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Differences between facilities in horse welfare profiles: slight differences in management/working conditions may be enough

L. Gueguen [a,b,c,1,*], R. Palme [d], P. Jego [b], S. Henry [b], M. Hausberger [a,e]

[a] UMR 8002 Integrative Neuroscience and Cognition Center, CNRS, Université Paris-Cité, 75006 Paris, France
[b] Univ Rennes, Normandie Univ, CNRS, EthoS (Éthologie Animale et Humaine)—UMR 6552, 35000 Rennes, France
[c] CCMSA, Caisse Centrale de la Mutualité Sociale Agricole, 93000 Bobigny, France
[d] University of Veterinary Medicine, Department Natural Sciences Biochemistry, Veterinär-Platz 1, Vienna A-1210, Austria
[e] Rhodes University, Dept Zoology and Entomology, Makhanda, South Africa

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A B S T R A C T

Many studies focus on animal welfare in terms of specific, either behavioural or physiological, indicators or on the impact of a particular management factor. However, an animal's welfare state results from the individual's perception of its general environment, which has consequences at both behavioural and physiological levels. Previous research on horses has shown that different riding schools could be characterised by different emotional/cognitive profiles of horses, in relation sometimes with one single management factor. In the present study, we aimed at determining if such facility-specific horse profiles could also be found in terms of welfare, i.e. facility-specific "welfare profiles", using a multifaceted approach where animals' welfare state was assessed based on detailed behavioural, health and physiological measurements. A total of 59 horses from three different riding schools, with a very similar global conventional management but differed slightly in terms of turn-out frequency and riding techniques were studied. A principal component analysis and statistical comparisons showed that, despite the close similarity in management between the three sites, the horses' welfare state was very different and specific to each structure. Thus, this study using behavioural, health and physiological measures, highlights the existence of facility horse welfare profiles and reveals that even apparently minor differences in management practices could have a major impact on the horses' welfare state. The quality of ridden work, which is often not taken into account in studies on horse welfare, could be a major issue.

Implications

This study is set in a context where much research focuses on animal welfare through individual and specific indicators. However, an animal's welfare results from its perception of its environment, influencing multiple indicators. The aim was to identify welfare profiles using a multidimensional approach. Results show that, despite similar living conditions, horses have different welfare profiles, potentially influenced by factors like weekly free turnout time and riding techniques. These findings highlight the importance of considering these subtle factors, especially since the facility with the best horse welfare profile was also the one with the fewest rider falls during lessons.

Introduction

Numerous studies conducted on domestic animals and more specifically on farm animals have shown that welfare defined as "a positive mental and physical state linked to the satisfaction of the animal's physiological and behavioural needs as well as its expectations, and varying according to its perception of the situation " (ANSES, 2018) is influenced by various management factors considered individually. This is the case for housing (for reviews; rabbits: Trocino and Xiccato, 2006; pigs: Hemsworth, 2018), feeding (for reviews; cattle: Llonch et al., 2018; sows and broilers: D'Eath et al., 2009) and social (for reviews; cattle: Grignard et al., 2000; farm animals: Rault, 2012) conditions. In horses, various studies have shown an impact of living conditions (housing, feeding, social life) on behavioural expressions of welfare (Kádár et al., 2023; Lesimple et al., 2016a; Mills and Riezebos, 2005), and on physiology or health (Correa et al., 2020; Popescu and Diugan, 2017; Schmucker et al., 2022).

* Corresponding author.
E-mail address: lea.gueguen.pro@gmail.com (L. Gueguen).
[1] Current address: MSA d'Armorique, Service Prévention des Risques Professionnels, 3, rue Hervé de Guébriant, 29412 Landerneau, France.

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Overall, it has been shown that a compromised state of welfare can be reflected both by behavioural (Mason and Mench, 1997), postural (Tallet et al., 2016), health (Lesimple et al., 2016b; Yngvesson et al., 2019) and physiological (Popescu and Diugan, 2017) changes. However, far fewer studies consider these aspects in a more global way. On-farm assessments have indeed shown differences between farms (cattle and buffaloes: Napolitano et al., 2005; dairy cattle: Andreasen et al., 2013), but mostly on very specific behaviours or physiological indicators and not by taking into account the overall state of welfare. Nevertheless, some studies have investigated the relationships between behavioural expressions of welfare and physiological measures, but the results are not always consistent between studies. What is more, the indicators measured in these studies are highly variable (Knierim and Winckler, 2009) and have not always been validated as welfare indicators (Scott et al., 2001; Winckler, 2019).

However, some, in particular physiological, indicators have proven useful. Thus, among the measurements that can be easily performed, blood count characteristics have proven interesting in a variety of species. The heterophile/leukocyte ratio is commonly used to assess the welfare of chickens under different rearing conditions (Altan et al., 2000; Davis et al., 2000; Elston et al., 2000; Nicol et al., 2006; Onbaşılar and Aksoy, 2005), with exposure to stress factors and/or corticosterone treatment resulting in a progressive increase of this ratio. It is also generally accepted that an increase in the neutrophil/lymphocyte ratio is more reliable than cortisol levels for assessing the impact of long-term environmental changes on chronic stress (Gross and Siegel, 1983; Swan and Hickman, 2014). A comparison between pigs living in impoverished environments and pigs living in environments enriched (newspaper, soil, balls and rope) showed a higher neutrophil/lymphocyte ratio in the animals living in the enriched environment (Backus and McGlone, 2018). In general, horses with impaired welfare exhibit physiological disorders and, in particular, a higher neutrophil/leukocyte ratio (Popescu and Diugan, 2017).

Anaemia (reduced red cell counts or haemoglobin levels) has also been found in riding school horses in developed countries and is repeatedly reported in studies on working equids in developing countries (Burn et al., 2010; Hausberger et al., 2016; Pritchard et al., 2009). In the latter, out of over 10 000 working equines observed, 20% avoided humans and over 13% appeared apathetic (Burn et al., 2010) and most were anaemic (Pritchard et al., 2009). In a study by Popescu and Duigan (2017), working horses exhibited a more impaired welfare and a higher neutrophil/lymphocyte ratio compared to breeding horses, supporting the findings of other studies that working modalities may have a major impact on welfare (e.g.(Christie et al., 2006; Hausberger et al., 2009; Lesimple et al., 2010; Lesimple et al., 2016a). Therefore, working conditions add to (and combine with) other management factors to influence the horses' welfare state and should be part of on-farm welfare assessments.

Previous research by Lesimple et al (2011) showed that different riding schools could be characterised by emotional/cognitive profiles of horses, leading to clusters of riding schools, and that changes in one single management factor (e.g. daily turnout) could be enough to influence these profiles. Based on this finding, the present study had two complementary aims: (1) determining if such facility-specific horse profiles can also be found in terms of welfare, i.e. facility-specific "welfare profiles", using a multifaceted approach where animals' welfare state is assessed based on detailed behavioural, health and physiological measurements, (2) providing a solid background for a longer–term study on effects of management changes on horses' welfare state.

In order to fulfil these aims, we re-analysed data from a study performed 15 years ago on three riding schools and for which several single aspects had already been analysed and published such as the human-horse relationship and its relation to possible chronic pain (Fureix et al., 2009, 2010), the impact of riding techniques on horses' chronic spine state (Lesimple et al., 2010); and the characterisation of "depressed-like" states (Fureix et al., 2012) or cortisol status (Pawluski et al., 2017). However, at that time, each factor had been considered separately and there was no search for facility differences or profiles, mainly because these three riding schools had similar general management practices. In the present study, we hypothesised that seemingly minimal differences in management practices may lead to different horse welfare profiles. Here, the riding schools had the same overall management practices but differed slightly in terms of turn-out frequency and riding techniques which can both be very influential (e.g. Lesimple et al., 2016a; Lesimple et al., 2016b). Since horses' welfare state can impact human safety (e.g. Luke et al., 2022), we also examined the incidence of falls during riding lessons.

Material and methods

Subjects and management conditions

This study was conducted in France in 2007 on 55 horses (14 mares and 41 geldings; aged 5–20 years, X ± SE = 11.9 ± 3.5) living in three different riding schools. On each site, only horses that had been living on the same site and under the same conditions for at least 1 year were observed.

Seventeen horses were observed (five mares and 12 geldings, aged 5–20 years, X ± SE = 11.4 ± 5.0) in Riding School A ("**RSA**"), 26 (five mares and 21 geldings, aged 7–16 years, X ± SE = 12.0 ± 2.3) in Riding School B ("**RSB**"), and 12 (four mares and eight geldings, aged 8–17 years, X ± SE = 12.6 ± 3.1) in Riding School C ("**RSC**"). Most (68%) horses were French Saddlebreds, equally distributed among the three centres; the remaining horses were of various breeds or unregistered. The horse ages' mean and SEs did not differ either between facilities, showing that the distribution of horses' ages were similar.

Horses' living conditions were overall similar in the three riding schools (Table 1): they were all housed in individual stalls with straw bedding and automatic drinkers, fed industrial pellets three times a day (5.2 ± 2.4 l) and given hay once a day (5.0 ± 1.6 kg). In each facility, horses worked 4–12 h per week (8.2 ± 2.7 h) in riding lessons for children and teenagers, with at least 1 day off per week.

Thus, differences between facilities were mostly restricted: (1) to the degree of turnout that went from a few hours per week in RSC to 1 day in RSB and 2 days in RSA (Table 1); and (2) variations in riding techniques as teaching strategies of riding instructors may vary with consequences on the horses' postures at work and spine state at rest.

Welfare assessment (reviewed in Lesimple, 2020)

Health measures

Body score. Each horse's body condition score (**BCS**) was assessed using Arnaud et al.'s scale (Arnaud et al., 1997). This scale evaluates the mass of fat deposits in five specific body locations (upper neck, withers, back of shoulder, ribs, tail tie) through palpation and by visual assessment of seven anatomical sites (upper edge of the neck, withers, back of shoulder, back line, ribs, croup, tail tie). Scores range from 0 (no fat deposits) to 5 (significant fat presence) per location, with the mean of these scores defining the BCS from 0 (emaciated) to 5 (obese).

Chronic health problems. Reported chronic problems (questionnaires): At each riding school, the caretaker involved in the daily care and health care of horses (i.e. the person most familiar to the horses) completed a questionnaire listing common chronic

Table 1
Horse management at the three riding schools (RSA, RSB and RSC). "Days" of turnout corresponded to an average of 6 h.

Item	Feeding and activity overview	RSA	RSB	RSC
FEEDING	Number of meals of hay per day (mean 1.0 ± 0)	1	1	1
	Amount of hay (kg) per day (mean 5.0 ± 1.6)	5 ± 1.7	3 ± 0.9	6.0
	Number of pellet meals per day (mean 3.0 ± 0)	3.0	3.0	3.0
	Amount of pellets (L) per day (mean 5.2 ± 2.4)	3 ± 0.8	7 ± 2.9	6 ± 0.8
TURNOUT	Number of days per week (mean 8.5 ± 3.9)	2 (week-end)	1 (week-end)	0.5
WORKING TIME	Number of hours per week (mean 8.2 ± 2.7)	11 ± 1.1	10 ± 1.7	9 ± 2.2

health problems in horses (e.g. lameness, cough, allergy, colic) (Knubben et al., 2008; Landman et al., 2004; Steenhaut et al., 2000). The caretaker indicated for each horse the presence (by ticking the box) or absence of each of the listed problems within the past 12 months, and noted any additional unlisted conditions.

Spine state assessment (see also (Fureix et al., 2010)): A non-invasive practitioner diagnosis, applicable to a large sample of animals, was employed here. A licensed chiropractor with 20 years of experience, who was unfamiliar to the study's horses, evaluated each horse's spine by manual palpation from head to tail. A second practitioner, trained and experienced, assessed one-third of the horses to test for agreement, which demonstrated high concordance between both practitioners (94.3 ± 3.7% consistency). The examination was based on manual palpation of bones and soft tissues to detect localised areas of spinal stiffness based on spinal mobilisation and palpable areas of muscle hypertonicity. Manual methods have been suggested to be effective in detecting back pain (Shearar et al., 2005; Wood et al., 2001), and assessments by practitioners were correlated with electromyographic measures in Lesimple et al. (2012). Horses were classified as totally unaffected, slightly affected (one affected vertebral site) or severely affected (at least two affected vertebral sites). The data also included the percentage of vertebral sites affected per horse.

Behavioural measures: observations in the home environment

While there is a range of validated indicators of compromised welfare (Lesimple, 2020), there is a crucial lack of indicators for positive welfare. Attentional state appears to be an interesting element for evaluating compromised, but also non-compromised welfare (Stomp et al., 2021). Therefore, in addition to the classical indicators of compromised welfare such as stereotypic behaviours, we also used several indirect measures of attentional state. These ranged from apathetic postures associated with reduced responses to sensory stimuli, to calm observational behaviours associated with positive states (Lesimple et al., 2012; Rochais et al., 2016b).

Sampling methods. All observations were made by a single observer (C.F.) and were recorded and then transcribed. Two main sampling methods (scan sampling and all occurrences) were used according to the type of indicators collected (Altmann, 1974). All observations (except for ear positions) were performed during three sessions (morning, afternoon, premeal,) distributed during the day: 0900–1100 h, 1400–1700 h, and half an hour before meals (respectively 0630–0730 h, 1130–1200 h, or 1730 –1800 h, depending on the schedule of the riding schools: RSA, RSB and RSC).

Scan sampling: The experimenter walked slowly and regularly (1 step per second) in the middle of the corridor, i.e. 2 m from the stalls in the stable, and approached each stall slowly in order to note: the position of the ears (axial ears, forward ears, backward ears), the orientation in the stall (towards the door, towards the walls) and the horse's behaviour (for example: eats hay, licks...). This quiet approach was intended to avoid reactions such as those observed when approaching the door suddenly (Hausberger and Müller, 2002). Measurements were taken every 2 min for three 30-min sessions. This was done on two successive days, resulting in 90 scans per horse.

All occurrences: The occurrence of depressive postures and stereotypies were extracted from focal continuous sampling sessions: each horse was observed in stall during 5-min sessions and depressive postures and stereotypic behaviours were recorded every time they occurred during these sessions. All horses were observed twice at three different times of the day (i.e. 6 times, with two sessions per period, hence 30 min per horse).

Welfare indicators (see also Lesimple, 2020). Stereotypic behaviours and abnormal repetitive behaviours: Stereotypic behaviours (**SB**), defined as "repetitive behaviours induced by frustration, repeated attempts to cope and/or brain dysfunction" (Mason et al., 2007), and other abnormal repetitive behaviours (**ARB**) have been shown to reflect inappropriate living conditions in horses (e.g. (Mills and McDonnell, 2005)) and chronic stress in a variety of species (Mason and Rushen, 2006). Stereotypic behaviours in horses are associated with altered cognitive abilities (review in Hausberger et al., 2019) and reduced fertility (Benhajali et al., 2014). Stereotypic behaviours are one of the most common and consensual indicators of compromised welfare (e.g. Lesimple, 2020).

The recorded stereotypic behaviours, presented in Supplementary Table S1, corresponded to those described in various studies (review in Mills and McDonnell, 2005). For a behaviour to be considered as SB/ARB, the behavioural sequence had to be repeated at least 3 times successively and observed at least 5 times, independently of the period of observation (Lesimple and Hausberger, 2014). For ease of reading, they will here all be grouped as "stereotypic behaviours". The data recorded were the number of SB and other repetitive ARB displayed by each individual (per half hour). Since the presence of stereotypic behaviours can reflect the effects of earlier conditions of life, we observed only horses that had been living in the riding schools for at least 1 year. Although in exceptional cases, some horses can keep performing them for longer than that, even after an improvement of life conditions, probably as a result of a genetic susceptibility, most studies converge towards a rapid decrease (or increase) in the prevalence and frequency of these behaviours after environmental changes (Lesimple et al, 2019).

Ears position: Ear positions have been used as indicators of welfare (and pain) in several earlier scientific studies. In horses, backward ear position is part of many pain scales (reviewed in Hausberger et al., 2016) and backward ear position, when the horse is feeding head down, is associated with negative chronic emotional states (e.g. (Henry et al., 2017; Lesimple et al., 2016a)). Three ear positions were noted: axial ears (perpendicular to the head-crest axis), forward ears (ears pointing forward with the pinnae of the ears clearly visible when looking at the horse from the front), or backward ears (ears pointing backward with the tips of both ears close together).

The instantaneous ear position of feeding horses was silently noted (only if they remained focused on feeding and did not react to the observer).

Orientation towards the wall: Horses' orientation towards a full wall in the home stall reflects a lack of interest towards their surrounding environment, which may be associated with potential health (e.g. anaemia, (Hausberger et al., 2016)) and/or "psychological" (Gleerup and Lindegaard, 2016) disorders. Therefore, horse's orientation towards a solid wall (back wall or partitions) or towards the outside (window or door tops) was noted.

Depressive posture (see also (Fureix et al., 2012)): Special attention was therefore given to this posture characterised by horses standing immobile with fixed neck and ears, a fixed and unfocused staring (no blinking), often associated with a stretched neck (jaw-open neck angle) and a similar height between the neck and the horse's back (neck - withers - back angle of about 180°). Such an attitude was easily distinguished from resting behaviour (during which eyes were at least partially closed and horses had a somewhat higher neck height) and observation of the environment (associated with mobility of neck, ears and eyes) (Fureix et al., 2012).

Observation behaviours (see also (Rochais et al., 2016a)): This calm state is characterised by the horse standing still (mostly at the stall door or window), neck held horizontally or slightly above horizontal (+/– 10°), with slow scanning of the environment and mobile, ears and neck (Rochais, et al., 2016a; Waring, 2003). This posture is distinct from the depressive posture in which the eyes are wide open for a long time, resting when the eyes are at least partially closed, and from vigilance (alarm) postures where the neck and tail are raised, ears and head are in a fixed position, which indicate high arousal states (Kiley-Worthington, 1976; Waring, 2003; Wolff et al., 1997).

Human-horse relationship tests (see also Fureix et al., 2009). Horses were subjected in their stalls to three standardised tests developed and commonly used to assess human-animal relationships (Hausberger et al., 2008; Waiblinger et al., 2006). The three tests selected allowed the evaluation of the reaction of horses to an unknown human at different levels of interaction, ranging from a passive presence of the human in the horse's home stall to more direct interventions (approach, contact, handling). All of them were performed by an experimenter unknown to the horses, thus allowing a more standardised approach (C.F. unknown to the horses at the start of the tests). Her observations were tape-recorded and later transcribed.

Data analysis. All human-directed behaviours were recorded *ad libitum* (positive behaviours: approaches, sniffing, licking, etc. towards the experimenter with ears forward; or negative behaviours: horse has its ears backwards with or without showing its teeth and stretched its neck towards the experimenter, sometimes approaching the experimenter).

For the present study, we considered only the number of positive and negative behaviours directed towards the experimenter during the tests.

Procedure (see Fureix et al., 2009). *The motionless person test ("MP"):* The experimenter entered the stall and stood motionless with her back against the closed door, facing inwards and looking at the ground. The test lasted 5 min.

The approach contact test ("ACT"): The experimenter positioned herself at 1.5 m from the subject and approached him perpendicularly, one step per second, up to the level of the shoulder. Then, she tried to touch the horse without forcing the contact. The horse was free to withdraw. The test ended when the experimenter succeeded in stroking the horse at least 2 s, or after three unsuccessful trials. This test was conducted at right and left horses' sides in a random order.

The halter fitting test ("Halter"): This test was used because horses were always fitted with their halter and tied up in their box for preparation before a riding lesson. The experimenter entered the box, holding a halter with her left hand. She approached the animal, walking slowly and regularly towards the horse's left shoulder, at approximately one step per second. When she was near the horse, *she stopped walking, put her right arm over the horse's neck and fitted the halter.*

Overall welfare assessment (Henry et al., 2017; Stomp et al., 2018, 2021)

In order to have a more comprehensive assessment of the chronic condition of individuals, a total chronic stress score (**TCSS**), adapted from Hausberger et al. (2012), was calculated for each horse based on the data obtained for the different measures previously described. TCSS calculation consisted in ranking the horses according to: (1) the number of stereotypic behaviours expressed during the 30-min focal sample sessions; (2) the percentage of scans spent with the ears backwards during feeding; (3) the percentage of time spent directed at a solid wall of the stall; and (4) the number of aggressive responses directed towards the experimenter during the human-horse relationship tests.

For all variables, the higher the value, the worse the welfare status and the higher the rank assigned to the horse. The TCSS was then calculated by adding the four ranks calculated for each horse so that in the end, the higher a horse's TCSS, the more compromised its welfare. For example, a horse ranked 9th lowest in the number of aggressive responses, 8th lowest in the number of stereotypic behaviours, 5th lowest in the percentage of scans spent with ears back during feeding, and 4th lowest in the time spent facing a solid wall had a TCSS of 26, which is higher, and thus reflects a lower welfare state, than a TCSS of 4 obtained by a horse ranked 1st lowest on all variables (see also Stomp et al., 2018).

Physiological measures

Faecal cortisol metabolites (see also Pawluski et al., 2017). Fresh faecal samples were collected immediately (less than 1 min) after defecation, directly from the bedding. Samples were collected between 1200 and 1300 h, after a day's rest (taking the 24 h delay in excretion of fCM in horses into account (Palme, 2019).

Each faecal sample was then kept frozen at –20 °C until further analysis. Extraction of samples followed the method described by Merl et al. (2000). Briefly, 0.5 g faeces plus 1 ml water and 4 ml methanol were vortexed for 30 min and centrifuged (2 500 g/15 min). One ml of the supernatant was mixed with 5 ml diethylether and 0.5 ml 5% $NaHCO_3$ for 10 s. Thereafter, the tube was inverted four times and the aqueous phase was frozen at –24 °C. Afterwards, the ether phase was decanted and dried down. The extract was re-dissolved in assay buffer and the concentration of 11,17-dioxodandrostanes (11,17-DOA), a group of cortisol metabolites, was measured with an 11-oxoetiocholanolone enzyme immunoassay (EIA), previously described (Palme et al., 1997) and successfully validated for use in horses (Möstl and Palme, 2002).

Haematological data. A blood sample was collected for each horse between 0800 and 0900 h, and taken within the hour to a veterinary laboratory for analysis (Laboratoire Vétérinaire Départemental Ille et Vilaine, Rennes, France). Samples were taken from the jugular vein, using a vacutainer© system with anticoagulant and sterile single-use blood collection cannulas. Haematological data analysed were the number of red blood cells (million/mm3), white blood cells (million/mm3), and platelets (thousand/mm3); percentages of neutrophils, eosinophils, basophils, lymphocytes, monocytes, haematocrit and haemoglobin (g/100 ml).

Possible impact of human actions on horse behaviour and welfare

Human actions during a routine task: stall cleaning

Routine daily caretaking tasks and the way they are performed may have large consequences on animals' reactions to humans or even welfare or cognition (Uetake et al., 2002). Video recordings (1 video per horse) were performed here during sessions of stall cleaning in order to characterise how the most familiar caretaker interacted with the horse in each facility. Stall cleaning occurred in all three riding schools during quiet periods (no riding lessons), between 0700 and 0900 h (according to the riding school's schedule). Video recordings were made using a camera handheld by the experimenter standing silently outside the stall. All caretakers were asked permission to be observed beforehand, and asked to behave in their usual way. Sessions of stall cleaning lasted 11–149 s. The data recorded in all occurrences were all the interactions directed towards the animal: visual (gaze > 1 s, eye contact < 1 s), vocal (any speech directed towards the horse) and tactile (any contact with the horse, whether with the hand or an object).

Measurement of human actions during riding sessions (Lesimple et al., 2010)

In 2007, we were only allowed to perform video recordings in two of the three riding schools (RSA and RSC). Moreover, some of the horses from these two sites were not ridden during the data collection for various reasons (e.g. injury, resting) and could not therefore be considered in the analysis. Thus, out of the 17 horses at RSA, 9 were filmed at work and out of the 12 horses at RSC, 8 were filmed at work. Two "beginners" (less than 50 h practice) lessons were video-recorded by the same observer (C.F.) using a JVC, Everio GZ-MG275 camcorder, which was on a tripod at a fixed place on the ground within the covered area used for lessons. The horses walked mainly along the wall on a path and the position of the camcorder enabled to film each horse-rider pair in a perpendicular position each time they crossed the camcorder "field of vision". The camcorder was at a distance of 25 m from the path. Horses' postures and riders' positions were recorded each time they crossed the camcorder's recording field. A mean of 10,74 ± 1,04 scans was obtained for each rider-horse dyad.

An earlier analysis of these data had shown that riders' actions differed between the two riding schools as a result of teachers' strategies and correlated with the horses' chronic spine state (Lesimple et al., 2010). Here, we considered mostly the riders' actions in order to see to which extent riding techniques may be involved in possible differences of welfare profile between facilities.

Observations of riders' positions focused on hands, considered as particularly prone to induce potential stress. Hands 'height in particular, when considering beginning riders, has an important impact on horses' back postures and chronic impact on spine state (Lesimple et al., 2010) and welfare overall (Lesimple et al., 2016a). Mouth escape responses involve high and hollow neck positions (Ridgway and Harman, 1999).

Hands height was considered as: high (more than 1 fist above the withers), middle (hand on withers) or low (hand under withers). Reins' length was also evaluated as it may determine a softer (longer reins) or harder (shorter reins) contact with the horse's mouth. Reins lengths were categorised as short (less than half the horse's neck length), medium (from half to the horse's neck length) or long (more than the horse's neck length); rein tension as tight (straight line between the rider's hands and the horse's mouth) or loose (curved line between the rider's hands and the horse's mouth).

Number of riders' falls

In order to explore potential associations between the welfare profiles of the facilities' horses and human safety, the number of falls during beginner sessions was recorded. Observations were conducted throughout the year (36 weeks), at the rate of one session per week, involving 10–15 horse/rider pairs each time.

Each fall from a horse was recorded in each of the three riding schools during direct observations of the riding sessions.

Statistical analysis

Statistical tests were carried out using R 4.2.0 software (alpha threshold set at $P < 0.05$), combined with RCommander software for principal component analysis (**PCA**). As our data were not normally distributed, we used non–parametric statistical tests (Siegel and Castellan 1988). To investigate potential welfare differences between horses at the three riding schools, Kruskall-Wallis (**KW**) tests were performed on the number of behaviours (e.g. number of stereotypic behaviours, number of aggressive behaviours) and the percentage of time spent on each behaviour (collected by scan sampling) for each horse, followed by Mann-Whitney posthoc tests with Bonferroni corrections in order to perform pairwise comparisons of the populations. In order to compare the number of horses that performed or did not perform a behaviour, chi2 tests were carried out. As these tests showed large differences between populations, we then performed a PCA in order to have a global representation of how horses grouped according to the welfare indicators. The PCA was then inspected, and the coloured clusters were plotted using hierarchical cluster analysis (Johnson, 1967).

In addition, in order to highlight each facility's horse welfare profiles, a visual analysis was performed by constructing radars for each riding school. Each radius of a radar represents the percentage of horses for each indicator. The bars to the left of the radar represent the percentage of horses with positive, and the bars to the right represent the percentage of horses with negative indicators.

Results

Horse welfare at the whole population level

High individual variations were observed but overall welfare assessment revealed a high prevalence of welfare problems, consistent with earlier studies on horses living in similar conditions.

No horse showed an abnormally low body score, but 27% (N = 15) of them were overweight (score of 4). A total of 73% of the horses (N = 40) were assessed as having back problems and 33% (N = 18) were reported as having recurrent health problems (lameness: N = 5, allergies: N = 4, eye irritations: N = 2 or sarcoid: N = 1).

Behavioural observations revealed that 65% of the horses (N = 36 out of 55) exhibited at least one type of stereotypic behaviour (1.9 ± 1.3 types) for 6.1 ± 6.8% of the time; 55% horses (N = 30) spent more than 50% of their time feeding on hay with their ears backwards; 18% horses (N = 10) exhibited at least once a depressed posture. All horses at each site observed the environment at least once, but they did so with very different frequencies (from 3% to 48% of scans).

Physiological analyses showed that many horses had abnormal blood counts: 42% of the horses (N = 23) presented abnormally high levels of neutrophil (above norm) of which 18% presented an abnormal neutrophil/lymphocyte ratio (i.e. outside the range 0.8–2.8: (Popescu and Diugan, 2013)) and 10 horses (18%) showed anaemia (haemoglobin level under norm). Faecal cortisol metabolite levels ranged from 1.7–24.4 ng/g, with 16 horses (29%) having abnormally low levels.

During the human-horse relationship tests: 73% (N = 40) showed at least one negative behaviour towards the experimenter during testing and 87%, (N = 48) showed at least one positive behaviour.

Comparison of the welfare state between populations (Supplementary Table S2)

No difference was found between facilities in terms of body score (Kruskal Wallis test, χ^2 = 0.48, df = 2, P = 0.79), human-directed behaviours whether positive (KW test, χ^2 = 1.64, df = 2, P = 0.44) or negative (KW test, χ^2 = 3.98, df = 2, P = 0.14) nor frequency of stereotypic behaviours (KW test, χ^2 = 3.441, df = 2, P = 0. 179) whether oral (KW test, χ^2 = 5.03, df = 2, P = 0.08) or motor (KW test, χ^2 = 1.95, df = 2, P = 0.38).

However, clear differences appeared between facilities in a number of other welfare measures both for positive and negative indicators (Fig. 1). Thus, the three sites differed in the number of reported chronic health problems (KW test, χ^2 = 22.57, P = 0.0001) and number of vertebral sites affected (KW test, χ^2 = 9.5, df = 2, P = 0.009) per horse. Differences appeared also in a number of behavioural measures: time spent with ears backwards during foraging (Kruskal Wallis test, χ^2 = 11.0, df = 2, P = 0.004), facing a wall (KW test, χ^2 = 26.1, P < 0.0001) or in observation behaviour (KW test, χ^2 = 11.0, P = 0.004), as well and in the number of horses exhibiting at least once a depressed posture (Chi-square test, X-squared = 6.39, P = 0.04). Clear differences appeared also in the total chronic stress score (TCSS) (KW test, χ^2 = 15.34, df = 2, P = 0.0005). Finally, the three sites also differed in terms of physiological characteristics, including faecal cortisol metabolites levels (KW test, χ^2 = 32.32, P < 0.0001) and haematological data such as lactic acid (KW test, χ^2 = 12.46, P = 0.002), white blood cells (KW test, χ^2 = 8.45, P = 0.015), basophils (KW test, χ^2 = 9. 72, P = 0. 008), haemoglobin (KW test, χ^2 = 28.26, P < 0.0001), neutrophil/lymphocyte ratio (KW test, χ^2 = 7.59, P = 0.02), and haematocrit (KW test, χ^2 = 28.45, df = 2, P < 0.0001).

Comparison between facilities revealed that RSC horses had more vertebral sites affected and more reported chronic health problems, more time spent facing a wall and had a higher TCSS score than both RSA and RSB horses (posthoc pairwise comparisons Mann Whitney tests with Bonferroni corrections: back problems: RSC vs RSA: P = 0.04 and RSC vs RSB: P = 0.03; chronic health problems: RSC vs RSA: P = 0.0003 and RSC vs RSB: P = 0.0002; time spent facing a wall: RSC vs RSA: P = 0.001 and RSC vs RSB: P < 0.0001; TCSS: P = 0.004 in both cases). More (35%, N = 9 out of 26) RSB horses were observed in a depressed posture (RSA: 5%, N = 1 out of 19 and RSC: N = 0 out of 12; X2 = 6.4, df = 2, P = 0.04) and/or presented anaemia (RSB: 62,5%, N = 10, RSA and RSB: N = 0; X2 = 13.6, df = 2, P = 0.001). Finally, RSA horses spent

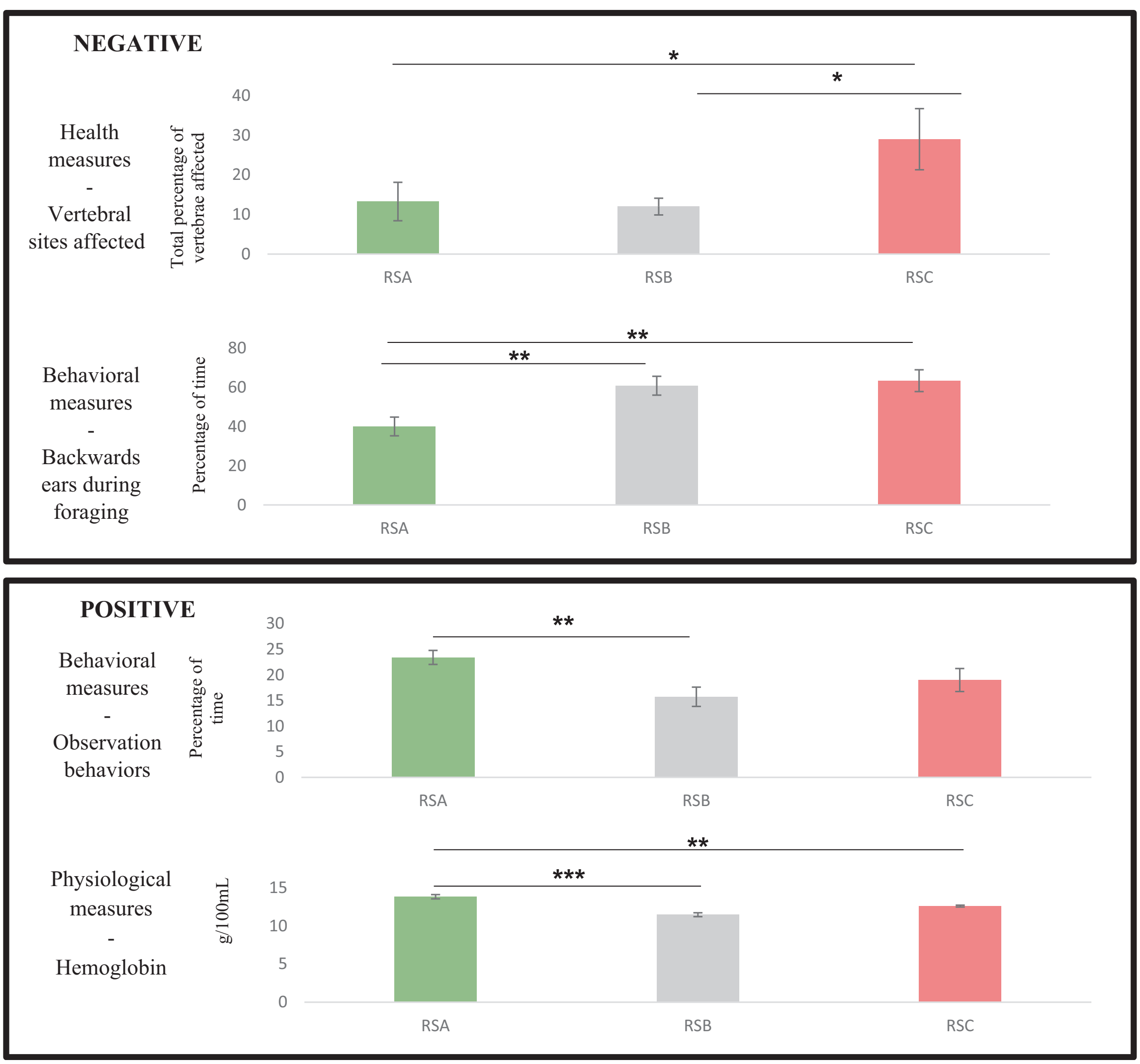

Fig. 1. Examples of differences observed between horses from the three riding schools (RSA, RSB and RSC): mean and SE of (a) number of affected vertebral sites, (b) time spent with ears back during foraging, (c) time spent in observation, and (d) mean haemoglobin level. *P < 0.05, **P < 0.01, ***P < 0.001, Kruskal-Wallis test and posthoc pairwise comparisons using Mann-Whitney tests with Bonferroni corrections.

less time with ears backwards during foraging than RSB and RSC horses (P = 0.01 in both cases) and more time in calm observation behaviour than RSB horses (P = 0.005); they also had higher faecal cortisol metabolites, haemoglobin, and haematocrit levels than RSB and RSC horses (faecal cortisol metabolites: RSB: $P = 3 \times 10^{-8}$ and RSC: P = 0.04, Haemoglobin: RSB: $P < 0.0001$ and RSC: P = 0.0095, Haematocrit: RSB: $P = 6.3 \times 10^{-6}$ and RSC: P = 0.0023).

Further differences could be observed between facilities in paired comparisons. Faecal cortisol metabolite levels were higher in RSC than in RSB (P = 0.0001). RSC horses also showed higher basophil, haemoglobin and haematocrit levels than RSB horses (basophil P = 0.017, haemoglobin P = 0.0023, haematocrit P = 0.0141), but had lower levels of lactic acid than RSA horses (P = 0.00059). RSB horses showed a higher neutrophil/lymphocyte ratio but lower counts of white blood cells than RSA horses (N/L P = 0.046, WBC P = 0.0082). Thus, it appears that each facility has a somewhat unique welfare profile.

Welfare profiles

In order to have a representation of the distribution of horses according to welfare indicators, a PCA was performed by combining behavioural, health and physiological measures. It revealed that the two first axes explained 31% of the variance (Fig. 2 and Table. 2).

Axis 1 (15.53% of variance) mostly opposed time spent in calm observation, higher haemoglobin and cortisol metabolites levels to indicators of poor welfare such as time spent facing a wall (Fig. 2). Axis 2 (14.35% of variance) mostly opposed indicators of poor welfare such as back and chronic health problems to more time spent in calm observation, positive reactions towards humans during tests and higher platelet levels.

Hierarchical analysis performed on these data revealed three clusters (Fig. 2): cluster 1 corresponded to horses that spent more time facing a wall and less time in calm observation; cluster 2 to horses with more back and other chronic health problems, as well as aggressive behaviours towards humans; and cluster 3 to horses with more positive reactions towards humans, more time spent in calm observation and better physiological markers. Moreover, each cluster was predominantly represented by one of the three particular riding schools. Thus, horses from cluster 1 mostly belonged to RSB (75%), from cluster 2 to RSC (75%), and from cluster 3 to RSA (90%) facilities respectively (Fig. 2). This analysis confirms a better welfare profile in RSA and two different profiles of compromised welfare expressions in facilities RSB and RSC, namely one more "depressed-like" (RSB), one more aggressive (RSC).

This appears especially clearly when welfare profiles (radar charts) are created for each facility: the RSB and RSC facilities comprised a higher percentage of horses with negative indicators, in agreement with the PCA clusters (which correspond to a rightward orientation in Fig. 3).

Welfare and riders' security

The observations of 36 1-h riding lessons for beginners in each centre revealed that the number of falls recorded, although relatively low (between 6 and 12), followed this gradient of welfare state between the three riding schools.

The number of rider falls was lowest at the RSA and highest at the RSC (χ^2 = 7.56, df = 2, P = 0.02) (Fig. 4).

Looking for explanatory factors for differences between facilities

Although, as mentioned above, there are some differences in the time spent in free turn out (per week) between facilities, these differences are rather small and may not be enough to explain the important differences in horses' welfare state between facilities. Other factors, such as the quality and amount of human actions during routine and riding procedures, may be involved.

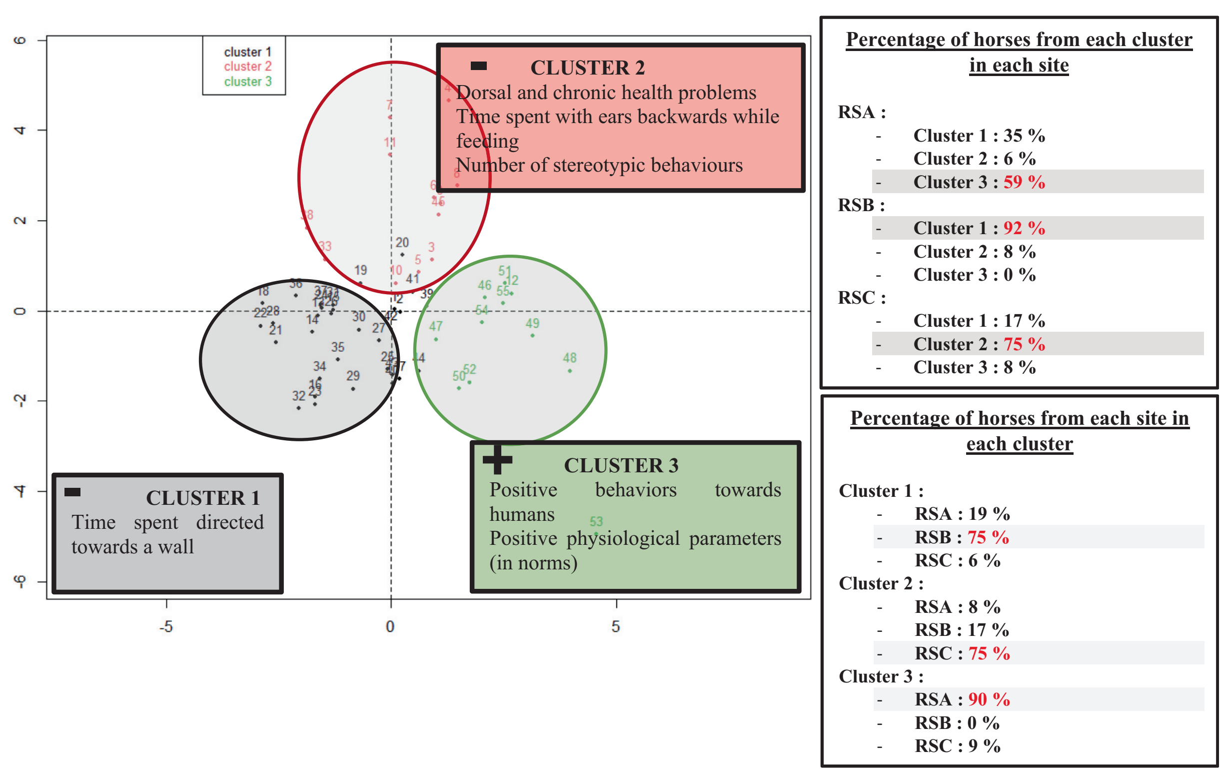

Fig. 2. Results of the PCA (Principal Component Analysis) performed on the behavioural, postural, health and physiological measures of the 55 horses (X = 1 (15.53%), y = 2 (14.35%)). Each cluster represents the majority of horses from the three equestrian establishments (RSA, RSB and RSC).

Table 2
All measurements taken on the horses included in the principal component analysis and their contribution to each of the two axes, with the variables that contributed most to the axes based on the threshold of 1/19 = 5.26.

Variables	Meaning of the variables	Axis 1	Axis 2
HEALTH MEASURES			
% vertebral sites affected	Percentage of vertebral sites affected/total spine	+0.002	+13.66
Reported chronic health problems	Sum of chronic health problems reported	+0.44	+20.27
BEHAVIOURAL MEASURES			
Ears backwards	Percentage of scans ears backwards while eating hay	−8.44	+10.38
Orientation towards wall	Percentage of scans spent facing a solid wall	−18.78	−2.0
Depressed postures	Number of depressed-like postures (all occurrences)	−0.90	−0.60
Stereotypic behaviours	Number of stereotypic behaviours (all occurrences)	−1.70	+5.43
Observation behaviour	Percentage of scans spent observing the environment	+8.10	−3.13
REACTIONS TO HUMANS			
Positive behaviours	Total number of positive behaviours during the human relationship tests	+3.51	−5.69
Negative behaviours	Total number of negative behaviours during the human relationship tests	+0.02	+0.14
PHYSIOLOGICAL MEASURES			
Lactic acid	Lactic acid level	−0.54	−0.13
White blood cell	White blood cell count	+3.21	−2.28
Platelets	Platelet count	−1.03	−12.47
Eosinophil	Percentage of eosinophils	+4.0	−3.99
Basophil	Percentage of basophils	+0.56	+8.94
Monocytes	Percentage of monocytes	−0.91	−0.21
Haemoglobin	Haemoglobin level	+17.90	+1.51
Neutrophil:lymphocyte ratio	Neutrophil / lymphocyte ratio	−7.55	−3.48
Faecal cortisol metabolites	Faecal cortisol metabolite levels after a resting day (evening)	+16.13	−0.90
MCV	Mean Corpuscular Volume (=hematocrit*10/erythrocyte)	+6.26	+4.77

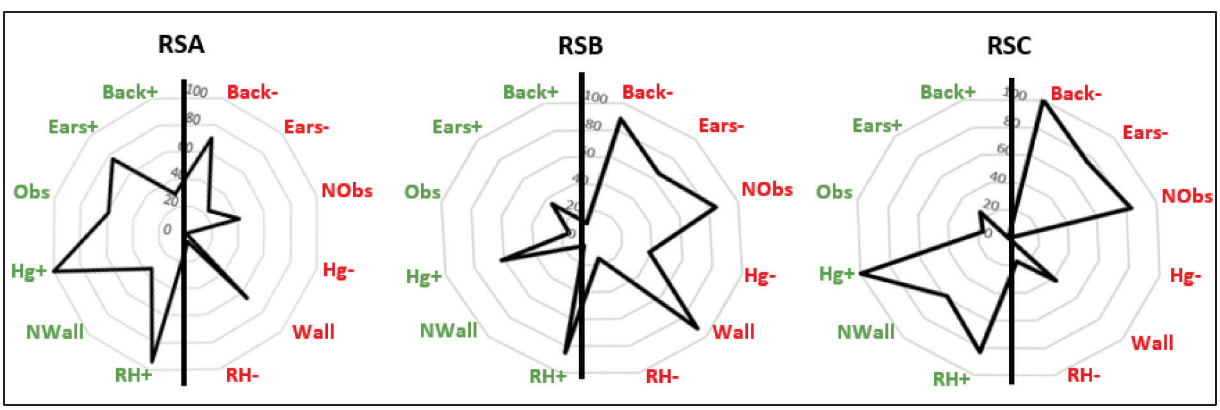

Fig. 3. Radar profile per riding school (RSA, RSB and RSC) based on an array of welfare indicator with each radius representing the percentage of horses exhibiting the characteristic for each of the welfare indicators (left: positive, right: negative). The bars on the left of the radar represent the percentage of horses with positive indicators (back+: no vertebrae affected, ears+: less than 50% ears back when feeding head down, obs: spending more than 25% of the time observing the environment, Hg+: haemoglobin level between 11 and 19 g/100 mL, NWall: less than 25% facing a wall, RH+: exhibiting at least one positive behaviour during the human relation tests,) and the bars to the right represent the percentage of horses with negative indicators (Back-: severe back problems: more than 4% of vertebral sites affected, ears-: more than 50% time spent ears backwards when feeding head down, Nobs: spending less than 25% of the time in observation of the environment, Hg-: haemoglobin level below 11 g/100 mL (anaemia), Wall: more than 25% of time spent facing a wall, RH-: no positive behaviour during the human relation tests) For example, in RSA 71% of the horses had back problems, in RSB and RSC, 89 and 100% of the horses had back problems.

Thus, analysis of human behaviour during stall cleaning revealed differences between facilities in the duration of the procedure (KW = 50, df = 2, $P < 0.0001$) with longest durations in RSA and shortest in RSB (RSA-RSB: $P < 0.0001$, RSA-RSC: $P = 0.0018$, RSB-RSC: $P < 0.0001$). Moreover, action modalities differed (number of vocal: KW = 35.512, df = 2, $P < 0.0001$; visual: KW = 21.112, df = 2, $P < 0.0001$; tactile: KW = 34.756, df = 2, $P < 0.0001$ actions) with fewer human utterances in RSB (RSB-RSA: $P = 2.3 \times 10^{-8}$, RSB-RSC: $P = 1.1 \times 10^{-5}$), more visual attention in RSC (RSC-RSA: $P = 0.007$, RSC-RSB: $P = 0.\ 0001$) and more tactile actions in RSC and more in RSA than RSB (RSA-RSB: $P = 2.6 \times 10^{-6}$, RSA-RSC: $P = 0.012$, RSB-RSC: $P = 2.2 \times 10^{-7}$). Thus, overall, RSC was the facility where the caretaker exhibited the most visual attention and tactile interactions during stall cleaning, RSA where stall cleaning sessions were the longest and the caretaker was moderately interactive with the horse, and RSB where stall cleaning was the fastest and without any interaction with the horse.

With regard to human actions in terms of riding techniques, Lesimple et al (2010) analyses had already revealed differences between RSA and RSC in riding techniques of beginners during riding lessons, due for some part at least to the riding teachers' respective teaching strategies with consequences for horses' welfare state. Hence, the more time the riders spent with high hands and tight reins, the more horses worked with a high and hollow/flat neck and the more they had back problems. The re-analyses of the riders' data additionally revealed that riders spent more time with short reins in RSC than in RSA (W = 58.5, $P = 0.009$), adding probably to the discomfort of the horses.

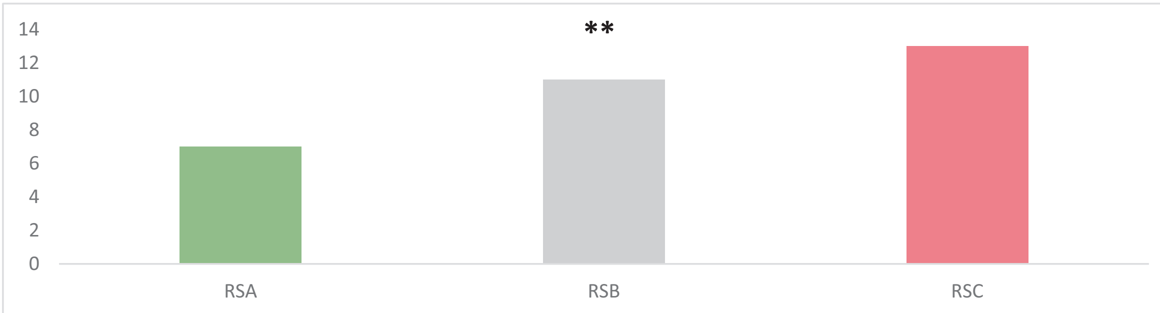

Fig. 4. Total number of falls from horses in 36 observed riding lessons for beginners in each riding centre (RSA, RSB and RSC), chi2 test, $^{**}P$ < 0.01.

Discussion

Overall, the welfare state of the whole population of instruction horses observed here was in line with the results of previous studies on horses living in similar conditions, whether health, behavioural or physiological parameters were considered. In particular, the three riding schools housed the horses mostly in single stalls with very limited turnout, which is known to increase the risks of developing stereotypic behaviours (e.g. (Lesimple et al., 2019; Lesimple et al., 2016a; Visser et al., 2008)). Similarly, a significant proportion of overweight horses were found at each site, which is in line with epidemiological studies on working horses in developed countries (e.g. (Giles et al., 2014; Jensen et al., 2016; Wyse et al., 2008); for a review: (Akinniyi et al., 2023; Hausberger et al., 2021) as a result of an imbalance between energy and fibre intake (e.g. Giles et al, 2014) which was similar in the three riding schools. The observed alterations of the physiological parameters were also convergent with earlier studies (e.g. (Popescu and Diugan, 2017)). Although some previous analyses had to some extent already mentioned these welfare issues for the studied population (Fureix et al., 2012; Lansade et al., 2018; Pawluski et al., 2017), the present results, based on re-analyses of the set of data, reveal that despite these management similarities, horse welfare differed in many ways between the three facilities studied, each of them presenting a different "welfare profile". This suggests that "subtle" differences in management practices may be enough for decreasing or increasing horses' welfare state.

The concept of "facility welfare profile"

Although there have been a number of studies aiming at understanding the multidimensional dimension of welfare (i.e. combining different types of measures such as behavioural and physiological parameters), most others have relied on surveys (i.e. subjective assessment by stakeholders), and few have focused on comparing facilities. The studies that have highlighted differences between sites generally focus on a particular behaviour, rather than considering the general welfare as a multifaceted aspect (cattle and buffaloes: Napolitano et al., 2005; Dairy cattle: Andreasen et al., 2013). The originality of this study is therefore to investigate the general state of welfare, both individually (considering each horse) and globally (within a stable). This is all the more important, as an animal's biological responses to stress factors may involve one or more of four channels: behavioural, autonomic, neuroendocrine and immunological (Moberg, 2000). Not all individuals react the same way to the same stimulus, and different stimuli can provoke different responses from the same individual. It is therefore important, when examining chronic stress in animals, to use a combination of objective and quantifiable measures, both behavioural and physiological (Crockett et al., 2000; Moberg, 2000; Rushen, 2000). Few studies have been carried out to analyse the impact of welfare status on physiological parameters in horses, cortisol being the most studied but also the most controversial indicator (e.g. Placci et al., 2019). Cortisol measures can be taken on different substrates (blood, salivary, faecal, hair) and even when using the same measures, results of studies may differ. Thus, Pawluski et al. (2017) and Fureix et al. (2012) showed that across the whole population of these same horses, horses with compromised welfare had lower levels of blood and faecal cortisol metabolites (chronic health disorders: Pawluski et al., 2017; depressed horses: Fureix et al., 2012) which was also observed in Visser et al (Visser et al., 2008). However, no differences were found between horses with gastric ulcers and their healthy counterparts (Malmkvist et al., 2012). Similarly, some studies on horse housing have found that moving from group housing with access to a paddock to individual stalls led to an increase of horses' cortisol levels (saliva: Erber et al., 2013; blood: Pessoa et al., 2016), whereas no difference was found in another study (salivary cortisol: Harewood and McGowan, 2005). In our study, the PCA showed a proximity between faecal cortisol metabolite levels, haemoglobin and Mean Corpuscular Volume (**MCV**) levels, and the time spent observing the environment, positive indicators were found at higher levels in RSA horses. Time spent in quiet observation of the environment reflects a positive attentional state (Waring, 1983), and is less frequent in horses with back problems (Rochais et al., 2016a). In the PCA, these parameters, as well as, the haemoglobin and MCV levels were opposed parameters indicative of compromised welfare such as the time spent with the ears backwards while foraging, the time spent facing a solid wall, and the N/L ratio, which highlights N/L ratio as an interesting indicator (Popescu and Diugan, 2017). There are few studies on haemoglobin and MCV levels and their potential interest for welfare assessment and they mostly deal with working conditions (racing), showing that an increase in these parameters is mainly due to physical stress during training (Bollinger et al., 2023; Filho et al., 2024; Rowicka, 2013). The present study suggests that the horses' living conditions could have an impact on physiological parameters, but also that, while cortisol is not very reliable, the use of metabolites, haemoglobin and MCV for assessing welfare were further validated here and present a much better option in terms of physiological measures.

When considering simultaneously all measures for the different welfare indicators, three welfare profiles emerged, each of them representing predominantly one of the three riding schools. Two of them, corresponding to riding schools B and C, were represented by mostly indicators of compromised welfare, while riding school A profile included also indicators of good welfare. Moreover, by calculating the TCSS (Total Chronic Stress Score, adapted from (Hausberger et al., 2012) and (Henry et al., 2017)), reflecting the level of chronic stress (Stomp et al., 2018), the results highlight an increasing gradient of chronic stress from RSA to RSB to RSC.

The profile associated with Riding School A corresponded to horses that were rather positive towards humans and with satisfactory physiological measures (i.e. within norms). In contrast, the profiles associated with riding schools B and C corresponded to horses that spent little time observing their environment and most time feeding with their ears backwards. In both centres, a high proportion of horses had back problems. They also had a higher proportion of horses with anaemia (haemoglobin level under norm) in riding school B and lower levels of basophils in riding school C. These results support further the strong relationship between behavioural expressions of welfare and the physiological state which is consistent with the fact that chronic stress has negative consequences at both physiological and behavioural levels that can therefore change in a co-occurrent way (Dantzer, 1978; Gregory, 2008). Thus, Riding School B horses' welfare profile corresponded mostly to behavioural apathy/depression and physiological anaemia. Descriptions of apathetic horses have all occurred in domestic situations where horses are subjected to social, spatial and/or feeding restrictions and potentially stressful work (Burn et al., 2010; Hall et al., 2008; Maurício et al., 2023; Popescu and Diugan, 2013; Ruet et al., 2019). Apathetic postures have been proposed as a depressive-like syndrome in horses, in association with decreased cortisol levels (Fureix et al., 2012, 2015) as in some other species (Camus et al., 2013; Fureix and Meagher, 2015). Depressive horses also show a reduced attention to their sound environment (Rochais, et al., 2016b), while horses with anaemia tend to withdraw from external stimulations by spending time facing a wall (Hausberger et al., 2016). In humans, there is evidence of an anaemia-depression link (Lee and Kim, 2020; Read, 1976; Xu et al., 2018), which would deserve further investigation in horses. Riding school C horses' welfare profile corresponded mainly to horses with chronic health problems, notably back problems, and also higher basophil levels (i.e. above norms). A recent study in humans has shown a correlation between back problems and elevated basophil levels (Özcan-Ekşi et al., 2024). RSC had the highest proportion of horses with back problems, which are associated with chronic behavioural and morphometric changes (e.g. Fureix et al., 2010; Sénèque et al., 2019). Horses with back problems show also an atypical resting EEG profile suggesting an impact even on the cognitive functions (Stomp et al., 2020a). Like all white blood cells, basophil cells play a part in the body's defence against external health risk factors, particularly infections and inflammation (Schwartz et al., 2016), often found in musculoskeletal disorders in humans (Barbe and Barr, 2006). Although some studies have also highlighted a positive correlation between lactic acid levels and back problems in horses (Jeffcott et al., 1982) and in relation to work intensity (Jung et al., 2019), we did not find any such relationship here. The highest levels of lactic acid were found in riding school A, where indeed horses spent more time working (on average 1 h more than in the two other centres), but where there were the least horses with back problems. This is an interesting result, since although the horses at this site worked more, this did not seem to decrease their overall welfare state, suggesting that quality of work may be more important than quantity. In Lesimple et al. (2016a) epidemiological study, working techniques (length of reins, height of rider's hands) but not the weekly time spent working had an influence on the emergence of stereotypic behaviours. Thus, one has to be careful before considering lactic acid alone in a welfare study.

How to explain the differences in welfare profiles?

The results of this study, combining behavioural and physiological data, clearly show that each equestrian facility had its own welfare profile, despite management conditions that were broadly similar.

Small changes in the horses' conditions of life may lead to rapid changes in behavioural and physiological welfare indicators. The daily broadcast of particular acoustic frequencies in the home stable for 3 weeks has been associated with important behavioural and physiological changes in racehorses (Gueguen et al., 2023). The frequency of stereotypic behaviours can change in the same horses according to whether they are in a stall which has an open window or not (Lesimple et al., 2019) or whether their hay is on the ground, in a slow-feeder on the ground in a haynet (Rochais et al., 2018). Finally, few studies have looked at the impact of changes in the life conditions on the overall welfare of horses, as most focus on one key indicator of welfare (usually stereotypic behaviours) or only on physiological parameters, or rely mostly on surveys and not direct observation.

In the present study, there were some minor differences in management practices, namely weekly turn-outs and riding instruction strategies. It should also be noted that the caretakers' behaviour towards horses during stall cleaning differed between the three sites in terms of vocal and tactile actions towards the horse, with more (but different) interactions overall in RSA and RSC and less in RSB. Although this seems a minor point as compared with the rest of the management conditions (on average just over 1 min of straw bedding per day), this may contribute to the different perceptions that horses have of their environment (Hausberger et al., 2008).

The number of weekly turnouts

The horses at site A went out every weekend (i.e. 2 days/week, 6 h per day), the horses at site B went out on Sundays (i.e. 1 day/week) and the horses at site C went out in groups for a few hours during the week (i.e. A few hours a week, with no specific rules). Thus, the gradient of free turnout follows the same gradient as the horses' welfare, although these differences were rather small. A recent study has shown that one single hour of free turnout (even without conspecifics) daily during 3 weeks greatly improved the behavioural and some physiological welfare parameters in horses housed in single stalls, although this duration may not have been enough to induce changes in blood formulae (Lesimple et al., 2020). The better welfare profile observed in RSA may therefore be at least partially explained by this increased time of turnout, especially as the horses observed had been living in these same facilities for more than a year. The higher levels of platelets and haemoglobin observed in this facility suggest that free outdoor exercise may induce a better oxygenation of blood, which would promote better physiological conditions and thus a better immune system.

Overall, many studies have underlined that permanent single−stall housing is clearly inappropriate, because of spatial and social restrictiona (for example: Cooper and Mason, 1998; Lesimple et al., 2011; Ruet et al., 2019…) and it is possible that the more the opportunity to be outdoors in free movement with social partners, the better the welfare state. There is however to our knowledge no study looking at such gradients (differences of just a few hours weekly) of turnouts and their possible consequences in terms of behaviour and especially physiology. Return from longer periods of outdoor turnout (pastures) may also lead to a rebound of chronic stress (Lesimple et al., 2020; Ruet et al., 2020), so at that stage, it is difficult to know whether these differences in turnout may explain the important differences observed in the welfare profiles.

Impact of human actions in and out of work

Lesimple et al. (2016a), on the basis of analyses of horses and riders' postures of 108 horse-rider dyads from 17 riding schools, found strong correlations between the time these beginning riders spent with high hands and the time spent by horses with high neck postures, which can lead to chronic problems (Lesimple et al., 2013).

In their earlier study on our population, Lesimple et al. (2010) found that the riding teachers of the two riding schools studied had opposite teaching strategies, focusing more on correcting the riders' posture (RSA), the other (RSC) on controlling the horse. Controlling the horse at an early stage of learning to ride may be very stressful given the imbalance and often lack of control on their hands of young beginning riders. Control involves shorter and more tensed reins and fear often leads to higher hands (personal observations).

It has been suggested for many years now that the way a horse is ridden can cause stress in the animal (e.g. McLean and McGreevy, 2010; ÖDberg, 1987). The rider's position has a strong impact on the horse's behaviour during work (e.g. Uldahl and Clayton, 2019), and defensive behaviours increase when the rider spends time with tensed reins (Eisersiö et al., 2023). When horses are ridden with tight reins and high hands, they tend to try and escape harsh bit actions by raising the neck, which, when repeated, may lead to chronic back problems (Cook and Kibler, 2018; Cook, 2003; Lesimple et al., 2010; Lesimple et al., 2016b). Working conditions have lasting effects and may influence the prevalence and type of stereotypic behaviours (Christie et al., 2006; Hausberger et al., 2009; Normando et al., 2002) or the emotionality (Hausberger et al., 2011). In the study by Popescu and Diugan (2017), working draught horses had a poorer welfare status than reproduction horses (breeding stallions and broodmares). In addition, the neutrophil/lymphocyte ratio was significantly higher in working horses than in breeding horses, confirming an increase in the N:L ratio in horses with poor welfare.

The prevalence of back problems is particularly high among ridden horses (see also Visser et al., 2014). In the present study, this was one of the most significant differences in welfare measures between the three riding schools and could be explained in particular by riding techniques (Dyson and Murray, 2003; Fonseca et al., 2006; Gillis, 1999; Lesimple et al., 2012). Several studies have demonstrated a link between rein tension and the horse's postural reactions (less time with a high neck with long reins, (Lesimple et al., 2010)) and behaviour (more defensive behaviour with short reins, (McGreevy and McLean, 2005) during the work session). Horses show also more relaxed and positive indicators when they are ridden in a more relaxed way (Stomp et al., 2020b).

As well as interacting with the rider during work sessions, the horse is also in daily contact with humans during routine procedures such as stall cleaning. This is a task to which little attention is paid, but which is of great importance from the horse's point of view. Indeed, Fureix et al (2009) have shown, on the same horses, that horses' reactions in the presence of the caretaker predicted their reactions to unfamiliar humans. To take this point further, we re-examined in more detail at how the caretakers interacted with the horses during this task and found differences between the three sites. Interestingly, RSC, where horses' welfare profile was the most impaired was also that for which interactions were most frequent. Horses are very sensitive to tactile stimuli, and tactile contact is not always perceived positively. Thus, repeated tactile contact by the caregiver may have been perceived negatively (Gueguen et al., 2022; Lansade et al., 2008, 2019; Rochais et al., 2014, 2023).

There are however a few limitations on our interpretations of the possible role of human actions. Thus, the possible correlates between human actions and horses' welfare state were not tested so that we can only suggest some hypotheses about them being determinant for horse welfare. Other subtle untested factors (e.g. background noise) may have differed between facilities and somewhat influenced the differences found. We found some differences in the behaviour of the three facilities' caretakers towards horses that did not seem to explain the differences observed in terms of horse welfare state. However, only one staff member was observed at each facility, who also knew the session was filmed so that these conclusions have to be considered with caution.

Impact on human safety

Interestingly, the results of our study show a similar gradient between the number of riders' falls during riding sessions and the horses' welfare state. Although we could not perform correlations between the number of falls and welfare indicators, given the relatively low prevalence of falls, we observed a lower number of falls in the facility where the horses' welfare state was better. This is consistent with the results of a study showing a negative correlation between horse welfare score and rider accidents: the horse's relative welfare score and its mounted hyperactive behaviour were negatively correlated (Luke et al., 2022). There may be several reasons for our finding: the time spent in free movement outdoors that can limit the expression of excitation behaviours by horses (e.g. Lesimple et al., 2011), the impact of riding techniques that may directly increase conflict behaviours through harsh or inappropriate actions or indirectly, because of chronic pain that may induce negative reactions from the horse (Björnsdóttir et al., 2014; Cook, 2003; Luke et al., 2023; Mellor, 2020; Uldahl and Clayton, 2019). In fact, horse behaviour, welfare and rider safety are interdependent and are not related to rider's level or horse's age (Luke et al., 2022; Ödberg and Bouissou, 1999). Several studies have shown that certain management practices generate more aggressive behaviour towards humans and more defensive behaviours during working sessions (Lesimple et al., 2011; Losonci et al., 2016; Rivera et al., 2002).

Conclusion

This study using behavioural, health and physiological measures highlights the existence of facility horse welfare profiles. It also reveals that even apparently minor differences in management practices could have a major impact on the horses' welfare state and that the quality of ridden work, which is generally not considered in studies evaluating horse welfare and its determinants, could be a major issue. This type of holistic approach to welfare assessment has many advantages over the use of one or two measures, and it has applications beyond horses. This promising approach therefore opens a whole new line of research on the welfare of domestic and other captive animals, that deserves to be developed further in future.

Supplementary material

Supplementary Material for this article (https://doi.org/10.1016/j.animal.2025.101520) can be found at the foot of the online page, in the Appendix section.

Ethics approval

Not applicable.

Data and model availability statement

The data/models were not deposited in an official repository.

The data/models that support the study findings are available from the authors upon request.

Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this work the author(s) did not use any AI and AI-assisted technologies.

Author ORCIDs

L. Gueguen: https://orcid.org/0000-0001-7434-8745.
R. Palme: https://orcid.org/0000-0001-9466-3662.
P. Jego: https://orcid.org/0000-0002-3874-9119.
S. Henry: https://orcid.org/0000-0002-1196-1507.
M. Hausberger: https://orcid.org/0000-0002-4867-5408.

CRediT authorship contribution statement

L. Gueguen: Writing – review & editing, Writing – original draft, Visualisation, Validation, Formal analysis, Data curation, Conceptualisation. **R. Palme:** Writing – review & editing, Resources, Investigation, Data curation. **P. Jego:** Writing – review & editing, Validation, Supervision, Conceptualisation. **S. Henry:** Writing – review & editing, Validation, Supervision. **M. Hausberger:** Writing – review & editing, Visualisation, Validation, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Data curation, Conceptualisation.

Declaration of interest

The authors declare no conflict of interest in this paper.

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