments where medial and lateral stability of the metacarpalphalangeal joint was necessary. Therefore, it can be postulated that the existence of the second and fourth interosseous muscle in these animals is a primitive trait that has evolved due their environment. *(May-Davis et al, 2019)*

Similar ligaments have also been found in other unguligrades. In the *Hippopotamus amphibious* and Suids (pigs) second, third and fourth interosseous are present. In theses species the axial muscles for the second and fifth toe are reduced, and in the third and fourth toes it is heavily developed. Another species these ligament-like muscles can be found is the *Acrocodia indica* (Malayan tapir). They have two muscles for each of the three toes. The second and fourth metacarpal insert on to the corresponding sesamoids as well as the extensor aponeurosis, while the axial muscles of those toes insert only on the sesamoids and their ligaments. *(Campbell, 1945)* The origin and insertion point in *Hippopotamus amphibious, Acrocodia indica* for the second, third and fourth interosseous muscle corresponds with that found in the aforementioned Equids. It has also been shown that interosseous muscles in the *Lama glama* (llamas) had most in common structurally with the interosseous muscles found in the study by May-Davis et al *(May-Davis et al, 2019)*. In camelid, much like in the modern breeds of horses, the fibres of the interosseous muscles have almost disappeared, leaving tendons running virtually uninterrupted from origin to insertion. *(Alexander et al, 2009)*

The ligamentous interosseous has evolved divergently in at the very least three different artiodactyl lineages and one perissodactyl lineage. This demonstrates their importance for the adoption and maintenance of unguligrade foot posture. *(Clifford, 2010)* Through the various studies done it can be stipulated that functionally the medial and lateral digits in prehistoric tridactyls, were important in stabilising the fetlock joint, and also in increasing agility and maneuverability. In the horses, ancestor, the Merychippus, these digits were also relevant in increasing traction over soft ground and savannas. *(Thomason, 1986)*

Methodology: Data collection

- 22 horses at Levende Landgoed NOVA were scanned. Out of these horses 9 are stallions, 12 are mares and 1 is a gelding.
- The horses were held by an experienced handler.
- Basic information for all horses were written down, including the name, age, sex, medical history (full form to be filled out can be found in Annex 1).
- The neck was sprayed down with 70% alcohol around the C2-C7 area to clean the surface of the skin and allow for clearer imaging. The ultrasound transducer gel was placed on the probe itself, and when needed more was added during the scan.
- The probe was placed in a transversal orientation perpendicular to the neck and parallel over the left TP of C2 and traversed dorsally until identification of the nuchal ligament lamellae attachment to the fan-shaped dorsal spine of C2. The dorsal aspect of the nuchal ligament lamellae on C2 was identified and this digitation followed to where it attaches to the nuchal ligament funicular cord.
- The neck was then further scanned from C2 to C6/C7. The attachment of the nuchal ligament lamella to the dorsal spine of the cervical vertebrae was identifiable between the articulating process joints.
- Next the fetlock joint was identified, the nodules of Metacarpal 2 and 4 will be felt to give an indication. The area is then also sprayed with 70% alcohol and baby oil was added to aid in the scanning.
- Due to time restrictions only the forelimbs were scanned, these are also the limbs that carry the greatest amount of the horse's weight, therefore the potential presence of these extra ligaments is most valid.
- The probe was then placed on a transverse plane so that the interosseous muscle(s) can be evaluated. When the muscles were hard to identify, it was also additionally be examined in the longitudinal orientation.
- Both forelimbs were scanned.
- All findings will be written down to be processed at a later date.

Methodology: Data processing:

Statistical analyses will be performed using SPSS. The data gathered will be nominal data, and the mode, or the most frequent number will be used. A null-hypothesis (H0) will be formulated and an alternative hypothesis (H1). H0 always remains true unless it can be proven with 95% confidence ($P \leq 0.05$) that it is false. In that case H1 becomes true. In order to test differences in the data X^2 test or chi-squared test and a cluster bar chart to depict the information.

Results

1. How many of the Caspian horses at the "Levende Landgoed NOVA" have a nuchal ligament lamellae attached from the second cervical vertebrae to the fifth/ sixth or sevenths vertebrae (trait 1)

Out of the 22 horses scanned all of them (100%) had a full nuchal ligament lamella, meaning all animals had lamella present from second cervical vertebrae till the 7th.

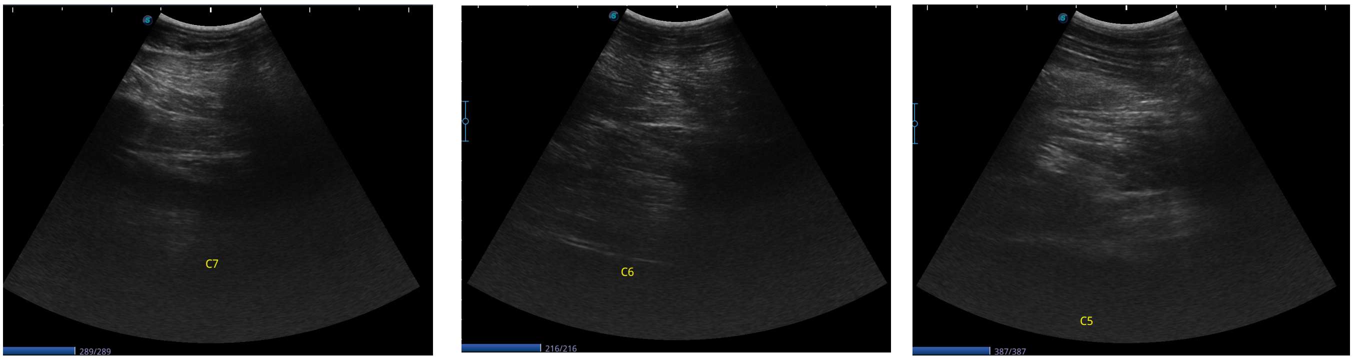
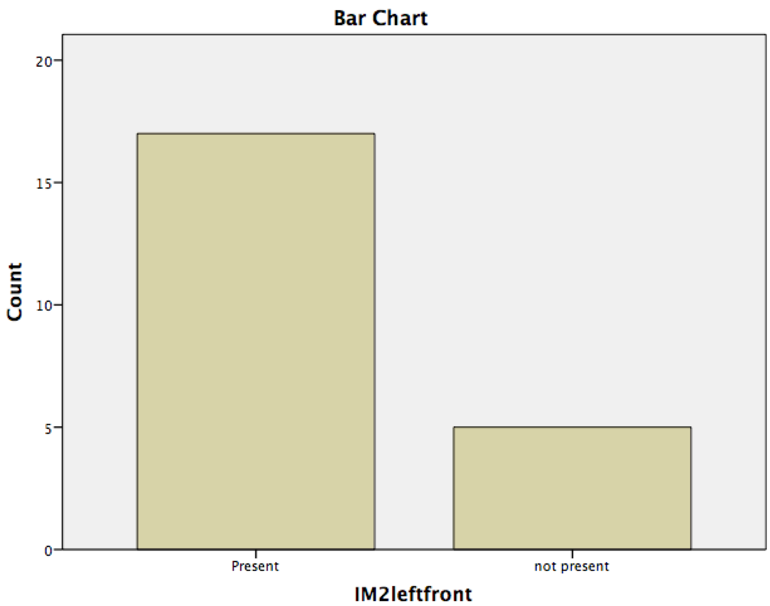


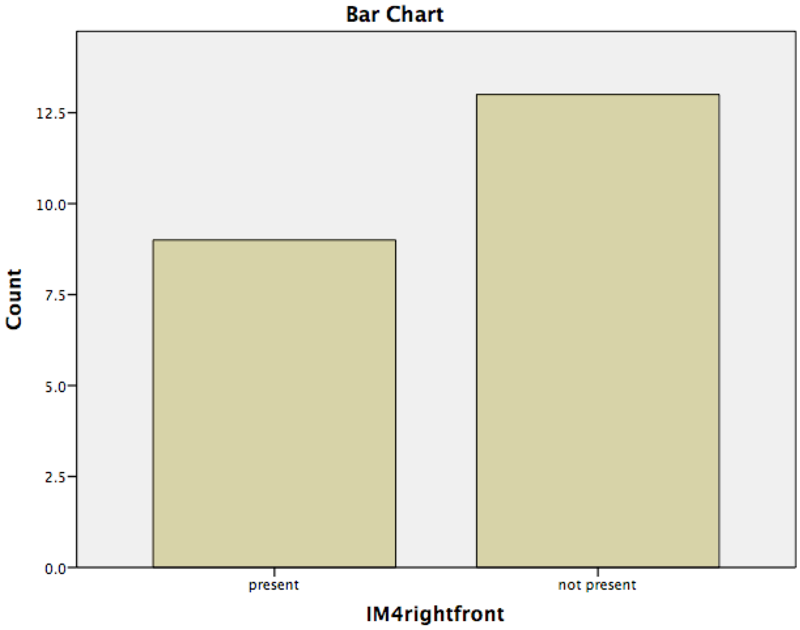
Fig 16: Scan of the Equine nuchal ligament lamella attached at C7, C6 and C5 respectively, in one of the horses studied.

2. How many of the Caspian horses at the "Levende Landgoed NOVA" have an interosseous muscles two and four present in their distal limbs (trait 2).

Out of the 22 horses looked at 17 (77%) had interosseous muscles two present in both forelimbs, and 8 (36%) horses had interosseous muscle four present on both forelimbs and one horse in which the fourth interosseous muscle was only visible on the right forelimb.



Graph 1: displaying the amount of horses with IM2 present and not present



Graph 2: displaying the amount of horses with IM4 present and not present

3. Is there a difference in the amount of Caspian horses that have Nuchal ligament lamellae and the presence of IM2 and IM4?

In order to look at differences chi-squared test is needed. However, since of the horses studied all horses had attachments of the nuchal ligament lamella at C2-C7, this was therefore a constant. No statistical analysis could be carried out.

100% of the horses had NLL attachments at C2-C7, 77% of the horses had IM2 present in both their forelimbs. Therefore the difference is 23% percent in the presence of these traits. While for the difference between the attachment of the nuchal ligament lamellae and presence of IM4 is 64% or when adding in the horse for whom the presence of IM4 could only be seen on one of the fore limbs, the difference then becomes 60%.

The difference between the presence of IM2 and IM4 can be analysed.

H0= there is no difference between the presence of IM2 and IM4
H1=There is a difference between the presence of IM2 and IM4

A chi-squared test was used, however, the results were deemed invalid, because the test showed that 50% of the cells did not have a sufficient number of cases. The table with the Chi-squared test can be found in the annex (Annex 24). Therefore the crosstabulation and the cluster bar chart was used to analyse the results via descriptive statistics.
9 horses who had IM2 present also had IM4 present and 8 Only had IM2 present. All horses who did not

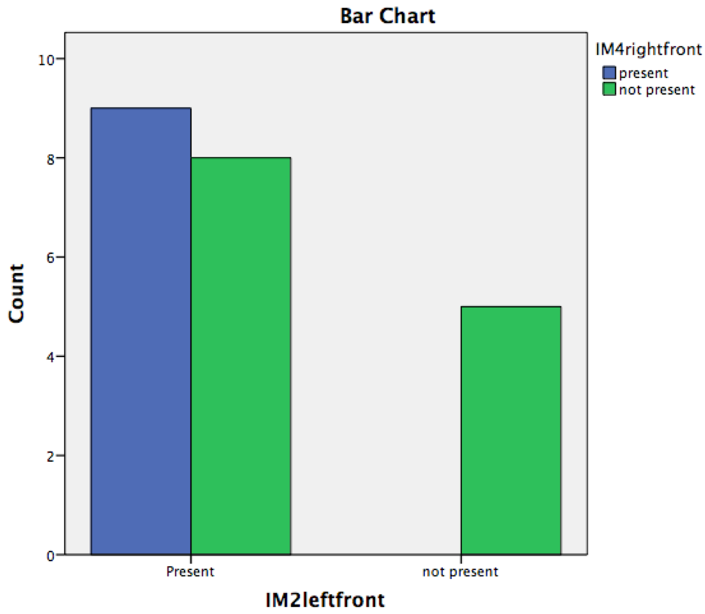
IM2leftfront * IM4rightfront Crosstabulation

Count

		IM4rightfront		
		present	not present	Total
IM2leftfront	Present	9	8	17
	not present	0	5	5
Total		9	13	22

Table 1: Crosstabulation displaying the difference between the presence of IM2 and IM4.

have IM2 also did not have IM4 present. There was a total of 5 horses who did not have both IM2 and IM4 present.



Graph 3: Cluster bar chart depicting the difference between the presence of IM2 and IM4.

4. Is there a difference between the sex of the individual and the presence of IM2 and IM4

H0= there is no difference between the sex of the horses and the presence of IM2
H1= there is a difference between the sex of the horses and the presence of IM2

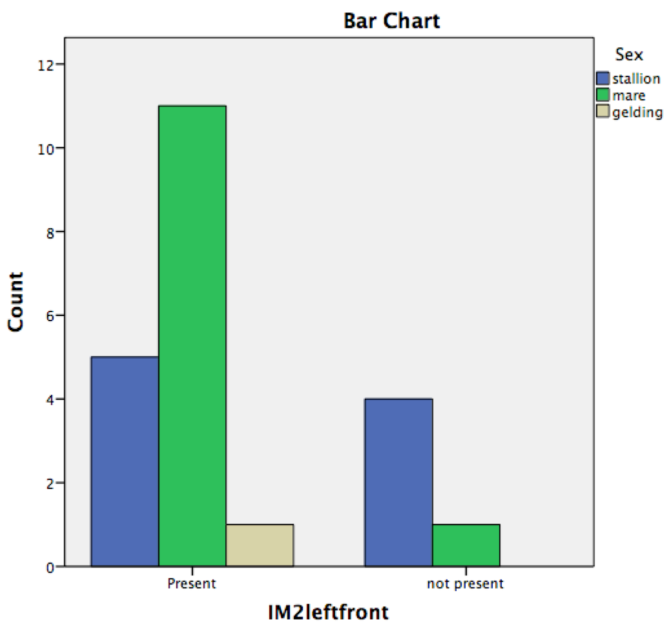
A chi-squared test was used, however the results were deemed invalid, because the test showed that a number of the cells did not have a sufficient number of cases. The Table with the Chi-squared test can be found in the annex (Annex 25).

IM2leftfront * Sex Crosstabulation

Count

		Sex			Total
		stallion	mare	gelding	
IM2leftfront	Present	5	11	1	17
	not present	4	1	0	5
Total		9	12	1	22

Table 2: Crosstabulation showing the difference between the presence of IM2 in mares, stallions and geldings.



Graph 4: cluster bar chart depicting the difference between the presence of IM2 in mares, stallions and geldings.

From the 9 stallions studied 5 had IM2 present, and 4 did not. From the 12 mares, 11 had IM2 present and 1 did not. The 1 gelding studied had IM2 present. Seeing as there was but 1 gelding used for the research, it is not representative. However, manipulating the results does not give a significant change in results.

H0= there is no difference between the sex of the horses and the presence of IM4
H1= there is a difference between the sex of the horses and the presence of IM4

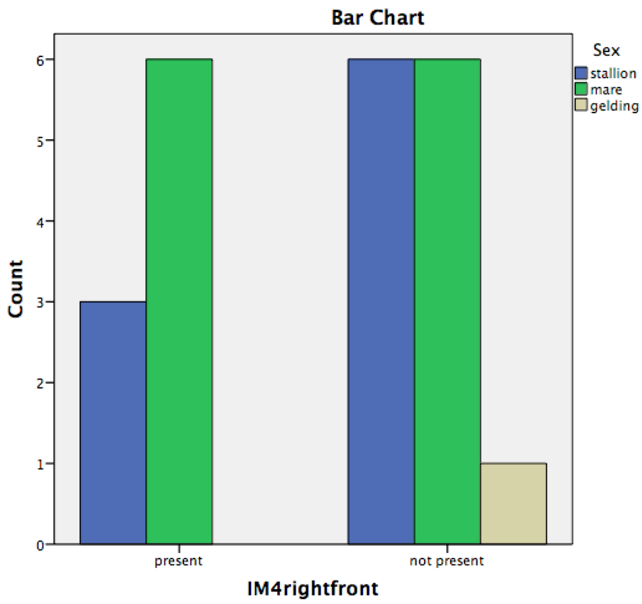
A chi-squared test was used, however the results were deemed invalid, because the test showed that a number of the cells did not have a sufficient number of cases. The Table with the Chi-squared test can be found in the annex (Annex 26).

IM4rightfront * Sex Crosstabulation

Count

		Sex			
		stallion	mare	gelding	Total
IM4rightfront	present	3	6	0	9
	not present	6	6	1	13
Total		9	12	1	22

Table 3 : Crosstabulation showing the difference between the presence of IM4 in mares, stallions and geldings



Graph 5: Cluster bar chart depicting the difference between the presence of IM4 in mares, stallions and geldings.

From the 9 stallions studied 3 had IM4 present, and 6 did not. From the 12 mares, 6 had IM4 present and 6 did not. The 1 gelding studied did not have IM4 present.

5. Is there a difference between the type of trait they posses and the medical history?
There was one horse with a medical history and this horse had IM2 present.

IM2leftfront * Medicalhistory Crosstabulation

Count

		Medicalhistory		
		no medical history	mikotoxines	Total
IM2leftfront	Present	16	1	17
	not present	5	0	5
Total		21	1	22

Table 4 : Crosstabulation showing the difference between the medical history and the presence of IM2

IM4rightfront * Medicalhistory Crosstabulation

Count

		Medicalhistory		
		no medical history	mikotoxines	Total
IM4rightfront	present	8	1	9
	not present	13	0	13
Total		21	1	22

Table 5 : Crosstabulation showing the difference between the medical history and the presence of IM4 right front

IM4leftfront * Medicalhistory Crosstabulation

Count

		Medicalhistory		
		no medical history	Mycotoxins	Total
IM4leftfront	present	8	0	8
	not present	13	1	14
Total		21	1	22

Table 6 : Crosstabulation showing the difference between the medical history and the presence of IM4 left front

The one horse with a medical history had IM4 present in the right forelimb and not in the left limb.

Discussion

In the introduction it was stated that there are 27 horses at Levende Landgoed NOVA, however at the time of research 23 horses were available or this research. During the making of the ultrasounds, for one horse the scans of the legs could not be made clearly and therefore these results were not used for the study. Also, seeing as the Caspian is a rare breed it was not possible to use any more than the amount of horses that were used. To compensate for the fact that the sample was not large enough, descriptive statistics was used to present the results. Nevertheless, crosstabulations and cluster bar charts give an accurate representation of the results. When looking at the results of the research it was found that there was no difference found in the presence of IM2 in the two forelimbs and one horse in which difference was found in IM4. This is the reason why when using the data collected for IM2 for analysis in order to answer the sub research questions, the data collected for the left front leg was used primarily. In the case of the IM4 ligament there was one horse where a difference was found in the presence of this ligament. Namely it was found that the IM4 was present in the right forelimb, and not in the left one. While this could be considered a potential example of the "human error" previously discussed, when looking for the IM4 in the left leg during the ultrasound of this horse the results were inconclusive.

The main research question was whether Caspian horses possess the primitive traits of having the full ligament lamellae and the interosseous muscles IM2 and IM4, and if these have an effect on their health and stability? Based on the genetical and historical research described in the literature review along with the results presented it can be concluded that the Caspian is indeed a primitive breed. The results further support the hypothesis that these traits are indeed primitive traits that have been unintentionally bred out in modern horses. 100% of the horses studied had a full Nuchal ligament lamellae attached from C2-C7. 77% of the horses had either only IM2 or also IM4 visible.

In previous studies two other breeds of *Equus cabballus* were found to have attachment points of the NLL at C6 and C7. The Dutch Konik and the Bosnian mountain horse (May-davis et al, 2014). Out of the 4 dutch Konik horses two (50%) had NLL attachments from C2-C7 and two did not. There was only 1 Bosnian mountain horse dissected for this research. The low amount of both breeds means a true comparison cannot be made in the commonality of this trait in these breeds versus what was found in the Caspian. In these breeds of horse IM2 and IM4 was also found to be present (May-Davis et al, 2019). While in the Caspian the amount that had IM2 present varies from the amount that had IM4 present, when looking at the Dutch Konik horse out of the 15 left fore limbs dissected 13 had both the IM2 and the IM4 present and from the 14 right distal limbs 13 had both IM2 and IM4 present. Also in the *Equus przewalskii*, *Equus asinus* and the *Equus quagga boehmi* in the limbs dissected all horses had either neither interosseous muscle present or both. This difference between the previous research on other breeds and the current one, could have various reasons.

From the horses studied more had the second interosseous muscle present then those who had the fourth interosseous muscle present. However, all horses who had IM4 also had IM2 present. Therefore IM2 could be hypothesised to be a more relevant muscle in the aiding of stability of the Caspian horses over the terrain that they originally roamed in Iran. As the Caspian originally roamed in the Mountains of Iran, the movements that they required in order to move between the rocks was considered almost deer-like (*Dalton, 1999)*. On this uneven terrain, the additional ligaments could have played a role in stabilising the horse. In order to get a better understanding, the effect of IM2 and IM4 on the stability of the horse should be separately analysed. The original research papers about the primitive traits by May-Davis et al discuss the common ancestor of the Dutch konik horse and the Bosnian mountain horses, the Tarpan. They correlated the grounds of the habitat that these horses roamed with the additional interosseous muscles they possessed. Throughout the evolution of the Dutch Konik, Bosnian Mountain Horse, Przewalski's horse, *Equus asinus* and *Equus quagga boehmi* the main habitat consisted of environments where medial and lateral stability of the metacarpi-phalangeal joint was necessary.

When originally found in Iran in 1965 by Louise Firouz, they found a total of 54 horses in Iran and the individuals were spread out over large distances that it was stipulated that from the remaining Caspians found they could not be considered "pure" Caspian breeds. *(Firouz, 1972)* The variations in IM2 and IM4 found, could therefore be a result of the horses used in this research hail from different "types" of Caspian horses in which the genetic material has been mixed with other domestic breeds of horses. Prior to DNA sequencing techniques four basic types of domestic breeds of horses was proposed; the Tarpan, the forest horse, draft horse and the oriental. This was based on the characteristics traits of these breeds due to their adaptations to habitat. *(Bennet, 1998)* While a potentially outdated classification of the horses, the Tarpan and the Oriental horses have clear differences in characteristics and environment, showing that the common link through these primitive traits was relevant for the adaption of both breeds.

Another potential reason for the differences in the results for IM2 and IM4 may be due to human error. These miniature horses posses very thin metacarpal and metatarsal bones (which was one of the reasons that their exact origins cannot be decisively placed, as the size of their bones could be confused with that of an Onager when found in various excavation sites.). This made scanning of the legs a difficult task to perform. In some of the horses, the ligament was very clearly seen and in certain horses it was somewhat inconclusive. While great care was taken to identify the ligaments in the horses leg correctly, this could not be stated with 100% certainty.

The effect of having these primitive traits has on their health and stability, could not be confirmed. With one exception, none of the horses had any known medical history. No instability coming from the neck region, torn ligaments in the legs or any other forms of lameness in the fore or hind limbs. This however, cannot be seen as a direct correlation between the possession of the traits and the health of the horses. Since the Caspian horses used had a low activity level, and lived in a more natural way in a heard in a pasture, this could also aid in minimising the chances of injuries to the ligaments. Despite the fact that no connection could be made between the possession of primitive traits and the medical history, the one horse which had a medical history was also the horse for whom IM4 was only found in one of the forelimbs. This horse had problem with Mycotoxins. This is a toxic by-product of mould that can occur in forages or concentrates. This toxin can result in the suppression of the immune system, thereby weakening the host and can lead to varying symptoms. While this is most likely due to coincidence, it is something to potentially look into in the future. Seeing as it can be considered inconclusive whether the left leg of the horse had the additional ligament or not when analysing the data in the results, it was deemed best to use the data from the right forelimb.

When looking at the differences in the legs the chi-squared test could not be used to illustrate a clear difference between the presence of IM2 and IM4 in the stallions, mare and gelding. In the crosstabulation it can be seen that almost all of the mares had IM2 present and half of the mares had IM4 present, while for the stallions both the IM2 and the IM4 was present in approximately equal amount. This could potentially show sexual dimorphism, however the relatively low availability of individuals and their diverse genetic background does not allow for a clear resolution. Also with the extent of this study it is unfortunately it is not clear whether the sex could have an importance in the passing down of the genetic variations for these traits.

During the research the dynamic patterns of free moving horses was observed. This analysis is not involved, because it would have changed the scope of this study completely. However our preliminary results are in accordance with what has been seen in other primitive breeds in terms of their stability and soundness. This was concluded based on analysis of their movement in walk, tort and canter was observed on a flat soft surface and walking up and down a sloped hard surface. Further analysis and research is still required.

Conclusion

The Caspian horse has been previously identified as an older breed its history dating back thousands of years. With the results presented in this thesis, the hypothesis that besides being an older breed it is also a primitive breed has been verified, showing primitive traits that have been unintentionally outbred through years of selection. 100% of the horses studied had a full nuchal ligament lamella, with attachments from C2-C7. 77% of the horses had IM2, and 36% of the horses had IM4 present. It has been found that out of the horses that had IM4 present all also had IM2 present. We identified a possible marker for sexual dimorphism, as we found differences in the presence of IM2 between the mares and the stallions. With the exception of one mare, all of them had IM2 present. However, for IM4 no such clear difference between the presence of IM2 and IM4 could be seen, meaning that sex most likely does not play a role in the presence of the trait. The connection between the traits and the medical history could not be concluded as almost all horses looked at where healthy, with no known injuries.

Through this study it is unfortunately not made clear where the variations in the presence of the trait in the interosseous muscle in the forelimb comes from. In order to fully understand these variations further analysis of the full genetic correlations would be required.

Recommendation

The theory of evolution and the phrase “survival of the fittest” coined by Darwin, show that through time animals adapt to better survive in their surroundings. With domesticated species, such as the horse, the changes that the breed has gone through cannot be considered adaptions to better survive but rather adaptions to better suit the needs of humans. When looking at modern breeds of horses, cases of lameness and ligament tears are extremely common. Some cases of lameness cannot be explained by issues in the limbs, and studies show various neck problems to be the root of the issues. *(Kirk et al, 1967)* Through treating only the symptoms, the bigger picture can be missed, which is that the horses are not necessarily better off by the breeding plans that are set out in terms of their health. Through looking at the traits, the relevance of the traits, and the extent to which these traits are seen in the primitive breeds, it can give a better understanding of the role of these traits and the implications when they are not present. Till now two members of the *Equus Caballus* have been found to posses these primitive traits. With the addition of this third breed, it gives more a clearer picture of the relevance of these traits. And a comparison of Caspian with the other two breeds found can lead to a further understanding of how these traits effect the horses stance, movement and stability. Through getting a better understanding of the extent of the presence of these traits a better idea can be formed of the extent of their importance, and how to best preserve it.

Another potential variable to research is the connection between the presence of IM2 and IM4 and the height of the horses. This would be of interest because Brenda Dalton wrote down her book titled “The Caspian horse” that there is a height difference in the breed of about 4 hands (41cm). She also writes that “the height factor cannot be explained by better husbandry or feeding, since animals with the same parentage, and under the same management can produce extremes in size.” This change in height could be due to the semi-wild Caspians interbreeding with other larger species of horses such as the Yabous. *(Dalton,1999)*

In order to gain a better understanding of the connection between the potential health risks for horses in further research the medical history of breeds that have these primitive traits can be compared with modern breeds that do not have these traits who are of the same age and training level. In the literature review it was described that while the interosseous muscle is named a muscle, in the modern breeds of horses it contains 10% muscle structure. *(Soffler and Hermanson, 2006)* It would be of interest to look into how the interosseous muscle IM2, IM3 and IM4 in the primitive breeds found is set up, in order to further understand the role of these structures and how they provide support for the limbs.

At Levende Landgoed NOVA a Akhal Teke horse was also present, an oriental breed related to the Caspian. Due to the connection between the breeds, it was of interest to scan this horse as well. Since it is just one specimen of the breed a conclusion could not be made, therefore it was also not added into the results. However, this horse also had a full ligament lamella with attachments at C2-C7 and Interosseous muscles two and four on both forelimbs. This is therefore another breed that should be investigated for these primitive traits. Thereby potentially give a better picture of these traits in the oriental horses.

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Annex:

Annex 1: Table showing overview of the results

		Nuchal ligament Lamellae	IM2 left front	IM2 right front	IM4 left front	IM4 right front	medical history	sex
1	**Parsagha Saadi**	C2-C7	Not present	Not present	Not present	Not present	no	s
2	**NOVA stables Padideh**	C2-C7	Present	Present	Not present	Not present	no	m
3	**NOVA stables Pahlavi**	C2-C7	Present	Present	Not present	Not present	no	s
4	**Henden Kaykay**	C2-C7	Present	Present	Present	Present	no	m
5	**NOVA Stables Key-Khosrow**	C2-C7	Present	Present	Present	Present	no	s
6	**NOVA Stables Yashira**	C2-C7	Not present	Not present	Not present	Not present	no	m
7	**NOVA Stables Yeganeh**	C2-C7	Present	Present	Not present	Not present	no	m
8	**NOVA Stables Yalenka**	C2-C7	Present	Present	Present	Present	no	m
9	**NOVA Stables Yashar**	C2-C7	Not present	Not present	Not present	Not present	no	s
10	**NOVA Stables Taher**	C2-C7	Present	Present	Not present	Present	mikotoxines	s
11	**NOVA Stables Shaka**	C2-C7	Not present	Not present	Not present	Not present	no	m
12	**NOVA Stables Mahnaz**	C2-C7	Present	Present	Present	Present	no	m
13	**NOVA Stables Adinda**	C2-C7	Present	Present	Present	Present	no	m
14	**NOVA Stables Azhar**	C2-C7	Present	Present	Not present	Not present	no	g
15	**NOVA Stables Bahira**	C2-C7	Present	Present	Not present	Not present	no	m
16	**NOVA Stables Azura**	C2-C7	Present	Present	Present	Present	no	m
17	**NOVA stables Quasama**	C2-C7	Present	Present	Present	Present	no	m
18	**NOVA stables Almas**	C2-C7	Present	Present	Present	Present	no	m
19	**NOVA stables Yunis**	C2-C7	Present	Present	Not present	Not present	no	s
20	**NOVA stables Palash**	C2-C7	Not present	Not present	Not present	Not present	no	s
21	**NOVA stables Yasmir**	C2-C7	Present	Present	Present	Present	no	s
22	**NOVA stables Bahadir**	C2-C7	Present	Present	Not present	Not present	no	s

Annex 2: Parsagha Saadi: 

Sex	Stallion
Date of birth	14-4-2006
Body condition score	5
Body weight	245kg
Discipline	Breeding
Activity level	Low
Medicsl history	No medical history
Parents (Mother x Father)	Parsagha Palifeh x Persicus Yussef
Grandparents (mothers side) (fathers side)	(Bytham Pari's Daughter x Bytham Razshir) (Persicus Fershteh x Schrab)
Nuchal ligament Lamellae	C2-C7
Interosseous muscles	Not present

Annex 3: NOVA Stables Padideh 

Sex	Mare
Date of birth	11-6-2010
Body condition score	6
Body weight	256kg
Discipline	Breeding
Activity level	Low
Parents (Mother x Father)	Parsagha Palifeh x Sirhowy Siamak
Grandparents (mothers side) (fathers side)	(Bytham Pari's Daughter x Bytham Razshir) (Sirhowy Assmanjuni x Persicus Kouchek Khan)
Nuchal ligament Lamellae	C2-C7
Interosseous muscles	IM2 both forelimbs

Annex 4: NOVA Stables Pahlavi: 

Sex	Stallion
Date of birth	16-6-2014
Body condition score	6
Body weight	256kg
Discipline	Breeding
Activity level	Low
Medical History	No medical history
Parents (Mother x Father)	NOVA Stables Padideh x Coalacre Shadon
Grandparents (mothers side) (fathers side)	(Parsagha Palifeh x Sirhowy Siamak) (Henden Rosana x Hopstone Shabdiz)
Nuchal ligament Lamellae	C2-C7
Interosseous muscles	only IM2 on both forelimbs

Annex 5: Henden Kaykay: 

Sex	Mare
Date of birth	8-5-2000
Body condition score	6
Body weight	318kg
Discipline	Breeding
Activity level	Low
Medical history	No medical history
Parents (Mother x Father)	Persicus Khorshgel Khanum x Runnymede Ibn Khaldun
Grandparents (mothers side) (fathers side)	(Mariam Khanum* x Zeeland*) (Hopstone Banafsheh x Forstals Barew)
Nuchal ligament Lamellae	C2-C7
Interosseous muscles	IM2 and IM4 on both forelimbs

Annex 6: NOVA Stable Key- Khosrow:

Sex	Stallion
Date of birth	3-6-2010
Body condition score	5
Body weight	220kg
Discipline	Breeding
Activity level	Low
Medical history	No medical history
Parents (Mother x Father)	Henden Kaykay x Coalacre Shadon
Grandparents (mothers side) (fathers side)	(Persicus Khorshgel Khanum x Runnymede Ibn Khaldun) (Henden Rosana x Hopstone Shabdiz)
Nuchal ligament Lamellae	C2-C7
Interosseous muscles	IM2 and IM4 on both forelimbs

Annex 7: NOVA Stables Yashira

Sex	Mare
Date of birth	16-10-2006
Body condition score	6
Body weight	300kg
Discipline	Breeding
Activity level	Low
Medical history	No medical history
Parents (Mother x Father)	Chippendale Yavash x Zarin Taze Majara
Grandparents (mothers side) (fathers side)	(Marida Malak x Cheleken Aval Pesar) (Sirhowy Arziz x Persicus Yussef)
Nuchal ligament Lamellae	C2-C7
Interosseous muscles	IM2 and IM4 Not present

Annex 8: NOVA Stables Yeganeh

Sex	Mare
Date of birth	6-6-2010
Body condition score	6
Body weight	245kg
Discipline	Breeding
Activity level	Low
Medical history	No medical history
Parents (Mother x Father)	Markazi Yasaman x Sirhowy Sjamak
Grandparents (mothers side) (fathers side)	(Chippendale Yavash x Chippendale Salaman) (Sirhowy Assmanjuni x Persicus Kouchek Khan)
Nuchal ligament Lamellae	C2-C7
Interosseous muscles	IM2 on both forelimbs present

Annex 9: NOVA Stables Yalenka

Sex	Mare
Date of birth	23-6-2009
Body condition score	7
Body weight	256kg
Discipline	Breeding
Activity level	Low
Medical history	No medical history
Parents (Mother x Father)	Markazi Yasaman x Persicus Amir
Grandparents (mothers side) (fathers side)	(Chippendale Yavash x Chippendale Salaman) (Taraneh x Zeeland)
Nuchal ligament Lamellae	C2-C7
Interosseous muscles	IM2 and IM4 on both forelimbs

Annex 10: NOVA Stables Yashar

Sex	Stallion
Date of birth	7-5-2017
Body condition score	5
Body weight	173kg
Discipline	Breeding
Activity level	Low
Medical history	No medical history
Parents (Mother x Father)	NOVA stables Yalenka x NOVA Stables Taher
Grandparents (mothers side) (fathers side)	(Markazi Yasaman x Persicus Amir) (Persicus Tehou x Miran Kazamir)
Nuchal ligament Lamellae	C2-C7
Interosseous muscles	IM2 and IM4 not present

Annex 11: NOVA Stables Taher

Sex	Stallion
Date of birth	18-6-2008
Body condition score	7
Body weight	230kg
Discipline	Breeding
Activity level	Low
Medical history	Mikotoxines
Parents (Mother x Father)	Persicus Tehou x Miran Kazamir
Grandparents (mothers side) (fathers side)	(Balsaghar x Secandar Gol) (Costessa Sherifa x Casbrook Kozzar Damzack)
Nuchal ligament Lamellae	C2-C7
Interosseous muscles	IM2 both forelimbs and IM4 on right front

Annex 12: NOVA Stables Shaka

Sex	Mare
Date of birth	31-5-2009
Body condition score	6
Body weight	225kg
Discipline	Breeding
Activity level	Low
Medical history	none
Parents (Mother x Father)	Markazi Shazadeh x Persicus Amir
Grandparents (mothers side) (fathers side)	(Marida Shayad x Chippendale Salaman) (Taraneh x Zeeland)
Nuchal ligament Lamellae	C2-C7
Interosseous muscles	IM2 and IM4 not present

Annex 13: NOVA Stables Mahnaz

Sex	Stallion
Date of birth	9-7-2017
Body condition score	4
Body weight	210kg
Discipline	Breeding
Activity level	Low
Medical history	No medical history
Parents (Mother x Father)	Almas x Mohtat
Grandparents (mothers side) (fathers side)	(Khosh x Ispil) (Mahin x Sahand)
Nuchal ligament Lamellae	C2-C7
Interosseous muscles	IM2 and IM4 present on both forelimbs

Annex 14: NOVA Stables Adinda

Sex	Mare
Date of birth	22-9-2006
Body condition score	6
Body weight	266kg
Discipline	Breeding
Activity level	Low
Medical history	No medical history
Parents (Mother x Father)	Markazi Azita x Markazi Shayan
Grandparents (mothers side) (fathers side)	(Marida Mush x Tandara Daric) (Marida Shayad x Chippendale Salaman)
Nuchal ligament Lamellae	C2-C7
Interosseous muscles	IM2 and IM4 on both forelimbs present

Annex 15: NOVA Stables Azhar

Sex	Gelding
Date of birth	17-5-2012
Body condition score	5
Body weight	266kg
Discipline	Carriage driving
Activity level	Low
Medical history	No medical history
Parents (Mother x Father)	NOVA Stables Adinda x Henden Nima
Grandparents (mothers side) (fathers side)	(Markazi Azita x Markazi Shayan) (Henden Khandan- Dokht x Persicus Nicky)
Nuchal ligament Lamellae	C2-C7
Interosseous muscles	IM2 on both forelimbs present

Annex 16: NOVA Stables Bahira

Sex	Mare
Date of birth	10-5-2011
Body condition score	6
Body weight	342kg
Discipline	Breeding
Activity level	Low
Medical history	No medical history
Parents (Mother x Father)	Henden Banou x NOVA Stables Shaqir
Grandparents (mothers side) (fathers side)	(Persicus Ai banou x Hopstone Shabdiz) (Markazi Shazadeh x Zarin Taze Majara)
Nuchal ligament Lamellae	C2-C7
Interosseous muscles	IM2 on both forelimbs

Annex 17: NOVA Stables Azura

Sex	Mare
Date of birth	25-5-2015
Body condition score	6
Body weight	205kg
Discipline	breeding
Activity level	Low
Medical history	No medical history
Parents (Mother x Father)	NOVA Stables Aïda x Parsagha Sassan
Grandparents (mothers side) (fathers side)	(Markazi Azita x Persicus Amir) (Sirhowy Atish Hajar x Persicus Yussef)
Nuchal ligament Lamellae	C2-C7
Interosseous muscles	IM2 and IM4 on both forelimbs present

Annex 18: NOVA Stables Quasama 

Sex	Mare
Date of birth	31-5-2016
Body condition score	4
Body weight	191kg
Discipline	Breeding
Activity level	Low
Medical History	No Medical history
Parents (Mother x Father)	(NOVA Stables Qitarah x Henden Nima)
Grandparents (mothers side) (fathers side)	(Parsagha Qhiraz x Coalacre Shadon) (Henden Khanandan-Dokht x Persicus Nicky)
Nuchal ligament Lamellae	C2-C7
Interosseous muscles	IM2 and IM4 both forelimbs present

Annex 19: NOVA Stables Almas 

Sex	Mare
Date of birth	30-3-2006
Body condition score	6
Body weight	324kg
Discipline	Breeding
Activity level	Low
Medical History	No medical history
Parents (Mother x Father)	(Khosh x Ispi
Grandparents (mothers side) (fathers side)	-
Nuchal ligament Lamellae	C2-C7
Interosseous muscles	IM2 and IM4 both forelimbs present

Annex 20: NOVA Stables Yunis 

Sex	Stallion
Date of birth	12-5-2019
Body condition score	5
Body weight	173kg
Discipline	Breeding
Activity level	Low
Medical History	No medical history
Parents (Mother x Father)	(NOVA Stables Yalenka x Henden Nima)
Grandparents (mothers side) (fathers side)	(Markazi Yasaman x Persicus Amir) (Henden Khanandan-Dokht x Persicus Nicky)
Nuchal ligament Lamellae	C2-C7
Interosseous muscles	IM2 on both forelimbs present

Annex 21: NOVA Stables Palash 

Sex	Stallion
Date of birth	17-6-2019
Body condition score	6
Body weight	132kg
Discipline	Breeding
Activity level	Low
Medical History	none
Parents (Mother x Father)	(NOVA Stables Padideh x NOVA Stables Key-Khosrow)
Grandparents (mothers side) (fathers side)	(Parsagha Palifeh x Sirhowy Siamak) (Henden Kaykay x Coalacre Shadon)
Nuchal ligament Lamellae	C2-C7
Interosseous muscles	Not present (Hard to see)

Annex 22: NOVA Stables Yasmir

Sex	Stallion
Date of birth	22-4-2018
Body condition score	5
Body weight	205kg
Discipline	Breeding
Activity level	Low
Medical History	none
Parents (Mother x Father)	(NOVA Stables Yusra x Parsagha Saadi)
Grandparents (mothers side) (fathers side)	(NOVA Stables Yalenka x Coalacre Shadon) (Parsagha Palifeh x Persicus Yussef)
Nuchal ligament Lamellae	C2-C7
Interosseous muscles	IM2 and IM4 on both forelimbs is present

Annex 23: NOVA Stables Bahadir

Sex	Stallion
Date of birth	10-4-2016
Body condition score	6
Body weight	266kg
Discipline	Breeding
Activity level	Low
Medical History	none
Parents (Mother x Father)	(NOVA Stables Bahira x NOVA Stables Key Sanjar)
Grandparents (mothers side) (fathers side)	(Henden Banou x NOVA Stables Shaqir) (Parsagha Shanaz x coalacre Shadon
Nuchal ligament Lamellae	C2-C7
Interosseous muscles	IM2 on both forelimbs present

Annex 24: Chi-squared test of the difference between the presence of IM2 and IM4

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	4.480[a]	1	.034		
Continuity Correction[b]	2.557	1	.110		
Likelihood Ratio	6.259	1	.012		
Fisher's Exact Test				.054	.049
Linear-by-Linear Association	4.276	1	.039		
N of Valid Cases	22				

a. 2 cells (50.0%) have expected count less than 5. The minimum expected count is 2.05.

b. Computed only for a 2x2 table

Annex 25: Chi-squared test of the difference between the sex and the presence of IM2

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	4.127[a]	2	.127
Likelihood Ratio	4.333	2	.115
Linear-by-Linear Association	3.649	1	.056
N of Valid Cases	22		

a. 4 cells (66.7%) have expected count less than 5. The minimum expected count is .23.

Annex 26: Chi-squared test of the difference between the sex and the presence of IM4

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	1.316[a]	2	.518
Likelihood Ratio	1.674	2	.433
Linear-by-Linear Association	.041	1	.839
N of Valid Cases	22		

a. 4 cells (66.7%) have expected count less than 5. The minimum expected count is .41.