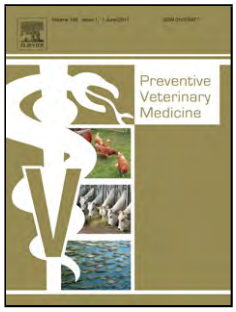

Welfare of horses from Mexico and the United States of America transported for slaughter in Mexico: Fitness profiles for transport and pre-slaughter logistics

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Welfare of horses from Mexico and the United States of America transported for slaughter in Mexico: Fitness profiles for transport and pre-slaughter logistics

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Abstract

Every year thousands of horses from Mexico and the United States of America (USA) are transported to slaughter in Mexico, but little is known about their welfare or pre-slaughter logistics. In this study, we recorded the origin, sex, age and condition of horses (121 journeys, 2,648 animals) upon arrival to an abattoir in northern Mexico, including transport details. Horse welfare was measured indirectly via individual scores for body condition, coat quality, lameness, ocular and nasal discharge, as well as reactivity to a chute restraint test, all

performed shortly after unloading. The average journey duration was 9.69 (±7.6) hours for horses from Mexico and 16.77 (±4.51) hours for horses from the USA (77% of all journeys). The prevalence of ocular discharge, nasal discharge, skin wounds, lameness and diarrhoea, were 23%, 12%, 11%, 9%, 1% (respectively) of all the horses observed, with no significant differences between Mexican and American horses ($P \geq 0.05$). During the chute test the American horses were calmer than the Mexican ones ($P<0.001$), who were more restless and aggressive ($P=0.001$). Likewise, vocalizations in their three variants during the restraint, neigh/whinny ($P=0.018$), nicker ($P<0.001$), and snort ($P=0.018$), were more common in horses from Mexico. In order to help characterize fitness for transport, a two-step cluster analysis was applied using the welfare indicators, suggesting the existence of four clusters (C) evaluated on arrival at the abattoir (from good to very poor fitness): good (profile C4, n=769, 29.1%), average (profile C1, n=799 horses, 30.2%), poor (profile C3, n=586, 22.1%) and very poor (profile C2, n=494, 18.6%). In fact, the C4 best welfare group had 0% lame, 0% nasal discharge, 16.4% ocular discharge, 7.9% skin wounds. Instead, the C2 poorest welfare group had 45.8% lame, 61.1% nasal discharge, 42.8% ocular discharge, and 19.9% skin wounds. Results show potential for using nasal discharge, lameness and ocular discharge as key indicators of horse fitness and welfare on abattoir. The study provides detailed scientific data to help establish strategies regarding optimal days of recovery post-transport and fast fattening for homogenization of weights between animals of different origins, logistic planning, and optimization of logistic resources to minimize the biological cost of long-distance transport.

Keywords: Horse welfare; Mexico; USA; Pre-slaughter logistics; Fitness for transport; Slaughter.

1. Introduction

Horse meat is produced and consumed worldwide, with Asia and the Americas being the biggest producers and Asia and Europe the most important consumers (Belaunzaran et al., 2015). At the same time, horse meat consumption is slowly increasing in several countries because of the availability of this interesting meat source but also due to its recognized nutritional value (Beldarrain et al., 2020). Since the 1960s, Mexico has consistently been one of the most important suppliers of horse meat on an international level (Jastrzębska et al., 2019). After 2007, production further increased after the USA prohibited horse slaughter (American Horse Slaughter Prevention Act), and live horses began being exported to Mexico (and Canada; see Taylor and Sieverkropp, 2013). Mexico became in a few years, one of the largest exporters of horse meat to the European Union. Nevertheless, in 2014 EU imposed a conditional ban on horsemeat imports from Mexico after an audit by the Food and Veterinary Office concluded that it was not possible to guarantee the reliability of producer's declarations or the traceability of the welfare of horses slaughtered for meat originating from the USA and Mexico (EUR-Lex, 2014). Therefore, exports to Europe were suspended, while smaller volumes are still sent to China, Vietnam and Russia. Conversely, the large volume of meat that is not exported is sold on the domestic Mexican market (Lozano, et al., 2020).

Pre-slaughter logistics are a vital part of modern meat horse production and include various stopovers between the original owner and abattoir (Stull, 2012). In fact, stopovers during transport are more the rule than exception at export stations, resting stations, sale yards, and markets, including multiple loading and unloading operations, handling for classifying by weight, gender, breed or commercial type, social mixing and double transports in one day, one week or one month (Miranda-de la Lama, 2018), all with potentially important effects

on horse welfare. Even under favourable conditions, horses are exposed to a range of potential stressors including loss of balance, isolation from the herd, forced proximity to unfamiliar or aggressive horses, novel or threatening surroundings, exposure to new pathogens, restraint, extremes in temperature, water and feed deprivation, and dust and particulate matter from the road (Friend, 2001; Miranda-de la Lama et al., 2014). The interaction between these stressors, with pre-existing health and wellness conditions and individual differences, can limit or affect an animal's fitness during journey and pre-slaughter operations. Therefore, according Cockram et al. (2019), fitness for transportation can be defined as the ability of an animal to cope with transport and pre-slaughter logistics. Therefore, fitness for transport should be assessed at the abattoir and practical indicators should be implemented on site which are valid (measure what they intend), repeatable (produce the same results after repeated observations of the same animal by the same and different observers), reliable (produce consistent results for different animals) and feasible (in terms of speed, cost and not compromising normal operating procedures) (Llonch et al., 2015). Recently there is interest in incorporating welfare indicators during meat inspection at abattoirs as a surveillance tool for animal health and welfare. In their 2009 report, the Farm Animal Welfare Council (FAWC) suggested the use of 'iceberg' or "key" animal-based indicators as a means of assessing overall animal welfare (Van Staaveren et al., 2017). These indicators should provide a picture of the overall welfare of the animal and function as a warning signal for underlying problems (FAWC, 2009).

The scientific literature regarding horse welfare in commercial abattoirs is restricted to a few studies in North America (Grandin et al.,1999; Friend 2000; Roy et al., 2015a; Roy and Cockram, 2015), South America (Werner and Gallo, 2008; Nivelle et al., 2020), Oceania

(Padalino et al., 2015), and Europe (Marlin et al., 2011; Roy et al., 2015b; Andronie et al., 2017). The transport and slaughter of horses is an important welfare issue that raises great social concern in the USA and Mexico, being a hot topic for non-governmental organizations, citizens and politicians in the region (de Aluja, 1998; Reece et al., 2000; Anderson, 2015; Monterrubio and Pérez, 2020). In 2019 at least 109 thousand horses of Mexico and the USA are transported to slaughter in Mexico (FAOSTAT, 2020). For many years, Mexican and international non-governmental organizations have provided anecdotal evidence that the pre-slaughter conditions of horses in Mexico is cruel (see GAIA, 2010; Human Society, 2011; Animal Angels, 2013), however, no large-scale scientific studies have been carried out to date. Applied research can help to provide detailed scientific data to help establish uniform national regulations regarding optimal days of recovery post transport and possible fast fattening for homogenization of weights between animals of different origins (as suggested by the EU's conditional ban), logistic planning, and optimization of logistic resources to minimize the horses biological cost of long-distance transport. The current study examined horses' from Mexico and the USA belonging to 121 journeys under commercial conditions upon arrival to an abattoir in northern Mexico using a series of animal-based welfare indicators. For the purposes of this article, the demonym used to designate horses from Mexico will be "Mexican" and for those from the USA, it will be "American". The aims of this study were 1) to recognize the current practices of the commercial transport and pre-slaughter logistics of Mexican and American horses slaughtered in Mexico, 2) to identify which key animal-based welfare indicators are most useful for evaluating the impact of transport on horse fitness, and 3) to identify clustering patterns regarding fitness of horses on arrival to the abattoir.

2. Material & Methods

The study was carried out in Zacatecas State (North-Central Mexico), where 60 % of all equids are slaughtered in Mexico at two abattoirs, both of which receive animals from the USA and nationally. Our research was conducted during the springer season (April to June) with an average of 20°C at a federally approved abattoir (Federal Inspection Type or TIF) in the municipality of Fresnillo, Zacatecas (23°11'08.9"N 102°51'49.6"W). The climate of this area is semiarid template (BS1kw), with rainfall rarely exceeding 355 mm per year, an average temperature of 15–18 °C, and altitude of 2121 m.a.s.l. The abattoir was selected based on the number of horses slaughtered per year (approximately 20,000 heads), the wide geographic sources of origin of the horses and because it meets the requirements for horse meat export according to Official Mexican Standards (NOM-008-ZOO-1994; NOM-009-ZOO-1994; NOM-033-ZOO-1995) concerning the operative regulation for TIF slaughter plants. The abattoir company did not take part in the analysis or interpretation of results. All procedures were conducted in accordance with the guidelines for the ethical treatment of animals in applied animal welfare studies (Sherwin et al., 2003).

2.1. Study description

This *ante-mortem* evaluation was implemented as a cross-sectional study in order to assess welfare and health conditions in Mexican and American horses that entered the abattoir chain through the ordinary planning. Data was collected from March until June 2016 for 121 journeys, for 2,648 animals with an average (± SD) live weight of 340.53±74.38 kg for horses and 164.97±42.31 kg for foals. Each journey was assessed without interfering with abattoir schedules on 10 sampling days each month of April, May and June (total 30 days). Each sampling day lasted from 09:00 h until 14:00 h with the goal of inspecting 3 to 5 journeys

per day. A journey was considered a group of horses of the same origin (adult horses or/and foals) coming from the same export station and belonging to the same slaughter group (same loading, transportation, unloading). Due to the large number of animals monitored it was not possible to determine the breed of each horse, although, to avoid biases we did not include giant breed horses, ponies, donkeys or hybrids such as mules or hinnies. All the animals either came from collection centres in Mexico or export stations of the USA, information which was available from the traceability system. Information on each shipment was obtained from the arrival's office, including the number assigned to each animal, commercial category (adult or foal), sex (male or female), origin (state or country of origin), transport and pre-slaughter conditions (including lairage time) and type of livestock vehicle. With this information, a database was created that enabled us to identify and locate each animal.

2.2. Arrival facilities at the abattoir

The abattoir was specially designed to slaughter horses, with a total area of 37,200 m^2, and operating from Monday to Saturday (0900–1400 h), with a slaughter capacity of 400 animals/day at a rate of 50–57 animals/h. There were two unloading areas, each one with a loadout (concrete ramp with metallic fences) and an arrival pen without a roof or water troughs. Both pens were connected by a small corridor of nonslip concrete floors (10 m) to a 94 m corridor (metallic fences) that led the animals to the animal reception module (roofed area with a weighing scale and chute). The admission office is located in this area, housing the official veterinarians and the origin, logistics and lairage records of all the animals. During lairage period the veterinarian and the operations manager plan and decide the slaughter order, according to the specific demands of the slaughterhouse and the physical and health conditions of the animals. If some horses were non-ambulatory horses, they were

euthanized or sent to the quarantine zone. According to the journey time and the number of animals per journey, the horses could be accommodated in 9 pre-slaughter pens (between 720 m^2 to 855 m^2 each with 60-70 m^2 of shade). Each pen was equipped with Polyshade™ roofing (high density polyethylene screen), drinkers and water were freely available, but no fodder. Any non-ambulatory or sick horses were housed in a quarantine zone that has a euthanasia area and four pens (37 m^2, 54.2 m^2, 59.8 m^2, and 61.4 m^2), each equipped with a roof, feeder, and water trough.

2.3. Assessment of animal-based welfare indicators

An assessment protocol was developed to register the welfare conditions of horses upon arrival to the abattoir based on an exhaustive literature review and preliminary observations of the research team (Table 1). The protocol was validated using 150 animals (those data were not used for subsequent statistical analyses). Once the group of animals was unloaded and led to the receiving module, each animal was assigned a number, a record sheet, and weighed. This individual record contained the characteristics of the horse, assigned lairage pen, transport and pre-slaughter information and sections for the evaluation of animal welfare. The assessment protocol was applied in two stages, the first stage was at the animal reception module (next to admissions office), where each animal was weighed on a chute with a scale, performing a reactivity or chute test (ChT). That consisted in restricting the movement of each horse for 30 seconds and scoring its behavioural reactivity at ChT (score of 3: 1–3 scale, where 1 quiet, 2 restless non-aggressive and 3 restless aggressive) and registering vocalizations (score of 4: 1–4 scale, where 1 none, 2 neigh/whinny, 3 nicker and 4 snort). Approximately three hours after weighing and registering the animals, the second stage of the assessment protocol was carried out in the lairage pens and included a body

condition score (BCS) assessed using a 5-point scale adapted from Grandin et al. (1999), where 1 was emaciated, 2 thin, 3 moderate, 4 heavy and 5 obese. Subsequently, the quality of the coat (score of 4: 1 presence of alopecia regions, 2 dry and opaque, 3 bright and soft and 4 long and opaque) and skin wounds (score of 2: presence or absence and number of abrasions, lacerations, and abscesses larger than 5 cm were considered) were assessed. We also noted nasal discharge (score of 4: where 1 without secretion, 2 slight, 3 moderate and 4 severe), ocular discharge (score of 4: where 1 without secretion, 2 slight, 3 moderate and 4 severe), and diarrhoea (score of 2: presence or absence). Lameness was scored on a 3-point scale adapted from AWIN (2015) with 1 = not-lame: can bear weight equally and fully on all limbs at rest and when walking; 2 = lame: the horse has imperfect locomotion but can walk. When walking the head rises when the pressure is placed on the lame foot. Raises pelvis raises as the lame hind leg hits the floor; 3 = non-ambulatory: the horse is unable to stand without assistance or is unable to bear any weight on one leg or shows halted movement. All stages of the protocol were carried out by the same observer and in the same order.

2.5. Statistical analyses

Data were entered into Microsoft Excel (Microsoft Corporation, 2010) and then analysed with the SPSS statistical package (IBM SPSS Statistic 22). Mapping was conducted by using QGIS version 2.18.15 (QGIS Delopment Team, 2015). Prevalence was defined as the proportion of horses with an occurrence of each animal-based indicator (lameness, nasal discharge, ocular discharge, diarrhoea and skin wounds), calculated as indicator frequency divided by the total sample (n=2,648). The characteristics of pre-slaughter logistics and the welfare conditions of horses from the USA and Mexico, were compared by the Chi-square test for qualitative variables and the U-Mann Whitney test for quantitative variables, due to

their non-parametric nature. The significance level was established at $p<0.05$. In order to identify and describe groups of horses with similar welfare conditions upon arrival at the slaughterhouse, a two-step cluster analysis was performed using the animal-based welfare indicators. Applying a two-step cluster approach, hierarchical followed by non-hierarchical, allowed to include both categorical and continuous variables in the formation of clusters, which may prove useful in applications where traditional clustering methods are inefficient (Hair et al., 2014). The two-step method has been used previously to examine horse fear profiling by Dai et al. (2015). Categorical variables included initially in the analysis were body condition score, coat quality score, ocular discharge, nasal discharge, lameness, behavioural, and vocalization during chute test, skin wounds, and **diarrhoea**. The log-likelihood distance measure was applied for clustering and the Schwarz's Bayesian Criterion was used to select the optimal number of clusters. Different combinations of these variables were explored in several cluster analyses and were accepted or discarded according to the overall silhouette measure of cohesion and separation. Variables selected for the final cluster solution were body condition, coat quality, lameness, nasal and ocular discharges. The optimal number of clusters was determined automatically, and a "cluster membership" variable was created that identified which horse belonged to which cluster. The final clusters were profiled by cross tabulating the variable "cluster membership" with a set of variables including all the animal-based welfare indicators, sex and age of animals, and the logistic issues. To identify the variables that discriminated between clusters, the contingency tables were used with their respective chi-square tests to compare ranges of independent samples.

3. Results

During the observation period no horses were found dead on arrival but 20 horses from the USA (0.75%) and 8 from Mexico (0.30%) were non-ambulatory at arrival. Overall, 49.1% of all animals were male (stallions and geldings), and 50.9% female. According to the commercial category, 84.7% were adults and the remaining 15.3% were foals.

3.1. Pre-slaughter logistics related to country of origin

Table 2 gives detailed information about the pre-slaughter conditions we evaluated. Although overall more adult horses were processed than foals ($P<0.001$), there were significantly more foals from Mexico as well as males ($P=0.003$), compared to the USA. Mean transport time for all animals was 15.24 ± 4.03 h, and lairage time at the abattoir was 4.71±0.13 h. Transport and lairage times were significantly higher for horses from export stations in the USA ($P<0.001$), where 77.19% (2044/2648) of the animals came from the USA, the remaining 14.7% (604/2648) were from Mexico. The details of the origin points of the animals are shown in Fig. 1; where Mexican horses come mainly from collection centres in the states of Zacatecas and Jalisco, to a lesser extent from Durango, Chihuahua and Sinaloa. While American horses come from both export stations in Texas and to a lesser extent in New Mexico. Mexican animals were mostly transported in gooseneck trailers while the journeys from the USA used a trailer + 48 foot-trailer ($P<0.001$).

The effects of transportation and pre-slaughter logistics on the welfare and health of horses according to their country of origin can be seen in Table 3. In the first stage of the protocol, at chute test we found that the American horses were calmer than the Mexican ones, who were more restless and aggressive ($P<0.001$). Likewise, the vocalizations in their three

variants, neigh/whinny, nicker, and snort, were more common in horses from Mexico ($P<0.001$). In second stage of the protocol, body condition score (no obese animals were observed) and ocular discharge, no significant differences were found between countries of origin ($P\geq0.05$). A similar effect was observed in coat quality, with the exception of long and opaque coat which was more common in Mexican horses ($P<0.001$). American animals had a higher proportion of animals with nasal discharge classified as slight, moderate, and severe ($P<0.001$). The prevalence of ocular discharge, nasal discharge, skin wounds, lameness and diarrhoea, were 23%, 12%, 11%, 9%, 1% (respectively), and no differences were observed between Mexican and American horses ($P\geq0.05$). Finally, among the animals with skin wounds, 72.4% had a single wound, 19.9% had 2, 6.7% had 3 to 5 wounds and 1.0% had multiple wounds, with no significant differences in this distribution between Mexico and the USA ($P\geq0.05$).

3.2. Clustering patterns of horse fitness for transport on arrival at the abattoir

The two-step cluster analysis suggested the existence of four clusters or profiles (C1, C2, C3, C4; Table 4) determined by four animal-based welfare indicators about body condition, coat quality, prevalence of lameness, ocular and nasal discharge. Profile was not correlated with age, sex or vocalizations. The distribution of animals into clusters was not homogeneous, with cluster 1 (C1) having 799 horses, C2 with 494 horses, C3 with 586 horses and C4 with 769 horses. Table 5 shows the horse profiles and their fitness for transport associated with pre-slaughter logistics to which they were exposed.

The group of horses in C1 were mostly thin or moderately body condition (according to the BCS), had a dry/opaque coat, no lameness, no nasal discharge, 18% had ocular discharge. In

terms of behaviour, C1 horses were mostly quiet during the chute test. In terms of pre-slaughter logistics, journey times were longer than 18 hours or also between 6 - 12 h, in trailer + foot-trailer, with a pre-slaught lairage of between 1 and 3 hours, mostly from the USA (especially from Texas: “Eagle pass” and New Mexico “Columbus”) although also from Mexico. Compared to the other three clusters, C1 had the greatest proportion of Mexican horses (24%). The C2 horses were mostly thin, with a dry/opaque or bright/soft coat, 20% skin wounds (the highest of all clusters), 42% ocular discharge, 46% lameness and 61% nasal discharge. They were mostly quiet in the ChT. Journey times for C2 were 6 - 12 h, in trailer + foot-trailer, with a lairage time of between 1 and 3 hours, and mostly of American origin (especially from Texas: “Eagle pass”).

The C3 horses were mostly thin (64%), with a bright/soft coat (85%) and 23% ocular discharges (highest of all clusters). In the ChT they were more restless individuals (non-aggressive + aggressive = 44.8%) although most were calm. Considering pre-slaughter logistics, journeys were > 18 h or 6 - 12 h and 12 - 18 h, in trailer + foot-trailer, with a lairage time of between 1 and 3 hours, followed by stays of 3 - 6 h. The C3 horses were mostly of American origin (especially from Texas: “Eagle pass”, “Presidio” and New Mexico “Columbus”). Finally, the C4 horses were all of moderate body condition with a bright/soft coat, no lameness, nasal discharge or diarrhoea, and the lowest incidence of eye discharges (16%) and skin wounds (<8%). Behaviourally they were mostly calm during the ChT test. These journeys for these animals were longer than 18 hours and also between 6 - 12 h, in trailer + foot-trailer but also truck + Gooseneck trailer, with a lairage period of between 1 and 3 hours and mostly American (especially from Texas: “Eagle pass” and New Mexico “Columbus”).

4. Discussion

Compared to other farm animals, spent horses come from a wide variety of sources (e.g., agriculture, police work, entertainment, therapy) and are eliminated for different reasons, including old age, poor physical condition, low commercial value, infertility, or behavioural disorders (Wallin et al., 2000). Thus, those journeys may include animals with a wide heterogeneity in size/weight, body condition and health, all affecting their "fitness for transport", but few studies have considered their health or welfare status. Most horses transported to slaughter are likely collected from multiple sources to accumulate enough to make a larger group for transport (Roy et al., 2015b). Consequently, they must be sent from their stables of origin to collection centres, auctions markets, exportation stations and later sent on to abattoirs. In addition, horses exported to Mexico are transported to export stations (authorized by the Mexican health authority -SENASICA-) located at border points in the USA territory. Once horses arrive at the border crossing, they are unloaded into holding pens for inspection (Engebretson, 2008). In these pens, horses can usually stay between 1 and 3 days. Following, the horses are transported or walked across the border where they are inspected by SENASICA at Verification and Inspection Points (VIP´s) and loaded into Mexican trailers and driven to the abattoir. Therefore, most of the horses in this study had undergone several journeys, social regrouping, exposure to novel environments, exposure to variable environmental conditions, repeated loading/unloading and handling. In this context, our study is the first to identify clustering patterns regarding fitness of horses on arrival to the abattoir.

4.1. Pre-slaughter logistics related to origin-country

An interesting result is the sex ratio of the slaughtered animals in both countries. A possible explanation may be related to the equine industry of the USA having a more pronounced female replacement than the emerging Mexican industry (Keaveney, 2008). Also, in Mexico meat from male horses has a higher economic value (DVM Victor Leyva, personal communication). Most of the Mexican horses came from states (Zacatecas and Jalisco) with a sporting tradition (*charreria*), while the rest of the animals were from states where horses are employed for work (stock, pack and draft power). The origin of the Mexican and American horses was much more heterogeneous.

When horses are transported for sport or recreation, they are often kept in single stalls with some form of restraint and protection on parts of their body or the lining of the stall. When horses are transported to slaughter, they are normally moved in loose groups (Friend 2001; Roy et al., 2015a). The size and design of horse trailers varies widely throughout the world, mostly due to the types of roads, weather conditions, commercial categories of the animals and the degree of specialization of the supply chains (Miranda-de la Lama, 2018). In the present study the trailers and infrastructure used to transport horses in Mexico was similar to the USA and Canada. Although Mexican regulations do not prohibit using double-deck trailers, as in the USA (Stull, 2012), in our study all the trailers had only one-deck, which helps to decrease wounds in the neck and the head and allow animals to stand upright (Stull, 2008).

Our results indicate that long journeys are also more of the rule than the exception. The journey times of horses exported from the USA to Mexico was almost 17 hours on average,

according to the information provided by the abattoir. However, this does not include previous journeys to the stables or other collection points, so it is likely that many horses have even longer accumulated journey hours. Some NGOs have documented journey times of up to 30 hours from auction markets in the USA to abattoirs in Mexico (Animal Angels, 2013). According to Padalino et al. (2015), longer journeys have a greater effect on horse health and require particular attention, with journeys longer than 10 hours possibly leading to psychological and physical exhaustion and sometimes even death. In Canada and the USA there are pre-transport and handling guidelines, as well as onboard vehicle requirements, but none of these exist in Mexico (Engebretson, 2008). Lairage is a common commercial practice that can be defined as a period of time where animals are given access to water but not feed, for hygienic reasons, before slaughter at the abattoir (Liste et al., 2011). In lairage, animals are expected to regain the energy lost during transportation. However, the rate which energy is gained depends upon the amount of stress from transportation and the conditions of lairage at the abattoir (Chulayo, Bradley, & Muchenje, 2016). From our results, the fact that the lairage period was less than 5 hours for animals from both countries is probably related to their poor physical condition when they arrive, since prolonging lairage can impose further stress on fatigued animals and increase non-ambulatory animals and mortality (Werner and Gallo, 2008).

In working horses, good body condition (heavy in our BCS) is strongly correlated with good coat condition (bright and soft) and both indicators are correlated with the absence of many clinical signs of disease (Popescu et al., 2014). Our study shows that regardless of the origin of the animals, about 50% of the animals studied had a soft and shiny coat, in addition to a moderate to heavy body condition. The remaining 50% had variations in coat quality and

BCS, which indicate health problems that can be derived or aggravated by transport, in addition to the health conditions prior to the journey. Both conditions have a combined effect, making it difficult to identify the cause at the abattoir. However, according to Butterfield et al. (2018), horses can lose weight and BCS after long-distance transport because body tissue is catabolized, particularly skeletal muscle and physiological ions are depleted, notably cations such as potassium and magnesium. Glycogen and other energy reserves are also depleted leading to hypoglycemia and dehydration. This would indicate that even animals with a moderate body condition were the most frequent, followed by the thin animals, in addition to a marginal prevalence of emaciated and heavy animals, and the notable absence of obese animals. These results could indicate that even animals with good fitness for transport may be affected in their body condition upon arrival at the abattoir. Therefore, animals with poor fitness before the journey should not be transported, even if their origin is Mexico. There is a potential conflict between the avoidance of the risk of suffering arising from a decision to not transport an animal that is not fit for transport and the financial loss associated with on-farm euthanasia compared with the potential return to a producer from transporting the animal for slaughter so that it can be sold for human consumption (Cockram, 2019).

In the USA and Canada, dehydration, fatigue, and skin wounds were identified as major welfare issues affecting horses transported for slaughter (Grandin et al. 1999; Roy et al., 2015a). However, from our results the presentation of nasal and ocular discharges may be due to a number of factors such as subclinical respiratory diseases, restraint in the “head-up” posture, leading to impairment of pulmonary clearance mechanisms, stress-related impairment of the immune response, presence of noxious gases and high concentrations of

airborne dust and bacteria, length and duration of journey, and body orientation during transport (Stull 1999; Oikawa et al., 2005). Our evaluations found that at least 24% of the horses had ocular discharges and 16% nasal discharges of varying magnitude. However, it was clear that animals from the USA were more likely to have nasal discharges compared to Mexican horses. This is probably due to multiple journeys, macro and micro environmental conditions and multiple stays in collection centres or auction markets and export stations that produce a marked stress response that favours immunosuppression in the most susceptible animals. Nasal and ocular discharges can be useful indicators to assess the state in which animals arrive at the abattoir, although they should not be the only ones used (Czycholl et al., 2019).

In this study, skin wounds (abrasions, lacerations, and abscesses) had the higher prevalence (11 %), with no significant differences between country of origin. This prevalence is higher compared to the 6% reported in Mexico by Sánchez-Casanova et al. (2014). Skin wounds are common in equids, and are usually associated with ill-fitting harness systems, poor handling by owners and handlers (de Aluja, 1998; Galindo et al., 2018), high or low densities during the journey (Padalino, 2015), handling during loading, unloading, fighting with other horses or even from sharp objects/obstacles in facilities or in the truck (Roy et al., 2015a). On the other hand, the prevalence of foot diseases that cause lameness in horses can reach 80% (Holzhauer et al., 2017). Recently, a study in the UK found that lameness had a prevalence of 36% and was the main reason horses were sent to the abattoir or euthanized (Pollard et al., 2020). In our study lameness was less than 9%, and no differences between country of origin. Although the prevalence reported in our study is lower than the reference prevalence, it must be taken into account that the horses that are sent to the abattoir have been selected for having

acceptable fitness for the journey. Therefore, the prevalence we report may be due to wounds during the journey or slight lameness that worsened during transportation (Bennett-Wimbush et al., 2014). Finally, less than 1% of the horses presented diarrhoea upon arrival at the abattoir. This result may be due to the fact that most of the animals came from journeys greater than 9 hours and were fasted (Friend, 2000; Stull, 2012). This indicator would have little applicability under the conditions of horses slaughtered in Mexico.

Temperament refers to an individual behavioural response that is repeatable over time in different situations, which includes variety of traits, such as willingness to take risks, exploration, aggressiveness, and avoidance of novelty (Freitas-de-Melo et al., 2019). In horses, individual temperament and reactivity traits may be assessed by their behavioural response during restriction in the chute (Calviello et al., 2016). In our study, we evaluated temperament using a ChT test, with the hypothesis that the behavioural response would indicate the impact of transport on the fitness of horses. Our results confirm this hypothesis, showing that travel fatigue can decrease or inhibit behavioural responses related to temperament. This was more evident in American horses, which were calmer and less vocal compared to Mexicans horses. Thus, behavioural responses attributed to temperament were affected by journey fatigue.

4.2. Clustering patterns of horse fitness for transport on arrival at the abattoir

The distances journeyed to slaughter from the USA to Canada and Mexico are likely to be very much longer than journeys from the point of entry into the EU to EU abattoirs (Leadon, 2012). It is important that horses be fit for transport, otherwise their health and welfare may be compromised during loading, transport, unloading, and pre-slaughter logistics (Vermeulen

et al., 2018). Measurements of impaired biological functioning, particularly those connected with decreased health and an altered behavioural response, can provide good corroborating evidence about whether welfare has been compromised (Duncan, 2005). In this context, valid, feasible and easy to assess indicators are fundamental (Losada-Espinosa et al., 2018). Ideally, veterinarians require a series of user-friendly criteria to detect animals with certain typologies related to animal welfare problems. Our results suggest the existence of four clusters or profiles of horses (C1, C2, C3, C4) characterized by the scores of body condition (BCS), coat quality (CQS), and the prevalence's of lameness, ocular and nasal discharges.

Traditionally, body condition and coat quality have been two of the most widely used indicators in welfare assessment protocols (i.e. Welfare Assessment Protocol for Horses - AWIN- or Standardised Equine Based Welfare Assessment Tool – SEBWAT-) in work and recreational horses (Hausberger et al., 2020). In our study, we found that these indicators were useful to visually characterize the fittest horses (C4), but they were less effective in profiling the horses with a lower fitness for transport, as occurred in profiles C1, C3 and C2 (from suitable to less apt, respectively). In other words, the lower the fitness for transport, the more heterogeneous was the body condition and coat quality. However, the prevalence of lameness, nasal and ocular discharges demonstrated greater sensitivity to discriminate between the fittest or most resilient profiles and the least fit. These measures could be considered key or iceberg indicators and integrated into specialized protocols to assess horse welfare at abattoir level. Although the prevalence of diarrhoea and skin wounds was much lower, they also indicate a similar trend. Diarrhoea was non-existent in the fittest animals (C4) and gradually increased in prevalence in the least fit (C2). Skin wounds were 8% in C4 horses but doubled in C2. The only variable that provided similar results among clusters was

the ChT test. These results indicate that this test is useful for evaluating post-transport fatigue but was not useful to distinguish associations with other welfare indicators or clear differences between profiles of horses at the abattoir (Reid et al., 2017). 37

The profiling used suggests a link between fitness to journey and horse health, as measured. Specifically, the indicators used to characterize profiles C1, C3 and C2 are related to two syndromes associated with long-distance transport in horses: shipping fever and exhausted horse syndrome. The clinical signs of shipping fever may include inappetence, lethargy, coughing, nasal discharge, dyspnoea, tachycardia, abducted elbows, reluctance to move and a stiff gait (Hurley et al., 2016). Exhausted horse syndrome includes elevated temperature, depression, anorexia (including water), dehydration, diarrhoea, colic, lameness and unwillingness to continue to walk (Foreman, 1998). The indicators we used appear sensitive enough to reveal animal welfare problems related to possible diseases, falling short of a diagnosis. It could be that diarrhoea, coat quality and body condition are related to some of these syndromes, but we could not confirm this. Ocular discharges were common in all four clusters, and although they are not directly associated with the clinical signs of shipping fever or exhausted horse syndrome, they may be exacerbated by fumes, noxious gases (i.e., carbon dioxide, carbon monoxide methane, ammonia), high temperatures, humidity and dust, all of which may be elevated at different times during a long distance journey (Oikawa et al., 2005; Miranda-de la Lama, 2018). Ocular secretions can be observed even several hours after transport, explaining their prevalence in our study. It is also possible that the prevalence of nasal discharge could be associated with the causes of eye discharge.

Within the pre-slaughter logistics chain, long-distance transport is considered a risky activity for the health and well-being of animals (Engebretson, 2008). Several scientific studies have shown that journey time challenges the fitness of animals, causing states of fatigue/exhaustion, heat distress, dehydration, and wound following trauma (Roy et al., 2015a). Even in healthy animals, journeys longer than 12 hours often have a major impact on physical condition and fitness (Padalino, 2015). Our results have corroborated these effects in C1, C3 and C4 horses. However, we found that journeys less than 12 hours characterized more than 50% of the animals in group C2. Coincidentally, this profile was the one that presented major health and welfare problems with greatly reduced fitness. Evidence of this is that the abattoir has prioritized their slaughter and that would explain why the horses went with a lairage period of less than three hours. The fact that the long-distance transport is not a great risk factor for C2 horses (is the profile with the least Mexican horses), would indicate that the origin and the pre-transport physical conditions of the animals are the decisive factors that affected their welfare. In the case of origin, 58% of the C2 horses come from Texas, especially from exports stations of Eagle Pass and to a lesser extent from Presidio, both origins are less than 870 km (between 9 and 12 hours of journey) away from the abattoir. The state of Texas has a long tradition of horse slaughter until its ban in 2007, however the capacity and infrastructure to stock and market slaughter horses has prevailed (Dodman et al., 2010). The strategic location of export stations in Texas allows to collect horses from the states in the Southeast, Southwest, Midwest and sometimes even from East Coast. Therefore, animals collected in Texas have one or more previous journeys that affect their fitness for final transportation to Mexico, even on horses that come from longer journeys like those in New Mexico (journeys of 1260 km or 14 to 18h). The associations between risk factors and welfare outcomes that were identified in this study have general applicability and

will provide information useful for guidance on the transportation of horses to slaughter in the region.

5. Conclusions

In conclusion, the cluster analysis showed that horses less fit for transport are more likely to suffer from lameness, nasal-ocular secretions, and skin lesions and indicate the existence of four horse profiles based on their fitness for transport evaluated on arrival to the abattoir: good (profile C4), average (profile C1), poor (profile C3) and very poor (profile C2). These profiles were defined by the scores of body condition and coat quality, and the prevalence of lameness, ocular and nasal discharges. However, the most useful indicators to define resilience or fitness for transport were nasal discharge, lameness and ocular discharge. These indicators were capable of explaining a proportion of the variation in the prevalence of different welfare outcomes on-abattoir highlighting their potential as key or iceberg indicators and can be integrated into specialized monitoring tools for welfare of slaughter horses. Finally, our results partially support the USA social concern about horse transport, since horses in the USA have obvious states of fatigue due to long-distance journeys and multiple logistic stops. Mexican horses exposed to much shorter journeys have health and welfare problems similar to horses in the USA. These results indicate the importance of making a common public policy for the transport of horses for slaughter in the two countries. Therefore, fitness monitoring on horse's arrival must be a priority at the abattoir level in the Mexican horse meat industry. This is important to ensure the safety of meat and the guarantee for horse meat consumers. The welfare of horses must be a shared legal and operational responsibility between the USA and Mexico.

Conflict of interest

All authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Fig. 1. Horse collection points and transport routes from Mexico (Collection points) and the USA (Export stations).

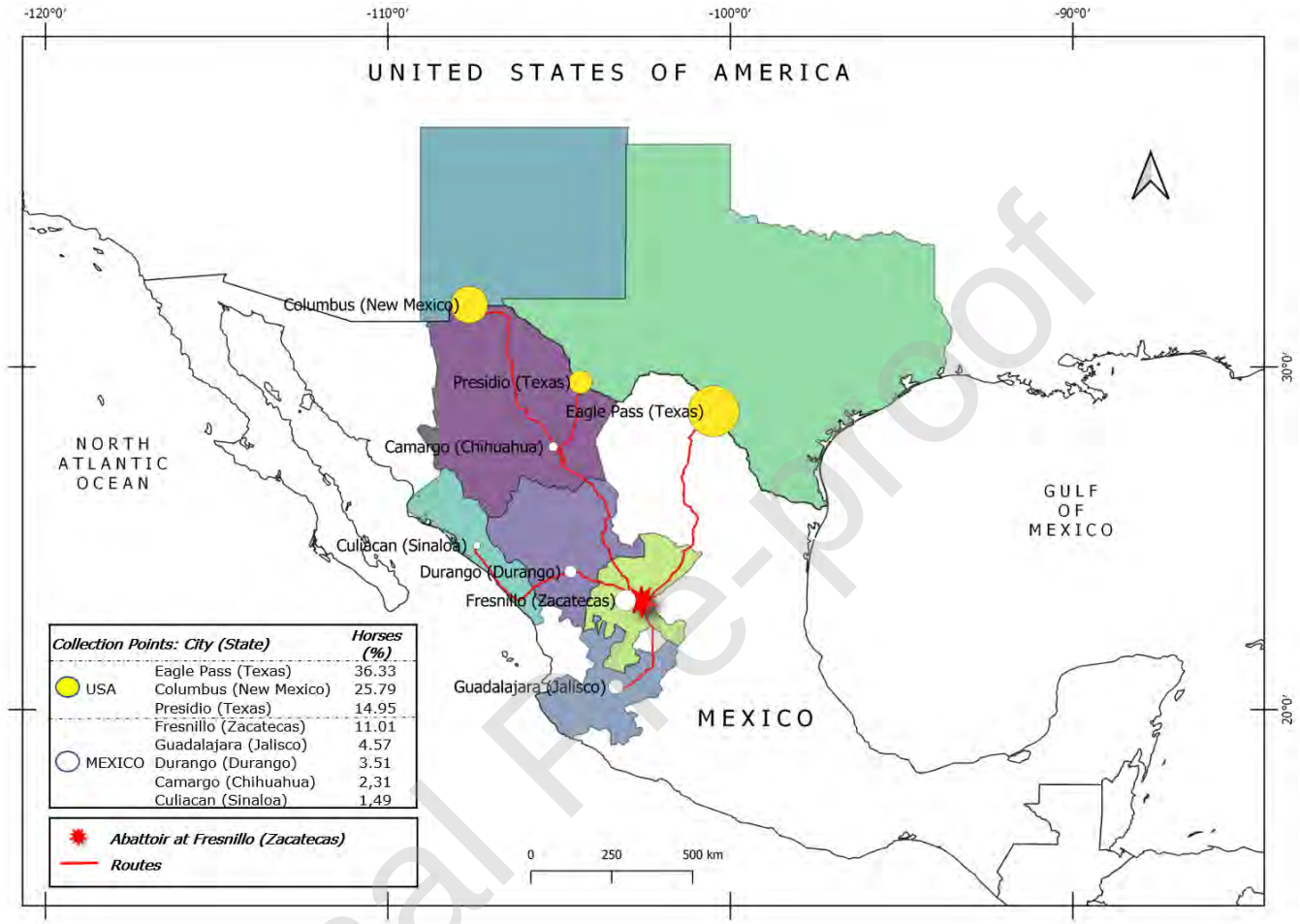

Table 1. Animal-based welfare indicators integrated into the transport fitness assessment protocol upon arrival at the abattoir.

Animal-based welfare indicators	*Score scale*	*Assessed at…*
First stage of the protocol		
Chute test: Behavioural reactivity	1-3	Animal reception module
Chute test: Vocalizations	1-4	Animal reception module
Second stage of the protocol		
Body condition score (BCS)	1-5	Lairage pen
Coat quality	1-4	Lairage pen
Skin wounds	1-2	Lairage pen
Nasal discharge	1-4	Lairage pen
Ocular discharge	1-4	Lairage pen
Diarrhoea	1-2	Lairage pen
Lameness	1-3	Lairage pen

Table 2. Characterization of Mexican and American horses slaughtered in Mexico according to sex, age and logistic issues (n=2648).

Variable	*Mexico (n=604)*	*USA* (n=2.044)	*Total* (n=2.648)	*P*
Age category (%) [a]				
Adult	77.81	86.84	84.78	< 0.001
Foal	22.19	13.16	15.22	
Sex (%) [a]				
Male	54.47	47.60	49.17	0.003
Female	45.53	52.40	50.83	
Time of logistic issues (h) [b]				
Journey time	9.69	16.77	15.24	< 0.001
Lairage time	3.37	5.10	4.71	< 0.001
Animals transported by… (%) [a]				
Trailer + Foot-trailer of 48-53 feet	51.99	99.86	88.90	
Truck + Gooseneck trailer 10 t	46.85	0.15	10.80	< 0.001
Truck + Small trailer 3 t	1.16	0.00	0.26	

P-values correspond to Chi squared (a) and U-Mann Whitney (b) tests, $P < 0.05$ denotes statistically significant differences.

Table 3. Assessment of animal-based welfare indicators at abattoir during pre-slaughter logistics of Mexican and American horses (n=*2648*) of two origins (Mexico vs USA).

Variables	*Mexico (n=604)*	*USA* (n=2.044)	*Total* (n=2.648)	*P*
Behavioural response at chute test -ChT- (%)				
Quiet	67.72	73.87	72.47	
Restless non-aggressive	12.42	15.95	15.14	< 0.001
Restless aggressive	19.86	10.18	12.39	
Vocalizations at chute test -ChT- (%)				
None	82.62	89.68	88.07	
Neigh/whinny	10.43	7.44	8.12	< 0.001
Nicker	5.30	2.25	2.95	
Snort	1.66	0.64	0.83	
Body condition score -BSC- (%)				
Emaciated	6.13	6.90	6.72	
Thin	37.42	38.60	38.33	0.440
Moderate	48.18	47.99	48.04	
Heavy	8.28	6.51	6.91	
Coat quality (%)				
Alopecia by regions	1.82	2.59	2.42	
Dry and opaque	39.74	39.68	39.69	< 0.001
Bright and soft	54.80	57.09	56.57	
Long and opaque	3.64	0.64	1.32	
Nasal discharge (%)				
No discharge	93.71	86.20	87.92	
Slight	3.64	6.60	5.93	< 0.001
Moderate	2.15	5.19	4.49	
Severe	0.50	2.01	1.66	
Ocular discharge (%)				
No discharge	76.32	76.81	76.70	
Slight	14.57	14.14	14.24	0.860
Moderate	7.28	6.75	6.87	
Severe	1.82	2.30	2.19	
Prevalence of complementary welfare issues (%)				
Skin wounds	9.11	11.79	11.18	0.061
Lameness	9.60	8.27	8.57	0.305
Diarrhoea	0.66	0.88	0.83	0.603

P-values correspond to Chi squared (a) test, $P < 0.05$ denotes statistically significant differences.

Table 4. Transport fitness profiles in Mexican and American horses slaughtered in Mexico based on animal-based welfare indicators (n=*2648*), with four clusters.

Variables	*C 1 (n=799)*	*C 2 (n=494)*	*C 3 (n=586)*	*C 4 (n=769)*	*P*
*Body condition score -BCS- * (%)*					
Emaciated	9.8	12.2	6.8	0.0	< 0.001
Thin	49.8	48.7	64.3	0.0	
Moderate	37.3	37.1	3.6	100.0	
Heavy	3.1	2.0	25.3	0.0	
*Coat quality score -CQS- * (%)*					
Alopecia by regions	0.0	0.8	10.2	0.0	< 0.001
Dry and opaque	100.0	50.9	0.0	0.0	
Bright and soft	0.0	47.5	84.5	100.0	
Long and opaque	0.0	0.8	5.3	0.0	
Prevalence of complementary welfare issues (%)					
Lameness *	0.0	45.8	0.2	0.0	< 0.001
Nasal discharge *	0.0	61.1	3.2	0.0	< 0.001
Ocular discharge *	17.8	42.8	23.5	16.4	< 0.001
Diarrhoea	1.1	1.4	1.0	0.0	0.022
Skin wounds	9.6	19.9	10.4	7.9	0.000
Behavioural response at chute test -ChT- (%)					
Quiet	77.5	77.9	64.2	70.1	< 0.001
Restless non-aggressive	11.0	15.0	19.6	16.1	
Restless aggressive	11.5	7.1	16.2	13.8	

* Variables that characterize the model in the two-step cluster analysis. *P*-values correspond to Chi squared tests. $P < 0.05$ denotes statistically significant differences.

Table 5. Transport fitness profiles of Mexican and American horses slaughtered at Mexico and their association with pre-slaughter logistics (n=*2648*).

Variables		*C 1 (n=799)*	*C 2 (n=494)*	*C 3 (n=586)*	*C 4 (n=769)*	*P*
Length of journey (%)						
0 - 6 h		13.4	6.7	11.6	12.4	
6 - 12 h		32.8	50.1	24.6	30.6	< 0.001
12 - 18 h		15.1	15.8	23.9	18.2	
>18 h		38.7	27.4	39.9	38.9	
Length of lairage (%)						
1 - 3 h		61.7	73.8	49.5	53.1	
3 - 6 h		16.4	10.8	25.6	20.4	< 0.001
6 - 12 h		13.0	9.9	18.1	17.9	
>12 h		8.9	5.5	6.8	8.6	
Country of origin (%)						
USA		75.8	82.2	76.3	76.1	0.036
Mexico		24.2	17.8	23.7	23.9	
Collection Point: City (State) (%)						
USA	Eagle Pass (Texas)	33.8	42.8	36.9	34.3	
	Columbus (New Mexico)	28.5	22.9	20.1	29.1	
	Presidio (Texas)	13.5	15.8	19.3	12.6	
Mexico	Fresnillo (Zacatecas)	11.4	7.1	11.1	13.3	< 0.001
	Guadalajara (Jalisco)	3.6	7.3	4.8	3.6	
	Durango (Durango)	4.6	2.2	3.8	3.0	
	Camargo (Chihuahua)	3.1	0.6	2.0	2.9	
	Culiacan (Sinaloa)	1.4	1.2	2.0	1.2	
Animals transported by… (%)						
Trailer + Foot-trailer of 48-53 feet		88.6	92.9	88.9	86.7	
Truck + Small trailer 3 t		0.3	0.2	0.5	0.1	0.027
Truck + Gooseneck trailer 10 t		11.1	6.9	10.6	13.1	

P-values correspond to Chi squared tests. $P < 0.05$ denotes statistically significant differences.