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HIGHLIGHTS

There is a need for sickness indicators in horses

We discuss pain / welfare scales and scores which can involve subjectivity

We present objective, visible indicators associated with health disruptions in horses

Much work is still needed but there are however warning signals of sickness states

Detecting horses' sickness: in search of visible signs

Martine Hausberger[1], Fureix Carole[2,3], Lesimple Clémence[1,2]*

[1]CNRS, Ethologie Animale et Humaine UMR Université de Rennes1 6552, Bâtiment 25, Campus de Beaulieu, 263 Avenue du General Leclerc, CS 74205, 35042 Rennes Cedex, France

[2]Université de Rennes 1, Ethologie Animale et Humaine UMR CNRS 6552, Bâtiment 25, Campus de Beaulieu, 263 Avenue du General Leclerc, CS 74205, 35042 Rennes Cedex, France

[3]Current address : Centre for behavioural Biology, Department of Clinical Veterinary Science, University of Bristol, Langford House Langford BS40 5DU, UK

* corresponding author: lesimple.c@gmail.com, phone: +33 2 23 23 30 45, fax: +33 2 23 23 69 27, UMR CNRS 6552 Ethologie Animale et Humaine, Université Rennes 1 Bâtiment 25, Campus de Beaulieu, 263 Avenue du General Leclerc, 35042 Rennes Cedex, France

Abstract

Assessing sickness in animals, by which we refer to non-specific states involving both physical discomfort and negative emotional states, is a real challenge. In this review, we demonstrate the need for clear and simple indicators of sickness in horses, a species in which suffering is largely underestimated. We provide a critical review of existing tools available to assess sickness in equids, which include composite pain scales and scores and welfare assessment scoring. Many such scales and scoring systems involve subjective assessments and lack of clear definitions. We discuss possible objective, visible indicators (qualitative and quantitative behavioural modifications and some postures) associated with sickness in horses, highlighting the two predominant modalities of expression (becoming unresponsive to environmental stimuli and “lethargic”, or becoming aggressive and hostile). Much work is still needed before an agreement can be achieved on the indicators of sickness in horses; there are however signs that, even if non-specific, should attract the owners’ attention on the horses’ welfare states.

Keywords: Horses; Illness; indicators; sickness assessment; welfare; pain.

1. Introduction

According to the Oxford Guide of the English Language (1984), sickness corresponds to a state of being sick, *i.e.* unwell, but also distressed or disgusted, bad, harmful, hostile. Several points deserve attention here: 1) sickness is non- specific; 2) the definitions refer to both physical discomfort ("unwell") and emotions (distress, hostility); 3) these last aspects indicate the interrelations between physical sensations and self-deprecation or anger. In fact, there is a complex relationship between sickness and stress, distress and pain (Ashley et al., 2005). Therefore the present review will also include aspects related to pain and chronic stress.

Sickness induces aversive experiences that range from mild discomfort to acute pain. Sickness is often accompanied by pain (Dantzer, 2004), an "unpleasant sensory and emotional experience associated with actual or potential tissue damage or described in terms of such damage" (International Association for the Study of Pain IASP, 1979). Discomfort or pain are ways of protecting the organism by prompting the avoidance of harm, promoting healing by inhibiting other activities that might cause further tissue damage (Bateson, 1991). "Suffering offers the best protection for survival" (Damasio, 1994). Pain grabs attention, interrupts associated behaviour: the more intensive and threatening the pain, the more disruptive of attention to anything else it is (Williams, 2002). Sick individuals may become lethargic, unresponsive to their environment and stop eating and drinking (Dantzer, 2004).

In humans, although clear pain scales exist, especially for non-verbal subjects (i.e. infants, elderly), underestimation of pain is widespread amongst health professionals whether in elderly (*e.g.* Forrest et al., 1989; Grossman et al., 1991) or in intensive care (Marquié et al.,

2003) units. Overexposure may raise the threshold of detection (Prkachin, 2002) as does the suspicion of overstatement of pain expression by the patient (Williams, 2002). Repeated exposure is one likely explanation as the phenomenon is observed more in more experienced staff (Prkachin, 2002; Prkachin et al., 2001, 2004). Another reason is that discomfort and pain are highly subjective experiences and that there is not necessarily a direct relation between the degree of expression of pain and the actual damage to the tissues.

While it is difficult to evaluate the degree of sickness in another human being, especially from another social community, it is still more so when trying to assess it in non-humans. Experience does not necessarily help: cats' emotional states are best evaluated by people who identify with them (*i.e.* thinking "pets are like us") whether or not they are cat owners (Thibault et al., 2006). Human beings also tend to look at other species as they would to conspecifics, with much emphasis on faces and much less on other body parts. This may be misleading in species where sickness expression may be through whole body or other body regions' postures (Leach et al., 2011).

Assessing sickness in animals is therefore a real challenge and requires the identification of reliable visual indicators of sickness, including discomfort or pain. As the idea is that practitioners, caretakers or owners detect at first sight a sick animal, visible indicators, especially behavioural and postural signals, are clearly needed.

But then, what is a reliable indicator? Referring to the Oxford guide to the English Language (1984), an indicator is "a thing that indicates something" (*i.e.* point out, be a sign of, show the need of), but also a "device on a vehicle showing when the direction is going to be altered". This last aspect makes an interesting parallel as sickness indicators are supposed to indicate changes from an homeostatic state of well-being/good health.

Sickness indicators should indicate the changes in behaviour and postures that correspond to changes in the internal state. The question arises on the reference taken to make comparisons: a healthy population? The individual itself? What is the reference for a health practitioner surrounded by unhealthy persons? What if the individual has never been seen in good health?

The question of the "reference" in domestic animals is complex, as life conditions have put such pressure on their abilities to adapt that whole populations may be in states of altered welfare and thus at risk of becoming sick. Although it is regularly argued that domestication/selection may have promoted an adaptation to the domestic constraints, domestic animals exhibit health and behavioural problems that show that they do not cope that well (*e.g.* Duncan, 1998; Fraser and Broom, 1990; Mason, 2010; Mason and Latham, 2004).

In the present review, we demonstrate the need for clear and simple indicators of sickness in horses, a species that experiences both the environmental constraints of farm animals and a working and affective relationship with humans. Suffering is largely underestimated in this species, mostly because the recognition of signs of discomfort is weak. We concentrate on sickness indicators, related to health problems and not on overall welfare assessments (psychological alterations), although, as mentioned earlier they are interrelated.

2. Issues in detecting changes due to sickness in horses

Sickness has, to our knowledge, not been a major focus of interest for scientists working on horse behaviour in natural conditions. There are anecdotal reports of infected wounds, lameness, loss of condition, dehydration and aging that may induce discomfort but mortality rates are low and are mostly due to early foals' deaths (Berger, 1983, 1986; Feist and Mc Cullough, 1975; Waring, 2003). In addition feral horses exhibit little signs of sickness: colic (sign of gastrointestinal pain), laminitis (foot inflammation) postures and stereotypic behaviours seem to be absent. Interestingly though, Przewalski horses kept in enclosures may develop laminitis (Budras et al., 2001). The precise causes of acute laminitis in the horse are not yet fully understood, but certain types of carbohydrate appear to trigger changes in the hindgut precipitating this disease (Mills, 2005). According to Bailey et al., (2004), "studies suggest that a large proportion of cases in the UK are linked to diet, thus laminitis may be considered a disease of domestication", and indeed the domestic situation can be very different from a naturalistic situation.

Most domestic horses experience social and spatial restriction, a high energy diet and demanding work. Some diseases are clearly characterized (*e.g.* Waring, 2003): tetanus is associated with a rigid, spread leg stance; laminitis with an arched body posture that takes the weight off the forefeet. The very well known "colic" includes frequent rolling, lying down - getting up, pawing, staring at flanks. Limb problems are detected when they induce lameness. All these symptoms are regularly encountered in the domestic situation to the point that riding centre owners may consider it normal that several horses exhibit colic at least once per year (Hausberger, pers. obs.). More problematic is the observation that some health problems are highly prevalent and remain undetected, hence worsened by the persistence of causal factors. Two clear examples are gastric ulcerations, that, according to Bell et al. (2007) affect 88.3 % of the ridden horse population, and vertebral disorders that affect at least 70 % of the ridden horse population (Fonseca et al., 2006; Jeffcott, 1979). In the absence of clear indicators, gastric ulcerations rely upon endoscopy in order to be detected, which also requires that the owner and practitioner are aware that this is a possible source of the problem encountered. Vertebral disorders that may be sources of chronic discomfort or pain (see Lesimple et al., This issue), are not easy to detect and most often are only detected when at an advanced stage where gait abnormalities or reactions to back touch are clearly visible. Moreover, radiographic imaging does not work easily in field situations as the horses' muscular mass is an obstacle to obtain clear images of the spine. Thus, vertebral disorders, even when suspected, remain difficult to diagnose (see Lesimple et al., This issue).

In any of the above mentioned cases, it is probable that before reaching such acute stages, horses have experienced some discomfort that, if detected, may have been alleviated. However, early signs may remain undetected because they are non-specific to a given pathology. Much attention will be given here to general expressions of sickness that may be common to preliminary stages of a whole range of health problems.

This is difficult even when the signals are very visible as shown in a recent study looking at stereotypic behaviours. Lesimple and Hausberger (2014) compared the responses to questionnaires of caretakers and owners asked whether their horses were performing any kind of abnormal repetitive behaviour (predefined with them) to ethological observations of the same 373 horses. The results show a huge discrepancy (5% reported versus 37% observed abnormal repetitive behaviours), even when only major well-known stereotypic behaviours (e.g. crib-biting) were considered. This underestimation may result from different factors (negation of a problem in their stables, attentional problems, lack of training, etc.) but overexposure to these behaviours is likely to be a major aspect: there was a correlation between the level of discrepancy and the proportion of stereotypic horses in the stable. In another study performed on 59 adult horses living in riding schools where ethological observations, blood sampling, spine assessment by practitioners and health questionnaires were performed, 34 % of the horses had a chronic health problem (e.g. lameness, respiratory problem), 73 % had back problems, 18% had anaemia while 66% of them performed abnormal repetitive behaviours (see also Fureix et al., 2010, 2013). Similar findings were obtained in a larger riding school population (N = 373, Lesimple et al., subm). In studies performed in developing countries, 89 % of the horses had limb abnormalities (Pritchard et al., 2005), and many (~10%) seemed to have anemia as evaluated through mucus membrane (Burn et al., 2010). When the majority of a population is showing similar behaviours and postures even if due to an altered internal state, it is certainly difficult to imagine that this majority is not the norm.

What is then a healthy horse? Horses living in the wild or in naturalistic situations may encounter stress but overall show little evidence of illness. Their time budget is spent predominantly eating and resting; locomotion is regular but mostly consists of slow walking; social interactions are rare but so is social isolation and social bonds are characterized mostly by quiet proximity; horses regularly scan the environment and remain attentive and ready to react to unusual stimuli (*e.g.* Waring, 2003). Ear and tail postures (Kiley-Worthington, 1976) and neck height are informative (to conspecifics) of the internal state of the animal (Waring, 2003; Wolff et al., 1997).

Despite rarely referring to the naturalistic situation, most sickness (including pain and welfare alteration) assessments in horses base their evaluation on changes in these parameters. But maybe precisely because these measures are taken without clear reference to a "norm", there is little consensus and a lack of reliability to the diverse scales and scores proposed. Detecting the first signs of discomfort may then induce a search for the cause of the discomfort and help identify sickness before it reaches advanced stages. However, in order to ensure validity, the search for indicators must be multidimensional, involving behavioural, health and physiological parameters.

3. Assessing sickness in horses: are there reliable visible indicators?

3.1. Sickness assessment: the example of pain and welfare scales and scores

3.1.1. The composite pain scales

According to Zimmermann (1984), pain in animals is "an aversive sensory experience that elicits protective motor actions, results in learned avoidance and may modify species-specific traits of behavior including social behavior". According to Robertson (2006), pain has been largely underestimated in animals, and in particular chronic pain should be looked at as a disease in itself. Several pain scales were developed for horses, most or all of them referring to behaviours and postures of head, neck, ears, tail, often in association with physiological parameters (Ashley et al., 2005). All were developed in a hospital context where animals were being operated for arthroscopy (Price et al., 2003), celiotomy (Pritchett et al., 2003), or abdominal repair (Graubner et al., 2011); were submitted to an orthopaedic-inducing pain (Bussières et al., 2008) or were suffering laminitis (Vinuela-Fernandez et al., 2011). Different pain-relieving drugs were administered and their effects used for testing the reliability of the chosen variables. Horses were generally assessed in their individual stalls, often with videorecordings. Scores of severity were given to each predetermined variable (*e.g.* no sweating = 0, excessive sweating= 3). Some indicators were developed taking into account the specificity of the variable, that is its degree of characterization of a particular type of pain (heart rate is not specific of a particular problem, while responses to palpation are indicative of the location and potentially the type of pain, hence are specific) and the sensitivity, which is indicative of the degree of pain (Bussières et al., 2008). Thus, in a study based on analgesic administration, the score obtained for the variable considered follows the same gradient as the level of analgesia given, which indicates the level of pain. In their study, pawing for example is very sensitive.

Ashley et al. (2005) produced the first review on the behavioural assessment of pain in horses and donkeys. Since then, more studies and new scales have been proposed. Most scales converge on the categories of items they take into consideration but in most if not all cases they rely upon subjective assessment, especially where behaviour is concerned (*e.g.* general subjective assessment of behavioural signs of pain in Graubner et al., 2011's scale). For example, in the Bussières et al., (2008) composite pain scale, it is unclear how an "exaggerated response to auditory stimulus" (score 1) differs from an "excessive to aggressive response to auditory stimulus" (score 2) and what "being bright" means (behavioural criterion). In that same scale, stupor and prostration have the maximum score in interactive behaviour (score 3 of pain) while in the behaviour ("appearance") category, restlessness and excitation have the highest scores compared to "being bright". While these proposals for scoring are probably based on clinical experience, a prior evaluation of the validity of the concerned variable would have strengthened its integration into a composite scale.

Most scales include physiological measures such as heart rate, respiratory rate, rectal temperature and sometimes cortisol. However heart and respiratory rates have poor reliability in terms of reflecting the severity of pain while temperature and cortisol reliability varies according to the type of pain (Graubner et al., 2011; Holton et al., 1998; Molony and Kent, 1997; Mormède et al., 2007; Pritchett et al., 2003; Price et al., 2003). Bussières et al. (2008) found that heart and respiratory rates were moderately specific and sensitive while rectal temperature was neither. This is especially interesting as heart rate is the first criterion mentioned by practitioners in evaluating the severity of pain in horses (Price et al., 2002).

In the Bussières et al. (2008) study, four out of the 15 elements measured appeared crucial: response to palpation in the sensitive zone, kicking at abdomen, interactive behaviour and posture (from normal walk to prostration). Despite the variety of the pain inducing situations involved in these different studies, some convergences are worth mentioning: restlessness and agitation as indicators of severe acute pain; rigid stance and reluctance to move, often associated with facing away, as a general indicator; a lower head carriage as an indicator of "depressive" state due to chronic unrelenting pain; aggression towards handlers or objects as an indicator of pain (Ashley et al., 2005).

While these scales do give some assessment of pain (van Loon et al., 2010), their validity is still undetermined. There is a need to test each variable involved and remove measures that did not prove reliable (*e.g.* heart rate, Graubner et al., 2011). There is also a need for clarification of variables. While Bussières et al. (2008) were very precise in evaluating pawing or kicking at abdomen by giving an objective measure of the number per five minutes, interactive behaviour is evaluated based on imprecise terms such as "pays attention to people", appearance is a mix of different postural elements and behaviours, and resistance to palpation is described as mild, resistant or violent, which may be perceived quite differently by different practitioners. Another evaluation of reaction to palpation determines only the presence/absence of reactions, while postural changes combine ears position and head level (Graubner et al., 2011). Most studies also include "appetite" as an indicator which reflects the horses' interest/disinterest in the available food; however no attempt is done to quantify it.

Specific behavioural or obvious postural alterations and subtle changes in time budget activities may be more representative of pain type and severity (Ashley et al., 2005). Pritchett et al. (2003) mentions that bouts of immobility and lack of responsiveness to positive stimuli occur more often than the generally accepted signs of visceral pain (restlessness, pawing, staring at flanks) in cases of gastrointestinal pain.

Horses in pain exhibit significant changes as compared to the healthy "naturalistic behavioural profile": they lose mobility, eating and social motivation as well as alertness. More emphasis should be placed on the development of quantitative evaluations of the postural and behavioural associated measures. Clear, measurable criteria would help limiting the effects of subjective perception. Using a clinical grading system, Vinuela-Fernandez et al. (2011) have shown that students from veterinary schools agreed more on elevated scores, while experienced practitioners agreed more on what a healthy horse is and not on the most severe cases.

3.1.2. A horse pain grimace scale?

One recent study (Dalla Costa et al., 2014) attempted to apply to horses the grimace scales already developed in rats (Sotocinal et al., 2011) and mice (Langford et al., 2010), by taking pictures of horses undergoing surgical castration, receiving analgesic either i) immediately before anaesthesia or ii) immediately before anaesthesia and then again post-surgery, and iii) of anaesthetised control horses (receiving analgesic immediately before anaesthesia but experiencing non-invasive, indolent procedures). They evaluated the pain level measuring six Facial Action units: stiffly backwards ears, orbital tightening, tension above eye area, prominent chewing muscles, mouth strained, strained nostrils and flattening of the profile. In parallel, behavioural observations were performed. They found an average accuracy of 73 %, which is less than in rodent studies, but the image qualities, with different angles and interference of the horses' eye blinks made definite conclusions difficult. Interestingly though, behavioural observations confirmed some of the above-mentioned findings that horses displayed a lowered level of alertness after operation. Postures with head lower than withers were not rare at that moment (Taylor et al., 2002). It would be interesting to test each facial feature separately in order to determine if accuracy could be improved. It is difficult to distinguish a "pain grimace" from an overall tension of the whole body (Salzen, 2002), which would mean that this "grimace", as in humans, could be found also in other high emotional contexts. Further studies are clearly needed here.

3.1.3. Sickness and welfare assessment scoring

In order to be able to assess the working equids in developing countries, the Brooke Foundation developed welfare assessments that were rapid, simple and useable by different (or even large numbers of) observers, and that could be performed in the field situation without disturbing the working activities (*e.g.* Pritchard et al., 2005; Burn et al., 2010). Forty-one measures of sickness were noted (eyes, teeth, body condition score, limb abnormalities, skin lesions, lameness, diarrhoea, etc.) and behaviour (reactions to human approach and presence) was scored in terms of presence or absence (e.g. alert versus apathetic) (Burn et al., 2010). This approach, developed with more than 2000 horses, revealed that lack of responsiveness was correlated with low body condition score, lesions of skin and deeper tissues and abnormal gait and that ridden horses, although more alert, tended to be more aggressive than draught horses. However this promising approach also lacks clearly defined parameters and scales. General attitude was not clearly defined and the horses had to be classified as either alert or apathetic (amongst which 8 % would be severely depressed). Despite training with the same guidance notes and pictures leading to 80% agreement with their trainers, there was only poor reliability between observers on aspect like "the apathetic general attitude" (Burn et al., 2010). The authors acknowledged that "assessment of alertness/apathy should be refined", which would allow a greater discrimination between stances relating to apathy and severe depression. They suggested that "a rigid stance, inattentive to the external environment, is often associated with chronic pain, and thus might be distinguishable from the more 'slumped' stance associated with depression/exhaustion" (Burn et al., 2010). Swann (2006) mentioned that the apathetic state, defined as non-responsiveness to external events, was observed only in working settings at the beginning of the working season in donkeys carrying bricks in hot temperatures, while it became permanent and associated with social withdrawal at the end of the season. Such donkeys simply appeared "lazy" to the owners. Further work on 715 Romanian horses by Popescu and Duigan (2013), led to the same conclusions that alertness and reactions to humans are major elements of expression of welfare states. Similar conclusions were reached by Fureix et al. (2012a)'s study on depressive-like horses.

Young et al. (2012) developed behavioural scoring associated with physiological measures (heart rate and cortisol) while testing horses with different stimuli (sound of electric coat clippers or fireworks) during grooming or social isolation. Using Principal Components Analysis they classified the horses in 4 categories from no stress to high stress (on the basis of the cortisol data and time spent in activities) to which were added the descriptions from the panel. Curiously, the "no stress" horses were performing repetitive oral behaviours, and the authors interpreted this finding by suggesting that stereotypic behaviours may have helped the horses reduce stress. It is unclear whether and to what extent acute reactions to stimuli, as observed here, are reflecting the welfare state of the animal. When a horse reacts strongly to a firework sound, does it mean it is experiencing a bad welfare? Is it reactivity stress or the expression of temperament traits? The fact that the high stress horses showed agitation, anxiousness and aggressiveness is precisely the opposite of the apathetic/depressed profile described in many above-mentioned studies. Further investigation is needed in order to more clearly establish the potential link between reactivity and chronic stress, although some studies argue that welfare alterations may lead to more excited personality profiles (Hausberger et al., 2011).

3.1.4. Can we assess sickness through scales and scores: conclusions

Despite very different and independent approaches, pain scale studies and behaviour scorings revealed the same general non-specific trends: extreme and durable pain or distress leads to apathetic (depressed-like?) states where the animals become indifferent to the environment. Several studies suggested that aggressiveness (or indifference) reflects pain (Ashley et al., 2005; Burn et al., 2010; Fureix et al., 2010; Popescu and Diugan, 2013; Pritchard et al., 2005). Loss of interest in food is mentioned in several studies on pain and novel behaviours (e.g. staring at flanks) have been associated with gastrointestinal pain (Ashley et al., 2005; Pritchett et al., 2003). However, all scales and scoring systems thus far involve subjective assessments and lack of clear definitions. The lack of precise definitions of behaviours and postures leaves a large part to individual interpretations, and only the extremes are really discriminant.

3.2. Can we rely upon behavioural or postural changes to detect sickness in horses?

Variables related to sickness (*i.e.* health disruption) will be considered here but vertebral disorders, given their prevalence and importance, will be considered in a joined paper (see Lesimple et al., This issue).

3.2.1. Changes in the behavioural repertoire

Novel behaviours typical of sick horses are mentioned in pain scales, such as staring at flank in cases of colic, but there is little clear evidence of changes in the behavioural repertoire *per se*. In a study on Arab broodmares housed in high density and without roughage available in the paddock, Benhajali et al. (2008) observed that the behavioural repertoire was restricted, lacking social behaviours, resting behaviours and rolling. When hay was made available, these behaviours reappeared and the horses had improved body condition and fertility (Benhajali et al., 2009, 2013). Although it is not certain that these mares were actually "sick", the fact that they proved healthier after the management change suggests it.

The lack of roughage, restricted time for feeding and inappropriate diet are common in domestic settings and can lead to potential gastric ulcerations, or at least digestive disorders that may lead to the expression of "abnormal behaviours" such as stereotypic behaviours (e.g. McGreevy et al., 1995, Nicol et al., 2002). As mentioned earlier, gastric ulcerations are present in most of the riding horse adult population. Horses have adapted to a diet rich in fibre (and poor in energy) and their digestive tract requires a quasi-continuous "filling". This is reflected by the huge part of the time budget devoted to feeding activities in natural conditions (e.g. Waring 2003). Commonly, domestic horses are fed in one to three meals of commercial pellets that are consumed in less than half an hour each, and no or restricted amounts of hay (e.g. 6-7kgs daily). When the litter is made of straw, this can add fibre, but many horses are on bedding made from wood-shaving or other substrates. Therefore horses may experience an empty stomach for many hours, while it seems, given the physiology of the digestive tract, that one hour with an empty stomach may already be a source of discomfort (Harris, 2005). Ponies fed only concentrates will eat their wood-shaving bedding (Houpt et al., 1988). Concentrates also reduce saliva production and increase gastric acidity, perhaps through the secretion of gastrin (Elia et al., 2010; Nicol et al., 2002; Wickens and Heleski, 2010). It has been proposed that oral stereotypies, such as crib-biting, may substitute for chewing hay in stimulating the production of saliva (Nicol, 1999). Gastric ulceration or inflammation is present in 60% of crib-biting foals compared to a prevalence of 20% in the non-stereotypic foals and its severity is higher, as revealed by endoscopy (Nicol et al., 2002). A relationship between gastric inflammation and oral stereotypies has also been found in adults (Mc Greevy et al., 1995). Crib-biting increases during and after meal consumption while antacids or naloxone reduce post-feeding crib-biting (Mc Bride and Cuddeford, 2001; Mills and Mcleod, 2002), up to 6-8 hours post-feeding suggesting that gastric pain is not the sole source of visceral discomfort. Clegg et al. (2008) suggest that there could be fermentative acidosis in the hindgut, as this time period corresponds to the arrival of food ingested in the caecum. According to Hemmings et al. (2007), visceral discomfort may play a role in the establishment of oral stereotypies through alteration of basal ganglia programming. Although feeding may not be the only factor involved in the emergence of oral stereotypies, these behaviours obviously reflect a potential state of sickness. Therefore their appearance has to be considered as an important indicator that the horse may be experiencing discomfort. Other behaviours such as vacuum chewing (Hausberger et al., pers. obs.) or yawning (Baenninger, 1987) may also be indicators of sickness that deserve further investigation. Stereotypic broodmares exhibit a lower fertility, which suggests that their health is altered (Benhajali et al., 2014).

3.2.2. Changes in time budget

In the above mentioned example of the Arab broodmares, in the absence of foraging opportunities, the horses increased time spent active walking while the usual locomotion, the slow exploratory walk, was reduced (Benhajali et al., 2008, 2009). Hence, the empty stomach and the associated discomfort may lead to some restlessness. Restlessness and agitation could be more characteristic of severe and acute pain (Ashley et al., 2005). Moreover the few social interactions exhibited were agonistic. Restlessness is mentioned in many clinical evaluations, especially in cases of digestive disorders, where horses tend to alternate lying down, standing up and rolling repetitively (*e.g.* Pritchett et al., 2003). Of course, the presence of abnormal behaviour and abnormal repetitive behaviours (which may reflect sickness) modifies the time budget. Thus, it has been shown that adult stereotypic horses living in stalls spent less time lying down and sleeping than non stereotypic horses living at the same place (Hausberger et al., 2007).

Although included in many pain scales, loss of appetite as an indicator is poorly defined. Since foraging is a behavioural priority for horses, lack of foraging behaviour when resources are available may be a good indicator of discomfort or pain. However, to date there has been no research to indicate this.

The time spent in certain orientations or postures as well as the time spent immobile may be good indicators of sickness, as these are features characteristic of the "apathetic, lethargic, depressed" horses observed both in hospital settings and in working conditions

(Burn et al., 2010; Fureix et al., 2012, 2015; Popescu and Diugan, 2013; Pritchard et al., 2005) but this is rarely measured (see further).

3.2.3. Alertness as an indicator of sickness

Most pain and welfare scales include “attitudes”, “alertness” or other similar wordings meaning responsiveness to environmental stimuli, including humans. Could this be a general sickness indicator?

3.2.3.1. Towards depression-like states

Sick horses are often described through words like “apathy”, “lethargy”, “depression” (Burn et al., 2010; Popescu and Diugan, 2013; Pritchard et al., 2005). As mentioned earlier, “apathetic” and “depressed” horses are often considered different in general attitude, but without reference to their differences. There is no standard measurement, although there is agreement that they are characterized by prolonged times of immobility associated with a rather “slumped” posture (weight on the forehand), head at or under withers level and a lowered interest for the environment (Burn et al., 2010, Fureix et al., 2012). According to Ashley et al. (2005), apathy is associated with fixed stare, dilated nostrils and a clenched jaw; depression and dullness are characterized by reduced alertness, self-isolation, facing away, lowered head carriage. These elements are included in different scales but mostly on the basis of subjective assessment and it is unknown if they are reflective of a state of sickness. Popescu and Diugan (2013) found that the 2.65 % of horses they considered as being in a “depressed mental state” (*i.e.* lower responsiveness towards environmental stimuli), were also those more prone to have body lesions.

We tried, as suggested by Burn et al. (2010) to characterize this condition and find out to which extent it can be analogous to human depression. In one study we labelled 24% of the horses (out of 59 horses living in three riding centres) 'withdrawn' as they exhibited bouts of inactivity while displaying a stationary, atypical, flat-necked posture, wide-open, unblinking eyes with a fixed gaze and no ears nor head movements (Fureix et al., 2012a) (**Fig. 1**). Even when not in this unusual posture, 'withdrawn' horses differed from control ("non-withdrawn") horses living in the same stables in several ways, they showed reduced responsiveness to standardized tactile stimulation using Von Frey filaments, less reaction to a human's sudden appearance at the stall door (Hausberger and Muller, 2002); less exploration and more behavioural signs of arousal (fear) when exposed to a novel object, and lower baseline levels of plasma cortisol (Fureix et al., 2012a). Due to analogies with some symptoms of human clinical depression, we hypothesised that withdrawn horses exhibit a depression-like condition, and tested this hypothesis by assessing anhedonia (the loss of pleasure), one of the most important symptoms of human depression (American Psychiatric Association APA, 2013), which has been successfully modelled in biomedical studies of rodents, particularly *via* inducing and recording reductions in sucrose intake (e.g. Papp et al., 1991; Willner et al., 1992). As predicted, the most withdrawn horses consumed the least sucrose, suggesting further depression-like conditions in these animals (Fureix et al., 2015). Like anhedonia, diminished ability to concentrate can also be part of clinical diagnosis of clinical depression in humans (Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition, APA, 2013) and our recent additional observations revealed that withdrawn horses paid a lower attention to auditory stimuli than controls (Rochais et al., subm.). Withdrawn horses also maintained withdrawn posture while at work (pers. obs.), supporting Swann's (2006) suggestion that in donkeys this syndrome may become chronic, thus independent of context.

3.2.3.2. A non-specific reliable indicator of internal state: the ears' position

All pain scales include ear positions as a variable, but they use different definitions. Overall though, ears pricked forward are considered signs of alertness and positive interactions, while ears pinned backwards appear as signs of sickness (discomfort or pain; Ashley et al., 2005; Dalla Costa et al., 2014; Graubner et al., 2011). Ear position is also an intrinsic part of identification of the horse's interactive state (*e.g.* Waring, 2003). However there is no clear definition of the different ears' positions, nor do the different scales and scores indicate the conditions in which ear positions have a reliable significance, nor how to evaluate it as part of the time budget. Ears are mobile and they can be backwards for various reasons including noises, negative interactions, discomfort and possibly sickness. In order to use this component as a reliable indicator of sickness, it is necessary to have established a standardized protocol and to have clear definitions. In a study performed on several hundred horses living either in natural or domestic conditions, we found that ear positions can be a useful tool in welfare assessment. For instance, horses that spend most of their time with the ears backwards in calm, non-interacting contexts were more prone to be sick (Fureix et al., 2010; Hausberger et al., subm.).

Ears held backward in a sustained way may indicate physiological disorders, hence sickness (Hausberger et al., subm.).

3.2.3.3. Testing orientation in the stall as a source of information

Positioning in the stall is one of the key parameters in Price et al. (2003) pain scale where being in the front is considered a positive sign, interpreted as a wish to interact. Overall, position and orientation within the stall may indicate that the horse is interested in its external environment and possibly ready to interact (Young et al., 2012). However, is the reverse true? Is staying at the back a sign of sickness? In a study performed on riding school horses (Fureix et al., 2009), we studied the orientation of the horses in their individual stalls. Fifty-five horses from three riding schools were observed in 2007 and 2008. Each horse was kept singly in a 3 m x 3 m individual straw-bedded stall in a barn, allowing auditory, visual, and limited tactile contact with conspecifics. Observations using Instantaneous scan sampling (N=90 scans per horse; Altman 1974) were used to assess horses' orientation in their stall: facing a wall, facing external cues (head towards a stall aperture), head out (through a window or in the stable corridor).

These behavioural observations revealed that while all horses could be seen at least once facing the wall, the time spent in this position varied greatly between individuals (3 to 91 % of the time budget, $\bar{X}$= 42.5 % +/- 21.5).

In parallel, we collected conventional haematological data. These analyses allowed us to identify horses with unusual levels of haematological parameters, for instance anaemic animals. Thus, 18% of the horses suffered from anaemia (see also Burn et al., 2010).

The time horses spent facing the wall and their haemoglobin level were negatively correlated: the more time they spent facing the wall, the lower their haemoglobin level was (Spearman correlation, N = 55, r_s = -0.51, $p < 0.01$). Facing the wall could be another way of "switching off" and avoiding contact with the environment or people. Ashley et al. (2005) mentioned that horses in pain tend to face away from handlers. Sickness may make horses "introverted" and hostile to external stimulations. As mentioned earlier, pain grabs attention, and the more intensive it is, the more disruptive of attention to anything else it is (Williams, 2002).

3.2.3.4. Interacting with others: aggressiveness as an indicator of sickness

According to Ashley et al. (2005), aggressiveness emerges from pain. Humans and pigs also become aggressive when unwell (*e.g.* Anderson et al., 2002; Day et al., 2008). Horses submitted to heavy work have negative behavioural responses to humans (Popescu and Diugan, 2013) and riding horses seem to be more aggressive than pack or draught horses (Pritchard et al., 2005). To our knowledge, the only proof that aggressiveness may reflect chronic discomfort/pain is that of Fureix et al. (2010) showing that horses presenting vertebral disorders were more prone to be aggressive towards humans. Following Ashley et al. (2005) suggestion that aggressiveness may reflect a state of general hostility, we studied whether aggressiveness towards humans could be associated with aggressiveness towards conspecifics as well.

Fifty-nine horses from three riding centres were observed in their individual stall for all occurrences of behaviours. Aggressive reactions could vary from simple threats (i.e. looking with ears laid back), threats to bite (i.e. showing the teeth in addition to simple threats) to threatening approaches (stretching the neck or approaching with ears laid back, sometimes with an attempt to bite, mouth open and teeth visible) (e.g. Hausberger and Muller, 2002; Waring, 2003). They were measured in two contexts: opportunistic recordings during 30 min focal observations where they could be directed towards the observer, another human or a conspecific walking in the corridor, and during specific human-horse relationship tests. Human-horse relationship tests were 5 standardized behavioural tests, routinely used in different studies on human – horse relationship

(Hausberger et al., 2008; Fureix et al., 2009, 2010). Aggressive reactions towards the experimenter were defined similarly to the above-mentioned reactions towards conspecific, and were summed across the five tests, yielding a total "aggression score". Fifty-nine percent of the horses displayed at least one aggressive reaction towards a conspecific during focal observations; 27% threatened humans present in front of the stall with one to 17 threats per horse and 71% threatened the experimenter at least once during the tests. Aggression towards conspecifics tended to be positively correlated with both the total aggression score test situation (N = 59, rs = 0.24, two-tail P = 0.072) and the number of times horses threatened humans walking in the corridor during the focal observation periods (N = 59, r_s = 0.22, two-tail P = 0.09).

Further observations of a subset of horses (N=29) at paddock revealed that the horses that threatened the experimenter at least once during the tests were also the most aggressive when in group (N1=19, N2=10, U=30, P<0.05) as were the horses that had threatened at least once humans during the observations in the box (N1=22, N2=7, U=20, P<0.02)

Increased aggressiveness towards humans (Ashley et al., 2005) and conspecifics (Benhajali et al., 2008) has been observed in a variety of negative contexts. In natural settings, aggressiveness is low in stable groups and increases when there are disturbances or restriction of resources (Fureix et al., 2012b). Przewalski horses that suffer foot pain have more agonistic interactions with conspecifics (Budras et al., 2011). Aggressiveness in domestic settings may be a non-specific indicator of sickness, triggered by discomfort and pain. It also shows more persistence across situations than friendly behaviours, suggesting that negative behaviours may be more reliable as a sign of poor well-being than positive behaviours as a sign of good well-being (Fureix et al., 2009). This generalization of aggressive behaviours between humans and conspecifics (and objects?, cf. Ashley et al., 2005) suggests that aggressiveness may reflect a "negatively biased mood" more than a real negative perception of the interlocutor. Indeed, sick horses tested in a cognitive bias task showed negative biases that may explain that all stimuli are perceived aversively (Henry et al., unpublished).

3.2.3.5. Conclusion:

Sickness alters the way horses "see the world": could alertness and interactive behaviours be non-specific but major indicators of sickness? Alertness is a term used in most scales to indicate the animal's responsiveness to environmental stimuli (*e.g.* Ashley et al., 2005; Popescu and Diugan, 2013). Although few studies have based their assessments on a clear quantitative approach, all observations converge to indicate that sickness modifies the way horses perceive and react to environmental stimuli. There are two predominant ways horses express their poor well-being: they may, especially in cases of chronic repeated pain, become unresponsive to environmental stimuli, motionless and lethargic or become aggressive and hostile. Sickness associated with pain and discomfort can lead to horses' self-attention and thus withdrawal from the environment with all senses: auditory (ears back, lowered reaction to sounds), visual (facing the wall, "empty gaze"), and tactile. Gustatory and olfactory responses have not been tested. Sick horses show fewer reactions towards positive stimuli (Pritchett et al., 2003), including sucrose (Fureix et al., 2015). Mobility (of body, ears, etc.) remains to be investigated as a potential indicator of non-sickness.

Healthy horses are regularly observed to be attentive to the environment, ready to react to stimuli and to be positive towards other living beings. Therefore the lack of responses may indicate a possible discomfort that, if untreated may transform into pain. However, definitions, thresholds and contexts still need to be determined.

3.2.4. Beliefs and « false friends » as obstacles to the detection of sickness in horse

Because the above mentioned indicators are non-specific, they are easily misinterpreted by owners and caretakers: apathetic equids can be considered "lazy", that is "unwilling" to perform their work (Swann, 2006), while aggressive horses are "badly behaved" and get punished or culled for this reason, especially as it is believed that it is a temperament trait. Knowing "laziness" and increased aggressiveness to be early signals of sickness would aid the detection of problems before they have transformed into more severe situations. Ear position is rarely considered outside an interactive context and as mentioned earlier, even visible abnormal behaviours may remain undetected. Long bouts of immobility in the stall (or paddock) are often interpreted as resting, probably because of the absence of awareness of the associated body posture (quite different between resting and being inactive, Fureix et al., 2012a).

On the other hand, some behaviours may be mistakenly considered as indicators of health. Thus, in folk psychology but also in many scientific reports, play behaviour is considered as a clear indicator of well-being. And indeed in young horses, play behaviour is a normal part of the behavioural development, and is impaired in cases of disease (e.g. Waring, 2003; Henry et al., 2009, 2012). However, adult play is almost absent from the repertoire of horses living in natural conditions as well as in domestic naturalistic settings, while being regularly reported by owners. We performed a study on 29 riding school horses living in individual stalls, fed concentrates and hay, and working every day for one to three hours where horses were observed when released in bare paddocks once a week in groups and independently were observed in their stall and had blood taken. The horses that played the most were those with the highest oxidative stress (a measure of susceptibility to diseases), the highest score of poor welfare and a highest aggressiveness towards humans. Players also had more vertebral disorders, were more often seen facing the wall and had a lower tactile reactivity when in their stall (Hausberger et al., 2012). Interestingly, similar findings have been obtained in primate species, and following Carr (1902), we propose that play behaviour in adults may serve as a way of "evacuating stress, hence toxins and getting oxygenation" (Blois-Heulin et al., 2015). Adult play is therefore an ambiguous behaviour that indicates that the animals are temporarily in a better situation, while being a potential signal of sickness.

Another ambiguous behaviour is yawning, which is generally considered to be associated with relaxed states, possibly leading to sleep. Opportunistic observations of high frequencies of yawning in frustrating or stress-inducing situations led us to investigate the potential link between yawning and stereotypic behaviours in horses (Fureix et al., 2011). One hundred and forty horses were observed in their individual stalls. Yawning and stereotypic behaviour frequencies both increased in the period prior to the meal when all horses did not get fed at the same time (frustrating situation). Yawners were also more prone to be stereotypic than non-yawners. High frequencies of yawning outside of a sleep/rest context may therefore be a potential signal that the horse is experiencing some discomfort, and in any case, does not appear to be a reliable indicator of good health.

4. General conclusion

Much work is still needed before an agreement can be achieved on the indicators of sickness in horses. However, there are signs that should attract the attention on the horses' wellbeing. Flattened backs and immobile postures have been described as a despair state when persistent. It may also result from learned helplessness where animals learn that they have no chance of escaping the pain or the repeated painful actions of riders (*e.g.* Hall et al., 2008). Ears' postures (when measured in an appropriate calm context) may reveal a range of sickness including back disorders (Hausberger et al., subm.). Aggressiveness and restlessness may be other ways of expressing discomfort and pain. It is important that reliable indicators are discriminated from "false friends" such as play or yawning and to get out the belief that aggressive horses are just nasty animals. Also, seeking indicators requires us to rely upon on clearly healthy populations while recognizing that the "norm" is not necessarily healthy.

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6. Ethical note

Reported studies performed by the authors were all complied with the French laws related to animal experimentation and the European directive 86/609/CEE and were approved by the University of Rennes 1 local Animal Care Committee any time approval was needed. Horse husbandry and care were under management of the riding schools: the horses used in this experiment were not research animals.

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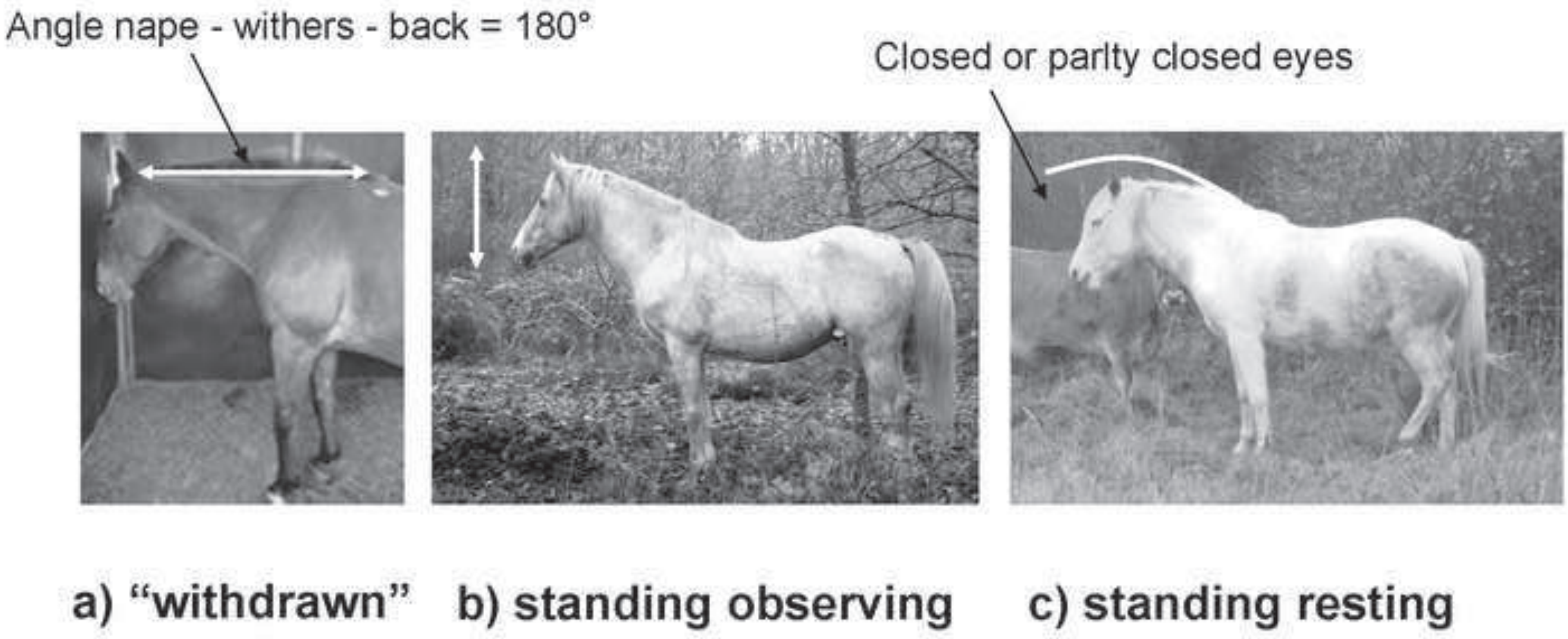
Figure Captions

Fig. 1 The posture of "withdrawn" horses. Pictures of a horse a) in a withdrawn posture, b) standing observing and c) standing resting. The withdrawn state is characterized by a stretched neck (obtuse jaw-neck angle) and a similar height between the horse's neck and back (a nape–withers–back angle of ≈ 180°). This posture is different from those associated with observation of the environment (for which the neck is higher), and resting, when eyes are at least partly closed and the horse's neck is rounder (Waring, 2003; Fureix et al., 2011). Note that the restricted size of the stall (3 m x 3 m) prevented the authors from taking a picture of the whole horse displaying the withdrawn posture, as we chose to use the same lens to limit shape distortion between images. From Fureix et al., 2012.

Figure 1



Angle nape - withers - back = 180°
Closed or parlty closed eyes
a) “withdrawn”
b) standing observing
c) standing resting