Universidade de Trás-os-Montes e Alto Douro

Donkey Welfare Assessment in Northeast Portugal

Dissertação de Mestrado Integrado em Medicina Veterinária

Zélia Cristina Pereira da Cruz

Orientador: Doutor Miguel Nuno Pinheiro Quaresma



Vila Real, 2020

Universidade de Trás-os-Montes e Alto Douro

Donkey Welfare Assessment in Northeast Portugal

Dissertação de Mestrado Integrado em Medicina Veterinária

Zélia Cristina Pereira da Cruz

Orientador: Doutor Miguel Nuno Pinheiro Quaresma

Composição do Júri:

Doutor Dario Joaquim Simões Loureiro dos Santos

Doutor Celso Alexandre de Sá Santos

Doutora Severiano José Cruz da Rocha e Silva

Vila Real, 2020

DECLARAÇÃO

NOME: Zélia Cristina Pereira da Cruz

C.C: 14652281

CORREIO ELECTRÓNICO: zeliacruz1@gmail.com

DESIGNAÇÃO DO MESTRADO: Mestrado Integrado em Medicina Veterinária

TÍTULO DA DISSERTAÇÃO DE MESTRADO EM MEDICINA VETERINÁRIA:

Donkey Welfare Assessment in Northeast Portugal

ORIENTADOR: Doutor Miguel Nuno Pinheiro Quaresma

ANO DE CONCLUSÃO: 2020

Declaro que esta dissertação de mestrado é resultado da minha pesquisa e trabalho pessoal e das orientações do meu supervisor. O seu conteúdo é original e todas as fontes consultadas estão devidamente mencionadas no texto, e na bibliografia final. Declaro ainda que este trabalho não foi apresentado em nenhuma outra instituição para obtenção de qualquer grau académico.

Vila Real, 2020

Zélia Cristina Pereira da Cruz

"Os sonhos quando são verdadeiros têm sempre pressa."

Mia Couto

AGRADECIMENTOS

Agradeço à Universidade de Trás-os-Montes e Alto Douro. A todos os professores, técnicos, auxiliares e demais funcionários, o meu obrigado.

Ao Doutor Miguel Quaresma pela sua orientação e disponibilidade. Por me ter acompanhado e não ter desistido de mim nas minhas inúmeras alterações de planos. Por me ter ajudado a realizar este trabalho, sem si não teria sido possível.

À AEPGA pela oportunidade de desenvolver este tema que tanto me apaixonou. Por me receberem como um membro da família dessa linda casa que é a associação. Um especial agradecimento à Belén e à Daniela, por terem sido as minhas companheiras de todos os momentos passados em Miranda do Douro, por me terem ajudado a desenvolver como profissional e como pessoa. Muito do que sou e vou ser é graças a vocês, levo-vos comigo sempre.

A toda a equipa da UNAM/ Donkey Sanctuary Mexico, por me terem aceite e ensinado tanto. Ao melhor encantador de mulas, Mauro Madariaga, por partilhar comigo os seus conhecimentos e por me inspirar a ser melhor e a querer aprender mais, com ou sem amuletos. Ao Dr. Omar, por ter sido um mentor e amigo durante o tempo que estive no México. Por todos os bons momentos passados na sua companhia, dentro e fora do trabalho. Ao Dr. Arturo que me recebeu e me fez sentir sempre em casa, mesmo estando a um oceano de distância dela.

Aos B&M, por serem o melhor grupo de amigos que eu poderia ter pedido, por serem a minha família transmontana e por me acompanharem nesta jornada. Aos meninos, agradeço que tenham cuidado de mim como a vossa pequena, 4ever papás. Às meninas, por serem as minhas irmãs e companheiras, obrigada por toda a amizade infinita. Com vocês cresci, aprendi e desaprendi. Agradeço e guardo comigo todas as noites de diversão e as tardes que as seguiram, sempre juntos. Assim continuaremos.

À minha família, que sempre me apoiou e acreditou em mim. À avó Leninha e avó Teresinha, por me tratarem sempre com todo o amor e por acenderem muitas velinhas por mim, que, estou convencida, passaram-me em muitos exames.

As minhas irmãs, por me apoiarem sempre. Por aturarem a minha má disposição matinal, por não me julgarem quando como duas tostas mistas, por me ligarem para saber se estou bem, por ouvirem todas as minhas histórias. Por todas as mensagens a dizer "transferi-te dinheiro", mesmo sem pedir. Por todos os bolinhos e cozinhados. Por serem um exemplo que eu quero sempre seguir. Por serem as minhas melhores amigas e por estarem sempre comigo, independentemente da distância.

Ao meu querido pai, por todas as propinas que pagou, viagens que fez a vila real, assados que me cozinhou. Por me ter dado uma boa educação e todas as oportunidades do mundo. Por deixar a menina pequenina que queria ser professora e cães seguir o seu sonho, com todo o apoio e amor. Agora sim. Acabaram-se as propinas!

Resumo

O burro de Miranda é uma raça oriunda do nordeste de Portugal, de uma zona denominada de Planalto Mirandês. Estes animais começaram por ser utilizados para trabalho, mas com o crescente envelhecimento da população a sua utilização é escassa e a raça enfrenta risco de extinção.

Uma grande maioria dos estudos de bem-estar em asininos são realizados em países em desenvolvimento, poucos foram publicados a nível da Europa e nenhum em Portugal. O presente estudo pretende colmatar esta falha, avaliando o Bem-estar em asininos, não só da raça Mirandesa, mas também aqueles que não têm raça definida.

Neste estudo foram avaliados os parâmetros baseados no protocolo AWIN foram avaliados em 2018 e 2019, no seguimento da campanha de bem-estar realizada pela Associação para o Estudo e Proteção o Gado Asinino (AEPGA).

Em termos populacionais as fêmeas foram o género predominante, seguidas dos machos castrados. Relativamente aos parâmetros de bem-estar, os problemas comportamentais foram os mais predominantes, encontrados em mais de 60% dos animais, em 2019. Na avaliação da condição corporal (CC) cerca de 50% dos animais apresentavam um CC ideal. Nos restantes constatou-se uma maior tendência para animais com excesso de peso. Na palpação externa das arcadas dentárias superiores, 23% dos burros mostraram dor ao exame.

Palavras-chave: Bem-estar Animal, Burros, Portugal, Planalto Mirandês

Abstract

The *Miranda* donkey breed had its origins in the northeast Portugal, in a place called *Planalto Mirandês*. In the beginning these animals were used for working, but with the on-growing aging population their use is scarce and the breed faces danger of extinction.

A vast majority of donkey's welfare studies are performed in developing countries, few were published in Europe and none in Portugal. The present study comes to bridge this existing gap, evaluating the welfare of donkeys, not only the ones with pedigree but also the ones without defined breed.

In this study were used parameters based in the AWIN protocol to evaluate the welfare in 2018 and 2019, integrated in the welfare campaign performed by AEPGA, the Portuguese association for the study and protection of the Asinine cattle.

In terms of population, females were the predominant sex, followed by geldings. Relative to welfare parameters, behaviour problems were the most common, found in 60% of the animals, in 2019. In the body condition scoring 50% of the animals presented an ideal BCS. In the remaining a tendency to an overweight condition was noticed. To the cheek palpation, 23% of all donkeys reacted painfully.

Keywords: Animal Welfare, Donkeys, Portugal, Planalto Mirandês

INDEX

P	Part I –Bibliographic revision				
1	. Int	roduction	.1		
2	. No	ortheast Portugal	. 2		
3	. Th	e donkey	.3		
	3.1	Origins	. 3		
	3.2	Donkey traits	.4		
	3.3	Donkeys in the world	.6		
	3.4	Donkeys in Portugal	.9		
4	. W	elfare	12		
	4.1	Animal welfare, history	12		
	4.2 A	nimal welfare, definition	14		
	4.3	Animal welfare, assessment	16		
	4.4	Animal welfare, equids and veterinary work	18		
5	. W	elfare assessment protocols	20		
	5.1	Welfare Quality®	20		
	5.2	The Animal Welfare Indicators Project (AWIN)	21		
P	art II	– Experimental work	27		
6	. Ot	jectives of the study	27		
7	. Ma	aterial and methods	28		
	7.1 D	Pata collection	28		
	7.2 W	Velfare assessment protocol	28		
	7.3 D	ata analysis	32		
8	. Re	sults	33		
	8.1 Y	ear of 2018	33		
	8.2 Y	Year of 2019	37		

APPENDIX I- Welfare assessment protocol	71
11. References	
10. Conclusions	
9.4 Health	
9.3 Behaviour	
9.2 Management	
9.1 Population	
9. Discussion	
8.3 Results 2018 - 2019	

Figure Index

Figure 1. Companionship behaviour	
Figure 2. Burro de Miranda	9
Figure 3. OIE welfare definition and the Five Freedoms	
Figure 4. Welfare Quality® principles and criteria	
Figure 5. Fat deposit areas	
Figure 6. Overgrown hooves	

Graphic Index

Graphic 1 Age variation in the year of 2018	
Graphic 2 Age variation in the year 2019	
Graphic 3 Classification of dental disorders in animals that responded positively t	o cheek
palpation	
Graphic 4 Sex variation in 2018 and 2019	
Graphic 5 Appropriate behaviour and signs of thermal stress, in 2018 and 2019	
Graphic 6 Comparison of body condition score, in the years 2018 and 2019	
Graphic 7 Comparison of good health parameters	

Table Index

Table 1 - Animal welfare timeline			
Table	2 - Awin indicators	22	
Table	3 - Donkey body condition score	29	
Table	4 - Dental classification chart	32	
Table	5 - Good health parameters: percentage of positive results	34	
Table	6 - Alopecia lesions, distribution in the year of 2018	35	
Table	7 - Good health parameters: percentage of positive results	38	
Table	8 - Alopecia lesions, distribution in the year of 2019	40	

Abbreviations List

- AEPGA Associação para o Estudo e Proteção do Gado Asinino
- BCS Body Condition Score
- BWT Bodyweight
- DE Digestive Energy
- DM Dry Matter
- FAUC Farm Animal Welfare committee
- IUCN The International Union for Conservation of Nature

Kg - kilograms

- kJ kilojoules
- MADC Matiéres Azotes Digestibles Cheval (Horse digestible crude protein)
- OIE World Organization for Animal Health
- PNDI Parque Natural Douro Internacional
- QBA Qualitative Behaviour Assessment
- RSPCA Royal Society for Prevention of Cruelty to Animals
- TMJ Temporomandibular joint

Part I – Bibliographic revision

1. Introduction

For centuries domestic animals have been of paramount importance in Mankind lives. After their arrival in Europe, donkeys have been used for their strength, resilience and behavioural traits. Their docile nature allowed them to be used in agriculture or as transport (Chirgwin *et al.* 2000; The Brooke 2011; Palo *et al.* 2016).

In Portugal there is a long tradition of using donkeys in agriculture, especially in the northeast. Besides from the two official Portuguese breeds, *Burro de Miranda* and *Burro Anão da Graciosa*, there are many crossbreed individuals, also used for the same purposes. Over the years a decrease in these animals' population has been noted, not just in Portugal but also in other European countries (Couto *et al.* 2016; FAO 2019a).

Although animal welfare is now a trending subject, for centuries the population has shown a growing concern towards it. One of the most famous quotes, by Jeremy Bentham, defines the beginning of the welfare characterization, "the question is not can they reason nor can they talk, but can they suffer" (Blosh 2012). Currently the definition of welfare is not a simple concept, rather it is the agreement between the Five Freedoms (FAWC 1993) and scientific, ethical, religious and economical views.

Due to their versatility, donkey's welfare problems can vary with their main usage and also with the country they live in (Burn et al, 2010; Passantino, 2011). In developing countries some of the most common alterations found are low body condition score and dental and hoof problems, though this is not necessarily the scenario found in Europe, more specifically in Portugal (Toit et al. 2008; Kumar et al. 2014; Dai et al. 2016). The variation in welfare problem's prevalence makes it hard to develop a fitting welfare protocol to every equid (Costa et al. 2016). Although many have been established for horses, only the AWIN project focused on developing a welfare assessment protocol specifically for donkeys (AHIC 2011; Minero et al. 2015).

The present study was developed to assess the welfare of the donkey population in the northeast Portugal, understand the biggest problems in order to better treat and prevent them in the future.

2. Northeast Portugal

In medieval times, in the Northeast of Portugal there was a land called the Land of Miranda (*Terra de Miranda* in Portuguese). This land was delimited by Sabor and Douro rivers and by the Spanish border. Nowadays it corresponds to the municipalities of Bragança, Freixo de Espada à Cinta, Miranda do Douro, Mogadouro and Vimioso. It is comprised by a diverse landscape, with long plains but also pronounced valleys and the Douro canyon (Meirinhos 2014). The vast plains have continuity in both sides of the border, in the Portuguese side it is called *Planalto Mirandês* and in the Spanish is called the *Planície Salamantina-Zamorana* (Palatão, 2011). More specifically, in the *Planalto Mirandês* are included the town halls of Vimioso, Miranda do Douro and Mogadouro (Jézéquel 2016). Other than the flat lands there are three rivers, tributaries to the Douro, in this region - the Angueira, Maçãs and Sabor (MAOT 2001; Rodrigues et al, 2009).

The northeast of Portugal is known for its severe weather - long cold winter and brief, though extremely hot summer. As the Portuguese saying dictates," nine months of winter, three months of hell" (*nove meses de inverno e três meses de inferno*). A land of strong cultural roots, it is home to the second Portuguese language, *Mirandês*, that although it has always been spoken, only in 1999 was decreed as an official dialect (Chazarra et al. 2011; Redentor 2002).

Another iconic item is the traditional wardrobe, *capas de honras*, meaning cape of honour. This piece of clothing originally had two types, one for the shepherds to use for daily work, another for Sundays and festive occasions. Both were made with a fabric produced from sheep wool named *burel*, although the first one was simple and the second filled with ornaments. From the last one comes the name and it is the only one produced nowadays (Meirinhos 2014). The land is also known for the *Pauliteiros*, a group of people that dance to the sound of traditional Portuguese instruments such the *gaita de foles, bombo* and *fraita* (Alge 2004). Many of these traditions are at risk of disappearing due to population decline and aging (INE 2019).

3. The Donkey

3.1 Origins

The donkey is a resilient, powerful, yet calm and bright animal. As so, Mankind saw the opportunity to explore these traits, thus began the domestication (Chirgwin et al, 2000; Palo et al. 2016). The domestic donkey (*Equus africanus asinus*) belongs to the genus *Equus*. This genus is composed by the eight-surviving species of equids, amongst them there is the domestic horse (*Equus caballus*), Asiatic wild ass (*Equus hemionus*) and African wild ass (*Equus africanus*) (Moehlman 2002; Rosenbom et al. 2015).

Although both belong to the same genus, divergence between horses and donkeys started 7.7 to 15.4 million years ago (Huang et al. 2015; Rosenbom et al. 2015). From this divergence two different species were formed, with differing traits, not only physical, but also physiological and behavioural. (Yilmaz et al, 2012; Huang et al. 2015).

Over the years, the collection of archaeological and linguistic data has been used to formulate various theories about where the domestication of the donkey took place (Blench 2004). One theory explored an Asian domestication and another the African domestication. Through DNA sequencing of archaeological findings, the first theory was excluded, confirming that the domestic donkey descends from the African wild ass (Beja-pereira et al. 2004). In Africa, the domestication is thought to have happened in one, or both, of the following ways: domestication of Nubian wild ass in Egypt or domestication in the northeast of Africa, in the Sahara region, approximately 5000 years ago (Beja-pereira et al. 2004; Blench 2004; Zeder 2008).

The original Asian and African species of wild asses have suffered a decay in its numbers, due to climate change and human impact. The IUCN is considering the Asian wild ass to be in an endangered state, with a decline of more than 50% of its specimen in the last 16 years. As for the African wild ass, the species is critically endangered due to the existence of no more than approximately 200 mature individuals. Both species have tendency to keep decreasing its numbers over the next years (Moehlman et al 2015).

Throughout the years, humans have selected the characteristics that would better fit their needs and many different breeds have been created with anatomical, physiological and temperamental differences (Orlando, 2015). Due to this variation in breeds, a donkey's height can vary between 80 to 160 cm, and their weight can sway from 80 until 480 kg (Kugler et al 2007).

3.2 Donkey traits

Donkeys are not small horses, consequently many physical and behavioural differences can be found (Burden et al. 2015). These differences can be as small as different chromosome numbers and as big as donkeys' ears (Burnham 2002).

3.2.1 Behaviour

Donkeys exhibit their behaviour in a different way than other equids, sometimes subtler, other times more vividly. For instance, when frightened all herbivores have a "fight-or-flight" response, donkeys more often choose to fight than horses although it is also common for them to freeze when scared or pressured (Burden et al. 2015). The bray is also very distinct from horses, it is heard in situations where donkeys feel threatened, when it's feeding time, or to reaffirm sexual interest (The Donkey Sanctuary 2018).

If retraced to its origin, donkeys lived in a poor environment, with scarcity of food and water, that is still imprinted in their behaviour today. Unlike other equids, the asinine tend to form smaller groups (The Donkey Sanctuary 2018). Nevertheless, donkeys are extremely social beings and they tend to form life-long companions. So, when the companions get separated it causes them high levels of stress which might even cause sickness (Burden et al. 2015). Some of these bonds can be noticeable in moments like grooming and it is common for donkeys to scratch one another to clean or when they have some parasites (figure 1). Other example is seeing them resting their head in the companion's back (The Donkey Sanctuary 1999).



Figure 1. Companionship behaviour

Although in folklore donkeys have been portrayed as not bright animals, studies have shown that donkeys actually have a better spatial cognition and preservation ability than horses (Osthaus et al. 2012).

3.2.2 Physiology and Anatomy

All equid species show anatomical differences which are imprinted in their DNA. Horses have 64 chromosomes, donkeys 62 and mules 63 (Burnham 2002). Besides from the bigger ears, donkeys have many unique characteristics in the head and neck region. Some are external and easily seen, like the thickness of the mandible (Burnham 2002), the bigger degree of anisognathia (Herman 2009), and the different location of the nasolacrimal duct – in the lateral part of the nostril in donkeys and ventral part in horses (Said et al. 1977). Others are not easily seen or found and can harden the veterinary's job. Puncturing the jugular is a common practice, either to administer drugs or collect blood, many authors have shown that the method to do so must be different from that in horses due to a more prominent cutaneous colli muscle (Burnham 2002; Herman 2009; The Donkey Sanctuary 2018).

Furthermore, when evaluating the musculoskeletal system, differences can also be found. As the common saying dictates, "no hoof, no horse", knowledge of the anatomy of normal hooves is crucial in all equids. First variation noticeable when looking at donkey's hooves is their upright position and more cylindrical shape, as opposed to horses more conical shape (Thiemann et al. 2013). Moreover, in healthy donkeys the line of hoof pastern axis should be parallel to the scapular spine (Grange 1995). When observing the sole the U-shaped surface, developed frog and the same thickness throughout the hoof wall are some of the donkeys contrasting features (Grange 1995). Studies have also shown that donkeys have one less lumbar vertebra and the number of the caudal vertebrae can vary from 15 to 17 (Jamdar et al. 1982).

Despite their capacity to thrive in more arid places, it is wrong to assume that donkeys have the same resilience as horses when it comes to colder temperatures. When their hair coat properties were analysed and compared, it was concluded that donkey's hair coat is lighter, shorter and thinner than that of horses, making them more predisposed to hypothermia condition during the winter season (Osthaus et al. 2017). Also, when administrating any drugs in donkeys, there must always be kept in mind the differences in metabolization and distribution (Matthews et al. 2005).

3.2.3 Nutrition

Obesity is a common problem amongst domesticated donkeys, nevertheless there is still a lack of studies to fully understand the nutritional requirements of this species (Martin-Rosset 2018). Donkey's digestive system is highly adapted to poor nutritional forages, being able to digest highly fibrous aliments. When donkeys and horses are fed the same way, the first can develop an obesity problem (The Donkey Sanctuary 2018).

When it comes to mature non-working donkeys, for maintenance energetic needs the digestive energy (DE) intake varies from 80 to 95 kJ per Kg of BTW, daily, which corresponds to 25% less than horse's daily DE need (Burden 2011). Although it is clear that energetic requirements can vary from one season to another, some authors show that DE necessity is bigger in winter (Wood et al. 2005) and others show that it is more notable in Spring (Carretero-Roque et al. 2005). In the same conditions, a donkey would need to ingest 1.3 to 1.7% of its bodyweight (BTW) in dry matter (DM), however, a same sized pony would need up to 2-2.5% of its BTW (Burden 2011). Protein needs are estimated to balance between 2.0 to 2.2 g of MADC per BTW ^{0.75} (Martin-rosset 2018). The nutritional requirements in different physiological states (growth, pregnancy, lactation) have not yet been studied for the asinine species (Martin-rosset 2018).

Not less important, water is also a part of donkey's nutrition. It comprises 60% of their body weight. As so they must always have access to water. Due to its origins, when compare to horses, donkeys can better withstand water deprivation and are able to rapidly recover from dehydration by ingesting large quantities of water in just a few minutes , up to 24 to 30 litres (The Donkey Sanctuary 1999).

3.3 Donkeys in the world

Since their domestication, animals have served many purposes, from the improvement of agricultural productions to social and cultural development. Donkeys are not an exception. (The Brooke et al. 2011). In 2016 the estimate number of donkeys in the world was of 50 million individuals, 34 % of all domestic equids (FAO 2019b).

In spite of some countries having found new roles for the asinine species (tourist transportation, milk production, donkey-assisted therapy, etc) its presence continues to be more notorious in less developed countries, being chosen for their aptitude to thrive in inhospitable places (Chirgwin et al. 2000; Beja-Pereira et al. 2004; Tadich et al. 2014; Palo et al. 2016;).

3.3.1 Donkey, an agricultural helper

Concerning agriculture, a lot of world's crops are still cultivated with the help of animals (Wilson 2003). Although this practice is more often carried out by populations without the means to acquire machines, animal power is sustainable in many ways. In one hand it facilitates fertilisation of the soil, directly from the animal or as a mean of transportation of another animal's manure (FAO 2010). On the other hand, modern agriculture faces many environmental challenges, being some of them the impact in global warming and increase if soil compaction. (Hamza et al. 2005; Rosset 2013) In the last example, the weight of the tractor compresses the soil, reducing its fertility, in this case animal power is a good solution, an increment of the soil fertility is noticeable in just a few years when the machine-animal change is made (Herold et al. 2009; Gantner et al. 2014).

3.3.2 Donkey, a transport system

Transport and agriculture are somewhat related. Donkeys can be used to transport goods to and from crop sites (instruments, seeds, fertilisers, harvests), and later to stablish trades. People with this means of transportation have a more effective trading system as opposed to those who must travel by foot. Some tasks usually portrayed by women in these countries, such as transportation of water and firewood, can be accomplished faster and more easily, donkeys help improve women's social status and welfare (Nengomasha et al. 2000; Mckenna 2007; FAO 2010; FAO et al. 2011). Donkey taxis are also common in highly touristic places (Zenebe et al. 1997; Thiemann et al. 2016).

3.3.3 Donkey, a breeding animal

In many countries breeding is still a common practice, most likely for one of three reasons. First, and most common where donkeys are used as working animals, is to create a replacing market of animals, for donkeys which arrive at the end of their working lives (Blench 2004). This also helps improve local economy, as opposed to international trades (Blench 2004; Fernando et al. 2004). The second reason is the use of reproduction as strategy to maintain breeds which are at risk of disappearing (Quaresma et al. 2014). The last reason is breeding for production of milk, meat and skin.

3.3.4 Donkey, a production animal

Since ancient times, donkey's milk has been used for its chemical properties. Hippocrates used it to cure infections, liver disease, poisoning, wounds and many other disorders (Karatosidi et al. 2013). Although effectiveness related to these disorders have not been proven, studies of the milk's quality and composition have been carried out, demonstrating that it is close to the human milk itself. Furthermore, its content in β -lactoglobulin is lower than in cows, being a good substitute for people with allergies, since β -lactoglobulin is one of the major allergens in milk (Polidori et al. 2012). Today, there are also many cosmetic products based on jennies milk (Karatosidi et al. 2013).

Though it is not common, meat consumption happens in some countries (Blench 2004). Studies show that donkey's meat has a high nutritional value, being composed by good quality proteins, vitamins and minerals (Aganga et al. 2003). In countries with a dense population of donkeys, it is an alternative to other types of meats. It is predictable that in countries with high rates of breeding, the slaughter and development of a meat market might increase (Camillo et al. 2017).

The Donkey Sanctuary estimates 1.8 million skins being traded in the world, per year, bearing in mind that the Chinese demand is around 10 million per year. This need comes from an old tradition that used donkey's skin to produce *ejiao*, medicine used for its' anti-ageing properties, libido stimulation and decrease in the occurrence of women reproductive diseases. The skin trade market has impact in the lives of people who live in different continents, as the flow of animal exportation is kept by contraband of stolen animals. (The Donkey Sanctuary 2017).

3.3.5 Donkey, a therapy resource

Over the last years there has been an increase in assisted therapy with equids, to treat physical and mental disorders (Borioni et al. 2012). Although hippotherapy (therapy with horses) has been extensively studied there are few authors that have taken interest in onotherapy (therapy with donkeys). Those who have, concluded that donkeys are more suitable for cases of

emotional, communicative and psychomotor disabilities, due to their calm, patient and affectionate behaviour (Karatosidi et al. 2013; Camillo et al. 2017).

3.4 Donkeys in Portugal

Donkeys arrived in Europe approximately in the year 2000 BC, passing through Morocco to Spain and Italy (Aranguren-Méndez et al. 2002; Kugler et al. 2007). European donkeys have two probable ascendants, the Nubian and the Somalian ass. Being the last one the most likely to have originated the majority of breeds (Mendez 2002). In 2018, there were 56 donkey breeds registered in European countries, 32.7 % of them were classified as critically endangered and 21.8% in endangered status. In Portugal there are two breeds, the *Burro de Miranda* and *Burro Anão da Graciosa*, both these breeds are considered at risk of extinction (Couto et al. 2016; FAO 2019a).

Other than the two-official breeds, there are many crossbreeds, in the Portuguese donkey population. Evan though most owners use donkeys for work, mainly ploughing and loading, their use is decreasing due to agricultural machinery. Others interests seem to be milk production, tourism, leisure and breed preservation, especially in the breed home-region (Quaresma et al. 2014).

3.4.1 Burro de Miranda breed

In Portugal the asinine population was abundant in 1940, with approximately 22,000 animals. Then a decrease in its numbers started and by the year of 1999 there were just about 5,000 individuals (Barbosa 2003).

In 1999 a study was made to evaluate donkeys' presence, socio-economic and ecological impact in what is known as the Douro International Natural Park (*Parque Natural do Douro Internacional* - PNDI). In the Northeast of Portugal there was a common type of donkeys similar to the Spanish *Zamorano-Leones*, known as the Donkey of *Miranda* (García 1999; Samões et al. 2000). In 2001 the Portuguese Association



Figure 2 Burro de Miranda

for Study and Protection of the Donkey (AEPGA) was created, with the intent to promote and protect donkeys in the land known as the *Planalto Mirandês*. A year later the *Burro de Miranda* (Figure 2) became an official donkey breed, the first in Portugal (AEPGA 2012).

This Portuguese breed is robust and characterised by a large body frame, bulky neck and abundant hair coat. Individuals are tall, measuring at least 125 cm of height at the withers, but can go up to 157 cm, with large limbs, voluminous joints and chestnut only present in the forelimbs. Their big head has a slight concavity in its otherwise straight profile, with the donkey's distinctive big ears and rounded tips. The straight back ends in a croup that tends to be taller than the withers (AEPGA 2012; Yilmaz et al. 2012; Quaresma et al. 2005, 2019).

The hair coat is more abundant in the face, ears, forelock, body-side and limbs. Added to an abundant mane, the hair is mostly brown with lighter aspects in the body-side, abdomen and white hair in the muzzle and around the eyes (Quaresma et al. 2005; AEPGA 2012).

Jennies start to be sexually active at 1.5 years of age and Jacks at 2. When they are 5 years old, they can be considered adult. This Portuguese breed's life expectancy goes up to 35 years of age (Quaresma et al. 2005; CAB 2019).

3.4.2 Legislation

With the ownership of an animal comes some responsibility, all the duties of an owner are inserted in the Portuguese and European laws. Some of these laws are in respect to identification, transporting, housing and welfare. As decreed in *Decreto- lei* $n^{o}123/2003$, all equids must be properly identified. In Portugal, equids are identified with a transponder in the left side of the neck, in accordance with the European Commission Regulation n^o 504/2008. In addition to the transponder all equids must possess an Identification Document, passport, in which there is a description of the animal and his corresponding identification number (Ministério da Agricultura 2013; CE 2014).

But the animals are not the only ones with the obligation to be properly identified, all equine owners must have a farm registering number, independently of the number of animals they own. Another duty of the owner is to declare an animal's death or disappearance, within the 30 days following the occurrence (CE 2014; Ministério da agricultura 2006).

When transported, all equids must be accompanied by their passport. All animals must be transported in equipment's that prevent any injuries and that can assure the animals safety. Handlers must be properly qualified to manage the animals, without resorting to violence or provoking unnecessary fear. Animals must have sufficient space, in accordance with their size and the duration of the journey and access to water and food (CE 2013).

According to *Decreto -Lei n.º 214/2008* and specifically to equids, where housing is concerned, there are some legislation to follow. In terms of public health, all stables must have a physical barrier to prevent the contact between equids and other species. Also, there must always be a quarantine zone and a disinfection area either for people or transports. Infrastructures must be made to ensure thermal isolation, control of ventilation, temperature and light. Windows must be located above the animal's back and the floor must be of easy cleaning and non-slippery. Appropriate drinking and eating conditions are also mandatory (Ministério da Agricultura 2009).

Since 1998 the European Union decreed the protection of animals in the Council Directive 98/58/EC. The rules would aim for the protection of farming animals, respecting the five freedoms. In the Treaty on the Function of the European Union (2009), the European Commission recognised animals as sentient beings and that animal welfare must be regarded in respect to legislation (CE 1998, 2007).

4. Welfare

4.1 Animal welfare, history

Since early in the history of human civilization animals play an important part in people's lives, and though their welfare is a trending subject nowadays, it has always been around (Table1). It goes as early as 500 BC that the talk about welfare starts (Violin 1990; Broom 2014). Although in the beginning some of the regulations' concerns were public health and not as much about animal wellbeing, it started to change once animals were considered sentient beings (Le Neindre 2009).

In 1996, DeGrazia explained sentience to be self-awareness and the animals' ability to feel happiness, fear or even pain. Later, in 2006, Broom reformulated this definition and considered sentient an animal that could evaluate the actions of others towards itself, remember its own actions and consequences, assess risks and benefits and have feelings and some degree of awareness (Duncan 2006; Broom 2014;).

Nowadays animal welfare is a complex issue. It is shaped by scientific, ethical, religious, economical and legal views, that can differ in different parts of the world. In the view of the World Organisation for Animal Health (OIE), animal welfare links both animal and human health, human wellbeing and the sustainability of socio-economic and ecological systems (OIE 2017).

To better assess animal's welfare without overlapping concepts and views, functions have been delegated, thus creating the Animal Welfare Science, Ethics and Law. Animal Welfare Science evaluates the animal's state, the effects of the environment and health in its life. Animal Welfare Ethics investigates how humans *should* treat animals as opposed to Animal Welfare Law, which states how humans *must* treat animals (The Brooke 2013d; Ryan et al. 2018).

Year	Person/place	Statement	Reference
500 BC	Pythagoras	Animals have souls	(Violin 1990)
200 BC	Indian Emperor Asoka	"The regulations I have given are that various animals must be protected "	(Dhammika 1993)
1635	Ireland government	"An Act Against Plowing by the Tayle and Pulling the Wool off Living Sheep"	(Blosh 2012)
1641	Massachusetts body of rights	. inhibition of mistreatment of animals with an owner. obligation of animals resting when needed	(Blosh 2012)
1700	Kant	Believed mistreating animals would lead to people losing empathy for other humans	(Fisher 2017; Potter 2005)
1789	Jeremy Bentham	Father of utilitarianism, "the question is not can they reason nor can they talk, but can they suffer".	(Blosh 2012)
1800	Darwin	Wrote a book entitled "The expression of the emotions in man and animals"	(Darwin 1872)
1824	Royal Society for Prevention of Cruelty to Animals (RSPCA)	Creation of the first organisation to fight for animal's rights	(Wooler 2014)
4.2 Animal Welfare, definition

For centuries there has been a growing concern about animal welfare, even before a scientific definition was stablished (Le Neindre 2009). In 1789, Jeremy Bentham explain that animals talking, or reasoning was not important, the fact that they could suffer should be a concern. Over the years there were four different approaches to what was the foundation of animal welfare: their ability to cope with their environment, animal's feelings, animal's experiences and animal's expression of natural behaviour (Fraser, 2003; Phillip, 2009;). Some of these ideas are intertwined, for example, an animal's ability to cope with its environment will depend on its nature (Broom 2011).

The most common is the *animal's capacity to thrive in the environment* it is introduced on. In 1976, Barry Hughes defined welfare as the harmonious relation between animal and its environment, physically and mentally (Le Neindre 2009). Later, in 1986, Donald Broom explained it as the capacity to cope with its environment; it could be satisfactory or not. (Broom 1986).

The *animal's feelings* perspective defends that welfare can be assessed if an animal's responses are based in emotion, although animal's feelings are not easily measured. Assessing an animal's welfare based on *past experiences* is in some levels related with the previous perspective (Fraser 2003; Bracke et al, 1999; Phillip, 2009).

The last one is based on the animal's ability to show its *natural behaviour*, whilst living in an environment as close to their natural one as possible (Fraser 2003).

In general, every definition has something in common: to assess welfare one must try to understand if an animal has quality of life (Appleby et al. 2014).

In 1993, the five freedoms to ensure any animals' welfare were stablished: freedom from hunger, discomfort, pain or fear and freedom to express normal behaviour (FAWC 1993). In 2008 it was considered by the OIE that a good welfare state means a healthy and comfortable life, alongside with good nutrition, the ability to express natural behaviour and the absence of negative feelings (such as pain or fear). This comes in agreement with the Five Freedoms' principle, as shown in Figure 3 (FAWC 1993; Manteca et al, 2012).



Figure 3. OIE welfare definition and the Five Freedoms

Although in the beginning animal welfare was more focused in small companion animals, it has developed quickly and nowadays there is a growing concern about farm animal welfare (Broom 1999). This is a very controversial subject although for many discussions it is important to remember that welfare measures the quality of life and not quantity, and also that the welfare assessment is a momentaneous characteristic and is subject to changes in time (Broom 1999; Appleby et al. 2014).

The increase in the world's population has a direct effect in animals' welfare, from farm animals and pets to wild fauna. This increase leads to a bigger demand of meat and a subsequent increase in cattle production industries, which stresses the need to develop sustainable forms of production without jeopardising meat-costs and animal welfare (Passantino 2011; Appleby et al. 2014). The growing population also leads to an increase of dog and cat's population, either as pets or strays, which can cause not only animal welfare problems but also public health issues, in the way that 61% human pathogens are zoonotic, and the increase of animals leads to an increase of the likelihood of zoonotic diseases (Davis 2011; Appleby et al. 2014).

4.3 Animal welfare, assessment

There are three parts that compose animal welfare assessment: collecting data, analysing it and acting accordingly (Le Neindre 2009). Animal welfare is a multidisciplinary subject that involves medical, ethological, biological and zootechnical parameters. (Blokhuis et al. 2019) As mentioned, in 1993 the Farm Animal Welfare committee (FAWC) defined what is now known as the Five Freedoms, (FAWC 1993). According to which, in order to be in a good welfare status, an animal must live with:

- Freedom from hunger and thirst- the animal must have access to fresh water and a healthy diet;
- Freedom from discomfort- the animal must have access to a shelter and a resting area;
- Freedom from pain, injury and disease- through prevention and when necessary diagnosis and treatment;
- Freedom to express normal behaviour- the animal must have enough space and company of its own species;
- Freedom from fear and stress- the animal must live under condition of no mental suffering.

4.3.1 Welfare parameters

The multidisciplinary view of welfare was adopted by many, such as Gonyou, who thought that "although the animal's perception of its condition must serve as the basis for wellbeing, much can be accomplished by involving behavioural, physiological and pathological studies" (Fraser et al. 1997; EFSA 2006;). When analysing all the different parameters, a negative indicator can mean poor welfare, but a positive indicator may not mean good welfare (Broom 1986; Le Neindre 2009; Blokhuis et al. 2019).

If, as Broom said, animal welfare is the animal's attempt to cope with its environment then it is a momentaneous characteristic, and it is subject to changes, therefore it can be improved. When doing so, one must ask the following questions "will the change improve animal health? Will it give the animals something they want?" (Broom 1999; Blokhuis et al. 2019) There are many ways and many protocols to assess animal welfare. But independently of which might be used,

and which criteria might be studied, they all must evaluate the following categories: health, behaviour and management.

i.Health

World Health Organization explains health as the state of complete physical, mental and social soundness and not just the absence of disease or injury. This comes very close to the general animal welfare idea (CE 2000). Unarguably health is imperative to a good welfare, but it is a complex concept and to evaluate it one must look at many parameters. Looking at morbidity and mortality, morbidity contributes to the analysis of each specific individual's welfare and mortality gives an overall picture of a farm's welfare status. Also, in farm animals, a decrease of production can be indicative of poor welfare but increase in production cannot necessarily be perceived as a good welfare sign (CE 2000; Le Neindre 2009).

Pain and suffering can also compromise an animal's welfare. By its definition, pain is a physical feeling caused by injuries or illness, which reflects health problems. In other hand suffering can be physical or mental and is related to unpleasant experiences. Pain can be detected through observation of animal behaviour or physical signs (i.e. changes in weight baring, anorexia, etc).

Physiological parameters can also be measured to assess welfare, changes in cardiac frequency, temperature, increase of hormones or acute phase proteins can be some indicatives of distress or disease.

ii.Behaviour

One of the best reasons to evaluate an animal's behaviour is that it is a non-invasive procedure. Also, through behaviour one can understand an animal's perception of its surroundings and experiences. Although, it is important to remember that every animal, and mostly every species, reacts a certain way to different stimuli, and what is perceived as a good reaction in one case may not be so in another (Dawkins 2003; Le Neindre 2009; Clive Phillip 2009; Broom 2014).

When an animal shows a great effort to avoid an object or situation, it can be due to a bad previous experience, so it indicates poor welfare. Some specific behaviours such as stereotypes (repeated movement with no obvious purpose) or self-mutilation can also be an indicative of poor welfare (Mills et al. 2005; Broom 2014). Broom stablished that in terms of behaviour, to

measure animal's welfare, it is important to evaluate the amount of normal behaviours that are shown or supressed, amount of aversion actions, indicators of pleasure and animal's attempts to cope with its environment (Broom 1986, 2014).

iii. Management

The environment in which animals find themselves can influence animal welfare. For example, appropriate housing and bedding can improve animal's wellbeing (Broom 2019). As mentioned above, some scientists have adopted a natural-living view, in which an animal that cannot express natural behaviour or live a natural life, cannot experience proper welfare. Others believe they can, if their needs are met, which is the human's obligation (Carr et al. 2018).

In all domestic animals, it is also the owner's responsibility to provide a healthy and balanced diet, bearing in mind that when it is not done properly, it will reflect on health and welfare (Davidson et al. 2003).

Furthermore, the zootechnic parameters are better stablished by legislation mentioned in 3.4.2.

4.4 Animal welfare, equids and veterinary work

"The Brooke", one of the world's leading organisations to improve working equids welfare, suggests that animal welfare should fit in everyday veterinary work. They recommend that when approaching an animal, a quick review of the five freedoms should be made, and when necessary coming up with quick and simple solutions (The Brooke, 2013).

Evaluation of body condition, mucous membranes, teeth and offering water are some of the ways to see if the animal experiences hunger and thirst. There are quick and easy fixes to these problems; rasping the teeth or advising the owner on nutrition are examples of simple ways to improve the animal's welfare. Changes in animals' routines can be made to upgrade their comfort and lower the likelihood of injury or pain. Carrying lighter burdens, resting in shades or wearing equipment that properly fits their bodies, not only helps animal's wellbeing but also helps them to work in a more efficient way. Usually, the purpose of a veterinary's consultation is to treat diseases, when doing so, it is important for the veterinary to avoid stressful situations and to adjust approaches to each individual. It is important to understand that equids welfare is

linked to the livelihoods of their owners and to the veterinary services available in the area (The Brooke 2013d; Luna et al. 2019).

Equids are very versatile; they can be used for many purposes. Their welfare problems are also diverse, depending on the species, their type of work and the area they live in (Burn et al, 2010; Passantino, 2011). Some common lesions are swelling of tendons and joints, skin lacerations and dental problems. In urban areas, displays of aggression are a common problem, whereas in rural zones, low body condition score and ectoparasites are more prevalent conditions (Burn et al. 2010; Amante et al. 2014).

Due to the heterogenicity of the conditions, the development of a protocol of welfare assessment is difficult (Costa et al. 2016). Some of the existing protocols for equids are the Australian Welfare Protocol, the Assessment Protocol for horses and the Animal Welfare Indicators for donkeys and horses (Wageningen UR Livestock Research 2011; AHIC 2011; Minero et al. 2015; Minero et al. 2015). These protocols measure indicators that can be animal based (i.e. behaviour, body condition score), resource based (i.e. housing type) and management based (i.e. time to exercise, number of meals a day) (Viksten et al. 2016).

The Donkey Sanctuary also developed the "hands-on donkey welfare tool". This helps veterinarians around the world to do a quick and easy welfare check. This "tool" is represented by a hand, and to each finger belongs a category. The thumb represents the behaviour, the index the body condition score (BCS), skin condition is represented by the middle finger, the ring finger the musculoskeletal system and the pinkie finger other signs of disease. The palm of the hand also characterizes the animal's life in general. Each parameter is evaluated from 1 to 5, in which 1 is the best score and 5 the worst evaluation, except for the BCS that is evaluated in the classic manner (also from 1 to 5, being 1 for thin animals, 3 for good condition and 5 for obese) (Galindo et al. 2017).

5. Welfare assessment protocols

5.1 Welfare Quality®

In 2004, a project was created with the intent of standardise animal welfare assessment and develop new ways to increase good animal welfare. In its launch only European countries participated in this initiative but were latter on joined by Latin American countries. The original name was "Integration of animal welfare in the food chain: from public concern to improved welfare and transparent quality" but it was later changed to what is now known as Welfare Quality (B) (Blokhuis et al. 2019).

The main objective of the project was to facilitate all welfare practices in pigs, poultry and cattle. Its development not only eased the welfare assessment and the solution of most common problems, but also boosted the scientific community in what animal welfare is concerned. It helped better define the parameters for good welfare, using mostly animal-based measures to each criterion, i.e. to assess the absence of prolonged hunger the body condition score would be measured.

Many publications were made resorting to the use of this protocol (Blokhuis et al. 2010, 2019). Protocols for all species included four principles, those principles were comprised of 12 criteria and to evaluate those criteria, 30 to 50 measures were developed. The principles are good feeding, good housing, good health and appropriate behaviour. The respective criteria is represented in Figure 4 (Blokhuis et al. 2010; Manteca et al. 2012).

As pioneer in the area, the Welfare Quality ® showed some limitations to its practice, the most important being the low diversity of species evaluated (Blokhuis et al. 2010).



Figure 4 Welfare Quality® Principles and Criteria (adapted from Blokhuis et al. 2019)

5.2 The Animal Welfare Indicators Project (AWIN)

As mentioned before, the welfare assessment protocols in Europe that centred the assessment in animal-based parameters, started around 2009, with the Welfare Quality® project. Few years later, the AWIN project was developed, between 2011 and 2015 (AWIN 2015). Its objective was to assess and improve animal welfare, resorting to human-animal relation, management and animal welfare indicators. Another difference between Welfare Quality® and the AWIN protocol is the targeted species, AWIN is centred in sheep, goats, horses, donkeys and turkeys. Also, it gives a special emphasis to pain evaluation, which is a common welfare problem. Since the AWIN protocol is very recent, not many publications have been completed, especially in donkeys, though some bibliography can be found. AWIN protocols have two levels of assessment, the first level is a quick yet feasible questionnaire. The second level is recommended for a more deep study of welfare, as it is slightly more time consuming and requires more animal handling situations (Minero et al. 2015). The AWIN related to donkeys is comprised of four welfare principles: good feeding, good housing, good health and appropriate behaviour. To which fit 12 welfare criteria and more than 20 welfare indicators that vary with the level of protocol used (Table 2). A common problem to attribute a certain indicator to a criterion is that most indicators can belong to a variety of criteria. To facilitate general welfare assessment, the AWIN protocol only correlates one indicator to a specific criterion (Minero et al. 2015).

Table 2 AWIN Indicators

Welfare principles	Welfare Criteria	Welfare indicators	
	Appropriate nutrition	Body condition score	
Good Feeding	Absence of prolonged thirst	Skin tent test	
		Water availability	
	Comfort around resting area	Bedding	
		Shelter dimensions	
Good housing	Thermal Comfort	Signs of thermal stress	
	Ease of movement	Not considered to animal in extensive	
		productions	
		Integument alterations	
	Absence of injuries	Swollen joints	
		Lameness	
		Prolapse	
Good health		Hair coat condition	
		Faecal Soiling	
		Discharges	
	Absence of disease	Cheek palpation	
		Abnormal breathing	
		Coughing	
	Absence of pain due to procedures	Signs of hoof neglect	
		Signs of hot branding	
Appropriate	Expression of social behaviour	Social interaction	
	Expression of other behaviours	Stereotypes	
behaviour	Good human-animal relationship	Human-animal relationship tests	
	Positive emotional state	Qualitative behaviour Assessment	

Both first and second level protocols were developed to not take too much time (five and 10 minutes respectively). To optimise time of evaluation there is a specific order to follow, first is the analysis outside without handling the animals, second is the evaluation outside of the parameters that need handling and last is the evaluation inside, of housing related parameters, such as bedding and shelter (Minero et al. 2015).

5.2.1 Good feeding

AWIN'S evaluation of good feeding is comprised of body condition score, skin tent test and water availability (Pritchard et al. 2008; Minero et al. 2015). Body condition scoring can be a part of different principles as there are various factors that can cause its decrease. There is the food availability and quality, but also health problems (diseases, dental problems). Skin tent and water availability evaluate the absence of prolonged thirst. Water is as important as food in a donkey's nutrition. Like in most animals, water comprises 60% of donkeys' body weight, and although they are adapted to a certain degree of water deprivation, it is still cause for welfare concern and can secondarily diminish nutrient intake as thirst depresses the appetite (The Donkey Sanctuary 1999; Duncanson 2010).

Other problems can result from feeding disorders. In donkeys, the development of hyperlipidaemia is common due to excessive weight combined with other predisposing factors (age, gender, pregnancy, sickness). In this condition, the lipolysis leads to an increase of triglycerides concentration in plasma, which leads to multiple organ failure (Burden et al. 2011; The Donkey Sanctuary 2018).

5.2.2 Good housing

Bedding, shelter dimensions and thermal stress are the three indicators for housing evaluation. Bedding is crucial to proper comfort, not only it absorbs moist but also softens the floor where donkeys lay. Studies have shown that animals prefer straw to shavings, when it comes to bedding (Ninomiya et al. 2008). Shelters should be at least 40 cm taller than the donkey's height at withers and the area should be between 5.5 to 9 m² per animal, accordingly to their size (SFC 2011). Looking for signs of thermal stress is important because donkeys' hair coat is not as waterproof as horses, so when exposed to adverse temperatures and heavy rain they are more propense to develop hypothermia (AWIN 2015; Osthaus et al. 2017).

5.2.3 Good health

Absence of injuries and disease are crucial for good health. When analysing the integument, it's important to look for deep or superficial injuries, as they can suggest trauma or give information about the amount of work the animal is doing and if the equipment is proper (Garrett 2018). Although all skin alterations are a sign of poorer warfare, not all injuries have the same severity, (i.e. alopecia is less severe than deep wounds) (Lombard et al. 2010). Also, the hair coat condition can be an ally to evaluate the general state of the animal, as it loses quality when an animal is sick or has poor nutrition. The suffering of unnecessary pain procedures, such as animal hot branding, has a negative reflection in the animal's welfare (Minero et al. 2015).

When looking at the musculoskeletal system it's important to do both static and a moving exam. Examining the hooves and looking for alterations, which can be a source of pain and stress. Swollen joints can be a sign of many inflammatory and infectious diseases, such as arthritis, which are painful and decrease animal welfare (Reed, Bayly, and Sellon 2010). Also, seeing the animal's movement is important to find several problems, located in the hooves, muscles and tendons or even neurological alterations (Fraser et al. 1990).

There are other indicators that suggest other infirmities. For example, when an animal is coughing, has a nose discharge or abnormal breathing it suggests a respiratory condition (Reed et al. 2010). Studies performed in Ethiopia showed that 5.5 % of working donkeys have signs of infectious diseases (Biffa et al. 2006). Some of the most common infectious diseases, which must be reported are the Equine Influenza, Glanders, Equine Herpesvirus, Equine Infectious Anaemia and Rabies (Reed et al. 2010; The Donkey Sanctuary 2018).

Although there is a number of diseases which are required to be reported, in Portugal there is no mandatory vaccination for equids, except sports horses, which must have the Equine Influenza vaccine, in order to participate in competitions (Federação Equestre Internacional 2019).

5.2.4. Appropriate behaviour

Assessment of an animal's behaviour is a big part of its welfare evaluation. The animal-human tests try to understand the quality of the relationship between the animal and its owner. This relationship relies highly on the owners view of animals, like empathy and perception of animal's pain. It is composed by three tests (Avoidance Distance, Walking Down and Tail tuck). When analysing it, is important to understand if a negative response is due to bad handling or to lack of it (Luna et al. 2019).

Aside from human-animal relation, the qualitative behaviour assessment (QBA) is an important tool, as it is a scientific method that assesses a donkey's emotional states (Minero et al. 2016). Although nowadays is common to use QBA for welfare assessment, for many years' scientist avoided it. After many studies that correlate behavioural traits with other physiological parameters, the scientific community validated this tool as a scientific measure (Fraser 2003).

The perception of animal's behaviour is hard to evaluate and requires experience (Minero et al. 2015). When an animal responds in a fearful way to a test it suggests that the animal has been poorly handled in the past, which we perceive has poor welfare. On the other hand, unresponsive animals can also be so due to exhaustion or an over-simulative daily routine (Pritchard et al. 2005).

The absence of social interaction can also be a sign of poor welfare as donkeys are very social beings, as all animals they should live in contact with other animals, preferably of the same species (FAWC 1993; Beaver 2019).

Part II – Experimental Work

6. Objectives of the study

With the increase of concern in animal welfare, it is clear that donkeys should also be a target of this worry, as they play an important role in the lives of their owners.

The main aim of the study was to not only assess the welfare of these animals, in the Northeast of Portugal, but also to do so resorting to animal-based parameters. To each animal, a welfare assessment was made, based in the first level of the Animal Welfare Indicators for Donkeys.

The welfare parameters assessed were:

- Good housing
- Good feeding
- Good health
- Appropriate behaviour

Following the initial assessment another objective was the identification of major welfare problems, in order to better understand, treat and prevent them in the future. Another was that, the results found in the present study could help contribute to the welfare of working and non-working donkeys in Portugal, as well as providing a better understanding of Portuguese asinine welfare status, as no studies were earlier performed.

Besides from welfare evaluation, another goal was population assessment, to give a general idea of the male: female ratio, as well as their distribution. Proper welfare can help improve not only animal's lives but also the lives of those who depend on them.

Expanding knowledge on animal welfare, as well as learning about proper donkey management and care was a secondary and more personal objective of this study.

While developing this project, other tools were acquired: learning about the costumes and traditions of the region, develop social skills and professional growth were some of them.

7. Material and Methods

7.1 Data collection

The study was carried out in the years of 2018 and 2019, integrated in the welfare campaign, conducted by AEPGA. The welfare campaign aimed to evaluate donkeys' welfare status, assess their main problems, as well as advising owners how to avoid them and teaching the communities on the daily basis necessities of their animals. Aside from the campaign, the association also created events in which owners could show their animals, giving them an extra incentive to keep them healthy. The welfare campaign was carried out by veterinarians, farriers and other members of the association. In a previously established date, all donkey owners and respective animals met with the team in a set location. Everyday a different village was visited.

To each animal, a welfare assessment was made, based in the first level of the AWIN for Donkeys. As all animals were evaluated in a common area, some indicators related to housing were not evaluated. Thus, being necessary to make some alterations to the original protocol. The protocol contained 30 questions, some related to animal identification, others to animal welfare. The assessment protocol can be consulted in Appendix I. Good feeding, good health, appropriate behaviour and good housing were all evaluated.

7.2 Welfare assessment protocol

Regarding animal identification the following information was collected: name, microchip number or NIN (National Identification Number), sex, age and village they lived in. Also, in case of females, it was recorded whether they were pregnant or not, in case they were the date of conception was also noted.

Regarding good feeding:

Body condition score was evaluated by both observation and palpation. It was scored from 1

to 5, as described in Table 3. Body sites, like neck, ribs, rump and hindquarters were carefully evaluated. Prominent bones were considered a sign of low BCS being highly noticeable in animals with a score of 1, unlike an animal with a score of 5, in which no bone protuberances were felt, and many fat deposits could be found. Some of the most common places to find fat pads are shown in Figure 5. (The Donkey Sanctuary 2018).



Figure 5. Fat deposit areas

SCORE	DESCRIPTION
1. Poor	Bone prominences easily seen and felt
	Lack of muscular development
	Ribs visible, belly tucked up
2. Moderate	Bone prominences seen
	Some muscular development
	Ribs are not as visible but easily felt
3. Ideal	Bone prominence not seen, but felt under muscle cover
	Good muscle development
	Ribs felt under muscle layer
4. Fat	Bone prominence only felt with firm pressure and not seen
	Fat layer on top of muscles
	Ribs hardly felt in the dorsal part, belly developed
5. Obese	Pockets of fat deposits found in neck, ribs and hips
	Overdeveloped belly

Table	3. Donkey	Body	Condition	Score	(adapted	from t	the Clinical	Companion	of the I	Donkey)
-------	-----------	------	-----------	-------	----------	--------	--------------	-----------	----------	---------

Regarding good housing:

Signs of thermal stress could be found in either extreme hot or cold situations. When an animal experiences heat stress, its respiratory rate is increased and other signs can be visible, like flared nostrils, apathy and sweating. In case of cold stress, low respiratory rate, apathy and shivering are common signs (Minero et al. 2015).

Regarding good health:

Integument alterations are common findings in working animals such as donkeys. In the protocol the presence of these alterations was noted, and were classified in four types: alopecia, skin lesion, deep wound and swelling. Distributed in the nine areas of the body: muzzle, head, neck, shoulder, back, girth/ribs, hindquarters, legs and hooves/coronets.

Swollen joints were commonly found by visual inspection and further explored by palpation.

Lameness was examined. First, observing the animal's posture while resting. Secondly, watching the animal walking in a straight line. Also questioning the owner whether the animals showed signs of lameness while or after working, were part of the exam.

Prolapses were evaluated by visually assessing the anus and vulva/ penis.

Discharges of nose, eyes and vulva/penis were scored as absent or present.

Hair coat condition was evaluated through the whole body, with special care not to confuse changing coat, due to changing season, with bad coat.

Faecal soiling was assessed by observation of the hindlimbs.

Cheek palpation consisted in the outside evaluation of teeth, by applying pressure in the cheeks against the upper teeth and pressuring the temporomandibular joint (TMJ).

Abnormal breathing was evaluated before any handling situations in order to prevent alterations provoked by stress.

Hoof neglect included overgrown hooves, cracks, or even wrong angulation.

Signs of hot branding were rarely found, although examination of neck and hindquarters was always conducted (Minero et al. 2015)

Regarding appropriate behaviour:

Social interaction was scored based in questioning the owner on the animal's environment, whether the donkey was in contact with other animals.

Human-animal relations were evaluated with three tests (Minero et al. 2015).

- **a.** Avoidance distance was conducted by walking from a three-meter distance from the donkey, with the arm slightly elevated. When approaching the animal if he tried to move away it was considered avoidance behaviour.
- b. Walking down side was the test in which the assessor was on the side of the donkey, and gently put his hand in the withers, then calmly walked down the side of the animal. If the donkey tried to flee or kicked it was considered a negative reaction.
- **c.** Tail tuck was evaluated alongside with the previous test, if the animal tucked its tail it was considered present.

Dental examination:

In addition, in 2019 further dentistry exams were conducted, such as lateral excursion and visual examination of the mouth, in order to conduct a list of animals which needed dental assistance. Animals were given a score of 1 to 3 regarding their needs for dental treatments such as rasping. Some of the abnormalities looked for consisted in enamel points, wear abnormalities (shear, step and wave mouth), over/ underjet, ramps, hooks and other disorders (diastemata, fractures, supernumerary teeth, etc) (Easley 2011; The Donkey Sanctuary 2019).

Table 4 shows dental classification parameters. Animals were classified as D1 when they showed no alterations of immediate concern. The classification of D2, when had few small changes that needed correction but where not causing eating disorders or discomfort. D3 corresponded to animals with abnormalities that had an immediate negative impact on the animal's welfare. For example, enamel points causing ulcers, wear abnormalities leading to inefficient food mastication. Also, if an animal showed various smaller alterations was considered a D3.

Classification	Enamel	Wear	Ramps	Hooks	Over/	Others	Observation
	points	abnormalities			underjet		
D1		-	-	-	-	-	
D2			small	small	-		Maximum 2 alterations
D3			big	big			

Table 4 Dental classification chart

7.3 Data analysis

Data collected was organised in Excel 2016 (Microsoft Corporation, USA). The statistical analysis was performed in the statistics programme JMP 7, 2007 (SAS Institute Inc, USA) in which a study of frequency, percentage distribution and variation was calculated, to all welfare parameters previously gathered.

8. Results

8.1 Year of 2018

8.1.1 Population

In the year of 2018 the population analysed was composed by 273 donkeys, 86% females (n=235), 10.3% geldings (n=28) and 3.7% jacks (n=10). Age was found to oscillate from less than one to 30 years of age (Graphic 1), although the most commonly found were donkeys of 4 (n=22) and 11 (n=19) years old.



Graphic 1 Age in the year of 2018

8.1.2 Good Feeding and Good Housing

Body Condition Score

In relation to the feeding evaluation, the measurements of the body condition score were, overall, satisfactory. Of the animals evaluated, 49.1% had a BCS of 3 (n=134). Fat animals were more common than thin ones, 17.6% of the animals had a BCS of 4 (n=48) and 17.6% (n=48) were evaluated as a 5. Thin animals represented an overall percentage of 15.4%. Of them 39 animals showed a BCS of 2 and three a BCS of 1. Furthermore, the body condition score of one of the animals was not evaluated.

Signs of Thermal Stress

Only two donkeys showed signs of thermal stress (0.7%), 267 did not bare any signs (97.8%) and four animals were not evaluated on this parameter.

8.1.3 Good Health

Concerning general good health, only 93 animals had all-positive evaluations. The remaining 180 donkeys had at least one negative or non-evaluated parameter. The indicator with less positive evaluations was the cheek palpation (64.8%), but this was also the one with least evaluations, only 241 animals were evaluated regarding this indicator. In other hand the indicator with the biggest percentage of positive results was *no signs of hot branding* (99.6%) followed by *no abnormal breathing* (97.8%). Further positive prevalence of *good health* parameters can be found in Table 5.

Table 5 Good Health Parameters: Percentage of positive results (percentage of animals that didn't show an alteration in each parameter, total n of 273 animals)

Parameter	Percentage of positive results
Lameness	93.0%
Abnormal breathing	97.8%
Haircoat condition	85.0%
Signs of hot branding	99.6%
Swollen joints	92.3%
Signs of hoof negelct	65.9%
Integument alteration	77.3%
Discharge-ocular	93.0%
Discharge-Nasal	96.3%
Discharge- vulva/penis	97.1%
Faecal Soiling	97.1%
Cheek Palpation	64.8%

Cheek Palpation

In the year of 2018, 32 animals were not checked for teeth disorders, 64 showed abnormalities (23.4%) and 177 didn't (64.8%).

Swollen Joints, Lameness and signs of Hoof Neglect

The joint evaluations showed that 92.3% of the donkeys didn't have swollen joints (n=252), 5.5% had swelling (n=15) and 6 animals were not evaluated. A total of 30.8% of the donkeys showed signs of hoof neglect (n=84), 65.9% didn't show (n=180) and 9 were not evaluated. When lameness was assessed, only 17 animals were lame (6.2%), and 253 were not (92.7%).

Faecal Soiling

Part of the welfare evaluation passes by looking for signs of diseases. Faecal soiling is the name given when there are signs of faeces in the hind legs of a donkey, it is suggestive of diarrhoea. Signs of faecal soiling were found in only 3 (1.1%) animals, with no signs found in 265 animals (97.1%), There were no results for the remaining 1.8% (n=5).

Integument alterations

In a broad approach, 211 animals did not show any type of integument alteration (77.3%) and 62 did (22.7%). Of the affected animals, some showed various types of lesion in different parts of the body. Alopecia was present in 19.4% of the animals (n=53) and absent in 80.6% (n=220). Regarding the number and areas where alopecia was found (there were 120 lesions) the most common places were back and ribs. Lesion distribution is shown in Table 6.

Area	N ^o Alopecia lesions	% alopecia lesions
Back	30	25%
girth & Ribs	30	25%
Head	12	10%
Hindquarters	9	7.5%
Hooves and coronet	1	0.8%
Legs	7	5.8%
Muzzle	2	1.7%
Neck	19	15.8%
Shoulder	10	8.4%

Table 6 Alopecia Lesions, distribution in the year of 2018

Skin lesions were present in only 7.3% of donkeys (n=20) and absent in 92.7% (n=253). The legs were the place where a bigger number of lesions were found, 39.2% of all skin lesions (n=9), followed by the head, with 17.4% (n=4). Hindquarters showed 13.1 % (n=3), the neck and shoulders each made 8.7% of skin lesion (n=2). In the hooves, back and ribs only one lesion was found in each (4.3%). Deep wounds were only found in one animal (0.4%), located in the legs.

The number of animals which showed swelling joints were 9 (3.3%). This problem was absent in 264 (96.7%). Three of these lesions were found in the head (33.3%), 3 in the hindquarters (33.3%), 2 (22.2%) in the neck and 1 in the legs (11.1%). Regarding the location where alterations were found the most common area was the back (n=34) followed by girth and ribs (n=31).

Hair coat condition and Signs of Hot Branding

Related to hair coat condition and hot branding, 3 and 6 animals were not evaluated, respectively. Only one animal was branded and the other 266 weren't (97.4%). Overall, only 13.9% of the animals showed unhealthy coat (n=38).

Discharges

Regarding this parameter, 247 animals didn't show any kind of discharges, either from the eyes, nose or sexual organs. Ocular discharge was found in 11 animals (4%) and was absent in 255 (93.4%). 7 were not evaluated. Nasal discharge was not evaluated in 5 animals, the others 96.3% didn't show signs of nasal discharge (n=263) and only 1.8% did (n=5). Discharges in the penis or vulva were found in 2 animals (0.7%), were absent in 265 (97%) and two donkeys were not assessed for this parameter.

Abnormal Breathing

Only three donkeys had signs of abnormal breathing (1.1%), 267 did not show signs (97.8%) and other 3 were not evaluated (1.1%).

3.1.5 Appropriate behaviour

Of the 273 animals evaluated, in the year of 2018, only 161 donkeys tested positively in all Appropriate Behaviour parameters (social contact, avoidance distance, walking down side and tail tuck).

Regarding the contact with other animals, 19 animals did not have any, either with donkeys or animals of other species (7%), other 4 were no evaluated (1.4%) and the remaining 250 had contact with other animals in a daily basis (91.6%).

When the avoidance distance was evaluated 46 animals reacted with avoidance behaviour (16.9%), 222 did not react to the test (81.3%) and 5 were not assessed (1.8%).

While performing the walking down test two things were evaluated a positive or negative response and tail tuck. 59 donkeys showed a negative response to the first test (21.6%), 207 had positive or neutral responses (75.8%) and in 7 no results were reported (2.6%). In addition, 36 animals reacted with a tail tuck (13.2%), 231 did not (84.6%) and 6 were not evaluated (2.2%).

8.2 Year of 2019

8.2.1 Population

In the year of 2019, 247 donkeys' welfare was assessed in 37 villages. Of the animals observed, 85.8% were females (n=212), 7.7% were geldings (n=19) and 6.5% were stallions (n=16). Ages varied from 1 to 31 years of age, the most common age was 3 (n=20), followed by 9 (n=18).



8.2.2 Good feeding and good housing

Graphic 2 Age in the year 2019

Body Condition Score

Under the good feeding parameter, 128 animals (51.8%) scored positively regarding BCS, with a score of 3, whilst 48.2% scored negatively (n=119). Of those, 8.9% were underweighted and 39.3% were overweighed. Evaluating underweighted animals, only one showed a BCS of 1 (0.4%) and 21 were found to have a BCS of 2 (8.5%), making up a total of 22 thin animals (8.9%). Of the overweighed animals, 74 showed a BCS of 4 (30%) and 23 a BCS of 5 (9.3%).

Signs of Thermal Stress

No signs of thermal stress were found in the 244 animals evaluated for this category (98.8%), for the remaining 3 there were no results (1.2%).

3.2.3 Good health

In what good health is concerned, there were only 66 animals that had only positive scores in all the good health parameters, the other 181 had one or more negative good health scores or were not assessed. The percentage of positive results regarding the good health parameters are shown in table 7.

Parameter	Percentage of positive results
Lameness	76.1%
Abnormal breathing	97.2%
Haircoat condition	88.7%
Signs of hot branding	97.6%
Swollen joints	98.0%
Signs of hoof negelct	79.8%
Integument alteration	76.9%
Discharge-ocular	96.8%
Discharge-Nasal	98.4%
Discharge- vulva/penis	98.0%
Faecal Soiling	87.4%
Cheek Palpation	59.9%

Table 7 Good Health Parameters: Percentage of positive results (percentage of animals that didn't show an alteration in each parameter, total n of 247 animals).

Cheek palpation

Regarding check palpation, 148 donkeys showed no alterations (60%), 58 showed abnormalities in the test (23.5%) and 41 were not evaluated on this parameter (16.6%).

Swollen joints, Lameness and Hoof Neglect

The data on lameness results only covers 213 donkeys (86.2%), of those 188 were not lame

(76.1%), the other 25 showed positive signs of lameness (10.1%). Only one animal showed signs of swollen joints (0.4%), 242 didn't show alterations (98%) and 4 were not evaluated (1.6%).

When evaluating the hooves, 46 animals showed signs of neglect (18.6%) (figure 4), four animals were not assessed (1.6%) and 197 didn't show any obvious signs of neglect (79.8%).



Figure 6. Overgrown hooves

Faecal Soiling

This sign was found in 23 animals (9.3%) and was absent in 216 (87.4%). 8 animals were not evaluated (3.2%). Prolapses were not found in any of the animals assessed, although result for 3 of these animals were not recorded (1.2%).

Integument Alterations

When evaluating integument, 190 donkeys showed no signs of alterations (77%), either alopecia, skin lesion, deep wounds or swelling, and 57 animals showed one or more alterations in one or multiple areas of the body (23%). Alopecia was the most common. It was present in 19.4 % of all animals (n=48), the other 199 didn't have alopecia anywhere in the body. Moreover, more than 113 lesions were found, being the most common places back, girth and ribs.

Area	N° alopecia lesions	% alopecia lesions
Back	20	17.7%
Girth & Ribs	20	17.7%
Head	5	4.4%
Hindquarters	27	23.9%
Hooves and coronet	0	0%
Legs	11	9.7%
Muzzle	1	0.9%
Neck	18	15.9%
Shoulder	11	9.7%

Table 8 Alopecia Lesions, distribution in the year of 2019

Skin Lesions were found in 6.9% of all animals (n=17), the remaining 93.1% had no signs of lesion (n=230). There was a total of 21 lesions found, most of them in the legs (n=6), followed by the shoulders (n=5) and head (n=3). In the back, neck, girth and ribs only two lesions were found in each. At last, the hindquarters were the area that showed least signs of this type of alteration. No animal displayed signs of deep wounds. And only two showed signs of swelling (0.8%), one in the muzzle area and anther one in the legs.

Hair coat condition and Signs of Hot Branding

Hair coat condition alterations were found in 26 animals (10.5%), Of the observed animals, 88.7% showed a healthy hair coat, 2 donkeys were not evaluated (0.8%). No animal evaluated showed signs of hot branding (97.6%, n=241).

Discharges

Of the 247 donkeys evaluated in the year of 2019, 233 didn't show discharges of any kind (eyes, nose, vulva/penis). Ocular discharges were the most commonly found, 6 animals presented them (2.4%), 2 were not evaluated (0.8%) and 239 didn't have any signs (96.8%). Three animals showed signs of nasal discharge (1.2%), one was not evaluated and 243 didn't have it (98.4%).

Although 5 animals were not evaluated for vulva or penis discharge, the remaining 242 were free of it (98%).

Abnormal Breathing

Of all the animals examined, no donkeys showed signs of abnormal breathing (97.2%) although there were no results for 7 donkeys (2.8%).

8.2.4 Appropriate Behaviour

In an overall evaluation only 107 animals scored positively in all behaviour-related tests. Social contact was the parameter with the best success rate; only one animal was evaluated as to not have any contact with other animals (0.4%), the remaining 246 (99.6%) contact with other donkeys or animals in a daily basis. In the avoidance distance test, 36% of the animals showed signs of avoidance behaviour (n=89), 61.9% did not react to the test (n=153) and 5 were not evaluated.

Whilst performing the walking down test 28.7% of the animals reacted negatively to the test (n=70), 67.2% had a neutral or positive reaction to the test (n=164) and 10 were not assessed. In addition, 32 animals performed a tail tuck (13%), 206 did not exhibited that sign (83,4%) and 9 animals didn't have the evaluation of this test (3.6%).

8.2.5 Other findings

Regarding teeth evaluation, only 141 animals were analysed. The following statistical results concern these animals, and not the total 247 donkeys evaluated in the overall welfare survey. Of the 141 donkeys, 41.1% were classified as a D1 (n=58), in the matter of dental disorders. Other 42.6% animals showed some abnormalities and were classified as D2 (n= 60). The remaining 23 donkeys had a more pressing need for dental treatment and where, therefore, classified as D3 (16.3%).

Furthermore, when comparing these results with those of check palpation, it was verified that of the 58 donkeys which responded positively, 36 were classified as D2 and 16 as a D3. The remaining 6 either were not evaluated or had a classification of D1 (graphic 3).



Graphic 3 Classification of dental disorders in animals that responded positively to cheek palpation

Also, in 2019, 15 animals had ectoparasites specifically lice. Regarding hoof problems, 17 animals had overgrown hooves in need of trimming, five had clubfoot, one had laminitis and another presented neurological problems.

8.3 Results 2018 - 2019

The population variation was similar in both years (graphic 4). In 2018 the positive results of appropriate behaviour (59%) were more positive than those of 2019 (43.3%), as shown in graphic 5.







Graphic 5 Appropriate behaviour and Signs of Thermal Stress, in 2018 and 2019

In 2019, there were more positive results regarding good housing (98.8%) and good feeding (51.8%) than in 2018 (97,8% and 49.1% respectively), this is visible in graphic 5 and 6. In relation to good health, each year had better results in 6 of the 12 criteria (graphic 7).



Graphic 6 Comparison of Body Condition Score, in the years 2018 and 2019



Graphic 7 Comparison of good health parameters

9. Discussion

9.1 Population

Sixteen villages were evaluated just in one of the years, the remaining 27 were evaluated in both years, although animals might not have been the same, either due to death or purchase of donkeys, or because owners didn't show to the annual check-up.

The percentage of females in both years was similar in 2018 (86.0%) and 2019 (85.8%). In proportion more geldings were evaluated than stallions in both years. Similar results were found in other studies completed in Europe, where there was also a clear majority of female population, followed by geldings (Quaresma et al. 2014; Dai et al. 2016). This gender tendency can be explained by the different behavioural traits, aggression is more likely to be found in stallions rather than in geldings, although, when castration is performed in more mature animals, stallion behaviour may persist (The Donkey Sanctuary 2018).

Still in relation with gender, contrasting results were found in studies performed in developing countries, where the majority of the donkeys were stallions (Kumar et al. 2014; Björkengren 2016; Tesfaye et al. 2016; Fsahaye et al. 2018). From what is documented, the difference in Mexican's gender population is due to the owners perception that jennies are more difficult to handle, the same opinion was found about male mules and stallions (Galindo et al. 2017).

Concerning age variation in the year of 2018 there was a bigger range of ages, the median value of age was 10.3 years old and in 2019 it was 10.8 years old. As in the present study, a wide age variation was found in different parts of the world (Kumar et al. 2014; Dai et al. 2016;), although most animals found were between the ages of 3 and 13 (Mclean et al. 2012; Tesfaye et al. 2016; Fsahaye et al. 2018;). In one hand the wide range of ages found in different parts of the world can be due to the necessity of owners to use animals for work. As such they use the animals until they die or cannot work anymore, hence the old ages. On the other hand, there is the need to replace them when it happens, for younger animals (Blench 2004). Also, most animals have ages between 3 to 13 years, this can be explained by the achievement of maturity of these animals, making them more apt for working and reproducing (Quaresma et al. 2005; Kumar et al. 2014;).

9.2 Management

In both years of welfare assessment, only two animals showed signs of thermal stress (graphic 5). Although the results are positive, thermal stress is a serious concern and sometimes overlooked in donkeys. Studies have shown that signs of heat stress caused by hot temperatures are more noticeable than those caused by cold temperatures (Matthews et al. 1998), some quick and easy parameters to evaluate can be rectal temperature, respiratory and heart rate (Pritchard et al. 2006), their increase alongside flared nostrils, apathic behaviour and sweating are suggestive of stress caused by high temperatures (Pritchard et al. 2005).

In developing countries welfare assessments showed that thermal stress is generally more common than in Europe, and donkeys are particularly more susceptible to this, when compared with horses or mules (Ali et al. 2015; Dai et al. 2016; Osthaus et al. 2017). Also there is a relation between the type of work performed by the animals and the prevalence of signs of thermal stress (Pritchard et al. 2005).

Although heat stress is not sometimes considered as important as other abnormalities, it is important to remember that it is not only a welfare risk but can also lead to serious health problems when unchecked, such as dehydration, organ disfunction and behavioural alterations. As so it is important to teach owners to identify thermal stress signs, so they can act on them, and prevent further suffering (The Brooke 2013a).

Proper feeding is vital to a healthy life and proper welfare. Regarding the body condition score, in both years, the majority of animals had a BCS of 3, although in 2019 the results were slightly better (51.8%) than in 2018 (49.1%) (graphic 6). Animals that scored 4 were more common in 2019 (30%) than in 2018 (17.6%) as opposed to animals with a score of 5 that were more frequent in 2018 (17.6% vs 9.30%).

Underweighted animals were more common in 2018 than in 2019. The number of donkeys scored as 1 were similar, 0.3% and 0.4% of all evaluated donkeys, in 2018 and 2019, respectively. But there was a significant difference between the donkeys with a condition score of 2 in the year 2018 (15.4%) and 2019 (8.5%).

The body condition score is the reflection of a proper or improper nutrition. As mentioned, when evaluating it, results showed that in the northeast of Portugal, the ideal score (BCS=3) was the most prevalent. Extreme scores were rare in both years (BCS=1 or 5). There was,

however, a bigger number of overweighed animals rather than underweighted. Other studies in Europe also showed that a majority of animals had a healthy body condition, although the tendency to over or underweight can vary regarding the type of work performed by each animal (Dai et al. 2016, 2017). For instance, dairy donkeys have superior energy requirements due to lactation and breeding needs, causing them to lean towards an underweighted condition (Galindo et al. 2017; Raspa et al. 2019).

The Portuguese tendency to donkey's overweight might be explained by the better quality of food available. Also in Europe, contrary to developing countries, animal power has been replaced by machinery and , as such, the animals are less used for work (Quaresma et al. 2014; Camillo et al. 2017). Most of the donkey's population are either companion or used for other objectives now trending in Europe, such as milk production or assisted therapy (Cox et al. 2010; Borioni et al. 2012; Mandrá et al. 2019).

In developing countries, the scenario differs, as it is more common to find a thin animal rather than an obese one, although the prevalence of ideal or skinny condition varies between countries or even between cities (Pearson et al. 1996; Burn et al. 2010; Kumar et al. 2014; Fsahaye et al. 2018). As mentioned, this difference can be related to the different food quality existing in European and developing countries (Starkey 1998; Björkengren 2016). A parallel study performed in Ethiopia showed that most of the working donkeys were highly infested with gastrointestinal parasites, mainly nematodes (Getachew et al. 2010). The high parasitic levels plus the lack of anti-helminthic treatments might be a causing factor of the low body condition scores. Other explanation might be the lack of dental care in less developed areas (Burden et al. 2010).

Although more common in less developed countries, in the present study 42 underweighted animals were found in 2018 and 22 in 2019. This can be due to wrong feeding management, diseases, teeth alteration or even social problems (The Donkey Sanctuary 2018). Also the food composition varies in each season, which can cause a change in nutrient intake and lead to a decrease in body condition (Couto et al. 2016).

In general, when evaluating an underweighted animal, prior to make nutritional changes it is important to find out what is causing the decrease in body weight condition. Weight loss can be secondary to other health problems as loss of appetite is a common clinical sign. Deficient food intake can be one of the sources of low BCS, either due to wrong nutritional management or dental problems. An improper work load can also originate weight loss (The Donkey Sanctuary 2018)

Although an untrained eye might look at a thin animal as less healthy than a fat one, obesity can lead to serious and life-threatening diseases. Obese animals show a higher basal insulin concentration, although it has not yet been proven that it increases insulin sensitivity in donkeys as it does in horses and ponies (Pritchard et al. 2019).

Hyperlipidaemia is one of the biggest concerns in European donkey's population. It consists in the increase of triglycerides in bloodstream. Although the incidence rate was thought to be of 3% in donkeys, a recent study showed prevalence of 11% in Great Britain (Burden et al. 2011). This is a problematic disease, with a mortality rate that can go as high as 80% (Reid et al. 1992).

The increase in body weight can also cause laminitis. Even though this disease is one of the most common causes of lameness in donkeys, its detection tends to be delayed due to donkey's stoicism (The Donkey Sanctuary 2018). Behaviour alterations might be the first noticed by the owners, like reluctance to move and longer periods laying. Later on, in the physical exam, an increase in digital pulse, hoof temperature and pain response to hoof exam might be indicative of this disease (Thiemann et al. 2013). It has many causes, some of them can be increase in grain consumption and improper hoof care (Abutarbush et al. 2014).

9.3 Behaviour

Animal welfare is directly linked with the way owners value animal life and their perception of their animal's feelings and experiences. The way an animal is treated has a direct input on its welfare, as so, evaluation of human-animal interactions is of upmost importance (Wemelsfelder et al. 2000; Luna et al. 2019;). When comparing the behaviour evaluation in both years, in 2018 the percentage of animals with all positive results (59%) was bigger than in 2019 (43.3%) (graphic 5).

In what concerned the avoidance test, in the year of 2019, there was a 36% prevalence of avoidance behaviour. Various authors explained this behaviour as a reflection of poor handling by the owner (Swann 2006). Although it is also important to recognise that tests were performed by someone strange to the animals, which might cause a negative reaction (Popescu et al. 2013).

The overall mainly positive results differ from those found in donkeys from developing countries, in which the negative response had a higher prevalence (Kumar et al. 2014). Although most of Portuguese population is kept for working purposes (Quaresma et al. 2014), the amount of work and the conditions of it are very different from those of poorer countries, which results in different welfare evaluations.

Walking down side and tail tuck reaction results were very similar in both years. In 2019 there was a slightly higher number of negative reactions, with 29% of the animals responding negatively to the walk down test and 13 % performing a tail tuck. These results resemble those found by Dai et al. in 2015, with most donkeys showing positive reactions when behavioural tests were performed. Although in this study reactions to human interaction were very positive, in what working animals are concerned, the absence of negative responses doesn't necessarily mean good handling (Pritchard et al. 2005), lack of responses might be due to exhaustion or over-stimulation (Rousing et al. 2001).

Even though in 2019 only one animal was socially excluded, in 2018, 7% of the animals assessed did not have contact with other donkeys or animals. The difference in results is explained by the different animal populations evaluated in both years. Social contact is an important part in welfare evaluation, especially since donkeys are by nature social beings (Burden et al. 2015).

9.4 Health

In the present study, when comparing good health results, both years scored better in six parameters. In 2018, the positive results were better when compared to those of 2019 in what concerned lameness (93%), abnormal breathing (97.8%), sign of hot branding (99.6%), integument alteration (77.3%), faecal soiling (97.1%), cheek palpation (64.8%) as presented in graphic 7. The 2019 assessment showed a higher prevalence of positive results in haircoat condition (88.7%), swollen joints (98%), signs of hoof neglect (79.8%), ocular (96.8%), nasal (98.4%) and penis/vulva (98.0%) discharges (graphic 7).

As in all equids, donkeys' teeth are constantly erupting throughout their life. Their unique mastication movements cause the even wearing of the teeth, so they do not overgrow. However, captive equids have different tooth wearing pattern than wild equids (The Brooke 2013a; Taylor

et al. 2015). This, alongside different eating routines, originates many teeth disorders. For this reason, routinely dental check-ups are necessary for a good health.

A fast way to detect most problems, without much animal manipulation, is through cheek palpation. It is performed by applying pressure on both cheeks against the cheek teeth. If an animal reacts it indicates pain, more likely due to enamel overgrowths in the maxillary teeth (Easley et al. 2011). When doing so in the Portuguese donkey population, in both years, 23% of all animals reacted painfully. Comparing to the results found in other studies performed in Europe, there was a bigger prevalence of cheek pressure response in Portugal rather than in Italy or the UK (Dai et al. 2016).

Regarding oral cavity's evaluation, of the 141 animals assessed almost 59% showed need for dental treatment. Studies performed by Rodrigues et al. showed that, in Portuguese and Spanish donkey population, cheek teeth disorders are more common than incisors' disorders. Also, the enamel overgrowths make up for 73% of all cheek teeth alterations (Rodrigues et al. 2012, 2013).

The results of the present study agreed with many others performed in both developed and developing countries, showing that dental disease is a common problem, transversal to working or non-working equids (Gallagher et al. 2008; Toit et al. 2008; Ramírez 2009; Assefa et al. 2018). It is more likely that rasping is not performed as often in poorer countries, as people do not have access to a veterinary or do not have the economic power to do so (Pritchard et al. 2005; Fsahaye et al. 2018).

Signs of diseases like discharges, prolapses or abnormal breathing were rare in both years. The most common were ocular discharges, found in 4% of the donkeys assessed in 2018. Results were more positive than those found in other studies. In Italy and the UK, ocular discharge showed a prevalence of 16% (Dai et al. 2016). Ocular problems had a similar incidence both in Chile (10%) and Ethiopia (17%) (Tadich et al. 2008; Fsahaye et al. 2018).

Either ocular or nasal discharges are not pathognomonic signs for any diseases, as they can be present in various infirmities, both local and generalized. These discharge can be present in respiratory problems, ocular disease and dental problems (The Brooke 2013b; The Donkey Sanctuary 1999, 2019), therefore, when noticed further investigation must be done, namely ophthalmic examination, respiratory auscultation and dental examination.
In 2018, 31% of the donkeys evaluated showed some level of hoof neglect. In the following year the prevalence was 19% for the same criterion, result similar to that found in the UK (Cox et al. 2010). Hoof care is vital to a proper welfare, as they bare all body weight. One of the most common hoof alterations found in 2019 were overgrown hooves. This problem can lead to lameness. Available studies on the epidemiology of lameness in donkeys are focused on working animals (Mendoza et al. 2018), one of those studies showed that musculoskeletal problems were more common in horses, and wounds or sarcoids were more commonly found in donkeys (Stringer et al. 2016).

The highest lameness prevalence was found in 2019, with 10% of all donkeys being lame, similar to the results found in Ethiopia (Amante et al. 2014). Lameness percentages varied in different studies. Amante et al. (2014) found that 12% of working donkeys showed some kind of lameness grade, in the same year, but in a different Ethiopian cities. Kumar et al. (2014) found slightly better results, with 10% of the animals being lame. Later on, a decrease in lameness prevalence was found, in 2016, by Tesfaye et al. and even better results were found in 2018, with only 4.4 % of the donkeys being lame (Fsahaye et al. 2018). These results do not differ much from those found in Europe, Cox et al. found that British donkeys had a lameness prevalence of 10%. Curiously, results uncovered by Dai et al., were the same as those of Mexico, 2% lameness diagnosed (Burden et al. 2010).

Although the prevalence of lameness, found in any of the studies mentioned, wasn't particularly high, when lameness is detected it is important to find the origin of the problem. Some of the most common causes can be laminitis, tendinitis or just improper trimming of the hooves.

Another problem, common to both years was integument alteration, with 23% of the population showing some kind of lesion. When analysing the integument alterations, results showed that alopecia is the most common integumentary alteration, found in almost 20% of the donkeys, with high prevalence in the back and girth and ribs. The second most common alteration found was skin lesion (7%), predominantly in the legs.

Diverging results are found in developing countries where wounds represented 80% of all integument alterations (Abutarbush et al. 2014). Although the local frequency changed, the back was also the most common place for wounds, (Abate 2017) this can be due to inappropriate harnessing, long hours of work and overweighed loads (Mclean et al. 2012).

Assessments were performed in a period when there was a high incidence of lice in Portugal. Although only 15 animals were diagnosed with these ectoparasites it is likely that many others might have had them. This can explain the high prevalence of alopecia lesions, also ectoparasites are more common in donkeys rather than in horses. This difference can be explained by the management differences between horses and donkeys, in which owners tend to neglect donkeys (Mekuria et al. 2010).

10. Conclusions

The results of the present study showed that, although there are some welfare problems to be amended in Portugal, most donkeys live a healthy and pain-free life.

The BCS was the main concern, with almost 50% of the donkeys with an improper body condition in both years. This can cause various health problems, that will, later on, decrease quality of life. Although BCS was one of the major welfare problems faced in developing countries, in Portugal it was found that animals tend to be overweighed, as opposed to poorer countries. Also, dental and hoof problems were commonly found in the Portuguese population.

In the year 2018, aside from the BCS, the problems with highest prevalence were improper behaviour, pain reaction to cheek palpation, hoof disease and integument alterations. In the year 2019 the same problems had the highest prevalence, with the exception of lameness, that was also commonly found. Though there were slightly better results in 2019, the conclusion that welfare was improved cannot be assumed, because the animals evaluated in 2018 were not the exact same evaluated in 2019. The results of 2019 are still positive, although there is room for improvement.

Furthermore, it would be interesting to continue welfare studies. In one hand, the study of the same population, with the exact same animals, can better evaluate the evolution of welfare in the region. On the other hand, the study of welfare in other parts of Portugal would also be of importance.

In future studies it would also be interesting to evaluate more criteria related with housing, such as shelter dimensions, clean beds and access to clean water. Also, it is vital to emphasize the importance of the evaluation of all welfare parameters in all the animals, as it was one of the flaws found in the present study. Another interesting change would be the assessment of the animals by the same person or systematize the assessment of more subjective parameters.

A better donkey welfare understanding can help improve the lives of the donkeys, not only in Portugal but also in other parts of the world. This can be done by educating the owners to what are basic daily needs and procedures, as to the importance of dental care and hoof trimming. Also teaching owners to identify signs of illness and pain ca help prevent escalation of an existing problem. These are some practical ways to improve animal welfare.

11. References

- Abate, Melkam. 2017. "A Survey on Major Health and Management Problems of Donkeys under Traditional Husbandry System at Selected Sites in Central Highland of Ethiopia." *International Journnal of Sciences: Basic and Applied Research* 31(2):28–42.
- Abutarbush, Sameeh M., Dirar M. Alqawasmeh, Zain H. Shaheen, Sarah F. Anani, and Margaret P. Ledger. 2014. "Equine Diseases and Welfare in Jordan : A Retrospective Study (1261 Cases)." *Jordan Journal of Agricultural Sciences* 10(3):493–503.
- AEPGA, Associação de Estudo e Proteção do Gado Asinino. 2012. "AEPGA Website." Retrieved June 30, 2019 (https://www.aepga.pt).
- Aganga, A. A., A. O. Aganga, T. Thema, and K. O. Obocheleng. 2003. "Carcass Analysis and Meat Composition of the Donkey." *Pakistan Journal of Nutritiom* (3):138–47.
- AHIC, Australian Horse Industry Council. 2011. Australian Horse Welfare Protocol.
- Alge, Barbara. 2004. "Continuidade e Mudança Na Tradição Dos Pauliteiros de Miranda." Universidade de Viena.
- Ali, Ahmed B. A., Mohamed Y. Matoock, Manal A. Fouad, and Camie R. Heleski. 2015. "Are Mules or Donkeys Better Adapted for Egyptian Brick Kiln Work ? (Until We Can Change the Kilns)." *Journal of Veterinary Behavior: Clinical Applications and Research* 10(2):158–65.
- Amante, Morka, Adisalem Hunde, Bojia Endebu, Eyob Hirpa, and Bedasso Mamo. 2014.
 "Health and Welfare Assessment of Working Equine in and around Nekemte Town, East Wollega Zone, Ethiopia." *American-Eurasian Journal of Scientific Research* 9(6):163–74.
- Ana Maria Santiago Palatão. 2011. "Património Geológico Dos Concelhos de Miranda Do Douro e de Mogadouro. Caracterização, Inventariação e Propostas Para a Sua Valorização." Universidade do Minho.
- Appleby, Michael C., Daniel M. Weary, and Peter Sandø. 2014. *Dilemmas in Animal Welfare*. CAB Intern. Oxfordshire.
- Aranguren-Méndez, J., M. Gómez, and J. Jorana. 2002. "Hierarchical Analysis of Genetic Structure in Spanish Donkey Breeds Using Microsatellite Markers." *Heredity* 89:207–11.

- Assefa, Zerihun, Ashenafi Mengistu, Alemayehu Fanta, and Manyahilishal Etana. 2018. "A Preliminary Study on Dental Health Problems and Associated Risk Factors on Donkeys in Ada ' a and Dugda Districts." *Academic Journal of Animal Diseases* 4(1):23–29.
- AWIN. 2015. "Development, Integration and Dissemination of Animal-Based Welfare Indicators, Including Pain, in Commercially Important Husbandry Species, with Special Emphasis on Small Ruminants, Equidae & Turkeys." CORDIS, EU Research Results. Retrieved July 25, 2019 (https://cordis.europa.eu/project/rcn/99394/factsheet/en).
- Barbosa, José Carlos. 2003. "O Gado Asinino Em Trás-Os-Montes. Contribuição Para o Conhecimento Da Sua Importância Socio-Económica." in V Colóquio Hispano-Português de Estudos Rurais. Bragança.
- Beaver, Bonnie V. 2019. Equine Behavioral Medicine. Academic Press, Elsevier Inc.
- Beja-pereira, Albano, Phillip R. England, Nuno Ferrand, Steve Jordan, Amel O. Bakhiet, Mohammed A. Abdalla, Marjan Mashkour, Jordi Jordana, Pierre Taberlet, and Gordon Luikart. 2004. "African Origins of the Domestic Donkey." *Science* 304:2.
- Biffa, Demelash and Moges Woldemeskel. 2006. "Causes and Factors Associated With Occurrence of External Injuries in Working Equines in Ethiopia." *The International Journal of Applied Research in Veterinary Medicine* 4(1):1–7.
- Björkengren, Julia. 2016. "Assessment of Donkeys ' Welfare in Addis Ababa and Ambo, Ethiopia." Swedish University of Agricultural Sciences.
- Blench, Roger. 2004. "The History and Spread of Donkeys in Africa." Pp. 22–30 in *Donkeys, people and development*, edited by D. Fielding and P. Starkey.
- Blokhuis, H. J., I. Veissier, M. Miele, and B. Jones. 2010. "The Welfare Quality Project and Beyond: Safeguarding Farm Animal Well-Being." Acta Agriculturae Scandinavica, Section A - Animal Science 60(3).
- Blokhuis, Harry J., Isabelle Veissier, Mara Miele, and Bryan Jones. 2019. "Safeguarding Farm Animal Welfare." Pp. 137–50 in Sustainability Certification Schemes in the Agricultural and Natural Resource Sectors: Outcomes for Society and the Environment. London, United Kingdom: Taylor & Francis Ltd.
- Blosh, Marie. 2012. "The History of Animal Welfare Law and the Future of Animal Rights." University of Western Ontario.

- Borioni, Nicoletta, Paola Marinaro, Silvia Celestini, Flavia Del Sole, Rachele Magro, Daniela Zoppi, Valentina Dall Armi, Federica Mazzarella, Alfredo Cesario, and Stefano Bonassi.
 2012. "Effect of Equestrian Therapy and Onotherapy in Physical and Psycho-Social Performances of Adults with Intellectual Disability : A Preliminary Study of Evaluation Tools Based on the ICF Classification." *Disability & Rehabilitation* 34:279–87.
- Bracke, M.B.M, Spruijt, B.M, and Metz, J.H.M 1999. "Overall Animal Welfare Reviewed. Part
 3: Welfare Assessment Based on Needs and Support by Expert Opinion." *Netherlands Journal of Agricultural Science* 47:307–22.
- Broom, Donald M. 1986. "Indicators of Poor Welfare." *British Veterinary Journal* 142(6):524–26.
- Broom, Donald M. 1999. "The Welfare of Vertebrate Pests in Relation to Their Management."Pp. 309–29 in Advances in Vertebrate Pest Management, edited by P. D. Cowan and C. J. Feare.
- Broom, Donald M. 2011. "A History of Animal Welfare Science." *Acta Biotheoretica, Springer* 59:121–37.
- Broom, Donald M. 2014. "Sentience and Animal Welfare."
- Broom, Donald M. 2019. "Animal Welfare Complementing or Conflicting with Other Sustainability Issues." *Applied Animal Behaviour Science* (June):0–1.
- Burden, F. 2011. "Practical Feeding and Condition Scoring for Donkeys and Mules." *Equine* veterinary education 24(11):589–96.
- Burden, F. A., N. Du Toit, and A. F. Trawford. 2011. "Hyperlipemia in a Population of Aged Donkeys: Description, Prevalence and Potential Risk Factors." *Journal of Veterinary Internal Medicine* 25:1420–25.
- Burden, Faith A., Nicole Du Toit, Mariano Hernandez-Gil, Omar Prado-Ortiz, and Andrew F. Trawford. 2010. "Selected Health and Management Issues Facing Working Donkeys Presented for Veterinary Treatment in Rural Mexico: Some Possible Risk Factors and Potential Intervention Strategies." *Tropical Animal Health and Production* 42:597–605.
- Burden, Faith and Alex Thiemann. 2015. "Donkeys Are Different." Journal of Equine Veterinary Science 35:376–82.
- Burn, Charlotte C., Tania L. Dennison, and Helen R. Whay. 2010. "Environmental and

Demographic Risk Factors for Poor Welfare in Working Horses, Donkeys and Mules in Developing Countries." *The Veterinary Journal* 186(3):385–92.

- Burnham, Suzanne L. 2002. "Anatomical Differences of the Donkey and Mule." *Anatomical Differences of the Donkey and Mule* 48.
- CAB, Centro de Acolhimento do Burro. 2019. "CAB Website." Retrieved July 1, 2019 (http://www.centroacolhimentoburro.pt/bem-estar/).
- Camillo, Francesco, Alessandra Rota, Lorenzo Biagini, Matteo Tesi, Diana Fanelli, and Duccio Panzani. 2017. "The Current Situation and Trend of Donkey Industry in Europe." *Journal of Equine Veterinary Science*.
- Carr, N. and M. Broom. 2018. Tourism and Animal Welfare. CAB International.
- Carretero-Roque, L., B. Colunga, D. G. Smith, M. Gonzalez-ronquillo, A. Solis-Mendez, and O. Castelan-Ortega. 2005. "Digestible Energy Requirements of Mexican Donkeys Fed Oat Straw and Maize Stover." *Tropical Animal Health and Production* 37(1):123–42.
- CE, Comissão Europeia. 1998. "Directiva 98/58/CE Do Conselho de 20 de Julho de 1998." Jornal Oficial Das Comunidades Europeias 23–27.
- CE, Comissão Europeia. 2007. "Tratado de Lisboa." Jornal Oficial Das Comunidades Europeias.
- CE, Comissão Europeia. 2014. "Regulamento (CE) N. o 504/2008 Da Comissão, de 6 de Junho de 2008." *Publications Office of the European Union*.
- CE, European Commission. 2000. The Welfare of Cattle Kept for Beef Production.
- CE, European Commission. 2013. "DECISÃO DE EXECUÇÃO DA COMISSÃO de 18 de Abril de 2013." *Jornal Oficial Da União Europeia* (8).
- Chazarra, Andrés, Antonio Mestre Barceló, Vanda Pires, Sofia Cunha, Manuel Mendes, and Jorge Neto. 2011. *Atlas Climático Ibérico*. Departamen.
- Chirgwin, J. C., P. De Roover, and J. T. Dijkman. 2000. *El Burro Como Animal de Trabajo*. edited by F. and A. O. of the U. N. (FAO). Roma.
- Clive Phillip. 2009. The Welfare of Animals. Springer.
- Costa, E. Dalla, F. Dai, D. Lebelt, P. Scholz, S. Barbieri, E. Canali, A. J. Zanella, and M. Minero. 2016. "Welfare Assessment of Horses : The AWIN Approach." *Animal Welfare*

25:481-88.

- Couto, M., A. S. Santos, J. Laborda, M. Nóvoa, and L. M. Ferreira. 2016. "Grazing Behaviour of Miranda Donkeys in a Natural Mountain Pasture and Parasitic Level Changes." *Livestock Science* 186:16–21.
- Cox, R., F. Burden, C. J. Proudman, A. F. Trawford, and G. L. Pinchbeck. 2010. "Demographics, Management and Health of Donkeys in the UK." *Veterinary Record* 166:552–56.
- Dai, Francesca, Emanuela Dalla Costa, Leigh Margareth, Anne Murray, and Elisabetta Canali.
 2016. "Welfare Conditions of Donkeys in Europe: Initial Outcomes from On-Farm Assessment." *Animals* 6(5):8–12.
- Dai, Francesca, Giulia Segati, Marta Brscic, Matteo Chincarini, Emanuela Dalla Costa, Lorenzo Ferrari, Faith Burden, Andrew Judge, and Michela Minero. 2017. "Effects of Management Practices on the Welfare of Dairy Donkeys and Risk Factors Associated with Signs of Hoof Neglect." *Journal of Dairy Research* 1–9.
- Darwin, Charles. 1872. *The Expression of the Emotion Sin Man and Animals*. edited by A. S. John Murray. London.
- Davidson, N. and P. Harris. 2003. "Nutrition and Welfare." Pp. 46–51 in *The Welfare of Horses*, edited by N. Waran. Edinburgh, Scotland, United Kingdom: Kluwer Academic Publishers.
- Davis, G. 2011. "One Health." Pp. 219–38 in Animals, Diseases, and Human Health: Shaping Our Lives Now and in the Future. Santa Barbara, California.
- Dawkins, M. 2003. "Behaviour as a Tool in the Assessment of Animal Welfare." Zoology 106(4):383–87.
- Dhammika, Ven S. 1993. "The Edicts of King Asoka." The Wheel.
- Duncan, Ian J. H. 2006. "The Changing Concept of Animal Sentience." *Applied Animal Behaviour Science* 100:11–19.
- Duncanson, G, R. 2010. Veterinary Treatment for Working Equines. Oxfordshire: CAB International.
- Easley, Jack. 2011. "Oral and Dental Examination." Pp. 28–34 in Focus on Dentistry. Albuquerque, USA.

- Easley, Jack and W. Henry Tremaine. 2011. "Dental and Oral Examination." Pp. 185–98 in Equine Dentistry, 3rd edition, edited by J. Easley, P. Dixon, and J. Schumacher. London, United Kingdom.
- EFSA, European Food Safety Authority. 2006. *Basic Information for the Development of the Animal Welfare Risk Assessment Guidelines*. Italy.
- FAO, Food and Agriculture Organization of the United Nations. 2010. Draught Animal Power.
- FAO, Food and Agriculture Organization of the United Nations. 2019a. "Domestic Animal Diversity Information System (DAD-IS)." *Trend in Risk Status*. Retrieved June 30, 2019 (http://www.fao.org/dad-is/trend-in-risk-status/en/).
- FAO, Food and Agriculture Organization of the United Nations. 2019b. "Food and Agriculture Organization, Statistics Division (FAOSTAT)." Retrieved June 11, 2019 (www.fao.org/faostat/).
- FAO, Food and Agriculture Organization of the United Nations and The Brooke. 2011. *The Role, Impact and Welfare of Working (Traction and Transport) Animals.* Roma.
- FAWC, Farm Animal Welfare Council. 1993. Report for Priorities for the Research and Development in Farm Animal Welfare. London.
- Federação Equestre Internacional. 2019. "Vaccination." Retrieved July 29, 2019 (https://inside.fei.org/fei/your-role/veterinarians/vaccinations).
- Fernando, Priyanthi and Paul Starkey. 2004. "Donkeys and Development: Socio-Economic Aspects of Donkey Use in Africa." in *Meeting the Challenges of Animal Traction: A Resource Book of the Animal Traction Network for Eastern and Southern Africa*, edited by A. T. N. for E. and S. Africa.
- Fisher, Naomi. 2017. "Kant On Animal Minds." ERGO 4(15):441-62.
- Fraser, Andrew and Donald Broom. 1990. *Farm Animal Behaviour and Welfare*. CAB International.
- Fraser, D. 2003. "Assessing Animal Welfare at the Farm and Group Level: The Interplay of Science and Values." Animal Welfare 12(4):433–43.
- Fraser, D., Weary, D.M, Pajor, E.A. 1997. "A Scientific Conception of Animal Welfare That Reflects Ethical Concerns." *Animal Welfare* 6:187–205.

- Fsahaye, Shushay, Niraj Kumar, Etsay Kebede, and Nigussie Abebe. 2018. "Health and Welfare Assessment of Working Donkeys in and around Rama Town, Tigray, Ethiopia." *Ethiopian Veterinary Journal* 22(1):26–39.
- Galindo, F., A. de Aluja, R. Cagigas, Huerta, L.A, and Tadich, T.A. 2017. "Application of the Hands-on Donkey Tool for Assessing the Welfare of Working Equids at Tuliman, Mexico." *Journal of Applied Animal Welfare Science* 1–8.
- Gallagher, J., F. A. Burden, and P. M. Dixon. 2008. "Post Mortem Survey of Dental Disorders in 349 Donkeys from an Aged Population (2005 – 2006). Part 1 : Prevalence of Specific Dental Disorders." *Equine Veterinary Journal* 40(3):204–8.
- Gantner, Ranko, Mirjana Baban, Pit Schlechter, The European, Draught Horse, and Domagoj Zimmer. 2014. "INDICES OF SUSTAINABILITY OF HORSE TRACTION IN AGRICULTURE." *Economy of Eastern Croatia Yesterday, Today, Tommorow* 3:616–26.
- García, José Emilio Yanes. 1999. *El Asno Zamorano Leonés, Una Gran Raza Autóctona*. edited by D. de Zamora. Zamora.
- Garrett, Chris. 2018. A Guide to Pack Saddles.
- Getachew, M., A. Trawford, G. Feseha, and S. W. J. Reid. 2010. "Gastrointestinal Parasites of Working Donkeys of Ethiopia." *Tropical Animal Health and Production* 42:27–33.
- Grange, Wiscombe. 1995. "Trimming Donkeys Feet." *EQUINE VETERINARY EDUCATION* 7(1):18–21.
- Hamza, M. A. and W. K. Anderson. 2005. "Soil Compaction in Cropping Systems A Review of the Nature , Causes and Possible Solutions." *Soil & Tillage Research* 82:121–45.
- Herman, C. L. 2009. "The Anatomical Differences between the Donkey and the Horse." *Veterinary Care of Donkeys*.
- Herold, Peter, Pit Schlechter, and Reinhard Scharnhölz. 2009. "Modern Use of Horses in Organic Farming." *Fédération Européenne Du Cheval de Trait Pour La Promotion de Son Utilisation*.
- Huang, Jinlong, Yiping Zhao, Dongyi Bai, Wunierfu Shiraigol, Bei Li, Lihua Yang, Jing Wu, Wuyundalai Bao, Zhencun Xing, Aoruga An, Yahan Gao, Ruiyuan Wei, Yirugeletu Bao, Taoketao Bao, Haige Han, Haitang Bai, Yanqing Bao, Yuhong Zhang, Dorjsuren Daidiikhuu, Wenjing Zhao, Shuyun Liu, Jinmei Ding, Weixing Ye, Fangmei Ding, Zikui

Sun, Yixiang Shi, Yan Zhang, He Meng, and Manglai Dugarjaviin. 2015. "Donkey Genome and Insight into the Imprinting of Fast Karyotype Evolution." *Nature Publishing Group* 1–10.

- INE, Instituto Nacional de Estatística. 2019. "Instituto Nacional de Estatística Website." Densidade Populacional ($N.^{o}/Km^{2}$) Por Local de Residência.
- Jamdar, M.N and Ema, A.M. 1982. "A Note of the Vertebral Formula of The Donkey." *British Veterinary Journal* 138(3):209–11.
- Jézéquel, Matilde Carrilho. 2016. "Sebes e Paisagem, Caso de Estudo : Planalto Mirandês." Universidade de Lisboa.
- Karatosidi, D., G. Marsico, and S. Tarricone. 2013. "Modern Use of Donkeys." *Iranian Journal of Applied Animal Science* (March).
- Kugler, Waltraud, Hans-peter Grunenfelder, and Elli Broxham. 2007. Donkey Breeds in Europe.
- Kumar, Niraj, K. K. Fisseha, N. Shishay, and Y. Hagos. 2014. "Welfare Assessment of Working Donkeys in Mekelle City, Ethiopia." *Global Veterinaria* 12(3):314–19.
- Lombard, J. E., C. B. Tucker, M. A. G. Von Keyserlingk, C. A. Kopral, and D. M. Weary. 2010. "Associations between Cow Hygiene, Hock Injuries, and Free Stall Usage on US Dairy Farms." *Journal of Dairy Science* 93(10):4668–76.
- Luna, Daniela and Tamara A. Tadich. 2019. "Why Should Human-Animal Interactions Be Included in Research of Working Equids' Welfare?" *Animals* 9(42):1–15.
- Mandrá, Patrícia Pupin, Thaís Cristina da Freiria Moretti, Leticia Alves Avezum, and Rita Cristina Sadako Kuroishi. 2019. "Animal Assisted Therapy: Systematic Review of Literature." *CoDAS* 31(3):1–13.
- Manteca, X., E. Mainau, and D. Temple. 2012. "What Is Animal Welfare ?" (The Farm Animal Fact Sheet, n°1).
- MAOT, Ministerio do Ambiente e Ordenamento do Território. 2001. Plano de Bacia Hidrográfica Do Rio Douro.
- Martin-rosset, William. 2018. "Donkey Nutrition and Feeding: Nutrient Requirements and Recommended Allowances- a Review." *Journal of Equine Veterinary Science* 65:75–85.

- Matthews, N., T. Taylor, and M. Hartsfield. 2005. "Anaesthesia of Donkeys and Mules." *EQUINE VETERINARY EDUCATION* 15(57):102–7.
- Matthews, Nora S., Tex S. Taylor, and Gary D. Potter. 1998. "Physiologic Responses during an Exhaustive Driving Test in Donkeys: Effect of Conditioning." *Applied Animal Behaviour Science* 59:31–38.
- Mckenna, C. 2007. Bearing a Heavy Burden. London.
- Mclean, Amy K., Camie R. Heleski, Melvin T. Yokoyama, Wei Wang, Amadou Doumbia, and Boubacar Dembele. 2012. "Improving Working Donkey (Equus Asinus) Welfare and Management in Mali, West Africa." *Journal of Veterinary Behavior: Clinical Applications and Research* 7(3):123–34.
- Meirinhos, Miguel Pires. 2014. "A Evolução Da Terra de Miranda : Um Estudo Com Base Nos Sistemas de Informação Geográfica." Universidade do Porto.
- Mekuria, Solomon and Rahmeto Abebe. 2010. "Observation on Major Welfare Problems of Equine in Meskan District, Southern Ethiopia." *Livestock Research for Rural Development* 22(3).
- Mendez, José. 2002. "caracterización y relaciones filogenéticas de cinco razas asnales españolas en peligro de extinción mediante la utilización de marcadores microsatélites: su importancia en los programas de conservación." Universidad Autónoma de Barcelona.
- Mendoza, F. J. and R. E. Toribio. 2018. "Donkey Internal Medicine Part II: Cardiovascular, Respiratory, Neurologic, Urinary, Ophthalmic, Dermatology and Musculoskeletal Disorders." *Journal of Equine Veterinary Science*.
- Mills, D. S. and M. Riezebos. 2005. "The Role of the Image of a Conspecific in the Regulation of Stereotypic Head Movements in the Horse." *Applied Animal Behaviour Science* 91:155–65.
- Minero, Michela, Emanuela Dalla Costa, Francesca Dai, Dirk Lebelt, and Philipp Scholz. 2015. AWIN Welfare Assessment Protocol for Horses.
- Minero, Michela, Francesca Dai, Emanuela Dalla Costa, and Leigh Anne Margaret Murray. 2015. AWIN Welfare Assessment Protocol for Donkeys.
- Minero, Michela, Emanuela Dalla, Francesca Dai, Leigh Anne, Margaret Murray, Elisabetta Canali, and Francoise Wemelsfelder. 2016. "Use of Qualitative Behaviour Assessment as

an Indicator of Welfare in Donkeys." Applied Animal Behaviour Science 174:147-53.

- Ministério da agricultura, do desenvolvimento rural e das pescas. 2006. "Decreto-Lei n.o 142/2006 de 27 de Julho." *Diário Da República n.º 144/2006* 5357–69.
- Ministério da agricultura, do desenvolvimento rural e das pescas. 2009. "Portaria n.º 634/2009 de 9 de Junho." *Diário Da República n.º 111/2009*, 3596–3602.
- Ministério da Agricultura, o Mar. do Ambiente e do Ordenamento do Território. 2013. "Decreto-Lei n.º 123/2013 de 28 de Agosto." *Diário Da República n.º 165/2013* 5198–5203.
- Moehlman, Patricia D. 2002. *Equids*: Zebras, Asses and Horses. Status Survey and *Conservation*. edited by patricia D. Moehlman. Switzerland: IUCN.
- Moehlman, Patricia D., N. Shah, and C. Feh. 2015. "Equus Hemionus, Asiatic Wild Ass, Asian Wild Ass." *The IUCN Red List of Threatened Species* 8235.
- Le Neindre, P. 2009. "Some Perspectives of the Assessment of Farm Animal Welfare in the European Union." *Agrociencia* XIII(3):28–34.
- Nengomasha, E. M., R. Anne Pearson, and A. Gebre Wold. 2000. *Empowering People through Donkey Power into the next Millennium*. london.
- Ninomiya, Shigeru, Masato Aoyama, Yumiko Ujiie, and Ryo Kusunose. 2008. "Effects of Bedding Material on the Lying Behavior in Stabled." *Journal of Equine Science* 19(3):53–56.
- OIE, World Organization for Animal Welfare. 2017. Global Animal Welfare Strategy.
- Orlando, Ludovic. 2015. "Equids." Current Biology, 973-78.
- Osthaus, B., Lianne Proops, S. Long, N. Bell, K. Hayday, and Faith Burden. 2017. "Hair Coat Properties of Donkeys, Mules and Horses in a Temperate Climate." *Equine Veterinary Journal* 50(3):339–42.
- Osthaus, Britta, Leanne Proops, Ian Hocking, and Faith Burden. 2012. "Spatial Cognition and Perseveration by Horses, Donkeys and Mules in a Simple A-Not-B Detour Task." *Animal Cognition* 16(2).
- Palo, Pasquale De, Aristide Maggiolino, Paola Milella, Nicola Centoducati, Alessandro Papaleo, and Alessandra Tateo. 2016. "Artificial Suckling in Martina Franca Donkey

Foals: Effect on in Vivo Performances and Carcass Composition." *Tropical Animal Health and Production* (48):167–73.

- Passantino, Annamaria. 2011. "Welfare Issues of Donkey (Equus Asinus): A Checklist Based on the Five Freedoms." *Journal of Consumer Protection and Food Safety* 6:215–21.
- Pearson, R. A. and M. Ouassat. 1996. "Estimation of the Liveweight and Body Condition of Working Donkeys in Morocco." *The Veterinary Record* 138:229–33.
- Polidori, Paolo and Silvia Vincenzetti. 2012. "Protein Profile Characterization of Donkey Milk." in *Creative commons attribution license*.
- Popescu, Silvana and Eva-andrea Diugan. 2013. "The Relationship Between Behavioral and Other Welfare Indicators of Working Horses." *Journal of Equine Veterinary Science* 33:1– 12.
- Potter, Nelson. 2005. Kant on Duties to Animals.
- Pritchard, Abby, Brian Nielsen, Amy Mclean, Cara Robison, Melvin Yokoyama, Susan Hengemuehle, Simon Bailey, and Patricia Harris. 2019. "Insulin Resistance as a Result of Body Condition Categorized as Thin, Moderate, and Obese in Domesticated U.S. Donkeys (Equus Asinus)." *Journal of Equine Veterinary Science* 77:31–35.
- Pritchard, J. C., A. R. S. Barr, and H. R. Whay. 2006. "Validity of a Behavioural Measure of Heat Stress and a Skin Tent Test for Dehydration in Working Horses and Donkeys." *Equine Veterinary Journal* 38(5):433–38.
- Pritchard, J. C., C. C. Burn, A. R. S. Barr, and H. R. Whay. 2008. "Validity of Indicators of Dehydration in Working Horses : A Longitudinal Study of Changes in Skin Tent Duration , Mucous Membrane Dryness and Drinking Behaviour." *Equine veterinary journal* 40(6):558–64.
- Pritchard, J. C., A. C. Lindberg, D. C. J. Main, and H. R. Whay. 2005. "Assessment of the Welfare of Working Horses, Mules and Donkeys, Using Health and Behaviour Parameters." *Preventive Veterinary Medicine* 69:265–83.
- Quaresma, M., A. M. F. Martins, J. B. Rodrigues, J. Colaço, and R. Payan-Carreira. 2014. "Pedigree and Herd Characterization of a Donkey Breed Vulnerable to Extinction." *Animal* 354–59.
- Quaresma, Miguel, Daniel Bacellar, Bélen Leiva, and Severiano S. Silva. 2019. "Estimation of

Live Weight by Body Measurements in the Miranda Donkey Breed." *Journal of Equine Veterinary Science* 79:30–34.

- Quaresma, Miguel, Miguel Nóvoa, António Monteiro, José Manuel Almeida, and Maria Portas. 2005. "A Raça Asinina de Miranda." *Revista Portuguesa de Ciências Veterinárias* 100:227–32.
- Ramírez, Velia Josefina Berumen. 2009. "Bienestar Animal En Équidos de Trabajo de Propietarios de Bajos Recursos Económicos En Aguascalientes." Universidad Autonoma de Aguascalientes.
- Raspa, Federica, Laura Cavallarin, Amy K. Mclean, Domenico Bergero, and Emanuela Valle.
 2019. "A Review of the Appropriate Nutrition Welfare Criteria of Dairy Donkeys: Nutritional Requirements, Animal-Based Indicators." *Animals* 9(315):1–16.
- Redentor, Armando. 2002. "I Parte- A Região de Bragança, Enquadramentos." Pp. 21–41 in *Epigrafia romana da região de Bragança*, edited by I. P. de Arqueologia. Lisboa.
- Reed, Stephen M., Warwick M. Bayly, and Debra C. Sellon. 2010. *Equine Internal Medicine*. Saunders, Elsevier Inc.
- Reid, SW and HO Mohammed. 1992. "Survival Analysis Approach to Risk Factors Associated with Hyperlipemia in Donkeys." *Journal American Veterinary Medical Association* 209:1449–1452.
- Rodrigues, J. B., P. M. Dixon, E. Bastos, F. San Roman, and C. Viegas. 2013. "A Clinical Survey on the Prevalence and Types of Cheek Teeth Disorders Present in 400 Zamorano-Leonés and 400 Mirandês Donkeys (Equus Asinus)." *Veterinary Record* 1–7.
- Rodrigues, Joana, Graciete Dias, and M. Isabel Caetano Alves. 2009. "Caracterização Do Património Geomorfológico Do Parque Natural Do Douro Internacional (NE de Portugal) Com Vista à Sua Valorização." *Associação Portuguesa de Geomorfólogs* VI:249–54.
- Rodrigues, João B., Stéphane Araújo, Fidel Sanroman-Ilorens, Estela Bastos, Fidel San Roman, and Carlos Viegas. 2012. "A Clinical Survey Evaluating the Prevalence of Incisor Disorders in Zamorano-Leonés and Mirandês Donkeys (Equus Asinus)." *Journal of Equine Veterinary Science* 1–9.
- Rosenbom, Sónia, Vânia Costa, Shanyuan Chen, Leili Khalatbari, Gholam Hosein, Ablimit Abdukadir, Chamba Yangzom, Fanuel Kebede, Redae Teclai, Hagos Yohannes, Futsum

Hagos, Patricia Moehlman, and Albano Beja-Pereira. 2015. "Reassessing the Evolutionary History of Ass-like Equids : Insights from Patterns of Genetic Variation in Contemporary Extant Populations." *Molecular Phylogenetics and Evolution* 85:88–96.

- Rosset, William Martin-. 2013. "Le Cheval : Un Animal Peu Polluant ? Point Sur Les Données Actuelles." 1–4.
- Rousing, Tine, Marianne Bonde, and Jan Tind Sørensen. 2001. "Aggregating Welfare Indicators into an Operational Welfare Assessment System: A Bottom-up Approach." *Acta Agriculturae Scandinavica, Section A - Animal Science* 30:37–41.
- Ryan, Shane, Heather Bacon, Nienke Edenburg, Susan Hazel, Rod Jouppi, Natasha Lee, Kersti Seksel, and Gregg Takashima. 2018. *Animal Welfare Guidelines*.
- Said, A. H., M. Shokry, M. A. Saleh, and A. A. Hegazi. 1977. "Contribution to the Nasolacrimal Duct of Donkeys in Egypt." *Anatomia Histologia Embryologia* 6:347–50.
- Samões, L., J. C. Barbosa, and C. Costa. 2000. *O gado asinino na área do parque natural do douro internacional. Razões da sua importância no meio rural.* Bragança.
- SFC, The Swiss Federal Council. 2011. Animal Welfare Ordinance.

Starkey, Paul. 1998. Improving Donkey Utilization and Management. Ethiopia.

- Stringer, A. P., R. M. Christley, C. E. Bell, F. Gebreab, G. Tefera, K. Reed, A. Trawford, and G. L. Pinchbeck. 2016. "Owner Reported Diseases of Working Equids in Central Ethiopia." *Equine Veterinary Journal* 1–6.
- Swann, William J. 2006. "Improving the Welfare of Working Equine Animals in Developing Countries." *Applied Animal Behaviour Science* 100:148–51.
- Tadich, T. A. and L. H. Stuardo Escobar. 2014. "Strategies for Improving the Welfare of Working Equids in the Americas : A Chilean Example." *Revue Scientifique et Technique* 33(1):203–11.
- Tadich, T., A. Escobar, and RA Pearson. 2008. "Aspectos de Manejo y Bienestar En Equinos de Tiro Urbano En El Sur de Chile." *Archivos de Medicina Veterinaria* 40:267–73.
- Taylor, L. A., D. W. H. Muller, C. Schwitzer, T. M. Kaiser, J. Castell, M. Clauss, and E. Schulz-Kornas. 2015. "Comparative Analyses of Tooth Wear in Free-Ranging and Captive Wild Equids." *Equine Veterinary Journal* 48(2):1–22.

- Tesfaye, Sitota, Benti Deressa, and Endale Teshome. 2016. "Study on the Health and Welfare of Working Donkeys in Mirab Abaya District, Southern Ethiopia." *Academic Journal of Animal Diseases* 5(2):40–52.
- The Brooke. 2013a. "Dehydration and Fluid Therapy." Pp. 124–32 in *The Working Equid Veterinary Manual*, edited by The Brooke. Stansted: Whittet Books.
- The Brooke. 2013b. "Ophthalmology." P. 184 in *The Working Equid Veterinary Manual*, edited by The Brooke. Stansted: Whittet Books.
- The Brooke. 2013c. "The Teeth- Ageing an Practical Approach to Dentistry." Pp. 225–52 in *The working Equid Veterinary Manual*, edited by The Brooke. Stansted: Whittet Books.
- The Brooke. 2013d. "Welfare, Behaviour and Handling of Working Equids." Pp. 23–49 in *The Working Equid Veterinary Manual*, edited by T. Brooke. Stansted: Whittet Books.
- The Donkey Sanctuary. 1999. *The Professional Handbook of the Donkey*. 3rd editio. Whittet Books.
- The Donkey Sanctuary. 2017. Under the Skin. Devon.
- The Donkey Sanctuary. 2018. "Head and oral Cavity" in *The Clinical Companion of the Donkey*. 1st ed. edited by L. Evans and M. Crane.
- The Donkey Sanctuary. 2018. "Hyperlipaemia and the Endocrine System" in *The Clinical Companion of the Donkey*. 1st ed. edited by L. Evans and M. Crane.
- The Donkey Sanctuary. 2018. "Infectious diseases" in *The Clinical Companion of the Donkey*. 1st ed. edited by L. Evans and M. Crane.
- The Donkey Sanctuary. 2018. "Nutrition" in *The Clinical Companion of the Donkey*. 1st ed. edited by L. Evans and M. Crane.
- The Donkey Sanctuary. 2018. "Respiratory System" in *The Clinical Companion of the Donkey*. 1st ed. edited by L. Evans and M. Crane.
- The Donkey Sanctuary. 2018. "Sedation, Anesthesia and Analgesia" in *The Clinical Companion of the Donkey*. 1st ed. edited by L. Evans and M. Crane.
- The Donkey Sanctuary. 2018. "The reproductive system" in *The Clinical Companion of the Donkey*. 1st ed. edited by L. Evans and M. Crane.
- The Donkey Sanctuary. 2019. "Diagnostic Methods." in The Clinical Companion of Donkey

Dentistry, edited by L. Evans and G. Lilly.

- Thiemann, Alex and Andy Foxcroft. 2016. "Working across Europe to Improve Donkey Welfare." *Veterinary Record* 179(12):298–300.
- Thiemann, Alex and Karen Rickards. 2013. "Donkey Hoof Disorders and Their Treatment." *In Practice* 35(March):134–40.
- Toit, Nicole du, Faith A. Burden, and Padraic M. Dixon. 2008. "Clinical Dental Findings in 203 Working Donkeys in Mexico." *The Veterinary Journal* 178:380–86.
- Viksten, S. M., E. K. Visser, and H. J. Blokhuis. 2016. "A Comparative Study of the Application of Two Horse Welfare Assessment Protocols." Acta Agriculturae Scandinavica, Section A - Animal Science 4702(June).
- Violin, Mary Ann. 1990. "Pythagoras- The First Animal Rights Philosopher." *Between the Species* 122–27.
- Wageningen UR Livestock Research. 2011. Welfare Monitoring System.
- Wemelsfelder, F., E. A. Hunter, M. T. Mendl, and A. B. Lawrence. 2000. "The Spontaneous Qualitative Assessment of Behavioural Expressions in Pigs : First Explorations of a Novel Methodology for Integrative Animal Welfare Measurement." *Applied Animal Behaviour Science* 67:193–215.
- Wilson, R. Trevor. 2003. "The Environmental Ecology of Oxen Used for Draught Power." Agriculture Ecosystems & Environment 8809(July 2003):21–37.
- Wood, Stephanie J., David G. Smith, and Catherine J. Morris. 2005. "Seasonal Variation of Digestible Energy Requirements of Mature Donkeys in the UK." Pp. 39–40 in *Equine nutrition conference hannover*.
- Wooler, Stephen. 2014. The Independent Review of the Prosecution Activity of the Royal Society for the Prevention of Cruelty.
- Yilmaz, Orhan, Saim Boztepe, and Mehmet Ertugrul. 2012. "The Domesticated Donkey, Species Characteristics." *Canaian Journal of Applied Sciences* 4(2):339–53.
- Zeder, Melinda A. 2008. "Domestication and Early Agriculture in the Mediterranean Basin : Origins , Diffusion , and Impact." *Proceedings of National Academy of Science* 105(33):11597–604.

Zenebe, Sisay and Tilahun Fekade. 1997. "The Role of Donkey Pack-Transport in the Major Grain Market (Yehil Berenda) of Addis Ababa." *ATNESA Animal Traction Network for Eastern and Southern Africa*.

APPENDIX I- Welfare Assessment Protocol

Avaliação de Bem-estar

- 1. Técnico da AEPGA (Nome):
 - Belen Leiva
 - Daniel Bacellar
 - Daniela Andrade
 - □ Miguel Nóvoa
- 2. Género
 - \square M
 - □ MC
 - \Box F
 - □ Outro

3. Fêmea (Gravidez)

- \Box Sim
- 🗆 Não

4. Data de Cobrição (caso esteja grávida)
5. Aldeia
6. Código Postal
7. Identificação Burro (Nome)
8. NIN (Animal)
9. Microchip (NIN ou Microchip)
10. Data de Nascimento

11. Contacto social

- □ Sem Contacto social
- \Box Contacto social
- \Box NA

12. Sinais de stress térmico

- □ Presentes
- □ Ausentes
- \Box NA
- □ Outro

13. Claudicação

- □ Claudicação não ambulatória
- □ Claudica
- □ Não claudica
- □ NA
- □ Outro

14. Avoidance distance

- □ Comportamento de evasão
- □ Sem evasão
- □ NA

15. Walking down side

- □ Negativo
- □ Positivo/Neutro
- \Box NA

16. Tail Tuck

- □ Presente
- □ Ausente
- \Box NA

17. Condição Corporal

- □ 1
- □ 2
- □ 3
- □ 4
- □ 5
- □ NA

18. Respiração anormal

- □ Presente
- □ Ausente
- □ NA

19. Condição do pelo

- □ Pouco saudável
- □ Saudável
- □ NA

20. Sinais the queimadura com ferro quente

- □ Presente
- □ Ausente
- \Box NA

21. Tumefação articular

- □ Presente
- □ Ausente
- \Box NA

22. Negligencia de cascos

- □ Presente
- □ Ausente
- \Box NA

23. *Tick all that apply.*

	Focinho	Cabeça	Pescoço	ombro	Costas	Cilha e costados	Garupa	Pernas	Banda coronaria e casco
Alopecia									
Lesão de pele									
Ferida profunda									
Tumefação									

24. Descarga ocular

- □ Presente
- □ Ausente
- \Box NA

25. Descarga nasal

- □ Presente
- □ Ausente
- □ NA

26. Descarga vulva e pénis

- □ Presente
- □ Ausente
- □ NA

27. Fezes nos membros posteriores

- □ Presente
- □ Ausente
- □ NA

28. Palpação da face

- □ Presença de anormalidades
- □ Sem anormalidades
- □ NA

29. Comentários e notas

APPENDIX II- Externship description

In the year of 2018 a 3 months externship was conducted in the University of Liége, Belgium. In the Equine hospital aside from the in-patient care there was a rotation between imaging, internal medicine and surgery. An abundance of cases was observed in a variety of specialities.

In January 2019, a month was spent with the UNAM and Donkey Sanctuary, doing ambulatory clinic, in various states of Mexico, delivering free veterinary caring to working equids.

The last externship was conducted in AEPGA, during March, April and May 2019. Besides the ambulatory practice, the development and evaluation of welfare was one of the most important activities, alongside with reproduction and preservation of the Portuguese breed, *burro de Miranda*.

Area	University of Liége	México	AEPGA
Neonatology	X		X
Dermatology		X	X
Orthopaedics	X	X	X
Neurology	X		X
Gastroenterology	X	X	X
Respiratory	X		
Dentistry	X	X	X
Farriery		X	X
Reproduction		X	X
Trauma		Х	Х