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1 Effects of the different transport phases on equine health status, behavior and welfare: a review

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- 10 Abstract:

11 The aim of this review is to provide practical outcomes on how to manage equine transport stress.

Many horses travel frequently during their life and transportation is one of the major causes of injuries, 12 13 health disorders and economic loss for horse breeding and the wider equine industry. There are a 14 number of recent publications in this area, but practical strategies aimed at reducing transport stress are still limited. The results published over the last twenty years are reviewed here in order to suggest 15 16 improved procedures to follow before, during and after a journey. Transport stress involves physical and mental stressors during handling, loading, transportation itself, unloading and adaptation to a new 17 environment. This article reviews how all these transport phases affect equine health status and 18 19 behavior and best practice management strategies. The suggested outcomes could be useful for equine 20 technicians, owners, breeders, grooms, and veterinarians to safeguard horse wellbeing.

21 **Keywords:** road-transport; stressor; health status; welfare; horses

23 **1. Introduction**

24 Transportation is an integral aspect of horse management, but transport stress is still an issue and many problems are associated with it. Horses are transported more frequently than any other type of 25 livestock (Friend, 2001). They are moved for many different reasons: in the past for war and, today, 26 27 mostly for competitions, breeding, pleasure activities, sale, or slaughter (Fazio et al., 2008). Three 28 thousand horses are transported daily in Italy (Giovagnoli, 2008). Around \$3 billion annually are spent 29 in transportation in the United States, with an estimated population of 9.2 million horses (American 30 Horse Council, 2005). It has been reported that a typical Texan horse owner transports an average of 31 2.5 horses, 24 trips (average 380 km per trip) per year (Gibbs et al., 1997; Gibbs et al., 1998).

Horses can develop loading, travelling or post-transportation problems. Many injuries to horses and owners occur during the loading procedure (Ferguson et al., 2001). Some horses move or kick inside the trailer, causing driving problems and fatal road accidents. Poor performance problems, health disorders and infectious diseases are the most common complications after transportation. Thus, assessing best practice transporting procedures for horses warrants comprehensive investigation (Cregier, 2010).

38 In the last twenty years many scientists have conducted research aimed at improving horse welfare 39 during transport and at reducing the incidence of related problems. Results are often conflicting, and 40 how best to manage transport stress is still a matter of debate. One reason for the conflict is that some 41 results are not comparable because the studies used different trucks or trailer models, in different 42 seasons, on different horse breeds. Moreover, it seems that the level of stress caused by transportation 43 is related to the temperament of the horse and its historical travel experience (Fazio et al., 2013), 44 orientation during travel, the provision for head movement, and factors such as driver skill, ventilation 45 and/or window placement.

This paper reviews approximately 20 years of publications concerning horse transport. The aim of this review is to split transport into its critical points, highlighting the effects that each phase has on horse behavior and pathophysiology. Suggestions for managing journeys to encourage practical outcomes that safeguard equine wellbeing before, during and after different types of transport are discussed.

51 52

2. Different means of transport: ship, train, truck, plane

The earliest form of water borne horse transport likely utilized a waterproofed hide stretched over a wooden frame; a form of transport thousands of years old. Specialized water-borne transport for horses has been in use for centuries, and has evolved with different types of ship/vessel construction for river, lake, or sea transport (Cregier, personal communication, 2014). Byzantine historian Theophanes

57 described such specialized horse transport in 762 AD. Chelandia were relatively small ships designed 58 to carry up to a dozen horses with a specific landing ramp (Pryor, 1982). Later, horses were carried 59 regularly by ships (Cregier, 1982), and sea transport was the only means of transport for horses until 60 the late 19th Century. Today sea transport is still in use because it is the cheapest form of transport, 61 particularly for slaughter horses travelling from South America to Europe (Giovagnoli, 2008). In 62 modern cargo ships, horses travel in boxes whose dimensions range from 4.5 x 4.5 m to 6.0 x 4.5 m and have access to a sand vard to exercise during the voyage (Waran et al., 2007). The key 63 64 disadvantages of sea transport are mainly duration and risk of injury (Judge, 1969). Long transfers by 65 ship are well accepted by horses, although after the journey animals can develop jet lag, medical 66 conditions associated with a change in management and conditions or caused by hierarchical conflicts associated with adapting to the new stall and social group (Cavallone et al., 2002). 67

68 The earliest mode of land transport was via horse-drawn wagon. Performance horses were drawn by 69 those of lesser value beginning sometime around the reign of Queen Anne in the early 18th century.

70 Transport by rail was very common from the mid-nineteenth century to the mid-twentieth century 71 with various wagon designs being used. The smaller horse car with two wheels on each end carried 72 eight horses. The larger car, supported by a four wheeled truck or bogie on each end, could carry 16 to 73 20 horses at speeds up to 145 km/h. Since more than one animal occupied a wagon, the risk of treading 74 on and kicks from cohorts was high. Thus, there was a special single horse van used for more valuable horses. The disadvantage of train transport has been mainly the rough, uncomfortable journey and, 75 76 often, prolonged waiting times at rail-heads for collection or delivery (Judge, 1969). Today, transport by rail is still in use in developing countries because it is a relatively low cost transportation method. 77

78 After World War II, following the building of interstate highway systems, road-transport became 79 the most popular means of transport (Friend, 2001). The 1960s and 1970s are known as the "Trailer 80 Age", because many different kinds of trailers and trucks were designed and built. Consequently, today 81 there are many different types of road vehicles used to transport horses, the major difference being 82 found between lorries or vans and trucks, and trailers. The "motorized horse box" or "horse lorry van" varies in capacity from 1 to 10, 12 or even 16 or more individually stalled or grouped horses. The most 83 84 common trailer is for two horses and it is usually attached to the towing vehicle at one point via a tow 85 hitch, which makes trailers less stable than lorries or trucks (Cregier, 1982). Trucks and trailers 86 generally have a rear and/or a side ramp for loading and unloading. In some countries (e.g., the US), 87 there may be no ramp and the horses are trained to "step up" into the trailer. In the U.K, Canada and 88 New Zealand, platform loading horse trailers were designed to reduce transport stress and increase 89 loading and unloading safety. The horses step onto the platform from the off side, reverse into the

trailer, and face away from the direction of travel to allow hind-quarter resting and freedom to balance
with their heads during transit (Equi BalanceTM).

The first reported air transportation of horses was during the early 1920's with the first race horses being flown across the Atlantic in 1946 (Judge, 1969). To date, air transport has become very common even though it is the most expensive form of horse transport. In the plane, horses travel in air stables or jet stalls designed to accommodate a maximum of three horses, side by side, separated by partitions (Waran et al., 2007). Air transport is used primarily for competitions and breeding purposes: in this manner stallions can be mated with mares in both the Northern and Southern Hemispheres in the same calendar year.

99 The most stressful phases of air transport are loading, unloading, taking off and landing (Stewart et 100 al., 2003). During the flight horses tend to have resting heart rate values and engage in resting 101 behaviors, indicating that they may settle better to air transport than road transport (Munsters et al., 102 2013). Quarantine regulations are generally applied after air travel to minimize biosecurity risks, 103 however, restraint in the quarantine boxes can cause an increase in heart rate associated with 104 environmental stress (Ohmura et al., 2012).

105 The vast majority of studies have focused on effects of transport stress in horses moved by road. It 106 is this mode of transport that is the focus of the remainder of this review.

107

108 **3. Different destination of transport: toward a slaughterhouse, a new stall or a competition**

Horses travel for different reasons, but the primary difference that can determine the kind of travelis the destination: a slaughterhouse or a different stall.

111

112 *3.1. Transportation of horses for slaughter*

113 Globally, many horses travel daily to slaughterhouses. Horsemeat consumption became popular in 114 Europe after World War II amongst people with lower-incomes. At the time beef was scarce, and old 115 or lame draft horses were slaughtered for affordable lean meat with a high iron content. Horsemeat has 116 become a delicacy associated with high prices in Europe (Stull, 2001), although the 2013 scandal 117 involving beef tainted with phenylbutazone containing horsemeat may have altered this profile. 118 According to the most recent data from the Food and Agricultural Organization (FAO) the largest 119 producer and consumer of horse meat in the world is China, which produced 170,848 tonnes of horse 120 meat in 2010. The second largest producer and consumer is Kazakhstan. In many European countries 121 horse meat is used as an ingredient in traditional meals, and is imported for this. In USA, even though 122 Congress cancelled federal funding to inspect horse meat destined for human consumption in 2007, 123 68,444 tonnes of horse meat was produced in the USA in 2010 for pet food (FAOSTAT). In Canada,

horsemeat exports, primarily to markets in Europe and Asia, exceeded \$60 million in 2011, and it is reported that more than 2,000 tonnes of horsemeat are consumed in Canada each year (www.equinecanada.ca). There are some countries (e.g., the United Kingdom) that have never accepted horsemeat as part of their diet (Reece et al., 2000).

In South America horsement is popular, Argentina is the largest equine meat exporter, and Chile
slaughters nearly 50 thousand horses annually (Werner et al., 2008).

Meat horses travel loose in the truck toward the slaughterhouse, and density during transport is variable. In high-density compartments horses can fall during shipment, resulting in injury or death, but injuries due to kicking may be less frequent (Whiting, 1999). In a lower density shipment, horses can escape biting situations and aggression, lowering the mentally stress for the horses (Collins et al., 2000), but aggressive behavior during transport may be related more to individual horse temperament than to conditions (Iacono et al., 2007a). Further studies are needed to define optimal travel density and environment.

Interestingly, the effects of travel on meat quality have been well studied in cattle and pigs (Ritter et al., 2008), but there are few studies in horses. After transportation increased blood lactate and glucose have been reported in horses after transport, factors which impair meat quality (Werner et al., 2008). The stress associated with transport, lairage and stunning also affects horse meat quality.

141 *3.2. Transportation toward a new stall*

There are an estimated 58 million horses worldwide, and the equine transport industry is a wealthy one. In industrialized countries horses reared primarily for pleasure or sport are most commonly transported professionally. Tamed horses travel in single stalls within the vehicle, whereas unhandled horses are more inclined to be transported in groups. It is common for mares to travel loose with their foal within a box inside the truck (Weeks et al, 2012).

Every time a horse travels to a new place, there is an associated adaptation period during which the horse needs to adjust to the stall, management and training styles, and diet. Because horses may also be exposed to pathogens which they have limited immunity to, standard-of-care recommendations include a quarantine period and gradual transitions to new diets. It is advisable to avoid abrupt dietary changes and prevent intense exercise sessions during the period following transportation (Waran et al., 2007).

153 *3.3 Travel before competition*

154 Despite many horses being transported specifically for performance purposes, surprisingly little is 155 known about the effect transportation has on performance and the results are often conflicting.

156 It has been suggested that for experienced horses, transport over short distances had little affect on 157 performance (Beaunoyer and Chapman, 1987). However, other research found that a 194 Km journey

158 could negatively affect the performance in a close race, particularly in front facing transported horses, 159 which displayed the effects of slight stress (Slade, 1987). The effect of transport on competition 160 jumping horses' performance was also studied. In this case, only horses with less travelling 161 experienced showed signs of major stress and reduced performance (Covalsky et al., 1991)

A recovery period of two hours after three hours travel was suggested on the basis of the increase of muscle enzyme concentration caused by the transport (Tateo et al., 2012). Travel longer than 8 hours is discouraged before a competition, as it could compromise racing performance and require some days of recovery (Linden et al., 1991).

166 After a 100 km transportation distance in a one-horse trailer, facing in the direction of travel, an 167 increase in the concentration of free triiodothyronine (T3) was recorded. Triiodothyronine (T3) is a thyroid hormone involved in growth, metabolism, heart rate and thermoregulation. Elevated levels of 168 169 T3 are commonly associated with sessions of intense exercise (Fazio et al., 2008a). Since T3 is 170 intricately associated with the other thyroid hormone, known as thyroxine or T4, by an endocrinology 171 feedback mechanism, it may be supposed that an elevated T3 value recorded at unloading before a race 172 could impair performance. But there has been no study to date that correlates the specific affect of 173 elevated T3 levels resulting from transportation with performance results.

The relationship between transport and performance needs more investigation. However, developing good scientific methodologies for definitively assessing the effects of transportation on performance is deemed very challenging due to the confounding effects of factors including horse temperament, position and orientation in the vehicle, fitness level of the horse, historical transportation experience and driver ability/skill level.

179

180 4. Phases of transport and their effects on horse behavior and physiology

181 The transport of animals is a complex procedure involving several potential stressors. The 182 transportation process includes the following critical factors which can all impact on stress levels: 183 handling, separation from familiar physical and social environments, loading, confinement, vibration, changes in temperature and humidity, inadequate ventilation, often deprivation of food and water and 184 185 unloading (Waran, 1993). Factors that induce stress during transport are mostly psychological (White et al., 1991), but physical factors such as the trailer motion, noise, the driver's ability and road 186 187 conditions also may play a major role (Jones, 2003). Confinement itself is stressful for horses (Mal et 188 al., 1991), but for many farm animals a stationary vehicle is generally considered to be less stressful 189 than a moving one (Tarrant, 1990). Indeed, during transportation, horses are subjected to changing 190 forces primarily due to acceleration, deceleration, and the turning movements of the vehicle (Waran 191 and Cuddeford, 1995).

7

Accordingly one survey reported horses have problems both in loading (53.4%) and travelling (51.5%). During travel the majority of problems were recorded when the vehicle first began to move (53%) or when it went around a curve (47%)(Lee et al., 2011). It has been reported that breed has no effect on the problem type, whereas the trailer orientation and the horses' mental association to negative experiences may be significant important factors in the etiology of trailering problems (Lee et al., 2011).

198 It should be highlighted that horses can associate the transportation itself with what they experience 199 during and after the journey. So it seems that horses used for sporting and recreational purposes with a 200 number of positive experiences of loading and transportation are less adversely affected by 201 transportation than horses with no experience and horses who have negative previously experienced 202 situations including falling or over-crowding (Leadon et al., 2008).

203

204 *4.1. Handling*

205 Handling refers to how animals are touched, moved and interacted with during husbandry 206 procedures. The handling methods (reinforcement or punishment) can have a significant affect on 207 animal welfare. It has been found that for horses sent for slaughter, handling without the use of sticks 208 or electric goads resulted in improved welfare and lower risk of poor quality meat (Broom, 2005). 209 Restraint is often part of the handling procedure. During restraint animals are often separated or 210 isolated from their conspecifics which is a known factor causing stress. Both handling and 211 transportation involve the interaction of animals with humans and it is important to know how animals 212 react to human behavior aimed at effectively moving and restraining them (Fazio et al., 2003). The 213 taming/training method (ethological or traditional), can have an ongoing influence on the horse-human 214 relationship, thereby having long term effects on the horses' behavior during subsequent handling 215 procedures (Casamassima et al., 2008).

216 Age, sex and physiological condition also influence the behavior of horses during handling and 217 transport; indeed handling young animals, such as foals and yearlings, which are usually not tamed 218 extensively, can be significantly more difficult and risky than handling older animals. Although it is 219 generally assumed that intact males are more difficult to handle than castrates, this difference may also 220 be age dependent. Rearing environment and previous experience are generally agreed to be of 221 considerable importance. Animals respond to challenges in their immediate environment through 222 several interacting mechanisms including behavioral, hemato-chemical, physiological and neuro-223 hormonal (Fazio, 2013). The response of animals to handling and transport can also depend on their 224 sensory capabilities, the visual field and flight zone. Some behavioral indicators of discomfort are 225 vocalizations, attempts to escape, kicking, or struggling. Identifying stressful situations by key

behaviors could be useful during handling and transport and would promote greater wellbeing inhorses (Siniscalchi et al., 2014).

Overall, it is generally more desirable to transport horses that are already well accustomed to handling so that they do not associate the stress of being handled with the process of being transported.

230

231 5.2. Loading (Injuries and Fear)

Loading is considered to be one of the most stressful components of transport (Waran, 1993). Loading fear comprises different stimuli, such as fear of entering an enclosed space, the height of the step leading onto the ramp and the instability and incline of the ramp (Houpt and Leib, 1993). It is these factors that result in inexperienced horses often exhibiting extreme evasive behavior and a strong reluctance to step up onto the ramp. Accustoming the foal to loading has been proven to reduce behavioral problems associated with loading and transporting later in life (Houpt, 1982).

238 The heart rate during loading is usually higher than the average heart rate during transport, 239 regardless of the level of experience. In fact, climbing a ramp is probably a frightening experience for 240 a naïve horse, and although horses may become accustomed to the situation, experienced horses are 241 still stimulated in some way. This elevation in heart rate can be ascribed partly to the energy expended 242 in climbing the ramp and partly to the emotional fear (Waran, 1993). Evasive behavior during loading 243 is typical of very young horses and the time taken to load is influenced by age. In fact, it is reported 244 that yearlings took more time to load (368 s) than 2-year-olds (29.5 s), 3-year-olds (21.5 s) and those 245 over 3 years-old (5 s) (Waran and Cuddeford, 1995).

246 As a fear response, many horses fight during loading which in itself is a source of stress and can 247 result in injury to the horse and/or handler. Behaviors such as rearing, pulling back, head-tossing, 248 pawing, and turning sideways are commonly exhibited. These behaviors are likely to be negatively 249 reinforced when the loading process is aborted by the handler (Baron, 1991). The combination of 250 loading a 'problem' horse and an owner who applies physical punishment can produce a very 251 dangerous situation. Rope burns, lost fingers, broken bones, or bruises and lacerations have been 252 reported as most common owner injuries. Lacerations to the head from banging into the trailer, scrapes 253 and cuts on the legs, broken legs from falling, or even a broken back if the animal falls backwards 254 while rearing are the most common horse injuries occurring during loading (Ferguson and Ruiz, 2001).

Some studies have been conducted to determine procedures which reduce loading fear. To reduce the likelihood of injury, horses that are difficult to load can be trained to load more willingly. Successful training procedures involve increasing the horses' confidence by breaking loading into simpler, separate tasks that can be accomplished in a relaxed mental and physical state. These tasks

include moving forward on command, stepping onto and backing off an unstable floor, and movinginto a confined space (Scoggins, 1996).

261 The Tellington-Touch Equine Awareness Method (TTEAM), developed by Linda Tellington-Jones 262 takes this concept of relaxed, progressive training one step further (Curcio-Wolfe, 1996). This method 263 uses non-aversive touch and commands in novel situations as a means for inducing behavioral changes 264 in horses. Horses are generally neophobic, and TTEAM is specifically designed to teach horses to 265 relax and function in the presence of novel and potentially frightening stimuli. Non-aversive retraining 266 methods (based on TTEAM) were effective in reducing loading time for horses with a history of 267 reluctance to load onto a trailer and were also associated with a decreased post-loading heart rate and 268 saliva cortisol levels (Shanahan, 2003).

Loading fear is innate in the horse, but some environmental stimuli can be attributed to exacerbating loading fear. One such example would be loading a horse directly from a brightly lit arena into a dark trailer (Cross et al., 2008).

People have used winches, whips, war bridles, chains, cattle prods, and a variety of other punitive methods to force horses to load. Although professional horse trainers do not openly advocate extremely aversive methods, most of their methods of loading horses include some form of negative reinforcement and the use of punishment in response to undesirable behaviors.

On the other hand, recent literature has proven that in particular for horses which refused to load, positive reinforcement (PR) provided the fastest training solution with the lowest levels of stress response (Hendriksen et al., 2011). It has been also reported that the use of applied equine training systems, based on positive reinforcement, results in increased probability of appropriate behavior being displayed during handling and loading procedures in loading problem horses (Slater and Dymond, 2011).

282

283 *5.3. Transportation*

284 Transportation involves many stressful factors. During the journey, the internal truck temperature, 285 relative humidity and level of environmental contaminants can change dramatically (Leadon et al., 286 1990). The horses in the vehicle may also have to adapt to unfamiliar factors including traveling 287 companions, confinement spaces, movement beneath their feet, acceleration and deceleration, 288 ascending and descending, taste of drinking water, exposure to vibrations and noise and so on. 289 Transport stress should be considered as a multi-factorial physical and emotional status, where the 290 sympathetic nervous system shifts from alert to fear many times and where the maximal effort is spent 291 in balance preservation. To maximize the wellbeing of any horse during transportation the following 292 factors should be taken into account and/or checked during the journeys.

293

294 5.3.1. Confinement and Isolation

Both confinement and isolation are stressful and may suppress feeding behavior during transportation (Mal et al., 1991).

Once loaded into the vehicle, the horse is placed in a restricted space, either due to being confined in an individual stall using partitions, or due to pressure on individual space exerted by the other loose horses with which it may be travelling. Tamed yearlings transported in individual stalls or loose had similar cortisol levels measured both during and after transport. In both situations transportation was a significant stressor (Garey et al., 2010).

302 Since transport often requires the animal to travel alone, the effects of transporting horses alone, in 303 company or with a mirror that provided surrogate companionship were investigated. Behaviors 304 (vocalizing, eating, head-tossing, pawing, and head-turning) and physiological parameters were 305 recorded. When traveling with a live companion significantly less time was spent vocalizing, head-306 turning, head-tossing and pawing and eating behavior increased. Heart rate and temperature also 307 significantly decreased when travelling with a live companion. Travelling with the mirror did not 308 significantly affect physiological responses when compared to travelling alone, but did significantly 309 reduce time spent turning the head, vocalising and head-tossing while eating behavior also increased 310 (Kay and Hall, 2009).

- 311 In general, the provision of surrogate companionship in the form of an unbreakable (e.g., stainless 312 steel) mirror is preferable to travelling alone, but where possible a live companion is recommended.
- 313

314 5.3.2. Effect of Density

315 During loose horse transportation, high stocking densities create a situation of constant struggle for 316 the horses. Medium stocking densities likely reduce injury and bruising during transportation, but also 317 increase transport costs. It was suggested that decreasing density would reduce the overall stressfulness 318 of long distance transport by allowing horses more opportunity to avoid aggressive horses, to stand in a 319 more comfortable position, to adopt their preferred travelling orientation, and perhaps allow them to 320 rest during periods when the truck is stopped (Whiting, 1999). It was recently shown that there was no 321 difference in the movements recorded in unrestrained horses transported in low or moderate density. 322 However, the movements of the unrestrained animals inside the truck were strongly influenced by a 323 small number (one or two) more active horses causing disruption of the group (Calabrese et al. 2009).

For individual horses stall size is important and there is legislation about the minimum space required during road transport according to age and type of horse, but minimum space allowances differ between countries (Waran et al., 2007). 327

328 5.3.3 Effect of fasting and water intake

329 Depending on the final destination, horses may or may not have the opportunity to feed and drink 330 en route. Sport horses transported to a racetrack are often allowed to feed on some hay, usually offered 331 in a net, because it has been shown that it does not impair performance, whereas horses transported to 332 a slaughterhouse are usually fasted to reduce the risk of soiling and resultant meat contamination 333 (Waran, 1995). Some studies suggest avoiding hay in the lorries in all cases, because it affects the air 334 quality. Dry hay is likely to pose a risk to horses prone to recurrent airway obstruction (RAO), which 335 could become symptomatic after allergen exposure: for these horses it is better to use wet/dampened 336 hay or pellets (Hotchkiss et al., 2007). However, if the hay is offered during the journey, it is generally 337 better to dampen it and to place it on the floor, to minimize the dust risk. Furthermore, this location 338 could stimulate travelling horses to eat with a lowered head position, which seems to be fundamental in reducing the development of respiratory diseases (Raidal et al., 1996). Regardless of whether or not 339 340 feed is provided during travel, weight losses are reported after a journey (Waran, 1993). Weight loss is 341 likely to be due to a combination of reduced feed and water intake and increased energy expenditure 342 and fluid loss through sweating (Smith et al., 1996). It is important to emphasize that horses always 343 tend to reduce feed and water intake during the journey, because they are less willing to eat and drink 344 in unfamiliar and stressful surroundings and from unfamiliar sources (Kay and Hall, 2009). Generally, 345 it is better if familiar water and food are offered to the horses during the journey, as well as during 346 planned rest periods. It has been proven that increasing the resting time and cleaning the interior of the 347 truck during rest stops decreases transport stress and respiratory insults (Oikawa et al, 2005).

During long trips water should be offered to the horses, while the vehicle is stationary, at least every 2 to 4 hours, especially when the environmental temperatures are high (Houpt and Leib, 1993). During a 24 hour trip, a stop is needed at least every 4 hours to provide the horse with the opportunity to urinate; horses urinate approximately six times daily and male horses in particular need to be able to stand in a particular posture which is difficult to do comfortably en route (Weeks et al., 2012).

In many codes of transport practice it is deemed acceptable to remove all access to water and food for up to 8 hours during transportation, but the effects of such long periods of fasting have not yet been well investigated.

356

357 5.3.4. Environmental Challenges

Temperature, humidity and ventilation inside the vehicle are critical aspects of a journey. Ventilation for horses during transportation has been a topic of research since the 19th century with links reported between level of ventilation and the occurrence of both heat stress and shipping fever.

361 Despite research findings, and proof that trailers are under-ventilated at all speeds (from 13 to 90 362 Km/h) and ventilation configurations, trailer design has not been modified (Purswell et al., 2006). The suitability of the trailer thermal environment is generally well assessed for other livestock but is still 363 364 lacking with regards to horses. The thermo-neutral zone (TNZ) in horses is also not well defined, but it 365 is estimated to be in the range between 25°C and 30°C. Thermal comfort depends not only on 366 temperature, but also on humidity and it is expressed by a calculated index. For horses the upper limit 367 of this thermal-index was set at 28°C by the Federation Equestre Internationale (FEI) for competitions. 368 The problem is that inside the truck this limit is not valid and it is often exceeded. In traditional 369 trailers, inside-to-outside temperature differences decrease with increased speed and the open vent 370 area, but it still ranges from 5.1°C to 9.5°C (Purswell et al., 2010). This means that horses should not be transported during hot and humid days, when outdoor temperatures exceed about 30°C. Since it 371 372 impractical to restrict horse transportation on days that exceed 30°C (particularly in hot arid countries), 373 future research should include new engineering and design solutions to improve thermal environment 374 and ventilation characteristics of trailers and trucks. Increasing vent and window area, increasing the 375 height of the vehicle and adding fans have all been suggested for vehicles transporting other species, 376 but should also be applied in horse transport vehicles (Mitchell and Kettlewell, 2008). The risk of heat 377 stress shock is increased dramatically when the vehicle is stationary and when more than one horse is 378 aboard the vehicle and under such circumstances it is advisable to park the vehicle in shaded areas with 379 all windows and ramps open during rest stops (Waran et al., 2007).

380 Animals produce CO₂ through respiration and ammonia with their urine, in addition to 381 microorganisms. As a result, the confined space inside the moving vehicle is not usually conducive to 382 a healthy environment. Consequently, good ventilation is vital to ensure not only acceptable air 383 temperature and relative humidity, but also low levels of airborne contaminants such as gases and dust. 384 Many studies in this research area were conducted by Leadon et al. (2008) who reported that air 385 usually enters the horse lorry through the windows or vents along the side, but that this air tends to 386 drop toward the floor and become contaminated. As a consequence the air quality in a lorry becomes 387 very poor. Further studies are needed on ventilation design to improve air circulation and air quality.

It is important to limit the concentration of noxious gases in the air because it has been proven that during transportation horse respiratory clearance is reduced following exposure to ammonia, nitric oxide and carbon monoxide. Those gases damage the pulmonary epithelial barrier, thereby increasing the permeability to bacteria (Traub-Dargatz et al., 1988). In a study reported by Smith et al. (1996), ammonia concentration was recorded in a standard 2-horse trailer. The mean concentration during a 24 hour journey was 0.8 ppm with no detectable odor. Although there is no recommended upper limit for exposure of equines to ammonia and other gases the safe human limit should be taken into account.

The current recommended exposure limit is 25 ppm for ammonia and 9 ppm for carbon monoxide (Pickrell, 1991). Endotoxins in air can also create inflammation of the airways. While it has been proven that stabled horses are exposed to 8-fold higher concentrations of endotoxin than pastured horses (Berndt et al., 2010) there are no reported studies of endotoxin levels in transportation vehicles.

399 Another critical equine health consideration is the risk of disease spread during and after 400 transportation. In the truck, animals from different farms often travel together, so pathogen 401 (bacteria/viruses/parasites) transmission risk is relatively high. Wherever possible, loading of animals 402 from different farms and of different ages should be avoided. Since trailers and trucks are saturated 403 with potentially harmful bacteria and spores after transporting horses, they must be disinfected prior to 404 reuse. However, effective disinfection is challenging especially in winter. An effective cleaning 405 procedure should involve cleaning with hot water, prior to removing excess water with a suitable 406 vacuum cleaner and the concentration of the disinfectant should be elevated at least by a factor of 3. Mechanical actions, such as scrubbing, will improve disinfection of vehicles as well as preliminary 407 408 disinfection prior to cleaning (Boehm, 1998).

Monitoring of the microclimate can be conducted with the use of probes located at various positions within the trailer to measure temperature, humidity and air movement. Monitors located in the vicinity of the horses head(s) should be used to measure concentrations of ammonia and other gases (Smith et al., 1996). The use of a commercial data logger inside the truck is strongly recommended with the collected data being used to modify the vehicle and management practices in manners that neutralize environmental challenges thereby improving animal welfare (Miranda-de la Lama, 2013).

Finally, to eliminate odors, neutralize some gases and disinfect hard surfaces, the use of vinegar, zeolite or baking soda as alternative microbiological agent can be used for routinely cleaning of the vehicle (Fong et al., 2011).

418

419 5.3.5. Direction of travel

420 Body orientation during travelling is an important matter of debate. Equine anatomical 421 conformation is such that 60% of the equine body weight is carried over the forelegs, and the 422 hindquarters are poorly designed for continual shifting of weight and bracing against directional 423 change (Cregier, 1982). It is probably for this reason that the most commonly observed body posture in 424 horses travelling in a forward facing direction involves standing with the front and hind limbs apart 425 and the forelegs stretched forward. This exaggerated limb position during transit likely helps the horse 426 to retain its balance by exerting inclined thrusts with one leg or the other, as occasion demands 427 (Roberts, 1990).

428 Inappropriate orientation, and consequential loss of balance, can cause injury during horse transport 429 (Whiting, 1999). There are different opinions amongst experts about travel positions that minimize 430 transport stress and optimize the horses' post-transport performance (Gibbs, 1999). Several studies have been performed in order to determine the effects of orientation on a horses' ability to maintain 431 432 balance during transport, but results have often been conflicting due to differences in trailer design and 433 lack of simultaneous comparisons. When transported in a 2-horse trailer, backward-facing horses had 434 fewer side and total impacts and losses of balance when compared with forward-facing horses (Clark 435 et al., 1993). Horses transported in a 2-horse lorry without a saddle compartment and facing backward 436 had a significantly lower heart rate, moved less frequently, and showed a greater tendency to rest their 437 rumps on a partition (Waran et al., 1996).

Comparison of three different positions (backward, forward and sideways) during a three hour journey for Standardbred trotters accustomed to travel, revealed that backward facing was the most ideal orientation. Backward-facing horses moved more but lost their balance less and were able to rest a hind quarter in a three leg position during the journey (Padalino et al., 2012).

However, since some horses have demonstrated a superior ability to maintain their balance in a
particular orientation, individual characteristics and other factors, rather than travel orientation alone,
may affect the ability of horses to maintain their balance during transport (Toscano and Friend, 2001).
Therefore, further studies are needed on the travel position.

446

447 5.3.6. Duration of travel: short *versus* long trip

In order to understand the effects of road transport on equine physiological and behavioral parameters many studies have been conducted and it seems that a key variable is the duration of the journey.

Long transport times likely have a strong effect on both equine physiological and endocrinal parameters. The effects of nine hours of transport on in-foal mares showed increased concentrations of cortisol and progesterone. Despite the increases, no early embryonic deaths were reported (Baucus et al., 1990).

After 24 hours transport, equine body weight decreased by 6% immediately after unloading with a 3% deficiency remaining at 24 h post transit. The white blood cell (WBC) counts, hematocrit and total proteins concentrations showed a progressive increase with the duration of travel, peaking at the termination of transport. Serum lactate, creatine kinase and aspartate aminotransferase concentrations increased during transport and in the early post-transit period. They returned to normal levels 24-h after unloading, whereas glucose concentrations tended to rise with the initiation of transport and did not decrease to baseline concentration within the 24 hour post transportation period (Stull and Rodiek,

2000). Plasma cortisol and the neutrophil:lymphocyte ratio also increased during transportation, as a
stress response. Those hematological and endocrinal changes may increase disease susceptibility and
influence energy availability for athletic performance following transportation of horses over long
distances.

Due to the long journey, stress-related respiratory diseases and even death have been reported in horses (Anderson et al., 1985; Oikawa et al, 1994; Racklyeft et al., 2000). It was observed that in healthy horses traveling in a trailer for 36 h (1,100 Km), the number of alveolar macrophages and their bactericidal function decreased and cortisol concentration remained elevated one week after transport; favoring the development of lower airway diseases (Laegreid et al., 1988).

According to European law, a journey is considered to be long if it lasts more than 8 hours,
requiring that some rest must be planned to minimize the risk of the above mentioned health problems
and to safeguard equine welfare.

474 Horses are transported mainly over shorter distances, in particular before competitions, so the475 effects of short journeys are also a topic of importance and relevance.

Comparing a short-duration (300 Km) journey and an exercise bout of cantering 1,500 m, similar
effects on serum enzymes and metabolic processes were reported in race horses (Codazza et al., 1974).

478 In a comparison of one hour vs. three hour journeys the number of movements recorded per 479 kilometer was greater during the short journey than the long one. More forward and backward 480 movements were reported, possibly as a result of the greater agitation shown by the horses at the start 481 of the journey. In addition, horses subjected to the short journey showed a higher serum cortisol 482 concentration at unloading, suggesting that they could not adapt to the travel in one hour (Tateo et al., 483 2012). Many authors have argued that horses need around 5 hours to adapt to a transportation 484 experience, and the first phase is always the most critical (Baucus et al., 1990; Iacono et al., 2007b). 485 Other studies have confirmed that the period of adaptation to a journey is longer than one hour which 486 increases the importance of animal management during short journeys, because the horse is under an 487 acute stress situation (Fazio et al., 2008b).

In conclusion, long and short journeys both affect equine behavior and endocrinology differently, but both require a restore time and appropriate management. Long journeys should be planned with frequent and long stops, during which the horses should be fed and watered, the male horses allowed to stale or urinate, and the truck cleaned. It is important to choose quiet and shaded rest areas. The horses could be more nervous than usual, so unload them only if the area is fenced and deemed 'safe'.

493

494 5.3.7 Effect of driver behavior

495 Driving style is another important factor which influences horse welfare during transportation. The 496 driver's ability could affect the movement of the vehicle and consequent balance ability of the 497 transported animals, in particular during accelerations, braking, cornering and any other difficult 498 maneuvers. There are two main components in driver behavior: style and skill, where "skill" is the 499 ability to control the vehicle and "style" is the way in which the vehicle is driven (West et al., 1993). 500 European rules require the driver to demonstrate skill and ability in transporting animals. Driver 501 behavior and road quality (motorway vs. minor road vs. city traffic) affect the behavior of transported 502 animals as has been shown for sheep (Cockram et al., 2004), cattle, young calves and pigs (Cockram 503 and Spence, 2012). In horses, heart rate seems to be highly correlated with muscular activity spent in 504 balance preservation, and both are strongly affected by the degree of experience of the driver. 505 Consequently, it has been suggested that vehicle condition (suspension, tire pressure), road quality and driver's professional ability are crucial factors in determining the magnitude of transport stress 506 507 (Giovagnoli et al., 2002).

508

509 5.3.8 Effect of noise and vibration

Animals can perceive high frequency sounds and it is possible that they are disturbed or scared by sounds that are inaudible to man. During loading, transportation and unloading, noises audible to humans can arise from different sources such as human voices, whips, animal vocalizations (including other species e.g. barking dogs), noisy machinery, alarm bells/klaxon, and compressed air brakes on vehicles. We cannot quantify easily all sources of ultrasounds, but animals are exposed to many 'annoying' noises. It has been proven that intensive noise results in a central nervous system excitation, which causes immune system suppression, fatigue and cell death (Minka and Ayo, 2009).

517 Vehicle vibration has been correlated with liver and muscle glycogen depletion and consequent 518 fatigue in bird (Warriss et al., 1999); this effect could be contribute to reduced animal performance or 519 horse meat quality after transportation.

520

521 5.4 Unloading

522 Unloading at journey's end may be another physical stressor. Some horses are particularly difficult 523 to unload, particularly if the ramp is very steep or if the animal is exhibiting lameness.

Heart rate tended to remain higher than 'at rest heart rates' up to 30 minutes after unloading, but it is difficult to apportion this to the unloading vs. the cumulative transport stress (Waran and Cuddeford, 1995). However, it has been recently proven that Quarter Horses were stressed more during loading into a truck and stepping off a 20 centimeter step than during unloading (Siniscalchi et al., 2014). 529

530 5.5 Adaptation period in a new environment

Little is known of horses' behaviors after a journey, and very often, behavioral alterations noted 531 532 may be a result of environmental change (Waran, 1993). The behaviors of horses transported both 533 short and long distances were studied. To allow the researchers to account for the confounding effect 534 of environmental change, a group of control horses was included in the investigation. These control 535 horses did not experience a journey but were relocated to unfamiliar stalls. Comparing the 3 groups 536 upon arrival in the new stalls, it was clear that horses which had made the journey sniffed less and 537 snorted later than the control group. In the first 2 hours following travel, horses were attracted 538 immediately to concentrated food and then started feeding on hay. After the long journey, the horses 539 performed more bouts of drinking, and drank earlier, than the short journey and control groups (Tateo 540 et al., 2012). The latter behaviors could be explained due to the slight dehydration caused by a long 541 journey, which could have had been a direct cause of the positive influence on drinking behavior 542 (Iacono et al., 2007a). It was in agreement with another study where horses, after 6 hours 543 transportation, tended to spend more time standing, playing or resting only after drinking and eating 544 (Waran, 1993).

After a journey, horses are usually more interested in feeding than in other behaviors, including exploration, rest, and play, which are usually concentrated in the post-feeding hours, probably to recover energy spent to maintain balance in the truck. Consequently, to guarantee favorable adaptation to a new stall, it is recommended that food and fresh water are offered to the horses at their arrival (Padalino et al., 2012).

550 A common practice to encourage rapid adaptation after a journey is to stall horses adjacent to a 551 familiar stable companions on arrival at the destination.

552

553 6. Major Pathology Connected with Travel

Another important research area relating to transport is the development of pathology and disease during and after travelling. Higher risks are connected with long transportation. These include acute colitis, laminitis, transit tetany, shipping fever and mild azoturia (Mansmann and Woodie, 1995). However, short and frequent transportation can also result in injuries and health disorders. Frequently traveled horses are likely to show disrupted feeding patterns, weight loss, and fatigue (Cregier, 1982).

Notably, each horse journey implies some risk of disease transmission. Safeguarding the horse industry against this danger is one of the major responsibilities borne by equine clinicians. The occurrence in 2002 of West Nile fever in a stallion in post-arrival quarantine and the catastrophic outbreak of equine influenza recorded in 2007 in Australia provide some examples of the risk and

- importance of quarantine periods. Clinicians also need to be aware of the potential problems relating tohorse transport and the way in which these problems may arise (Herzolz et al., 2008).
- 565

566 6.1. Traumatic Injuries

567 The second (second to paddock/yard) most common source of injuries to horses is the transportation 568 trailer (Darth, 2014). At loading leg injuries associated with to the loading ramp are very common. 569 During the journey halter rubbing at the poll or muzzle and tail rubbing are specific types of abrasion. 570 To avoid these problems, it is useful to use specialized protections such as head bumper or soft 571 wrapping around the halter and protective bandaging on the tail. Long tie-ropes are suggested to allow 572 lowering of the head, but protective screens must be in place to eliminate the risk of biting between 573 neighboring horses. Wither wounds can be caused though contact with the vehicle ceiling While leg 574 wounds most commonly occur as a result of loss of balance after braking and cornering. Rapid and 575 extreme braking has been reported to result in vertebral fracture and dislocation in horses facing 576 forward and restrained with short tie-ropes (Masmann and Woodie, 1995).

A recent Australian survey reported that 72% of horse owners blamed the horse's behavior for travel incidents. However, the author highlighted that many incidents are actually related to the driver ability particularly on winding country roads. Among the most commonly reported behaviors were scrambling and slipping during cornering – both of which are exacerbated by wet flooring. Injuries resulting directly from horse-horse interactions and conflict were also common as were injuries caused during loading and unloading (Noble, in print).

583 Injuries during transportation can also be related to road accidents; 2500 known transport accidents 584 involving horses have occurred in the USA over the past 30 years. Often the blame is attributed largely 585 to 'poor' design of vehicles or inappropriate use of vehicles (e.g. overloading). While traumatic 586 injuries are very common; correct transporting procedures and appropriate levels of care could reduce 587 their prevalence.

588

589 5.2 Respiratory illness post transport

Recent transportation is frequently associated with the development of pleuro-pneumonia or *shipping fever*, which is a mixed bacterial pneumonia with varying involvement of the pleural space (Raidal, 1995). Although its etiological role is uncertain, *Streptococcus zooepidemicus* has been frequently isolated from pneumonic lesions in those cases (Mair and Lane, 1989). *S. zooepidemicus* is an ubiquitous bacterium in the upper respiratory tract and a secondary invader. It may be activated when host susceptibility to respiratory infection is increased by stress associated with events such as transportation. It has been suggested that transportation predisposes the upper respiratory tract and the

597 lower airways to invasion by the bacterium, with resultant occurrence of episodic pyrexia and acute 598 pneumonia (Oikawa et al., 1994). Pleuro-pneumonia has a 5% incidence after long transportation, with 599 a significant negative affect on horse welfare, largely because it associated with a relative high case 600 fatality rate (Wilkins, 2003). Early identification and appropriate treatment results in survival rates in 601 the range of 43 to 76%, but only approximately 60% of affected horses are able to resume their athletic 602 career (Copas, 2011).

603 The initial clinical signs of shipping fever can be insidious with the most common symptoms being 604 fever, depression, and sometimes stiffness. A lack of coughing or nasal discharge is not uncommon 605 and clinical signs are not specific. Factors that may contribute to transport-related respiratory disease 606 in horses are: presence of subclinical respiratory diseases, prolonged restraint in a "head-up" posture 607 that affects the pulmonary clearance mechanisms, stress-related impairment of the immune response, 608 the presence of noxious gases, high concentrations of airborne dust and bacteria in the truck, poor 609 ventilation, length and duration of journey and any mentioned emotional and physical stressor, which 610 results in immune system suppression (Oikawa et al., 1995). Recent studies have also proven that an 611 imbalance in oxidative status could be a related cause in the development of respiratory disease in 612 horses (Po et al., 2013), but validation is required to ascertain the link with transportation.

In the traditional hauling of horses, it is common to place hay in a net at the horses' nostrils to allow feeding and to keep the horse entertained. Unfortunately, normal, good quality hay contains many dust particles and small mold spores that can be inhaled. Dampening the hay and placing it on the trailer floor, or replacing hay with pellets reduces the risk of significant air contamination.

617 To reduce the insult to the respiratory system, it is important also to choose a good bedding material 618 which is not dusty but which is also very absorbent.

619 Since it has been proven that horses restrained with their heads elevated for 24 hours developed an 620 accumulation of purulent airway secretions and bacteria, which increased the pulmonary risk (Raidal et 621 al., 1996), cross tying horses should be limited and it restrain the horses with a rope which is long 622 enough to allow the horse to lower its head. Horses may be secured by the "log and rope" method, that 623 allows head movements up and down as well as side to side. Using the "log and rope" method it is 624 feasible to provide hay at floor level thereby allowing the horse to eat in a natural position (with head 625 lowered). Another approach is to anchor the tie-rope alongside the withers. The operator then has the 626 option to route the tie rope through a second ring positioned such that the horse cannot worry the 627 neighboring horse. In well-designed trucks where the stall dividers are well designed and there is no 628 risk of horses biting travelling companions, horses can travel untied.

629 Another preventative measure for reducing shipping fever is to avoid transporting horses which are 630 undergoing drug therapy. For example, phenylbutazone can mask the early signs of pneumonia while

631 corticosteroids are known to further decrease the horses' defense mechanisms and may also increase632 the risk of laminitis (Mair and Lane, 1989; Racklyeft et al., 2000).

Increasing the rest time and frequency and cleaning the interior of the vehicle during rest stops
 reduces transportation stress and respiratory insults which may lead to respiratory diseases (Oikawa et
 al., 2005).

Diagnosis of early stage pleuro-pneumonia and other respiratory diseases associated with transport is an area that deserves investigation and is not widely reported in the literature. Infrared thermography (IRT) has been shown to have merit in the early detection of bovine respiratory disease complex before it was otherwise manifested (Schaefer et al., 2012). There are also many other reported examples of the use of IRT for early disease detection across many species including humans.

641 Since recent significant findings have linked hydrogen peroxide, a common oxidant marker, in 642 exhaled breath condensate to *R. equi* pneumonia in foals (Crowley et al., 2013), the latter method 643 could become useful to test the travelled horses at unloading, to evaluate the pulmonary antioxidant 644 status and likelihood of subclinical pneumonia.

645

646 *5.3 Dehydration, laminitis and colic syndrome*

Even when water is available, horses tend to dehydrate during a journey which predisposes them toother pathologies.

Dehydration status can range from mild to severe after transporting and is largely dependent on the travel conditions (e.g. environmental condition and journey duration). Despite an increased physiological requirement for water, drinking behavior is generally suppressed by the travel stress (Mars et al., 1992).

The earliest stages of dehydration are difficult to determine clinically, because a horse could have up to 5% dehydration without showing any significant clinical signs. For the athletic horse 2-3% dehydration can affect performance, so preventing even slight dehydration may be extremely important for racehorses or any horse travelling to competition.

Dehydration can also cascade into more serious metabolic situations. Moderate dehydration can initiate abnormalities in blood flow to the hooves thereby inducing laminitis. This problem is probably often accentuated by the hoof inflammation caused by removing shoes (a practice for reducing kick injuries) from a horse that normally wears shoes. Other compounding factors that may significantly increase the risk of laminitis could be the duration of the journey relative to the state of fitness of the horse, the level of carbohydrate intake maintained during the journey and any potential endotoxic disorders initiated by the travel event itself. Preventive measures would include: not changing the

shoeing status of the horse, adding frog support to higher risk horses, and reducing carbohydrate intakebefore and during transportation (Masmann and Woodie, 1995).

666 Diarrhea can also be caused by transport stress and increases the risk of laminitis, since it is always 667 associated with endotoxemia.

668 Severe dehydration could induce the development of large colon impaction. In fact, there is a higher 669 risk of colic in horses that have more than six transports/year compared with horses that are not 670 transported and those transported fewer than six times per year (Tinker et al., 1997).

Another potential risk caused by dehydration is the reported decrease in renal function which is particularly relevant for horses due to undergo medical treatments (MacAllister and Tailor-MacAllister, 1994).

674 There are several techniques which are often implemented to minimize the risk and extent of 675 dehydration. One involves the familiarization of horses with an aqueous normalizing substance, such as apple flavoring which reportedly offsets any difference in water taste during transportation and at 676 677 the destiantion (Mars et al., 1992). Mineral oil or electrolyte-enriched water via naso-gastric tube has 678 also been successfully implemented, pre-transport, for the prevention of gastrointestinal impaction. 679 Care should be taken with the administration of both mineral oil which can affect the absorption of 680 other nutrients and electrolyte which should not be given in a concentrate form (such as paste) close to 681 a stress event (including race, extreme exercise or transportation). Electrolytes should be always 682 administered in an iso-osmotic solution (Pagan, 2005). The recommendations for stopping frequency 683 during transportation are every 4-6 hours and over-nighting horses at least every 12-16 hours. During 684 stops and over-nighting, to prevent dehydration and correlated pathologies, the horses should have the 685 ability to exercise and to be examined by a veterinarian who can administer fluids as required. Oral 686 electrolytes and water can be easily administered via naso-gastric tube to mild or moderately 687 dehydrated horses or intravenously if dehydration is severe. A 450-500 kg horse's stomach can 688 tolerate 6-8 liters of electrolyte-enriched water every 15 minutes for 1 to 2 hours (Masmann and 689 Woodie, 1995).

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- 691

692 6. Transport and Oxidative Stress

Oxidative stress (OS) occurs when the oxidant/antioxidant imbalance results in excess production of reactive oxygen metabolites (ROMs) and leads to cellular and tissue damage. Oxidative stress plays an important role in diseases such as cancer, laminitis, neurological disease and heart and pulmonary diseases (Dunlap et al., 2006).

697 The imbalance occurs during and after stressful events such as travel, exercise and intensive 698 management in both humans and animals (Kirschvink et al., 2002). Racehorses are often transported 699 long distances and endure maximal exercise during training and races and have been shown to suffer 700 frequently from oxidative stress (Hargreves et al., 2002). It has been found that in horses oxidative 701 stress has an effect on the development of airway disease, such as chronic obstructive pulmonary 702 disease and exercise induced pulmonary hemorrhage (EIPH) (Soffler, 2007; Kirschvink et al., 2008). 703 However, the potential role of oxidative stress in the pathogenesis of transport pneumonia is still 704 unclear.

705 A recent study was conducted to evaluate oxidative stress indicators in ten horses, transported 528 706 km by road. Physical (rectal temperature, respiratory and heart rates), hematological, biochemical and 707 oxidative stress (malonedialdehyde (MDA) and superoxide dismutase (SOD) parameters were 708 measured before and after transportation. The transportation induced significant increases in 709 respiratory and heart rates, and in haematocrit and glycemia levels While the other clinical, 710 haematological and biochemical parameters remained unchanged. The occurrence of oxidative stress 711 induced by a 12 hour journey was evident by a significant increase in plasma MDA concentrations 712 coupled with a significant reduction in plasma SOD activity compared to baseline values (Onmaz et 713 al., 2011). The effects of an 8-hour journey by road on plasma total antioxidant status (PTAS) and 714 general clinical appearance were also investigated in horses and showed that the average PTAS 715 increased soon after unloading and remained elevated even after 24 hours stall rest (Niedźwiedź et al., 716 2013)

One approach to reducing the affect of oxidative stress during horse transportation would be to supplement their diets with antioxidants prior to transporting, which has been assessed in other production animals (Adenkola et., 2011). In goats it is reported that long distance travel affected their oxidant/antioxidant status and decreased their excitability and grazing behavior after unloading. In contrast, goats supplemented with ascorbic acid before the journey, did not display similar behavioral changes after unloading and their oxidant/antioxidant systems remained in balance after the journey was completed (Minka and Ayo, 2013).

Further study is needed to investigate the relationship between transport stress, oxidative imbalanceand respiratory pathologies development.

726

727 **7. Conclusions**

To safeguard horse welfare during transportation it is important to minimize transport stress.

A horse which is disturbed when first coaxed into a transport vehicle may show various signs of disturbance, but most of these signs will disappear by the tenth transport experience (Broom and

Johnson, 1993; Schmidt et al., 2010). Thus, loading and transport training with appropriate provision of positive and negative reinforcement are strongly recommended (McGreevy and McLean, 2007). Sport horses should be transported occasionally for pleasure riding, decreasing the association between the truck and the competitions. As transport is a stressor even for experienced horses, it may be reasonable to give a rest period (a period with no travelling) to "frequent traveler horses".

To limit health problems post transportation, it is important to examine the health status of the horses before traveling, to give provide them with electrolytes and antioxidants (Vitamin E, C and selenium) and to optimize the environmental conditions inside the truck.

During long distance journeys it is essential to plan rest stops (of at least thirty minute duration) every 4 hours for watering, feeding, urination (particularly for male horses), and cleaning the vehicle. Horses should not be loaded more than 18 hours without being unloaded and being able to do some physical exercise. Upon arrival at the destination it is important to offer fresh water and high quality food, since it is apparent that drinking and feeding are the horses' highest priority needs after transportation.

While significant research has been conducted and reported allowing the development of best practice management guidelines, further research is required to more fully understand the relationships between transport stress, immune system status and risk of post transport disease development. Transport stress is caused by a mosaic of stressors and the travelling horse's wellbeing can be best improved only through a multi-factorial approach.

750

751 Conflicts of Interest

752 The author declares no conflict of interest.

753

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How to manage equine transport stress.

How each transport phase affects horse behavior and heath status.

How to prevent the principal pathologies connected with transportation.

Practical outcomes useful to safeguard the travelling horse welfare.