Managing Horses in Groups to Improve Horse Welfare and Human Safety

Reactions to Mixing and Separation

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Doctoral Thesis
Swedish University of Agricultural Sciences
Uppsala 2010

Acta Universitatis agriculturae Sueciae
2010: 87

Cover: First encounter on pasture (*photo: E. Hartmann*)

ISSN 1652-6880
ISBN 978-91-576-7532-3

Print: SLU Service/Repro, Uppsala 2010

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Abstract

The aim of this thesis was to investigate whether specific anecdotal concerns related to keeping horses in groups are supported by science and, if so, provide scientifically based recommendations that could be implemented in practice.

The aim of studies I and II was to identify methods for mixing unfamiliar horses that could minimise aggressive interactions and associated risk of injury. Results of study I revealed that pre-exposure of young horses in neighbouring boxes tended to lower contact-aggression (e.g. kicks, strikes) and biting behaviour in particular was reduced when the same pair of horses subsequently met in a paddock. This was not found when older horses were mixed (study II). Aggressive behaviour received by a new horse was not significantly different in meetings when it met one other horse compared to meeting two unfamiliar horses at the same time.

Removing a horse from a group of four in study III was generally unproblematic. Most horses approached the handler when she was catching the horse and while standing with it in the middle of the paddock. Thus, potential risk may be higher in situations when the handler remains relatively stationary, as other horses of the group have time to approach. Rank did not influence the number of horses following to the paddock gate and interactions between horses were rare.

Since horses naïve to social separation may be more difficult to handle away from the group, the objective in study IV was to investigate whether the initial presence of a companion horse would modify responses to separation. Results revealed no significant differences in heart rates and the number of training sessions required when the horses were subsequently trained in the absence of the partner compared to horses trained alone from the start.

In summary, results give little support for the original areas of concerns about mixing and separating horses. Risk of injury to both horses and humans should not be overestimated when handling horses in groups, but being aware of potential risk situations and being able to react accordingly is likely to increase horse welfare and human safety.

Keywords: equine, behaviour, welfare, housing, mixing, aggression, injury, separation, habituation, learning

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To my parents

We can never really know what it is to be a horse. Our best hope is to be as objective as possible and to balance our emotional attachment to the horse with our tradition of rational enquiry. To do this, we need to redefine the way we think about the horse, using sciences of behaviour.

Andrew McLean (Academic horse training, 2008)

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List of publications

The present thesis is based on the following papers, referred to by their Roman numerals (papers also correspond to studies I to IV):

I Hartmann, E., Winther Christensen, J., Keeling, L.J. (2009). Social interactions of unfamiliar horses during paired encounters: Effect of pre-exposure on aggression level and so risk of injury. *Applied Animal Behaviour Science* 121, 214-221.

II Hartmann, E., Rundgren, M., Keeling, L.J. (in press). Comparison of 3 methods for mixing unfamiliar horses (*Equus caballus*). *Journal of Equine Veterinary Behavior: Clinical Applications and Research*.

III Hartmann, E., Søndergaard, E., Keeling, L.J. Identifying potential risk situations for humans when removing horses from groups. Manuscript.

IV Hartmann, E., Christensen, J.W., Keeling, L.J. (in press). Training young horses to social separation: Effect of a companion horse on training efficiency. *Equine Veterinary Journal*.

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Introduction

The group housing of farm animals is widely applied in practice, and scientists have investigated numerous aspects of it to help improve animal welfare and human-animal interactions at the group level. However, compared to the amount of scientific literature available in farm animals such as cattle, pigs and laying hens, less has been done when it comes to the management of horses kept in groups. This is despite the fact that keeping horses in groups is now recognised to best fulfil their physical and behavioural needs, especially their need for social contact with conspecifics. Although husbandry conditions for horses have improved over the last decades, many horses do not have a social life, mainly due to their owners concerns about group housing.

This thesis, therefore, aimed at providing some insight into whether specific concerns related to keeping horses in groups are justified and, if so, provide scientifically based solutions that could be useful in practice to improve horse welfare and human safety.

Group housing of horses

Horses are adapted to live in social groups. The social behavioural repertoire of feral and free-ranging horses has been extensively described (Feh, 2005; McDonnell, 2003; Waring, 2003; Goldschmidt-Rothschild & Tschanz, 1978; Feist & McCullough, 1976). The same social behaviour patterns can also be observed in domestic horses under human husbandry conditions when given the opportunity (VanDierendonck *et al.*, 2004; Christensen *et al.*, 2002b; McDonnell & Haviland, 1995). Thus, domestication has had little if no effect on sociality (Waran, 2001).

Because horses are highly social animals by nature, housing them in groups is recommended. For example, previous studies have highlighted the benefits of group housing horses compared to individual housing on horse-horse (Bourjade *et al.*, 2008; Christensen *et al.*, 2002a) and horse-human relationships (Søndergaard & Ladewig, 2004; Rivera *et al.*, 2002). Furthermore, it has been shown in horses as well as in other social species that lack of social experience early in life can affect adult social behaviour (Bøe & Færevik, 2003; Olsson *et al.*, 1999; Veissier *et al.*, 1994). Christensen *et al.* (2002a) have found that depriving 2-year old horses of social contact with conspecifics during a 6-month period resulted in increased aggression as well as less submissive behaviour when later mixed with other horses. Showing undesirable social behaviour when horses are introduced to other horses may reduce their chances of being kept in groups. Although, it is only through group living that horses can develop appropriate social skills (Ladewig *et al.*, 2005). Other studies have pointed out that confinement and lack of social contact are associated with the development of stereotypies such as weaving, box walking or crib-biting (Henderson, 2007; Cooper & Albentosa, 2005; Bachmann, 2002; Nicol, 1999; McGreevy *et al.*, 1995b). Providing horses with visual contact to a stable companion or even a mirror can reduce the incidence of these repetitive behaviours (Mills & Davenport, 2002; McGreevy *et al.*, 1995a). Stereotypic behaviours or other abnormal behaviours that occur in stabled horses have never been observed in their free ranging counterparts (Feh, 2005).

Evidently, modern stable design should accommodate the horses' social needs. This is, for instance, acknowledged in the Swedish animal welfare regulations and guidelines on the keeping of horses (DFS 2007:6, Ref. L 101). It states that horses should be kept with other members of their species (chapter 2, section 1), and that horses up to 12 months of age shall be given free exercise together with at least one other horse (chapter 5, section 1). However, the majority of horses are still housed singly for large parts of the day for ease of management, monitored feeding and exercise regimes. Elite performance horses in particular are often confined in single boxes with limited or no physical contact to conspecifics (Henderson, 2007). This was also evident in a Swedish survey from Svala (2008), where 49 yard owners (approximately 1000 horses) were interviewed, showing that stabling in individual loose boxes and single turnout in paddocks was common practice for competition horses (dressage, jumping, eventing, trotting). Bachmann & Stauffacher (2002) reported from a survey in Switzerland (622 respondents, 2536 horses) that 83.5% of horses were kept individually and only 16.5%

were housed in groups. A Danish survey indicated similar conditions (157 respondents, 1698 horses). Most horses were housed individually in boxes (65.1%) or tie stalls (10.9%), and only 24.1% of horses were kept in groups (Søndergaard *et al.*, 2004). The question remains of why group housing of horses is not widely applied in practice? Although there is already a considerable amount of scientific documentation on the benefits of group housing horses compared to the more traditional housing in single boxes, there are still some unanswered questions and concerns.

Recent research has shown that injuries from fights or play in group housed horses have rarely been a problem (Jørgensen *et al.*, 2009; Lehmann *et al.*, 2006; Grogan & McDonnell, 2005). Nevertheless, one of the main arguments by horse owners against keeping horses in groups is the risk of horses injuring each other during social interactions, especially when meeting for the first time. A different argument against group housing is the perceived difficulty of removing a horse from its group. It is claimed that horses in a group may interfere when one member of the group is being removed, thus putting the handler in a vulnerable position. Another common claim is that horses not used to being separated from peers may be more difficult to handle away from the group and will react more to being temporarily alone than horses kept singly. Indeed, all events inducing social separation in gregarious species are likely to provoke behavioural and physiological changes (Alexander & Irvine, 1998; Mal *et al.*, 1991). Therefore, it is a matter of finding appropriate training methods to habituate naïve horses to tolerate social separation rather than restricting full social contact as a precautionary measure to avoid undesirable behaviour.

Introducing new group members

Domestic horses are faced with social challenges throughout their lives, given that social companionship may change repeatedly due to ownership transfer, horses being moved to another yard or being regrouped. Change of group membership is a natural process in the wild (Boyd & Keiper, 2005; Waring, 2003; Khalil & Kaseda, 1998; Kaseda *et al.*, 1997). For instance, fillies and colts disperse between two to three years of age to join mixed juvenile groups or bachelor groups, and in the case of young females also other harem bands (Feh, 2005). Individuals will leave voluntarily or will be chased away, usually by the harem stallion (Waring, 2003; Goldschmidt-Rothschild & Tschanz, 1978). Nevertheless, harem bands are rather stable over time and may not be readily open to admitting strangers (Waring, 2003).

Social cohesion within the band is maintained through a social hierarchy (measured as dominance or avoidance order) and through affiliative relationships (Boyd & Keiper, 2005; Feh, 2005; Fraser, 1992). The more stable the social grouping and the stronger the social bonds between individuals of the same social unit, the more horses display minimal aggression to promote group cohesion (Waring, 2003). It is the frequency of mixing, space restrictions and limited feed resources that constitute a social challenge for domestic horses compared to natural conditions (Knubben *et al.*, 2008; Fürst *et al.*, 2006). This may account for the higher rates of aggression often reported for horses under human husbandry (Waran, 2001).

In general, meeting unfamiliar conspecifics usually leads to immediate overt aggression (Erhard & Mendl, 1997; Tennessen et al., 1985; Addison & Baker, 1982). This is because the group's social structure becomes disrupted and individuals seek a new one or defend their old position. Since dominance status in horses is positively correlated with time of residency (VanDierendonck *et al.*, 1995), newcomers are normally at a disadvantage in that resident horses are likely to behave aggressively towards them (Alexander & Irvine, 1998). Responses of resident animals to unfamiliar conspecifics, in particular agonistic behaviour, have been widely studied in pigs in so called resident-intruder tests (D'Eath, 2002; D'Eath & Pickup, 2002; Erhard & Mendl, 1997), but also in cattle (Bøe & Færevik, 2003; Knierim, 1998; Kondo *et al.*, 1984), hamsters (delBarco-Trillo *et al.*, 2009), rabbits (Farabollini *et al.*, 1991) and primates (Brent *et al.*, 1997). Mixing unfamiliar animals does not only result in changes in behaviour, it can also elicit physiological changes such as an increase in cortisol levels and heart rate (Pollard *et al.*, 1993; Zayan, 1991). Moreover, since reactions to intruders are usually aggressive, overt aggression can increase the risk of physical injury which remains a major welfare concern. However, as the mixing of unfamiliar animals cannot always be avoided, the question arises of how introduction of newcomers can best be carried out so that the level of aggression and associated risk of injury is minimised.

Different mixing methods have been investigated, such as pre-exposing animals in neighbouring pens to permit first contact through pen partitions before allowing free interaction in the same enclosure (Jensen & Yngvesson, 1998; Fraser, 1974). Other methods include introducing one individual versus introducing pairs or groups of animals into an established group (Gygax *et al.*, 2009; Knierim, 1998), chemical intervention with sedative drugs or chemicals to mask individual smell (Luescher *et al.*, 1990), or mixing animals repeatedly to adapt them to future grouping processes (Raussi *et al.*, 2005). To my knowledge, no experimental work has been done to study methods of introducing horses to each other, thus management relies on anecdotal knowledge and subjective experience. In practice, methods vary widely; some suggest allowing horses to familiarise in neighbouring boxes (Zeeb & Pollmann, 1996) while others confront the newcomer with the entire group at once (Pollmann, 2006). Therefore, finding methods that minimise aggression level at mixing and lower associated injury risk would be useful in practice.

Pre-exposure or direct introduction

During pre-exposure, it is assumed that animals have the possibility to gain information about the unfamiliar opponent through visual, olfactory, auditory and restricted physical assessment through pen partitions. This is likely to modify social interactions when the individuals meet freely later on. The idea of information gathering originated from game theory models. These models examined how different assessment strategies might be used by each contestant to adjust the costs and benefits to engage in a fight with the perceived value of a resource to reduce energy expenditure and risk of injury or even death (Arnott & Elwood, 2009; Maynard Smith, 1974).

That opponents assess each other prior to contests has been confirmed in experimental tests in, for example, rainbow trout (*Oncorhynchus mykiss*) by Johnsson & Åkerman (1998). The authors could show that rainbow trout that had the chance to observe the fighting ability of future opponents by watching their success against other individuals, settled conflicts faster and with less aggression than those that were confronted with a previously unseen fish. Another approach was taken by Jensen & Yngvesson (1998) who studied the effects of exposing pigs in neighbouring pens for 24 hours to allow first assessment before mixing. They found that contests were significantly shorter in pigs that had met in neighbouring pens compared to control animals with no such prior exposure. Similar results were obtained in a study by Fraser (1974) where vigorous biting at mixing decreased when pigs were previously housed in neighbouring pens.

The benefits of pre-exposing unfamiliar animals in neighbouring pens have also been confirmed in zoo- and laboratory mammals. Burks *et al.* (2004)

studied pre-exposure in female elephants (*Loxodonta africana*). First, elephants were allowed to have visual, auditory and olfactory contact by placing them in opposite stalls across a hallway. Later, limited tactile contact was permitted by putting the animals in adjacent stalls before they were placed together. This sequential introduction method managed aggression more effectively than the non-sequential introduction method which was based on subjective decisions by caretakers. In the sequential method, empirical behavioural data were collected and animals were only allowed to proceed to the subsequent introduction step when there was a significant reduction in aggression. delBarco-Trillo *et al.* (2009) tested the effect of familiarity in hamsters (*Mesocricetus brandti*) kept under laboratory conditions. Familiarity was obtained by housing pairs of male hamsters, matched by age and weight, in a cage that was divided by a wire-mesh barrier. The barrier allowed hamsters to see, hear, smell and touch each other, but it prevented them from physically fighting. The hamsters were housed in this cage continuously for 2 days. For testing, one male was placed in an arena either together with an unknown partner or with the familiar cohabitant. The familiarity achieved through pre-exposure resulted in a decrease in the number of fights, the percentage of time spent fighting as well as an increase of the latency to engage in a fight (delBarco-Trillo *et al.*, 2009).

In summary, giving unfamiliar animals the opportunity to familiarise in an environment that allows first assessment, yet restricts physical contact has been proven successful in reducing aggression under controlled conditions. Therefore, this approach may also serve as a valuable tool when introducing unfamiliar horses to each other.

Paired encounter or direct group introduction

The arrival of one horse at a time at a yard may be the most common situation in practice. So the question arises as to whether or not it is advisable to introduce a newcomer to a resident group at once, or to let the new horse meet each group member separately. Indeed, there is controversy among horse owners as to which method has the greatest potential to lower aggressive encounters, and there is no scientific based recommendation available. Results from a German survey revealed that the new horse was immediately put together with the entire resident group in 65% of the yards (64 respondents, 1165 horses) (Pollmann, 2006). In the remaining yards, the newcomer was introduced to some resident group members separately, taking into account the rank of the resident horse or its general friendliness towards strangers. Unfortunately, data from Pollmann (2006) did not specifically give information on why yard owners preferred a certain mixing method over another. Possibly, the owners' decisions may have been based upon merely positive previous experiences when introducing the newcomer to the entire group at once.

Brent *et al.* (1997) compared the effect of introducing one chimpanzee (*Pan troglodytes*) to a group directly with mixing it with one other individual (paired encounter). Brent *et al.* (1997) recorded lower levels of aggression when exposing one chimpanzee to the group directly. The authors speculated that the higher aggression level during paired encounters was a result of the more defensive behaviour of the resident animal due to the lack of social support which would otherwise be present in group introductions. Social support could lower stress responses by the mere presence of, or interactions with familiar conspecifics (Wiepkema & Schouten, 1990). Furthermore, a resident animal could join a group member and help in defeating an intruder, or it may want to stop the partner from interacting with the intruder. This behaviour, when a third animal is actively intervening in a dyadic interaction, is termed 'intervention'. Interventions have mainly been studied systematically in primates (Roeder *et al.*, 2002; Petit & Thierry, 1994), whereas quantitative studies in equines are sparse. Schilder (1990) recorded interventions in captive zebras and VanDierendonck *et al.* (2009) studied interventions in a herd of Icelandic mares and geldings. There seems no consensus about the possible functions of interventions. One suggestion is that they may serve to safeguard already existing social relationships (Schilder, 1990; VanDierendonck *et al.*, 2009).

Studies of triadic interactions when unfamiliar animals meet would be of interest as they could give insight into how a situation is perceived by the individuals involved. This would be relevant from a practical perspective when introducing a new horse to a pair of resident horses or to more than two horses at once.

Disrupting the contact to the group

Leaving the group

As in other group living species, horses in free ranging populations form relatively cohesive units and show synchronised activity and coordinated patterns of movements to promote group cohesion (Waring, 2003; Fraser, 1992). In a domestic setting, when horses are kept in relatively stable social groups, it is, therefore, likely that at least some horses initially follow a horse that is being removed from its group. These horses may simply follow at a distance, but they could also physically interact with the horse being removed and the handler may get accidently in the way, or they may interfere in the catching process by approaching the horse and handler in a threatening manner.

Apparently, the potential problems or risks associated with catching and removing a horse from its group have hardly received any scientific attention. Although Jørgensen *et al.* (in press) put forward that the risk for the human of removing a horse from its group is possibly overestimated. They observed different occasions when a familiar person (the owner or caretaker of the horse) entered the enclosure of a group of horses, caught one horse and led it away from the group and out of sight from peers. Ninety-six percent of the target horses followed the handler without resistance. In 75 % of the tests (total 100 tests), other horses in the group did not interact with the target horse and handler, and in only three cases, horses threatened the horse being led. Verrill & McDonnell (2008) have studied compliance with catching in 104 domestic and semi-feral horses and ponies approached at pasture. They focused on the effect of human-to-horse eye contact on catching outcome; hence, no further reference was made to other conditions that could have influenced the catching process. Almost all subjects were either consistently approachable or unapproachable regardless of maintaining or avoiding eye contact (Verrill & McDonnell, 2008).

Considerably more work has been done with regard to group decision-making processes around collective movements in animals. The underlying causes for individuals to make the decision to follow or not to follow an initiator of movement are debated in the scientific community. It has been proposed that possibly a combination of different rules, including social status and affiliative relationships affect following behaviour (King, 2010; Petit & Bon, 2010). Bourjade *et al.* (2009) stated that no individual can be identified as a consistent leader. Horses rather share their decision to move through several pre-departure behaviours (behaviour performed prior to the departure of the initiator of movement). Furthermore, Bourjade (personal communication, 2010) pointed out that the action of following may be more relevant than the action of leading, because in most cases leaders are incidental. Thus, collective movements can simply be triggered by one individual moving away from the group regardless of its leadership qualities (Pillot *et al.*, 2010).

The underlying mechanisms for collective movements have yet to be studied in domestic horses kept in groups where there is more forced movement initiated by humans when individuals are being removed from peers. Other factors may be influential in this process, such as group stability, motivation to follow in expectation of feed or handling experience. A previous positive interaction with humans has been shown to increase the likelihood of horses approaching a human (Hausberger *et al.*, 2008) and this has also been shown in farm animals (Hemsworth, 2003). Studies in horses mainly assessed the reactions of a single horse approaching a motionless person (Lansade *et al.*, 2004; Seaman *et al.*, 2002), whereas experimental approaches transferred to a group situation are rare (Søndergaard & Halekoh, 2003). Although this would be highly relevant to study since a horse's motivation to approach a person may also be affected by how other horses of the group react to that person, as was pointed out by Søndergaard & Halekoh (2003). Given that positive handling increases the likelihood of horses approaching a human entering a paddock, it could then be speculated that this could also affect their motivation to follow. On the other hand, one could also argue that increased experience of leaving and returning decreases the horses' interest to follow a horse that is being removed from its group. This highlights the need for further investigation of reactions of horses towards a human when horses are kept in groups.

Learning to be alone

Living in groups has definite survival value for horses and other social species because of mutual vigilance and protection against predators (Manning & Dawkins, 1998; Waring, 2003). Consequently, any situation that is different from the horses' natural tendency to rejoin the group (i.e. being isolated from conspecifics and from the protectiveness of the group) can be experienced as negative. For example, behavioural signs of stress caused by disrupting contact to peers can be reflected in increased locomotion and vocalisation (Lansade *et al.*, 2008; Harewood & McGowan, 2005; Bagshaw *et al.*, 1994; Mal *et al.*, 1991), but also in physiological changes such as increased heart rate and cortisol levels (Boissy & Le Neindre, 1997; Hopster & Blokhuis, 1994; Pollard *et al.*, 1993; Carbonaro *et al.*, 1992).

Lansade *et al.* (2008) cited neighing (whinnying) in horses to be specific to a situation of separation and isolation and it correlated with defecation, moving around in the pen and taking a vigilant posture. Correspondingly, first time stabling in isolation elicited marked behavioural responses (increased vocalisation and locomotion) compared to when the same horses were kept in a group prior to isolation (Harewood & McGowan, 2005). Other stress-related behaviours like pawing and snorting were all displayed significantly more frequently in individually than in pair housed horses (Visser *et al.,* 2008). However, the magnitude of the stress response can vary considerably between individuals due to experiences during early development, genetics, temperament or age, and is continuously modulated (Moberg, 2000). Jørgensen *et al.* (in press), for instance, recorded a higher arousal and increased locomotion in young horses (1 to 4 years old) compared to older horses when they were placed out of sight from remaining group members. The authors explained this difference by the lower level of experience to social separation in these young horses.

Behavioural responses to social separation influence the horses' manageability. For example, if horses react with undesirable behaviour (e.g. stopping, balking, rearing) in attempts to re-unite with the group, they may be difficult to handle and jeopardise not only their own, but also the handlers' safety. Moreover, it had been proposed that more reactive horses tend to take longer to learn a task (Visser *et al.*, 2003; Heird *et al.*, 1986; Fiske & Potter, 1979) and that stress can impair learning ability (Murphy & Arkins, 2007; Nicol, 2002; Mendl, 1999). Heird *et al.* (1986) compared the performance of sixteen horses in a maze task, grouped according to reactivity. Horses scored as highly reactive required more trials to reach learning criterion and achieved a lower percentage of correct responses than did the less reactive horses. Horses studied by Visser *et al.* (2003) were tested repeatedly in two instrumental conditioning tasks. Their results indicated that high levels of reactivity may have been associated with non-performance in some horses, i.e. horses that did not complete daily test sessions had higher mean heart rates and expressed more agitated behaviours (pawing, neighing).

At some point, a horse's reaction towards separation and temporary isolation from conspecifics should diminish to allow successful learning and safe handling to take place. Therefore, appropriate training methods are warranted that lower such reactions and habituate horses to social separation. Habituation is a simple form of non-associative learning and leads to a decrease in responsiveness to a stimulus after repeated exposure (McGreevy, 2004; Cooper, 1998).

One approach to reduce reactions to separation is to test animals in the presence of peers. For example, Færevik *et al.* (2006) tested calves' reactions to separation in a novel environment once alone and once together with a companion. The calves' reactions were less pronounced when they were with a familiar partner than when they were alone. McGee & Smith (2004) provided evidence that even the presence of humans can reduce stress responses in foals temporarily separated from their dam. Furthermore, Boissy & Le Neindre (1990) demonstrated that learning performance in an operant task was improved when conspecifics were present. Thus, there is clear evidence that behaviour of individual animals can be influenced by the presence of conspecifics (Nicol, 1995). It is argued that this could also aid the habituation process of training naïve horses to tolerate being separated from their social group.

Moreover, an animal's perception of a frightening stimulus or situation could be switched to a more positive outcome by engaging the animal in an alternative behaviour, such as feeding to replace an undesired response (counter conditioning). Counter-conditioning has been reported to be an effective behaviour modification technique in horses (McGreevy & McLean, 2005; Gough, 1999).

Aims of the thesis

The aim of this thesis was to address experimentally some of the anecdotal assumptions related to keeping horses in groups and to develop practical solutions that may help to safeguard horse welfare and human safety. The specific aims were:

- To evaluate whether different mixing methods (pre-exposing horses in neighbouring boxes before mixing them in a paddock, and introducing a single horse to a pair of resident horses instead of one by one) have the potential to lower aggressive interactions and associated injury risk between horses meeting for the first time.

- To identify situations that constitute a risk for the handler when a horse is being removed from its group by studying social interactions between horses and between horses and the handler. Furthermore, to study whether the number of horses following differs depending on the rank of the horse being led or whether a single or a pair of horses is being removed.

- To study two methods of training horses to tolerate social separation (initially with a familiar companion horse or alone from the start) and evaluate which of the methods is most effective with regard to the number of training sessions required to succeed in the learning criterion and in terms of decreasing stress levels as measured by heart rate.

Materials and methods

Animals, management and housing

Three of the studies (I, III and IV) were carried out at the research centre Foulum in Denmark. The horses used in these studies were obtained from different Danish breeders, and they were returned to the studs after completion of the studies. They were all Danish Warmblood and not older than 2 years (fillies). The common practice at the studs was to wean the foals at around 4 months of age and to keep them in single sex and same age groups until they were 3 years old, when they were started to be trained or were sold. The fillies were relatively unhandled upon arrival at the research facility, as handling at the studs was limited to veterinary treatment and hoof care. Thus, before any experimental testing could start, the horses were trained to respond to lead pressure, based on a combination of negative (release of lead pressure) and positive (feed reward) reinforcement. They were furthermore habituated to the test environments and to wearing heart rate equipment in study IV. Since horses were reared in groups, they were naïve to social separation from peers.

Study II was carried out at the Department of Clinical Sciences at the Swedish University of Agricultural Sciences in Uppsala, Sweden. In contrast to the Danish Warmblood fillies used in the other three studies, the mares used in study II were older (6 to 18 years) and of a different breed (Swedish Standardbred). Therefore, their level of experience with regard to human handling and training, including their experience of meeting other conspecifics was presumably very different. Most horses had been used for breeding, riding or racing purposes before arrival at the research facility.

Mixing young horses (study I)

The 2-year-old Danish Warmblood fillies (n = 20) were kept in groups of 10 horses each on grass pastures for 24 hours/day. The two groups were separated by a 2-m wide corridor between pastures which did not allow any physical contact over the fence. Consequently, only horses in the same group were considered to be familiar with each other.

There was free access to water on pasture and the horses received supplementary feed (barley and molasses) only during habituation training. All horses were unshod.

Mixing older horses (study II)

Sixteen Swedish Standardbred mares were used (mean age 11 ± 4.4 SD), of which half had been kept at the research facility for at least one year before the start of study II (called 'resident' horses). These horses were used for training veterinary students. They were kept in a group on grass pasture during the days and in a stable in single boxes during the nights. Therefore, all resident horses were considered to be familiar with each other.

The other half of the horses were purchased from private owners before the start of testing. They were called 'unfamiliar' horses because they were all unfamiliar to the resident horses. The unfamiliar horses were kept in a separate part of the stable, out of sight from resident horses during the nights. During daytime, pairs of unfamiliar horses were kept together outside, but there was no physical contact with the resident horses although visual and auditory contact could not be avoided. The unfamiliar horses left the facility after test completion and new horses were brought in (2 horses arrived at a time).

All horses were fed hay or hay silage and concentrate feed (barley) and had free access to water. They were all unshod.

Removing horses from groups (study III), training to social separation (study IV)

A total of 32 Danish Warmblood fillies were used in study III and IV, of which 18 horses were yearlings and 14 horses were 2 years of age. They had no previous experience of being separated from peers which was an important pre-requisite for participation in these two studies. The horses were split into 8 groups, containing 4 horses each, approximately one month before testing. Allocation to groups was done based on stud origin, sire and age: 6 groups contained 2 one-year-olds and 2 two-year-old horses; 2 groups contained 3 one-year-olds and one 2-year-old horse.

The groups were kept 24 hours/day on grass pastures, measuring 3000 m^2 each. There was free access to water and supplementary feed (mixture of hay and straw) was provided daily on pasture after the experimental testing. The horses were all unshod.

Test environments and testing

The horses were habituated to the test environments and test procedures before the start of the studies. All experimental tests were video recorded by cameras placed outside the test areas.

Study I and II

Study II was a follow up experiment of study I to evaluate whether the two mixing methods tested in the latter would give similar results when testing horses of different age, breed and experience. The experimental procedures were therefore kept identical, and test environments were similar, too.

Experimental tests in study I and II were conducted in single boxes indoors and in a paddock outdoors, comparable to conventional housing facilities for horses. The boxes measured 9 m^2 each. The partition between the boxes contained an opening that allowed horses to put their head in the neighbours' box (at 1.2 m off the ground in study I, 1.4 m in study II). The remaining parts were constructed of solid planks (up to 1.2 m) and vertical bars (spaced 8 cm apart) in study I, whereas in study II, there were no bars and limited physical contact was only possible through the opening. Boxes did not contain any bedding and 1 kg of hay was provided in the front corner furthest away from the opening. The test paddock was a grass paddock in study I (20 m x 40 m) and a sand paddock (20 m x 20 m) in study II, fenced in by electric wire and metal bars, respectively. Test boxes were located 20 m (study I) and 40 m (study II) away from the test paddock.

To evaluate which mixing method had the potential to lower aggressive interactions, horses in study I experienced two treatments: two horses met in neighbouring boxes for 5 minutes (B), subsequently the same horses met in the test paddock for 10 minutes (BP), and two horses only met in the test paddock for 10 minutes without prior box exposure (P) (figure 1). In study II, a third treatment was added: two resident horses met one unfamiliar horse in the test paddock for 10 minutes (PP). This test was preceded by another 10-minute test (Pre-PP) during which only the resident horse pair was released in the test paddock (figure 1). The resident horses were allocated to pairs according to similar age.

A total of 60 meetings were arranged in a balanced order in study I, whereby always one horse from one group met six horses from the other group and vice versa. Each horse experienced treatments 1 and 2 three times, and it was alternated between treatments so that none of the horses experienced a treatment twice in a row. Horses were tested once per day (5 pairs per day) and had a one-day break between tests.

A total of 62 tests were carried out in study II, whereby each unfamiliar horse was tested once per day, experiencing the three treatments twice in a balanced order. Resident horses also experienced treatments 1 and 2 twice, but since they were used as pairs in the third treatment, each resident horse experienced this treatment four times.

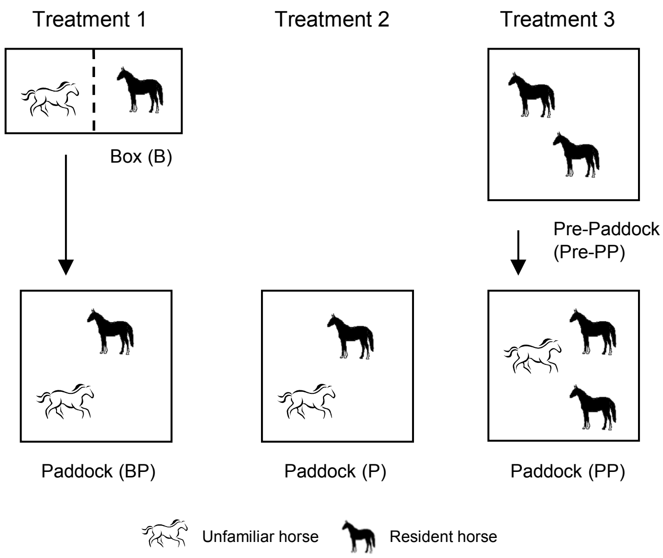

Figure 1. Layout (not scaled) of treatments. In study I, horses met in neighbouring boxes (B), afterwards in the paddock (BP), and horses only met in the paddock (P). In study II, treatments 1 and 2 were identical to study I. In treatment 3, two resident horses met one unfamiliar horse in the paddock (PP) after the resident horses had been together in the same paddock (Pre-PP).

When horses were allowed to make first contact in neighbouring boxes, they were led by one handler each into the test boxes. They were turned around to face the door before being released at the same time. Handlers stepped away from the door and stood motionless for the duration of testing (5 minutes). After the box test, horses were immediately led to the outdoor test paddock. They were placed apart from each other in opposite corners of one side of the paddock (when two horses met). However, when three horses met in the paddock in treatment 3 (study II), the unfamiliar horses' position in the paddock corners was alternated; consequently all horses entered the paddock in a randomised order, but the unfamiliar horse was never placed in between resident horses. Horses were always turned around to face the fence and were released simultaneously. Handlers left the paddock and waited motionless outside (10 minutes).

All social interactions between horses were analysed in the same manner in study I and II to make results comparable. Social encounters in tests where three horses met in study II were split into interactions occurring between resident and unfamiliar horses, and interactions occurring between resident horses only. This was done to make the data comparable with the meetings where only two horses met. A social interaction consisted of a sequence of single behaviours by the sender horse, the receiver horse and the sender's response to the receiver's behaviour. Single behaviours were categorised into aggressive, submissive and non-aggressive behaviour (table 1). Aggressive behaviour was further subdivided into non-contact and contact aggression. Contact-aggression (e.g. kick) was argued to constitute an increased risk of injury as physical contact may occur compared to the behaviours assigned to non-contact aggression (e.g. kick threat).

Table 1. *Overview of the single behaviours within each behaviour category and sub-category*

Category	Sub-category	Behaviours
Aggressive behaviour	Contact aggression	Bite, kick, strike, push
	Non-contact aggression	Ears laid back, threat approach, bite threat, kick threat, strike threat, attack, chase, follow aggressively, present rump
Submissive behaviour		Avoid, retreat, flee
Non-aggressive behaviour		Approach, body sniff, genital sniff, nasal sniff, follow friendly, social groom

We were aware of the distinction one can make between defensive (e.g. kick) and offensive (e.g. bite) aggression (Feist & McCullough, 1976). However, we were only interested in whether or not aggressive behaviour carries a potential injury risk. Thus, it was argued that a kick, whether it was given in defence or offense, increases the risk of physical injury.

Other variables were analysed, for example the latency to first physical body contact in the paddock (study I, II) and whether horses used the opening in the box (study II). If horses interacted through the opening, interactions were categorised into aggressive (one horse responded with submission to a received aggression or with counter-aggression), non-aggressive (both horses showed no aggression) or mixed interactions (one horse was aggressive or submissive and the other horse showed no aggression). Furthermore, it was recorded in both mixing studies whether horses showed inappropriate aggressive behaviour, meaning that a sender horse responded with further aggression to a receiver's submission. This was classified as inappropriate because it would contradict the hypothesis that horses display the minimal amount of aggression a situation requires.

It was also of interest to study the occurrence of intervention behaviours when three horses were allowed to interact in a paddock (study II). Interventions were defined to occur when a third horse (intervener) positioned itself between two other horses. The intervener usually exhibits aggressive behaviour towards one or both horses of the dyad. For each intervention, the type of social interaction that was intervened was recorded, i.e. non-aggressive or aggressive approaching interactions, non-aggressive and aggressive interactions, and neutral which was defined when horses of the dyad did not seem to interact.

Horses were always checked for injuries after testing if the social interactions had involved any physical contact.

Feeding tests were carried out to determine the dominant horse of the resident horse pair in study II. Horses were allowed to feed concentrates from a container which was large enough to give the two horses access at the same time. Tests lasted for 2 minutes and were repeated 5 times during the study. Observations were made of aggressive behaviour resulting in one horse displacing the other horse from the feed container. The horse that displaced the partner most frequently was considered to be the dominant horse.

Study III

The aim of study III was to identify potential risk situations for the handler when separating a horse (the target horse) from its group. A risk situation was defined by the closeness of loose horses in the group or by any physical contact with them.

Each horse from a group of four was removed once alone (single treatment) and once together with a companion (pair treatment). This was replicated after a 4-week interval during which the horses were used for study IV. In total, 96 tests were conducted; 64 according to the single, and 32 according to the pair treatment. Pairs of horses were allocated at random, with the constraint that pairs consisted of one yearling and one 2-year-old horse. The exceptions were two pairs that consisted only of yearlings. The horses were separated from their group in a random order, and tests rotated between groups in a balanced order. In the pair treatment, horses were always led by one handler each.

A test was divided into 5 phases, consisting of approach, catch, post, walk, and gate (figure 2). In phase 1, the target horse was approached by the handler in normal walking speed. When the handler reached the horse (phase 2), the lead rope was attached to the halter and the horse led to a post placed in the centre of the paddock. At the post, horse and handler remained there for 30 seconds (phase 3) before the handler led the horse back to the gate (phase 4). The person operating the gate opened it when horse and handler were within 5 m of the gate (phase 5).

The post was set in the middle of the paddock to standardise the distance horse and handler had to travel to reach the paddock gate. At the post, the handler was not allowed to initiate any contact with the target horse or the other loose horses around. However, the handler was allowed to displace a horse by raising an arm or by pushing a horse physically away during any phase to increase or maintain distance. When the target horse had been taken out from the paddock, it was walked 5 m away from the gate and was returned to its group after 1 minute. The same test procedures were applied when taking out two horses at the same time. When two horses were led out simultaneously, it was aimed to keep a distance of at least 2 m between horses during all phases.

Social interactions between horses (based on behaviours used in study I and II), the horses' behaviour towards the handler and the handler's actions towards the horse (swing, push) were recorded. In addition, the number of horses close (< 2 m, 2 m - 5 m) to the handler and horse being led was documented.

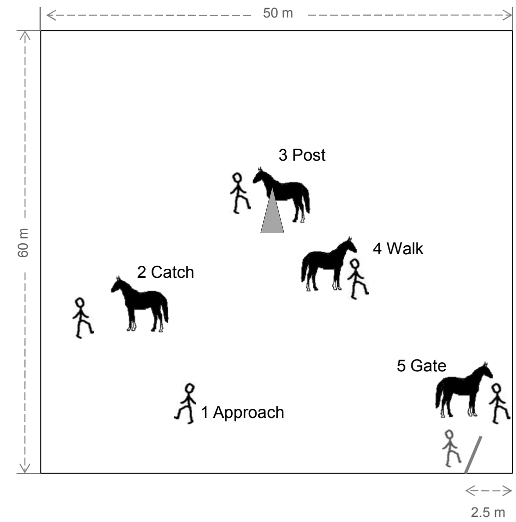

Figure 2. Overview (not scaled) of the 5 different phases when removing a single horse from its group. One extra person was responsible for opening and closing the paddock gate.

Similar to study II, feeding tests were conducted to identify the horse highest in rank during a competitive situation at the feed trough. This was done at group level, and 3 tests were carried out in each group at different times during the study. Each test lasted 5 minutes. The horse that monopolised the feed trough longest and displaced the other three horses from the group most frequently was recorded as the dominant horse.

Study IV

To study the effect of training horses to tolerate social separation, horses were divided into two groups: half of the horses (n = 16) were trained singly from the start, the other half (n = 16) were initially trained together with a familiar companion (n = 8 pairs). If these horses reached learning criterion (step 3) as a pair, they proceeded being trained alone, repeating the training steps.

The training was split into 3 training steps. In training step 1, the handler stayed with the horse at the feed container placed in the stable corridor (figure 3). In step 2, the horse was led to the arena and allowed to feed from the container while the handler was present.

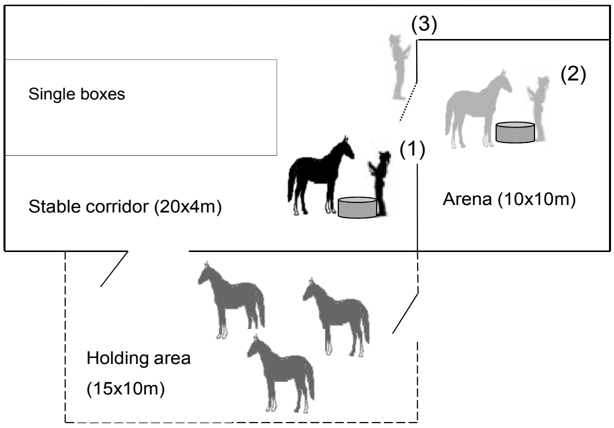

Figure 3. Layout (not scaled) of the three training steps for horses trained alone in study IV. Horses of the group that were not trained were kept in the holding area outside the stable building.

In training step 3, the handler led the horse to the feed container and left it there alone, waiting outside the arena in sight of the horse. Training steps for horses trained in pairs were identical, but two feed containers were provided. The same pairs of horses were used as in study III.

A horse succeeded in a training step when it was feeding for at least 90 seconds out of 120 seconds during one session. It was then allowed to proceed to the next training step. If a horse did not succeed, it had to repeat the same step. The decision of whether or not a horse succeeded was taken immediately after each session, based on the time spent feeding. The timing was done by a person other than the handler. Horses were returned to the holding area between training sessions. Five sessions were performed per day and horse, and a maximum of 20 sessions were allowed to reach the final learning criterion (step 3, alone in arena). If a horse did not reach this criterion, it was classified as having failed to complete the learning task. A horse was defined as being habituated to social separation when it succeeded in passing step 3.

Heart rate was recorded throughout the training sessions to give an indication of the horses' physiological response. It was recorded with Polar Equine RS800 (Polar Electro OY, Kempele, Finland), consisting of an Equine Wearlink, a W.I.N.D. transmitter and a wristwatch receiver.

Statistical analyses

The statistical methods used in this thesis are described in detail in each paper, but a brief summary of the main methods used is presented below.

In studies I, II and III, horses acted as their own control, that is, the same individuals were repeatedly tested in each of the different treatments (cross-over design). Although in the mixing studies (I, II), the different treatment groups were considered to be independent because horses never met another horse twice. Data were treated as dependent when within treatment comparisons were made, for example in study II for behaviours exchanged through the opening in the box. Data were also treated as dependent in study III for comparisons of different phases made within the single treatment (one horse was removed from the group), and for comparisons between the single and pair treatment (two horses were removed).

Non-parametric tests were used predominantly throughout the studies as behaviour frequencies recorded were not normally distributed. Mann-Whitney U-tests (U) were applied, and Wilcoxon signed rank tests (W) in the case of paired observations (dependent data). Parametric tests (t-test and paired t-test) were applied when data fitted a normal distribution (study II, III).

To compare the latency to physical contact between horses in the different treatment groups, Mood's median test and log-rank test (Mantel-Haenszel) were applied in study I and II, respectively, accounting for censored data (some horses did not make contact). Log-rank test was also used in study IV for comparison of the total number of training sessions the horses received when trained singly or alone after receiving the pair training, as some horses never succeeded in the final learning criterion (training step 3). The Chi-square (or Fishers' exact test) was used for comparison of the number and the type of first contact that horses made in paddock meetings in study I and II, and for the number of horses succeeding or failing in the final learning criterion in study IV.

Scatterplots with corresponding correlation coefficients (Pearson's product-moment correlation) were used in study I to determine whether the behaviour shown in boxes would correlate to behaviour shown when the same horses met in the paddock, and whether more aggressive horses also showed more inappropriate aggression. A binomial test was applied in study

I to test whether individual aggression level was independent of behaviour shown by the opponent horse.

Statistical analyses were performed using Minitab (version 15, release 2007, Minitab Inc., State College, Pa.) and R software for statistical computing (release 2007, R Foundation for Statistical Computing, Vienna, Austria).

Summary of results

In the paragraphs below, the most important results from each study are presented; more detailed information can be found in the respective papers.

Study I and II

No physical injuries were recorded during the 60 paddock tests in study I. In study II (46 paddock tests), only two injuries (minor superficial skin damage with hair loss) were a result of an aggressive interaction between a resident and an unfamiliar horse during one paddock meeting when three horses were mixed.

In study I, pre-exposure of young horses in neighbouring boxes for 5 minutes significantly reduced biting in the subsequent paddock meetings compared to meetings where horses did not experience the box exposure. Furthermore, there was a tendency for reduced 'contact aggression' (bite, kick, strike, push). This effect of pre-exposure could not be replicated with older horses in study II.

In study II, horses made use of the opening placed in the partition of the boxes by putting their head into the neighbour's box (74% of total 82 social interactions) and most interactions were non-aggressive compared to aggressive (both horses showed aggression) and mixed interactions (one horse was either aggressive or submissive while the opponent showed no aggression). In study I, a significant correlation was found for 'bite threat' shown in the box which correlated with 'contact aggression' in the subsequent paddock meeting.

Physical contact was made less often in meetings when horses were pre-exposed in boxes than in meetings when horses met immediately in the paddock (study I). Horses were also slower to come into contact after pre-exposure than when meeting directly in the paddock. This was not observed when testing the older horses in study II, as physical contact was made in very few of the paddock tests and treatment did not affect latency to contact.

Table 2. *Frequencies of aggressive behaviours (median, quartiles Q1, Q3) shown per paddock test and the total number of paddock tests performed when mixing unfamiliar horses in study I and study II*

	BP	P	PP
Study I (total tests)	30	30	-
Median (Q1, Q3)	13 (3, 20)	13.5 (8.5, 20)	-
Study II (total tests)	16	15	15
Median (Q1, Q3)	7 (4.3, 11.8)	6 (2, 16)	11 (6, 16)

When comparing the effect of mixing two horses in the paddock with mixing three horses at the same time in study II, no significant difference in aggression level was found (table 2), nor when subdividing aggression into contact aggression and non-contact aggression. However, the behaviours 'attack' and 'flee' were recorded significantly more frequently in triadic compared to dyadic meetings. Unfamiliar horses did not receive significantly more aggression when meeting two resident horses at once than when meeting a single resident horse (when resident horses acted as 'sender' and sender's response to behaviour of the unfamiliar horse). Furthermore, there were no significant differences in the resident horses' aggressive behaviour when being together as a pair compared to when meeting the unfamiliar horse.

There were significant differences in individual aggression levels across each horse's six meetings in study I, indicating that individual aggression level was influenced by the behaviour of the opponent horse. Inappropriate aggressive responses (aggressive behaviour given in response to a receiver's submissive behaviour) were shown by 12 out of 20 horses in study I, and by 9 out of 16 horses in study II. In study II, mainly resident horses were showing it, whereby in study I, all horses could be considered as residents with regard to time of residency at the research facility. Resident horses in study II were also responsible for most of the aggression given to unfamiliar horses in all tests.

A total of 68 interventions were recorded in study II. These were solely initiated by resident horses and almost always by the dominant horse of the pair (91.2%). The intervener horse directed interventions more often to the unfamiliar horse than to the other resident horse. The intervention behaviour 'choose interposition' (when a horse positioned itself between two other horses) was most frequent whereas intervening 'via other behaviour' (when the intervener (threat) bites or (threat) kicks towards one horse of the dyad) was least frequent. Most interventions occurred between horses that did not seem to be interacting socially (51.5%) and between horses that were approaching non-aggressively (36.8%). They occurred least frequently during aggressive approaches (8.8%) and non-aggressive interactions (2.9%). No interventions were recorded during aggressive interactions.

Study III

There were significantly more horses within 2 m of the handler when a single horse was separated from the group during the phases approach, catch and during the 30 seconds waiting phase at the post than during walking the horse to the gate and at the gate.

Very few social interactions (total 24) were observed between horses and between horses and the target horse, of which 16 were aggressive and only 2 involved physical contact (bite). Aggressive behaviour directed to the handler was recorded twice (presentation of rump during catching the target horse) and non-aggressive interactions (sniffing) were significantly more often observed at the post than during the phase catch. Although other horses of the group were close to the handler during most phases, physical contact initiated by the handler was only necessary 13 times while catching a horse. Contrary to this, the handler displaced horses frequently by swinging her arm, but there was no difference in frequencies between the 5 phases.

Rank of the horse being led did not influence the number of horses following from the post to the paddock gate. The rank of the target horses did not either affect the number of horses within 2 m of the target horse during any of the other phases. A greater proportion (of the two loose horses) followed when two horses were removed compared to the proportion (of the three loose horses) following when a single horse was taken out.

Study IV

Twelve horses (out of 16) that were trained alone from the start and eight horses (out of 14) that were trained first in pairs and afterwards alone succeeded in the final learning criterion (to be alone in arena). There was no treatment effect on the number of required training sessions, nor an effect on heart rate.

However, heart rate was significantly lower when horses were trained together compared to when the same horses of the pair were subsequently trained individually. Heart rate in horses trained alone from the start tended to be higher than in horses trained in pairs. When analysing heart rate averaged over all sessions, for those horses that started the training alone and reached the final learning criterion, heart rate was significantly lower than in horses that did not reach criterion. There was no such difference in horses succeeding in the learning criterion that were trained individually after experiencing the pair training.

When analysing heart rate of horses in their first training session in step 1, heart rate was significantly lower in horses trained alone from the start that succeeded in the final learning criterion compared to horses that failed. Again, this difference was not apparent in horses trained alone after being trained with a companion. Heart rate in the horses' last training session during step 3 among horses that passed the final learning criterion was not significantly different between singly trained horses and horses trained alone after the pair training.

General discussion

In this thesis, management practices of horses kept in groups were studied with specific emphasis on reactions of horses to mixing and to separation from the group. Results from studies I and II point to solutions of how the process of mixing unfamiliar horses could be arranged so that aggressive interactions and associated injury risk can be minimised. Results from study III indicated that potential risk for a human when removing a horse from a group may be reduced by avoiding being stationary. In study IV, results further showed that training horses to tolerate separation from peers initially together with a familiar companion may not be more efficient than training horses alone from the start.

Mixing horses

Effect of pre-exposure or direct introduction

Based on findings from previous resident-intruder studies, where it was shown that pre-exposing animals in neighbouring pens reduced aggressive interactions when the same individuals met freely afterwards (delBarco-Trillo *et al.*, 2009; Burks *et al.*, 2004; Jensen & Yngvesson, 1998; Fraser, 1974), we hypothesised that the same positive effect could be replicated when using horses. In study I it was found that pre-exposure of young unfamiliar horses in neighbouring boxes reduced biting behaviour and tended to reduce contact aggression (bite, kick, strike, push). Thus, the results support findings reported in the studies cited above, even though our duration of pre-exposure was relatively short (5 minutes) compared with, for instance, the 10-minute exposure in delBarco-Trillo *et al.* (2009) or the 24 hours and 4 days applied by Jensen & Yngvesson (1998) and Fraser (1974), respectively.

However, when study I was replicated with older horses of a different breed in study II, pre-exposure in neighbouring boxes failed to have an effect on aggression levels shown in the subsequent paddock meetings. The underlying reasons, why the results from study I could not be confirmed, can only be speculated upon. A likely reason may indeed be related to the duration of pre-exposure which may have been too short to have an effect in these older horses. Another reason may be that the benefit of pre-exposure is generally less in mature horses socially experienced in meeting unfamiliar conspecifics. Thus, the older horses in study II had possibly more experience in reacting adequately to social signals than the fillies worked with in study I. This may be supported by findings from a recent study by Bourjade *et al.* (2008) who have shown that adult horses influenced the social behaviour of young. They introduced adult horses of the same sex into groups of either young male or female horses (1 and 2 years of age). High frequencies of agonistic interactions were recorded among the young, but fewer agonistic interactions were observed when adult horses were present. Correspondingly, Rutberg & Greenberg (1990) observed in Assateague feral ponies that younger mares tended to be more aggressive when establishing rank while aggression declined when mares grew older.

Social interactions occurring during pre-exposure were not recorded and therefore not part of the statistical analyses in the studies by delBarco-Trillo *et al. (2009)* or Jensen & Yngvesson (1998) so little is known about the actual level of interaction prior to mixing. However, delBarco-Trillo *et al.* (2009) acknowledged that their subjects (male hamsters) spent most of the time by the wire-mesh screen investigating each other during the familiarisation period. Only Burks *et al.* (2004) recorded behaviours during pre-exposure of female elephants as they based their decisions on whether or not to proceed with the mixing process on these observations. We recorded social interactions between horses during pre-exposure in both mixing studies. Evidently, the horses did engage socially in the boxes and interacted through the opening in the box partition, whereby non-aggressive interactions, including greeting behaviour (sniffing) were most frequently exchanged through the opening in study II. This supports the theory that animals do assess each other during pre-exposure.

Nevertheless, interacting in boxes is probably not a substitute for gaining full physical contact to the opponent which may be supported by the fact that in our studies, horses continued interacting when placed in the paddock. Christensen *et al.* (2002a) pointed out that horses need access to full physical contact in order to establish relationships to other horses, which cannot be achieved by simply housing them next to each other. This was based on their observations that previously singly housed horses did not associate more often (nearest neighbour recordings) with previous box neighbours than with unknown horses, whereas previously group housed horses associated significantly more often with their former group mates than unknown horses. Also, delBarco-Trillo *et al.* (2009) highlighted that familiarity obtained during pre-exposure did not seem to decrease motivation to interact with the opponent when subjects were put together in the same enclosure.

If full contact is necessary to establish a relationship, it would be valuable if one could make a prediction of the course of the subsequent encounter in the paddock by observing social interactions in boxes. This would have practical consequences as it may be possible to assess the risk of injury. In study I it was shown that bite threat performed in the box correlated with contact aggression (bite, kick, strike, push) in the paddock, although the correlation was low. On the one hand, this may be due to external factors that could have influenced social interactions during the paddock meetings and that were not present when horses met in neighbouring boxes. An example would be motivation to join peers as these were in visual distance of the test horses when placed in the paddock. Furthermore, test environments differed considerably in terms of space allowance. Consequently, instead of trying to correlate behaviours, behavioural data recorded during box meetings could alternatively be used to adjust the duration of pre-exposure. This approach was taken by Burks *et al.* (2004) as they only placed pairs of elephants in the same physical space when there was a significant decrease in aggression during pre-exposure which included first visual and later limited tactile contact. This would imply that horses are only placed together in the same physical space when behavioural observations reveal that aggression had significantly decreased in neighbouring boxes, or, in other words, when friendly interactions outweigh aggressive behaviours.

Effect of paired encounter or direct group introduction

Whether there is a difference in the frequency of aggressive behaviour received by a new horse when it is introduced to a pair of resident horses directly compared with when it meets each resident horse separately was investigated in study II. It was found that the level of aggression received by the new horse was not significantly different in either of these two mixing methods. Brent *et al.* (1997) observed that direct introductions of one chimpanzee to another were characterised by more aggressive interactions than when one chimpanzee was introduced to the entire group. This would support our conclusion to confront the new horse with the group directly, as the total aggression received by the newcomer would potentially be minimised. Studies in farm animals are lacking this comparison of introduction method mainly because it is less relevant in practice. Usually mixing occurs at group level and animals are rarely introduced singly into established groups. This is different in horses as the most common situation in practice is that only one new horse arrives at a time at a yard. The argument against introducing a single horse directly to the group would be that we observed more attack and flee behaviours when the three horses met which implies increased locomotion and potentially higher stress levels.

Our results cannot be extrapolated to larger groups as we only allowed three horses to meet in a paddock, and we only introduced one horse to a pair of resident horses. Group size has been suggested to correlate with the number of social interactions when grouping cattle (Bøe & Færevik, 2003). Differences in frequencies of social interactions may be expected when more than one individual is introduced to a group. These individuals will usually form sub-groups, regardless of familiarity prior to introduction and this tends to reduce the involvement of subgroup members in social interactions with resident animals (Durrell *et al.*, 2003; Knierim, 1998).

There were no significant differences in the resident horses' behaviour when they were together as a pair compared to when they were together with the unfamiliar horse. Contrary to our finding, Farabollini *et al.* (1991) reported that the dominant animals of groups of four female rabbits directed aggression significantly more often to group mates when an unfamiliar conspecific was introduced compared with stable conditions when no female intruder was present. Although in study II it was also the dominant horse that tended to directed most aggression to the unfamiliar horse, there was no increase in aggression towards the familiar horse. This difference in results in the two studies regarding the aggression between group mates may be related to species specific differences in social behaviour, but may also be related to group size, as only two resident horses were present in our study whereas in the study conducted by Farabollini *et al.* (1991), four animals were confronted with an unfamiliar intruder. This would also relate back to the explanation given above that group size is likely to influence the level of social interactions. If more partners are present, there are more possibilities for social interactions, and social relationships may be more easily disrupted.

Interventions, that is, when a horse was positioning itself between two other horses to intervene in an ongoing interaction or to prevent a social encounter were observed frequently when three horses were placed in the paddock. Most interventions were directed to the unfamiliar horse and not to the other resident horse. One could speculate that this is plausible from an evolutionary perspective as directing aggression towards a familiar partner could jeopardise the relationship and this would not be expected in this resident-intruder situation where residents are likely to stick together. This may support the theory that interventions could take place to safeguard an existing relationship with a familiar partner (VanDierendonck *et al.*, 2009; Schilder, 1990). However, this can only be speculated upon as, with our data, it was not possible to determine what intention the intervener horse could have had by showing such behaviour. Also, we termed a behaviour intervention even when horses of the dyad were not clearly interacting. Thus, it may even be possible that some of the interventions were redirected aggression as meeting unfamiliar conspecifics could be a source of unpredictability and frustration (Giersing & Andersson, 1998).

The dominant horse of the resident horse pair was responsible for most of the interventions, but there was no evidence that this occurred during aggressive interactions. That we recorded no interventions in aggressive interactions may be explained by the short duration of aggressive encounters which probably makes interfering physically more difficult (Schilder, 1990). Since only few interventions were recorded in aggressive interactions by VanDierendonck *et al.* (2009) and Schilder (1990), this may explain why they could not find that the decision to interfere was influenced by rank relationships. Rank relationships are certainly more pronounced in horses kept under human husbandry due to, for instance, space allowance and restricted resources compared with the more free ranging conditions under which interventions were studied by VanDierendonck *et al.* (2009) and Schilder (1990). Clearly, more insight is needed before any conclusions can be drawn related to intervention behaviour that may be applicable in a resident-intruder situation.

Aggression level and associated injury risk

Horses behaved aggressively when placed together in all paddock tests, but injuries (minor superficial skin damage) were only recorded during one test with three horses. This low injury incidence is in line with the fact that those behaviours involving physical contact (e.g. strike, kick) were rare compared to non-contact aggressive behaviours (e.g. threat to kick).

There is limited published data for comparison of injury rate caused by aggressive interactions in groups of horses. Jørgensen *et al.* (2009) drew specific attention to injury incidence in horses before and one day after grouping. They found that injuries were only superficial (hairless spots or swelling), and that there was no damaged skin. Social interactions were not recorded immediately after grouping by Jørgensen *et al.* (2009), thus the authors proposed that minor injuries could have also been the result of rough play instead of aggressive interactions. In another recent study conducted by Søndergaard & Christensen (2009), no serious injuries were registered in groups of mares where group membership remained unchanged or was shifted between groups. Although horses in the unstable groups showed more threatening behaviours than horses kept under stable social conditions, the frequency of physical aggressive interactions was not significantly different between groups. Data are also consistent with results obtained by Søndergaard & Turner (2008) who introduced unfamiliar horses (mares) into groups of two familiar mares and no injuries were noted at any point during the study period.

Our results with regard to low injury incidence are in line with the studies cited above, given that all horses were experienced with being kept in groups before participating in the studies. Since group housed horses have more opportunities to practice their social skills compared to horses reared singly, their social behaviour may be more sufficiently developed. Thus, they seem to be less prone to injuries caused by aggressive interactions (Ladewig *et al.*, 2005), even when group membership changes frequently (Søndergaard & Christensen, 2009).

Nevertheless, despite the presumably sufficient social experience of horses used in studies I and II, some inappropriate aggressive responses (aggression as a response to submissive behaviour shown by an opponent) were recorded. This would not be expected given the adaptive value of displaying the least amount of aggression a situation requires to reduce energy expenditure and risk of injury (Waring, 2003). An explanation could be that the continued aggression was caused by the inability of the submissive partner to increase or maintain sufficient distance due to restricted space allowance. Despite the occurrence of inappropriate aggression, the horses were flexible in their behaviour towards meeting unfamiliar conspecifics, as aggressive responses were modified in each meeting (study I). However, horses tested in study I also showed clear individual differences in total aggression level. This supports anecdotal reports from horse owners and handlers that characterise individuals as 'aggressive' or 'friendly' when confronted with unfamiliar conspecifics. It also supports the theory that aggression may be a specific individual characteristic, referred to as a personality or temperamental trait. In pigs, different studies have documented consistent individual differences in aggressiveness towards meeting unfamiliar conspecifics (D'Eath & Lawrence, 2004; D'Eath, 2002; Erhard *et al.*, 1997; Hessing *et al.*, 1993), but this still needs to be confirmed in horses.

Removing a horse from the group

Potential risk situations

It is an anecdotal assumption that it is risky for the human to move among a group of horses because of interference from other horses when removing an individual. From the handler's perspective, risk may be overestimated as separating young horses from a group of four in study III (total 96 tests) was generally unproblematic. This corresponds to results reported by Jørgensen *et al.* (in press). Those authors evaluated the procedures of 100 horses being separated from their group (mean group size 8 horses) by a familiar handler and also concluded that the procedure of catching and separating a horse was unproblematic for the handlers. In our study, no horses or handlers were injured, although some aggressive behaviour between horses was observed. There were few occasions when a handler had aggressive behaviour directed towards her by another horse. The majority of the interactions between horse and handler were friendly (sniffing).

There did, however, seem to be some differences in the potential risk between the different phases of catching and separating the horse. Risk was regarded as higher when the handler was relatively stationary during the phases catching the target horse and while waiting at the post in the centre of the paddock. That the handler was relatively stationary during these two phases presumably allowed other horses of the group to approach and remain very close (within 2 m) to the handler and the target horse on the lead. That more horses were also close to the handler during the 'approach' phase points to the fact that horses of the group were often already close to the target horse before the handler entered the paddock. Least risk, as estimated by horses that were further away, e.g. when walking to the gate, was to be expected. This is because while moving horses spread out as they follow the target horse at different speeds. Consequently, moving with a horse on a lead in a group of horses may be safer than being stationary with it.

The ease of catching and removing a horse from its group may be partly attributable to the handling experience our horses had gained prior to testing, as habituation to handling is likely to benefit the horse-human relationship (Hausberger *et al.*, 2008; Mal & McCall, 1996). Verrill & McDonnell (2008) and Jezierski *et al.* (1999) reported increased compliance when catching horses in an open field following positive experiences with humans. In farm animals, it has also been shown that handling can shorten the animals' latency to approach a test person or to engage in physical contact with the experimenter (Hemsworth *et al.*, 1996).

A common assumption is that accidents to humans happen near the gate to the paddock. This was not confirmed in study III which may be due to the extra person who was responsible for opening and closing the gate, allowing the handler and the caught horse to exit quickly. The horses that are following are likely to catch up if the handler would have to stop to open the gate, as horses did when the handler was stationary at the post. In this study, despite having a person to help, three horses managed to escape at the same time as the target horse was being led out. In the study conducted by Jørgensen *et al.* (in press), two horses escaped through the gate before handler and horse had left the paddock. This escaping in itself could be argued to constitute a risk moment for the handler and probably also for the horse that is now running loose outside the paddock.

Following the horse being removed

Most of the time horses followed (from the post in the centre of the paddock to the gate) the horse that was being led out and there seemed to be an effect of the proportion of the group that was being removed. A higher proportion of horses followed when a pair of horses (0.5 of the group) was removed compared to when horses were removed singly (0.25 of the group removed). It is difficult to extrapolate to other group sizes, but from an evolutionary point of view it is adaptive for horses, as for other social species, to stay or move with the majority of the herd to maintain cohesiveness (Petit & Bon, 2010). Thus, the recommendation of whether to remove one, two or more horses at the same time from the group will possibly depend on the group size. The proportion of horses removed at any one time should preferably be less than half, although this assumption would need to be confirmed.

Bourjade & Sueur (2010) and Bourjade *et al.* (2009) proposed that the probability of each group member joining a movement is influenced by the number of individuals already moving. This would support our finding as proportionally more horses followed when two horses were led compared to a single horse. Pillot *et al.* (2010) concluded, after studying following behaviour in groups of sheep (*Ovis aries*) kept in a 25 m circular arena, that witnessing a movement of a conspecific was enough to initiate movement in other group members. One sheep of a group of four was trained to approach a panel after hearing a sound, and when this individual started moving, it systematically triggered a collective movement of the remaining naïve group members. This may also be seen in the light of results reported by Bourjade *et al.* (2009). They studied Przewalski horses (*Equus ferus przewalskii*) living under semi-free conditions, and recorded a collective movement of the entire group in 93 % out of 145 start attempts initiated by one horse that started walking away from the group. Another explanation of why at least some horses followed the horse being led out in study III may also be due to the closeness of these horses prior to walking the target horse to the gate. As mentioned earlier, being stationary at the post allowed other horses of the group to approach and remain close to the handler and the horse on the lead. Ramseyer *et al.* (2009) suggested that short distances between individuals promoted recruitment for following a first mover when testing the effect of spatial distribution on decision making to follow in groups of 19 ewe lambs (*Ovis aries*) at pasture. Animals first recruited were those that were close to the first mover and also its preferential partners (Ramseyer *et al.*, 2009). The question arises as to whether the space available for the sheep studied by Pillot *et al.* (2010) was a confounding variable and it would have been interesting to elaborate on this further.

The number of horses following the target horse from the post to the gate was not influenced by its rank, that is, when it was the highest ranked horse of the group or a lower ranked individual. The common held belief that more horses would follow a horse high in rank could thus not be supported. This corresponds with findings from Pillot *et al.* (2010) who could not show that dominance was a prerequisite for the initiator of movement to being followed in sheep. King (2010) and Petit & Bon (2010) have recently put forward that dominant status per se may not be confined to the act of leading and thus being followed. Nevertheless, there is continuous debate in the scientific community about leadership qualities in group-living animals, and leadership is also poorly defined among horses. Motivation to follow is certainly influenced by multiple factors, especially in situations when human activity is involved.

Training to social separation

Staying together as a group is adaptive for horses. Horses that have not learned to leave the group and to be subsequently alone will possibly experience this situation as stressful. Appropriate training is likely to reduce responses to social separation. Two training methods were tested in study IV to evaluate which method has the best potential to reduce stress levels: training a horse alone from the beginning or training it initially in the presence of a familiar companion.

Training horses alone or initially with a companion

The hypothesis that training horses initially with a companion would be more beneficial than training horses alone from the start could not be supported. This conclusion is based on the results that heart rate in horses increased when switching from the pair training to the individual training and that there was no difference in the number of training sessions required. Instead, it seemed that horses trained with a companion had to re-learn being in the test situation in the absence of the partner.

The pair training was, nevertheless, probably experienced as less stressful. Horses trained in pairs had lower heart rates than during the subsequent individual training, but heart rate was also lower compared with horses trained alone from the beginning. This calming effect of a companion was expected and is in accordance with results from other studies (Færevik *et al.*, 2006; Boissy & Le Neindre, 1990). Our results may also indicate that the subsequent absence of the partner may have overshadowed the primary reinforcer food, making social company the prioritised one, a least in naïve and non-food deprived horses.

It could be questioned whether the presence of companion horses that were already habituated to social separation would have revealed different results than using naïve partners. The possibility of learning from experienced conspecifics was demonstrated by Christensen *et al.* (2008) when differentiating between a habituated companion horse and an untrained companion during training horses to react calmly to a frightening test stimulus. The authors found that when these horses were subsequently tested in the absence of the habituated companion in a post-test, they had lower heart rates, received lower reactivity scores and had shorter latencies to return to feeding than the horses previously paired with an untrained conspecific. One could also question whether the presence of a trained partner could have been beneficial in our study with regard to fewer training sessions required during the pair training.

Horses trained initially in pairs did not proceed quicker through the pair training than the horses trained alone from the start. Therefore, it can be concluded that while the presence of the companion horse appeared to alter the physiological stress response (heart rate) towards the test situation, it did not influence performance. This is in contrast to results from, for example, Boissy & Le Neindre (1990) who found that learning performance was higher in heifers exposed to the test stimulus in the presence of social partners, although partners only acted as 'spectators'. Our result could, therefore, be a consequence of horses in pairs disturbing each other in the feeding situation during training step 3 (the pair of horses was left alone in an indoor arena where two feed containers were placed in the centre). Redgate & Davidson (2007) and Holmes *et al.* (1987) have suggested that the presence of another horse during feeding may facilitate feeding, but may also potentially be stressful. Thus, our horses would have required more training sessions to meet our feeding-based habituation criterion.

Predicting learning outcome

Since heart rate in horses trained alone from the start and who succeeded in the final learning criterion (being alone in an indoor arena) was lower during their first training session than the heart rate in horses that failed to learn the task, heart rate could potentially be used to predict learning outcome. This was previously also suggested by Christensen *et al.* (2006). Furthermore, other studies in horses have demonstrated that the most fearful or reactive horses take longer to learn, e.g. Heird *et al.* (1986), Lindberg *et al.* (1999) and Fiske & Potter (1979). This further supports the conclusion that individuals with low heart rates during training in the current study learn better or, alternatively, that fast learners more quickly become calmer.

Methodological considerations

Below, the methods described in the thesis will be briefly discussed in terms of their potential benefits and constraints.

Duration of testing

The horses were tested in environments that represented conventional housing conditions for horses. Yet, all horses were habituated to the test environment before the start of the studies, otherwise one would not know whether responses to testing would be confounded by exposure to a novel environment. Some of the young horses worked with in study I showed strong reactions to being confined in a box (e.g. increased locomotion, kicking box walls, pawing, rearing) at the start of the habituation training. This reaction to first time stabling is not unusual and has also been reported in studies conducted by Visser *et al.* (2008) and Harewood & McGowan (2005). For that reason, exposure time in boxes was limited to 5 minutes. By extending the time spent in the box, more habituation sessions would have been needed to exclude reactions related to confinement occurring later on, but this was practically not possible. Since study II was a follow up experiment, the duration of exposure time in the box was kept the same as in study I, even though the horses were used to being kept in boxes already before the start of the study.

Furthermore, observations made during a pilot study showed that horses meeting for the first time started to interact immediately after being placed in neighbouring boxes. This was also observed when horses were released together in a paddock. Therefore, a 10-minute exposure in the paddock seemed long enough to be able to observe these immediate responses to mixing. That there may be more peaks of social interactions, particularly aggressive encounters later in time cannot be excluded. Social relationships may be settled after only few social interactions, but it can also take several days before a relationship becomes fixed and overt aggression gets replaced by more subtle threatening gestures. Whether injury incidence would have been higher when horses were left together for longer periods can only be speculated upon.

Feeding tests to evaluate dominance

Feeding tests were carried out in studies II and III to identify the most dominant horse of a pair of horses and of a group of 4 horses, respectively. Using a competitive situation at a feed trough is a convenient and relatively quick way of gaining insight into dominance relationships. Although it may not reflect complex relationships (e.g. triangular) and it may not exactly be the same dominant-subordinate relationship in other situations and activities (Lehmann *et al.*, 2003; Waring, 2003; Ellard & Crowell-Davis, 1989).

Since we were only interested in determining the horse highest in rank, carrying out feeding tests seemed justified according to a study conducted by Ellard & Crowell-Davis (1989). They evaluated dominance relationships in mares by comparing data from field observations with results obtained from paired and group feeding tests. The horse that was determined as being highest in rank was identical in both of their tests and was also the most dominant horse when agonistic interactions from field observations were analysed. Our behavioural recordings made during feeding tests also corresponded well with the handlers' assessment during daily interactions with the horses.

One critique often mentioned in relation to the use of feeding tests as a means to determine dominance relationships is that the outcome may largely depend on the horses' motivation to compete for feed at that time. To circumvent this, the feeding tests were repeated at different time points throughout the studies. The tests revealed consistent results reflecting that horses were possibly always sufficiently motivated to compete for feed.

The use of feed during training

Feed is frequently used when testing animals in a frightening test situation as motivation to feed is a useful indicator of how stressed an animal may be. For example, Christensen *et al.* (2006) found that the longer it took a horse to return to feeding the more stressed it was while confronted with a frightening test stimulus. Indeed, we could confirm the advantage of using feeding behaviour as an indicator of stress as those horses that failed to feed calmly during training sessions in study IV also had increased heart rates.

Using feed as a positive reinforcer can also be useful in facilitating learning and is widely used in behaviour modification in animal training. Since we knew that separation from the group would be initially experienced as aversive, we wanted to offer a positive consequence which was the provision of feed.

Another benefit of using feed was that it allowed us to have some control over the position of the horses when they were left alone in the indoor arena during the last training step 3. Additionally, it was also meant to keep them more stationary in general as increased locomotion would have confounded heart rate measures.

Apart from the potential benefits of using feed in a test situation, the use of it could also be criticised. One reason is the difficulty in controlling for feeding motivation. In our case, there could have been a motivational conflict between attempts to re-unite with the group and sufficient motivation to feed. However, we assumed that horses were equally motivated. First, this was argued to be the case because the feed was highly attractive (barley mixed with molasses and hay) and none of the horses showed any aversion to it before the start of the study. Second, because all horses were kept under the same feeding regimes, i.e. were pastured with similar access to grass and supplementary feed, but also had similar exercise levels. Supplementary feed (mixture of straw and hay) was provided daily in the home paddocks, but was always given after testing to avoid satiation before the test sessions. Another way of avoiding satiation and ultimately assuring sufficient feeding motivation was by keeping training sessions short (2 minutes) and limiting the number of sessions to a maximum of 5 sessions per day. Furthermore, concentrates were mixed with hay to avoid fast feeding.

A final consideration is that competition over feed may have taken place when a pair of horses was left alone in the indoor arena in the last training step 3. We expected that horses would feed from separate containers as they were taught to do so in the two preceding training steps. However, we found that some horses fed from the same container, others displaced each other from the container and horses changed position between containers. Nevertheless, feeding was only interrupted for very short periods of time and all pairs, except one, succeeded together as a pair in this training step 3.

Effect of sex, age and breed

All studies were meant to be experimental under controlled conditions to increase accuracy, repeatability and interpretation of results. Therefore, it was desirable to get horses for each study that had been reared under similar conditions in groups, were of the same sex, similar age and breed. The question remains of whether horses of different sex, age and breed would respond differently when exposed to similar test conditions.

We have no reason to believe that other horses, in general, would behave differently. If so, then it is presumably more a difference in frequency and intensity of behaviours shown than it is in the occurrence of behaviour per se. For instance, if horses meet for the first time, they are likely to have aggressive encounters, whether they are mares, geldings or stallions (Jørgensen *et al*., 2009; Vervaecke *et al*., 2006; Christensen *et al*., 2002a; Alexander & Irvine, 1998). Vervaecke *et al.* (2006) did not find significant differences in aggression in groups of mares compared to gelding groups nor did Jørgensen *et al.* (2009). Stallions are likely to show more intense interactions than geldings as castration reduced their aggressive tendencies (Waring, 2003; McDonnell & Haviland, 1995; Feist & McCullough, 1976).

Age may be more likely to affect behaviour than sex itself. In this thesis, mares of different age were tested in study I (1 to 2 years old) compared with study II (6 to 18 years). We could not confirm the effect of pre-exposure in study II which may be due to the age difference and the presumably different social experience in these older mares. This may support findings from Rutberg & Greenberg (1990) who recorded reduced involvement in aggression when mares grew older. That age presumably is an influencing factor on social interactions was also confirmed recently by Bourjade *et al.* (2008).

Whether breed has a significant effect on social interactions is questionable. According to a study conducted by Lloyd *et al.* (2008), variability of the personality traits dominance (aggression towards conspecifics and people) and sociability (motivation to seek close contact to conspecifics) between different horse breeds was low. Thus, differences in our two mixing studies may be more related to age rather than breed. In contrast, breed differences may be more pronounced in terms of an individual's reactivity level as proposed by Wolff *et al.* (1997) and Lloyd *et al. (2008).* Thus, effect of breed on behaviour may be more relevant when studying horses' responses to training or potentially stressful situations as was done in study IV.

Effect of handling

The training of horses to tolerate leaving the group and to being subsequently alone was done in the presence of a handler (study IV). The handler had the horse on a lead rope and was always present, even when the horse was released in the indoor arena for the last training step 3. Here, the handler was waiting at the entrance of the arena, visible to the horse.

We were aware that by having the horse on a lead in training steps 1 and 2 it was forced to remain with the handler at the feed container. If a horse had been allowed to move away at every attempt to rejoin the group, certainly the chances of the horse returning to feeding would have been low. Having the horse freely during the first two training steps was not an option due to safety considerations, and it would probably not reflect a training situation commonly applied in practice. Furthermore, having the horse on a lead was not confounding in terms of evaluating learning success. However, not all horses were easily prevented from turning away from the feed container and the results showed that it was these highly agitated horses that were more likely to fail in the final learning criterion.

Perspectives for future studies

In this thesis, some aspects related to group housing of horses have been studied. These were chosen because of specific concerns horse owners may have against keeping horses in groups, such as those related to mixing unfamiliar horses and separating horses from groups. It would be highly relevant to increase the scientific enquiry in these areas, as several very interesting questions arose during my PhD project.

In the following paragraphs, some possible directions for future research will be highlighted, based on results from my studies. Ideally, future research should offer practical solutions that could ultimately contribute to improved horse welfare and human safety.

Meeting unfamiliar conspecifics

Even if the ambition is to have stable groups of horses, some mixing of unfamiliar horses is inevitable. Identifying mixing methods that have the potential to reduce aggressive interactions and associated injury risk has an important practical value. Therefore, evaluating the effects of different mixing methods on aggression level, including pre-exposure in boxes and paired or group encounters in outdoor enclosures clearly warrants further research. In particular, it is recommended to study the effect of different durations of pre-exposure in neighbouring boxes on aggressive behaviour shown in a subsequent paddock meeting. It is also worth to consider collecting behavioural data during the pre-exposure so that the decision of whether or not to proceed with placing animals in the same enclosure is based on objective measures, i.e. mixing could be delayed until there is a significant reduction in aggressive interactions.

Furthermore, it is necessary to address the effects of mixing methods on aggression level in the period following mixing, for instance, when previously unfamiliar horses have been left together for several hours or days. This is of interest as it would give insight into the time frame horses require before they become familiar with each other which is characterised by a replacement of overt aggression with more subtle interactions and affiliative encounters. Future research may also focus on studying the social interactions between newly introduced horses that differ in age as this may have a profound impact on reactions towards unfamiliar conspecifics.

Further investigation of the resident-intruder effect in horses and its implications in practice are needed. For example, to test whether interactions vary when two or more horses are placed together in an area that is new for all horses.

Although there has been much work done on horse temperament, to my knowledge, a horse's aggressive tendencies towards conspecifics and its general sociability (motivation to seek close contact to companions) has hardly been studied experimentally. Yet, being able to identify these characteristics could benefit individuals by adjusting mixing methods, especially for those individuals that are less flexible in their responses to changes of social partners.

It is well known that there can be strong social ties between horses, especially between mares, and this would potentially influence social interactions when introducing new horses into established groups. The strength of social bonds may be specifically reflected in intervention behaviours. The interfering horse may want to alter a dyadic interaction between another familiar horse and an intruder or stop it from continuing and in that way prevent a weakening of the bond to a preferred social partner. Other theories of the possible intentions for intervening have been proposed by several authors, however, more insight is needed particularly on the circumstances in which interventions occur and the functions they serve when grouping horses.

The horses used in my studies were all reared and kept in groups before participating in the experimental testing. The lack of social contact with conspecifics early in development has been shown to affect behaviour during social encounters later in life. Thus, the question arises as to how horses with inappropriate social behaviour towards conspecifics respond to mixing and whether repeated grouping could improve their social skills. Alternatively, it may be possible to determine what minimal social experiences horses would require to allow them a normal social life.

Subjective experiences from some horse owners suggest increased aggressiveness in mares towards conspecifics during oestrus. However, very few studies have investigated the effects of hormonal fluctuations on behaviour in mares. Therefore, more studies are needed to evaluate whether the stage of the oestrus cycle affects the frequency of aggressive behaviour in mares. Studies are also needed that distinguish between the effect of social novelty and hormonal status on aggressive behaviour. This would be relevant in practice to know whether mixing with other horses should be avoided during oestrus or whether it could be done at any stage of oestrus cycle.

Separation from conspecifics

Another area worth further exploring is the effect of a trained companion horse on learning performance in a naïve subject. In study IV, two naïve horses were trained to tolerate social separation. However, this did not seem to lower the number of training sessions required nor did it lower heart rate when the same horses continued to be trained in the absence of the partner. Contrary to this, there have been studies conducted showing that the presence of a trained companion may benefit more in terms of learning outcome than using an untrained partner. Therefore, it would be interesting to explore whether the study we conducted would reveal different results in terms of fewer sessions needed to succeed in the initial pair training. Whether the experience gained during the pair training could be transferred to the training situation when the horse is subsequently trained in the absence of the experienced partner remains open for testing. Moreover, if the aim is to use conspecifics during training, the quality of the social relationship between the horses may be relevant in terms of learning outcome and social transmission of behaviour.

Very few studies have investigated ways of safely approaching horses kept in groups. Considering the practical relevance, it would be important to elaborate more research in this area and to study, for example, the influence of group size and group composition on horse-horse and horse-human interactions.

Conclusions and practical applications

The current study has provided some preliminary insights into reactions to mixing and to social separation in horses experienced with group housing. Although more research is necessary, the results do highlight some solutions that may be useful in practice.

- Injury incidence was low and injuries were only superficial when mixing unfamiliar horses. Thus, the risk of horses injuring each other immediately after being placed together in a paddock, i.e. within 10 minutes, is possibly overestimated.

  If horses are experienced with being reared and kept in groups, there is no reason why they cannot be mixed with unfamiliar conspecifics. Although injury risk may be low, careful observation of all social interactions occurring at all stages of mixing is nevertheless important in order to be able to intervene if aggressive encounters would escalate.

- Pre-exposing horses in neighbouring boxes does not inhibit aggression when the same horses meet in a paddock afterwards, but it has the potential to lower aggression of the type that involves physical contact at least in young female horses and immediately after mixing.

  It is recommended to offer young horses the opportunity to familiarise themselves in neighbouring boxes before the pair is placed together in an outdoor area. Boxes should allow some restricted physical interaction through bars or an opening in the box partition.

- Introducing an unfamiliar horse to either a single resident horse or a pair of resident horses had no significant effect on aggression received by the newcomer.

  It could be recommended to introduce a single new horse to a pair of resident horses at once as this is likely to result in lower total aggression received by the horse being introduced.

- Being stationary when removing a horse from the group puts the handler and potentially the horse being led in an unsafe situation as other horses can gather around and can interfere.

  If the aim is to remove a horse from its group for training purposes or other handling practices, it is recommended to enter, catch and walk the horse out of the paddock directly and avoid being stationary for longer periods. Having help to open the paddock gate to be able to pass quickly or having a well designed gate that can easily be opened and closed would also be useful.

- It was not effective to habituate naïve young horses to social separation initially with a familiar companion because these horses seemed to have to re-learn being in the training situation in the absence of the partner. Nevertheless, the pair training was beneficial since horses trained in pairs were less stressed as measured by heart rate.

  Most horses that are naïve to being separated from group members are likely to experience social separation as stressful. Therefore, appropriate habituation training is essential to ease handling and safeguard horse welfare and human safety. Although training young horses in the presence of a companion horse may be beneficial in other training situations, in the case of training horses to tolerate social separation it may be worth considering training young horses alone from the start.

Svensk sammanfattning

Gupphållning av de flesta av lantbrukets djur används ofta i praktiken, och forskning har undersökt många aspekter av detta för att bidra till förbättrad djurvälfärd och interaktion människa-djur på gruppnivå. Jämfört med den mängd vetenskaplig litteratur som finns rörande lantbruksdjur så som nötkreatur, svin och fjäderfä har dock förhållandevis lite gjorts när det handlar om hur man hanterar hästar som hålls i grupp. Detta trots att grupphållning av hästar anses vara det bästa alternativet för att uppfylla deras fysiska och beteendemässiga behov, speciellt behovet av social kontakt med artfränder. Även om förhållandena för hästskötsel har förbättrats under de senaste decennierna är det fortfarande många hästar som inte har ett socialt liv, huvudsakligen på grund av ägarens oro inför grupphållning. Syftet med denna avhandling är därför att undersöka om vissa vanliga orosmoment är berättigade som sådana och att, om så är fallet, erbjuda vetenskapligt baserade lösningar som kan användas i praktiken för att förbättra hästens välfärd och människans säkerhet.

De fyra studierna (artikel I - IV) som presenteras i denna avhandling är en del av ett nordiskt samarbetsprojekt vid namn: Grupphållning av hästar under nordiska förhållanden: Strategier för att förbättra hästens välfärd och människans säkerhet.

I den första delen av avhandlingen (artikel I och II) var målet att identifiera metoder som har potential att minska aggressiva interaktioner när man för samman obekanta hästar (i detta fall ston), eftersom många hästägare hävdar att skaderisken är hög i just denna situation och att grupphållning på grund av detta oundvikliga moment är problematiskt.

Resultaten av studie I visade att unga hästar (Danskt Varmblod) som stod i intilliggande boxar där de kunde interagera med varandra under en kort tid (5 minuter) tenderade att visa mindre kontaktaggression (beteenden där risken för fysiska skador är större såsom sparkar) när de sedan träffade varandra lösa i en paddock i 10 minuter. Denna effekt kunde inte verifieras med äldre hästar av annan ras (Varmblodig travhäst) i studie II. Dessa hästar visade inte mindre aggression efter att har mötts i boxarna än de gjorde när de inte hade haft denna möjlighet. I studie II testades även en tredje metod där en obekant häst mötte två andra hästar som kände vararandra. Aggressionsnivån i dessa möten var inte högre än när den obekanta hästen endast mötte en häst i paddocken. Detta innebär att aggression som mottas av den obekanta hästen möjligen kan minimeras om hästarna introduceras enligt den senare metoden.

Skador under testerna var sällsynta; bara en lättare skada noterades under totalt 106 möten mellan obekanta hästar i paddocken. Slutsatsen av studie I och II blir att den omedelbara skaderisken kan vara överskattad.

En annan vanlig åsikt är att det är farligare för en person att hämta en häst ur en grupp än att närma sig en ensam häst. Därför var syftet med studie III att utvärdera hästars reaktioner mot varandra och mot en person i samband med att en eller två hästar leddes ut från gruppen, och att identifiera situationer där hästen eller människan löpte risk att bli skadade. Utöver detta undersöktes om dessa reaktioner var annorlunda när det var en häst med hög rang som togs ut ur gruppen jämfört med en häst med lägre rang. Från det ögonblick som personen gick in i paddocken, där en grupp på 4 hästar gick, tills personen gick ut ur hagen med hästen (totalt 5 olika bedömningssituationer) var det signifikant fler hästar nära personen och den ledda hästen när dessa stod relativt stilla. I denna studie inträffade detta under själva infångandet och under väntan vid en stolpe i paddockens mitt. Rang hade ingen betydelse för antalet hästar som följde efter eller kom nära hästen (< 2 m), och interaktioner mellan hästarna var sällsynt. När två hästar leddes ut följde de lösa hästarna oftare efter, jämfört med när en ensam häst togs ur gruppen.

Slutsatsen är att eftersom riskfyllda situationer hänger samman med närheten till lösa hästar i gruppen skulle säkerheten kunna förbättras såväl för personen som hästen genom att bibehålla ett avstånd till de andra hästarna samt att minska tiden av stillastående i paddocken.

Det är väl känt att hästar som är ovana vid att lämna gruppen kan vara mer svårhanterliga på grund av isoleringen från andra hästar. Detta påverkar säkerheten för både häst och människa, och det är därför viktigt att hitta träningsmetoder som minskar reaktionerna på separationen för att underlätta träningen. Således var syftet med studie IV att undersöka om närvaron av en annan oerfaren men bekant häst kunde förändra responsen på separationen, sänka stressnivåer (mätt som hjärtfrekvens) och öka träningens effektivitet (mätt som antal träningstillfällen som krävdes för att nå målet: att vara ensam i en inomhusarena och äta lugnt från en foderhink). Resultaten visar att hästar som tränades i par hade lägre hjärtfrekvens jämfört med när samma hästar senare tränades ensamma. Däremot tycktes inte parträningen ge någon fördel vad gällde antalet träningstillfällen som krävdes, då detta antal inte var signifikant lägre än hos hästar som tränats ensamma från början. Istället verkade det som att hästar som först parträнats måste lära sig testsituationen på nytt då de tränades utan sin partner. Resultaten visar också att hästar som hade låg hjärtfrekvens under det första tillfället av social separation tycktes ha bättre inlärningsförmåga vilket innebär att träningsbarheten kunde förutsägas.

Sammanfattningsvis visade resultaten av projektet att riskerna för både häst och människa inte ska överskattas men inte heller negligeras när hästar hålls och hanteras i grupp. Projektet har gett en viss insikt i hur riskfyllda situationer kan hanteras men många frågor kvarstår om hur obekanta hästar kan introduceras för varandra men också hur hästarna kan vänjas vid att vara ensamma i samband med att de används. En ökad medvetenhet om riskerna och en insikt i hur beskrivna situationer bör hanteras och en förmåga att agera rätt bidrar till en ökad säkerhet.

References

Addison, W.E. & Baker, E. (1982). Agonistic behavior and social organization in a herd of goats as affected by the introduction of non-members. *Applied Animal Ethology* 8, 527-535.

Alexander, S.L. & Irvine, C.H.G. (1998). The effect of social stress on adrenal axis activity in horses: the importance of monitoring corticosteroid-binding globulin capacity. *Journal of Endocrinology* 157, 425-432.

Arnott, G. & Elwood, R.W. (2009). Assessment of fighting ability in animal contests. *Animal Behaviour* 77, 991-1004.

Bachmann, I. (2002). Pferde in der Schweiz: Prävalenz und Ursachen von Verhaltensstörungen unter besonderer Berücksichtigung der Haltung und Nutzung. *Dissertation*, Zürich.

Bachmann, I. & Stauffacher, M. (2002). Housing and exploitation of horses in Switzerland: A representative analysis of the status quo. *Schweizer Archiv für Tierheilkunde* 144, 331-347.

Bagshaw, C.S., Ralston, S.L. & Fisher, H. (1994). Behavioral and physiological effect of orally administered tryptophan on horses subjected to acute isolation stress. *Applied Animal Behaviour Science* 40, 1-12.

Boissy, A. & Le Neindre, P. (1990). Social influences on the reactivity of heifers: implications for learning abilities in operant conditioning. *Applied Animal Behaviour Science* 25, 149-165.

Boissy, A. & Le Neindre, P. (1997). Behavioral, cardiac and cortisol responses to brief peer separation and reunion in cattle. *Physiology & Behaviour* 61, 693-699.

Bourjade, M., Moulinot, M., Henry, S., Richard-Yris, M.A. & Hausberger, M. (2008). Could adults be used to improve social skills of young horses, *Equus caballus*? *Developmental Psychobiology* 50, 408-417.

Bourjade, M. & Sueur, C. (2010). Shared or unshared consensus for collective movement? Towards methodological concerns. *Behavioural Processes* 84, 648-652.

Bourjade, M., Thierry, B., Maumy, M. & Petit, O. (2009). Decision-making in Przewalski horses (*Equus ferus przewalskii*) is driven by the ecological contexts of collective movements. *Ethology* 115, 321-330.

Boyd, L. & Keiper, R. (2005). Behavioral ecology of feral horses. In: Mills, D.S., *et al.* (Eds.) *The domestic horse*. Cambridge University Press, pp. 55-82.

Brent, L., Kessel, A.L. & Barrera, H. (1997). Evaluation of introduction procedures in captive chimpanzees. *Zoo Biology* 16, 335-342.

Burks, K.D., Mellen, J.D., Miller, G.W., Lehnhardt, J., A., W., Figueredo, A.J. & Maple, T.L. (2004). Comparison of two introduction methods for African elephants (*Loxodonta africana*). *Zoo Biology* 23, 109-126.

Bøe, K.E. & Færevik, G. (2003). Grouping and social preferences in calves, heifers and cows. *Applied Animal Behaviour Science* 80, 175-190.

Carbonaro, D.A., Friend, T.H., Dellmeier, G.R. & Nuti, L.C. (1992). Behavioral and physiological responses of dairy goats to isolation. *Physiology & Behavior* 51, 297-301.

Christensen, J.W., Ladewig, J., Søndergaard, E. & Malmkvist, J. (2002a). Effects of individual versus group stabling on social behaviour in domestic stallions. *Applied Animal Behaviour Science* 75, 233-248.

Christensen, J.W., Malmkvist, J., Nielsen, B.L. & Keeling, L.J. (2008). Effects of a calm companion on fear reactions in naive test horses. *Equine Veterinary Journal* 40, 46-50.

Christensen, J.W., Rundgren, M. & Olsson, K. (2006). Training methods for horses: habituation to a frightening stimulus. *Equine Veterinary Journal* 38, 439-443.

Christensen, J.W., Zharkikh, T., Ladewig, J. & Yasinetskaya, N. (2002b). Social behaviour in stallion groups (*Equus przewalskii* and *Equus caballus*) kept under natural and domestic conditions. *Applied Animal Behaviour Science* 76, 11-20.

Cooper, J.J. (1998). Comparative learning theory and its application in the training of horses. *Equine Veterinary Journal, Suppl.* 27, 39-43.

Cooper, J.J. & Albentosa, M.J. (2005). Behavioural adaptation in the domestic horse: potential role of apparently abnormal responses including stereotypic behaviour. *Livestock Production Science* 92, 177-182.

D'Eath, R.B. (2002). Individual aggressiveness measured in a resident-intruder test predicts the persistence of aggressive behaviour and weight gain of young pigs after mixing. *Applied Animal Behaviour Science* 77, 267-283.

D'Eath, R.B. & Lawrence, A.B. (2004). Early life predictors of the development of aggressive behaviour in the domestic pig. *Animal Behaviour* 67, 501-509.

D'Eath, R.B. & Pickup, H.E. (2002). Behaviour of young growing pigs in a resident-intruder test designed to measure aggressiveness. *Aggressive Behaviour* 28, 401-415.

delBarco-Trillo, J., McPhee, M.E. & Johnston, R.E. (2009). Nonagonistic familiarity decreases aggression in male Turkish hamsters, *Mesocricetus brandti*. *Animal Behaviour* 77, 389-393.

Durrell, J.L., Beattie, V.E., Sneddon, I.A. & Kilpatrick, D. (2003). Pre-mixing as a technique for facilitating subgroup formation and reducing sow aggression in large dynamic groups. *Applied Animal Behaviour Science* 84, 89-99.

Ellard, M.E. & Crowell-Davis, S.L. (1989). Evaluating equine dominance in draft mares. *Applied Animal Behaviour Science* 24, 55-75.

Erhard, H.W. & Mendl, M. (1997). Measuring aggressiveness in growing pigs in a resident-intruder situation. *Applied Animal Behaviour Science* 54, 123-136.

Erhard, H.W., Mendl, M. & Ashley, D.D. (1997). Individual aggressiveness of pigs can be measured and used to reduce aggression after mixing. *Applied Animal Behaviour Science* 54, 137-151.

Færevik, G., Jensen, M.B. & Bøe, K.E. (2006). Dairy calves social preferences and the significance of a companion animal during separation from the group. *Applied Animal Behaviour Science* 99, 205-221.

Farabollini, F., Albonetti, M.E. & Dessì-Fulgheri, F. (1991). Response to intruders in female rabbit colonies is related to sex of intruder and rank of residents. *Behavioural Processes* 24, 111-122.

Feh, C. (2005). Relationships and communication in socially natural horse herds. In: Mills, D.S.*, et al.* (Eds.) *The domestic horse. The evolution, development and management of its behaviour.* Cambridge University Press, pp. 83-109.

Feist, J.D. & McCullough, D.R. (1976). Behavior patterns and communication in feral horses. *Zeitschrift für Tierpsychologie* 41, 337-371.

Fiske, J.C. & Potter, G.D. (1979). Discrimination reversal-learning in yearling horses. *Journal of Animal Science* 49, 583-588.

Fraser, A.F. (1992). *The behaviour of the horse.* C.A.B. International, Wallingford.

Fraser, D. (1974). The behaviour of growing pigs during experimental social encounters. *Journal of Agricultural Science* 82, 147-163.

Fürst, A., Knubben, J., Kurtz, A., Auer, J. & Stauffacher, M. (2006). Group housing of horses: veterinary considerations with a focus on the prevention of bite and kick injuries. *Pferdeheilkunde* 22, 254-258.

Giersing, M. & Andersson, A. (1998). How does former acquaintance affect aggressive behaviour in repeatedly mixed male and female pigs? *Applied Animal Behaviour Science* 59, 297-306.

Goldschmidt-Rothschild, B. & Tschanz, B. (1978). Soziale Organisation und Verhalten einer Jungtierherde beim Camargue-Pferd. *Zeitschrift für Tierpsychologie* 46, 372-400.

Gough, M.R. (1999). A note on the use of behavioural modification to aid clipping ponies. *Applied Animal Behaviour Science* 63, 171-175.

Grogan, E.H. & McDonnell, S.M. (2005). Injuries and blemishes in a semi-feral herd of ponies. *Journal of Equine Veterinary Science* 25, 26-30.

Gygax, L., Neisen, G. & Wechsler, B. (2009). Differences between single and paired heifers in residency in functional areas, length of travel path, and area used throughout days 1-6 after integration into a free stall dairy herd. *Applied Animal Behaviour Science* 120, 49-55.

Harewood, E.J. & McGowan, C.M. (2005). Behavioral and physiological responses to stabling in naive horses. *Journal of Equine Veterinary Science* 25, 164-170.

Hausberger, M., Roche, H., Henry, S. & Visser, E.K. (2008). A review of the human-horse relationship. *Applied Animal Behaviour Science* 109, 1-24.

Heird, J.C., Lokey, C.E. & Cogan, D.C. (1986). Repeatability and comparison of two maze tests to measure learning ability in horses. *Applied Animal Behaviour Science* 16, 103-119.

Hemsworth, P.H. (2003). Human-animal interactions in livestock production. *Applied Animal Behaviour Science* 81, 185-198.

Hemsworth, P.H., Price, E.O. & Borgwardt, R. (1996). Behavioural responses of domestic pigs and cattle to humans and novel stimuli. *Applied Animal Behaviour Science* 50, 43-56.

Henderson, A.J.Z. (2007). Don't fence me in: Managing psychological well being for elite performance horses. *Journal of Applied Animal Welfare Science* 10, 309-329.

Hessing, M.J.C., Hagelso, A.M., van Beek, J.A.M., Wiepkema, R.P., Schouten, W.G.P. & Krukow, R. (1993). Individual behavioural characteristics in pigs. *Applied Animal Behaviour Science* 37, 285-295.

Holmes, L.N., Song, G.K. & Price, E.O. (1987). Head partitions facilitate feeding by subordinate horses in the presence of dominant pen-mates. *Applied Animal Behaviour Science* 19, 179-182.

Hopster, H. & Blokhuis, H.J. (1994). Validation of a heart-rate monitor for measuring a stress-response in dairy cows. *Canadian Journal of Animal Science* 74, 465-474.

Jensen, P. & Yngvesson, J. (1998). Aggression between unacquainted pigs - sequential assessment and effects of familiarity and weight. *Applied Animal Behaviour Science* 58, 49-61.

Jezierski, T., Jaworski, Z. & Gorecka, A. (1999). Effects of handling on behaviour and heart rate in Konik horses: comparison of stable and forest reared youngstock. *Applied Animal Behaviour Science* 62, 1-11.

Johnsson, J.I. & Åkerman, A. (1998). Watch and learn: preview of the fighting ability of opponents alters contest behaviour in rainbow trout. *Animal Behaviour* 56, 771-776.

Jørgensen, G.H.M., Borsheim, L., Mejdell, C.M., Søndergaard, E. & Bøe, K.E. (2009). Grouping horses according to gender - Effects on aggression, spacing and injuries. *Applied Animal Behaviour Science* 120, 94-99.

Jørgensen, G.H.M., Fremstad, K.E., Mejdell, C.M. & Bøe, K.E. (in press). Separating a horse from the social group for riding or training purposes - a descriptive study of human-horse interactions. *Journal of Applied Animal Welfare Science*.

Kaseda, Y., Ogawa, H. & Khalil, A.M. (1997). Causes of natal dispersal and emigration and their effects on harem formation in Misaki feral horses. *Equine Veterinary Journal* 29, 262-266.

Khalil, A.M. & Kaseda, Y. (1998). Early experience affects developmental behaviour and timing of harem formation in Misaki horses. *Applied Animal Behaviour Science* 59, 253-263.

King, A.J. (2010). Follow me! I'm a leader if you do; I'm a failed initiator if you don't? *Behavioural Processes* 84, 671-674.

Knierim, U. (1998). The behaviour of heifers after single or group introduction to the dairy herd. *KTBL-Schrift* 382, 115-120.

Knubben, J.M., Fuerst, A., Gygax, L. & Stauffacher, M. (2008). Bite and kick injuries in horses: Prevalence, risk factors and prevention. *Equine Veterinary Journal* 40, 219-223.

Kondo, S., Kawakami, N., Kohama, H. & Nishino, S. (1984). Changes in activity, spatial pattern and social behavior in calves after grouping. *Applied Animal Ethology* 11, 217-228.

Ladewig, J., Søndergaard, E. & Christensen, J.W. (2005). Ontogeny: preparing the young horse for its adult life. In: Mills, D.S., *et al.* (Eds.) *The domestic horse. The evolution, development and management of its behaviour.* Cambridge University Press, pp. 139-149.

Lansade, L., Bertrand, M., Boivin, X. & Bouissou, M.F. (2004). Effects of handling at weaning on manageability and reactivity of foals. *Applied Animal Behaviour Science* 87, 131-149.

Lansade, L., Bouissou, M.F. & Erhard, H.W. (2008). Reactivity to isolation and association with conspecifics: A temperament trait stable across time and situations. *Applied Animal Behaviour Science* 109, 355-375.

Lehmann, K., Ellendorff, F. & Kallweit, E. (2003). Dominanzverhalten bei Pferden - Eine Literaturstudie. *Landbauforschung Völkenrode* 53, 241-260.

Lehmann, K., Kallweit, E. & Ellendorff, F. (2006). Social hierarchy in exercised and untrained group-housed horses: A brief report. *Applied Animal Behaviour Science* 96, 343-347.

Lindberg, A.C., Kelland, A. & Nicol, C.J. (1999). Effects of observational learning on acquisition of an operant response in horses. *Applied Animal Behaviour Science* 61, 187-199.

Lloyd, A.S., Martin, J.E., Bornett-Gauci, H.L.I. & Wilkinson, R.G. (2008). Horse personality: Variation between breeds. *Applied Animal Behaviour Science* 112, 369-383.

Luescher, U.A., Friendship, R.M. & McKeown, D.B. (1990). Evaluation of methods to reduce fighting among regrouped gilts. *Canadian Journal of Animal Science* 70, 363-370.

Mal, M.E., Friend, T.H., Lay, D.C., Vogelsang, S.G. & Jenkins, O.C. (1991). Behavioral responses of mares to short-term confinement and social isolation. *Applied Animal Behaviour Science* 31, 13-24.

Mal, M.E. & McCall, C.A. (1996). The influence of handling during different ages on a halter training test in foals. *Applied Animal Behaviour Science* 50, 115-120.

Manning, A. & Dawkins, M.S. (1998). *An introduction to animal behaviour.* fifth edition, Cambridge University Press.

Maynard Smith, J. (1974). The theory of games and the evolution of animal conflicts. *Journal of Theoretical Biology* 47, 209-221.

McDonnell, S.M. (2003). *A practical field guide to horse behavior.* first edition, The Blood-Horse.

McDonnell, S.M. & Haviland, J.C.S. (1995). Agonistic ethogram of the equid bachelor band. *Applied Animal Behaviour Science* 43, 147-188.

McGee, S. & Smith, H.V. (2004). Accompanying pre-weaned Thoroughbred (*Equus caballus*) foals while separated from the mare during covering reduces behavioural signs of distress exhibited. *Applied Animal Behaviour Science* 88, 137-147.

McGreevy, P.D. (2004). Learning. In: McGreevy, P.D. (Ed.) *Equine behavior: a guide for veterinarians and equine scientists.* Saunders, Elsevier Limited. pp. 85-118.

McGreevy, P.D., Cripps, P.J., French, N.P., Green, L.E. & Nicol, C.J. (1995a). Management factors associated with stereotypic and redirected behavior in the thoroughbred horse. *Equine Veterinary Journal* 27, 86-91.

McGreevy, P.D., French, N.P. & Nicol, C.J. (1995b). The prevalence of abnormal behaviors in dressage, eventing and endurance horses in relation to stabling. *Veterinary Record* 137, 36-37.

McGreevy, P.D. & McLean, A. (2005). Behavioural problems with the ridden horse. In: Mills, D.S., *et al.* (Eds.) *The domestic horse. The evolution, development and management of its behaviour.* Cambridge University Press, pp. 196-211.

Mendl, M. (1999). Performing under pressure: stress and cognitive function. *Applied Animal Behaviour Science* 65, 221-244.

Mills, D.S. & Davenport, K. (2002). The effect of a neighbouring conspecific versus the use of a mirror for the control of stereotypic weaving behaviour in the stabled horse. *Animal Science* 74, 95-101.

Moberg, G.P. (2000). Biological responses to stress: Implications for animal welfare. In: Moberg, G.P.*, et al.* (Eds.) *The biology of animal stress: Basic principles and implications for animal welfare*. CABI Publishing, pp. 1-21.

Murphy, J. & Arkins, S. (2007). Equine learning behaviour. *Behavioural Processes* 76, 1-13.

Nicol, C.J. (1995). The social transmission of information and behaviour. *Applied Animal Behaviour Science* 44, 79-98.

Nicol, C.J. (1999). Understanding equine stereotypies. *Equine Veterinary Journal, Suppl.* 28, 20-25.

Nicol, C.J. (2002). Equine learning: progress and suggestions for future research. *Applied Animal Behaviour Science* 78, 193-208.

Olsson, I.A.S., de Jonge, F.H., Schuurman, T. & Helmond, F.A. (1999). Poor rearing conditions and social stress in pigs: repeated social challenge and the effect on behavioural and physiological responses to stressors. *Behavioural Processes* 46, 201-215.

Petit, O. & Bon, R. (2010). Decision-making processes: The case of collective movements. *Behavioural Processes* 84, 635-647.

Petit, O. & Thierry, B. (1994). Aggressive and peaceful interventions in conflicts in Tonkean macaques. *Animal Behaviour* 48, 1427-1436.

Pillot, M.H., Gautrais, J., Gouello, J., Michelena, P., Sibbald, A. & Bon, R. (2010). Moving together: Incidental leaders and naïve followers. *Behavioural Processes* 83, 235-241.

Pollard, J.C., Littlejohn, R.P. & Suttie, J.M. (1993). Effects of isolation and mixing of social groups on heart rate and behaviour of red deer stags. *Applied Animal Behaviour Science* 38, 311-322.

Pollmann, U. (2006). Datenerhebung in Offenlaufställen für Pferde. *DVG Fachgruppe Tierschutzrecht*, pp. 126-138.

Ramseyer, A., Boissy, A., Dumont, B. & Thierry, B. (2009). Decision making in group departures of sheep is a continuous process. *Animal Behaviour* 78, 71-78.

Raussi, S., Boissy, A., Delval, E., Pradel, P., Kaihilahti, J. & Veissier, I. (2005). Does repeated regrouping alter the social behaviour of heifers? *Applied Animal Behaviour Science* 93, 1-12.

Redgate, S.E. & Davidson, H.P.B. (2007). The effects of the presence or absence of a familiar horse during feeding. In: Hausberger, M.*, et al.* (Eds.) *Horse behaviour and welfare*. EAAP Publication 122, Wageningen Academic Publishers, pp. 65-69.

Rivera, E., Benjamin, S., Nielsen, B., Shelle, J. & Zanella, A.J. (2002). Behavioral and physiological responses of horses to initial training: the comparison between pastured versus stalled horses. *Applied Animal Behaviour Science* 78, 235-252.

Roeder, J.J., Duval, L. & Gosset, D. (2002). Aggressive and neutral interventions in conflicts in captive groups of Brown Lemurs (*Eulemur fulvus fulvus*). *American Journal of Physical Anthropology* 118, 253-258.

Rutberg, A.T. & Greenberg, S.A. (1990). Dominance, aggression frequencies and modes of aggressive competition in feral pony mares. *Animal Behaviour* 40, 322-331.

Schilder, M.B.H. (1990). Interventions in a herd of semi-captive plain zebras. *Behaviour* 112, 53-83.

Seaman, S.C., Davidson, H.P.B. & Waran, N.K. (2002). How reliable is temperament assessment in the domestic horse (*Equus caballus*)? *Applied Animal Behaviour Science* 78, 175-191.

Svala, C. (2008). Hur hålls hästarna i Sverige och vilka är motiven. *Ett* projekt genomfört med stöd av Jordbruksverkets satsning på Livskraftigt hästföretagande 2008. *Sveriges Lantbruksuniversitet*, Alnarp.

Søndergaard, E. & Christensen, J.W. (2009). Stable versus unstable groups of horses: effects on social behaviour and injuries. In: Søndergaard, E., *et al*. (Eds.)*, Proceedings of the 21st Nordic Symposium of the International Society for Applied Ethology,* Bjerringbro, Denmark, p. 20.

Søndergaard, E., Clausen, E., Christensen, J.W. & Schougaard, H. (2004). Housing of horses: Danish recommendations. *DIAS Report*, Special Edition.

Søndergaard, E. & Halekoh, U. (2003). Young horses' reactions to humans in relation to handling and social environment. *Applied Animal Behaviour Science* 84, 265-280.

Søndergaard, E. & Ladewig, J. (2004). Group housing exerts a positive effect on the behaviour of young horses during training. *Applied Animal Behaviour Science* 87, 105-118.

Søndergaard, E. & Turner, K.G. (2008). Timeframe for a novel horse to become familiar in a group. In: Krüger, K. (Eds.), *Proceedings of the 2nd International Equine Science Meeting,* Regensburg, Germany, p. 39.

Swedish Animal Welfare Agency's administrative provisions and guidelines on the keeping of horses (2007). *DFS 2007:XX, Ref. L 101.*

Tennessen, T., Price, M.A. & Berg, R.T. (1985). The social interactions of young bulls and steers after re-grouping. *Applied Animal Behaviour Science* 14, 37-47.

VanDierendonck, M.C., de Vries, H. & Schilder, M.B.H. (1995). An analysis of dominance, its behavioural parameters and possible determants in a herd of Icelandic horses in captivity. *Netherlands Journal of Zoology* 45, 362-385.

VanDierendonck, M.C., de Vries, H., Schilder, M.B.H., Colenbrander, B., Þorhallsdóttir, A.G. & Sigurjónsdóttir, H. (2009). Interventions in social behaviour in a herd of mares and geldings. *Applied Animal Behaviour Science* 116, 67-73.

VanDierendonck, M.C., Sigurjonsdottir, H., Colenbrander, B. & Thorhallsdottir, A.G. (2004). Differences in social behaviour between late pregnant, post-partum and barren mares in a herd of Icelandic horses. *Applied Animal Behaviour Science* 89, 283-297.

Veissier, I., Gesmier, V., Le Neindre, P., Gautier, J.Y. & Bertrand, G. (1994). The effects of rearing in individual crates on subsequent social behaviour of veal calves. *Applied Animal Behaviour Science* 41, 199-210.

Verrill, S. & McDonnell, S. (2008). Equal outcomes with and without human-to-horse eye contact when catching horses and ponies in an open pasture. *Journal of Equine Veterinary Science* 28, 309-312.

Vervaecke, H., Stevens, J.M.G., Vandemoortele, H., Sigurjónsdóttir, H. & De Vries, H. (2006). Aggression and dominance in matched groups of subadult Icelandic horses (*Equus caballus*). *Journal of Ethology* 25, 239-248.

Visser, E.K., Ellis, A.D. & Van Reenen, C.G. (2008). The effect of two different housing conditions on the welfare of young horses stabled for the first time. *Applied Animal Behaviour Science* 114, 521-533.

Visser, E.K., van Reenen, C.G., Schilder, M.B.H., Barneveld, A. & Blokhuis, H.J. (2003). Learning performances in young horses using two different learning tests. *Applied Animal Behaviour Science* 80, 311.

Waran, N.K. (2001). The social behaviour of horses. In: Keeling, L.J., *et al.* (Eds.) *Social behaviour in farm animals*. CABI Publishing, pp. 247-274.

Waring, G.H. (2003). *Horse behavior.* second edition. William Andrew Publishing.

Wiepkema, R.P. & Schouten, W.G.P. (1990). Mechanisms of coping in social situations. In: Zayan, R., *et al.* (Eds.) *Social stress in domestic animals*. Dordrecht Kluwer Academic Publishers, pp. 8-24.

Wolff, A., Hausberger, M. & Le Scolan, N. (1997). Experimental tests to assess emotionality in horses. *Behavioural Processes* 40, 209-221.

Zayan, R. (1991). The specificity of social stress. *Behavioural Processes* 25, 81-93.

Zeeb, K. & Pollmann, U. (1996). Verhaltensgerechte Pferdehaltung: Gruppenauslaufhaltung. *Tierhygienisches Institut Freiburg*, Germany.

Acknowledgements

Thanks to the Swedish Research Council for Environment, Agricultural Sciences and Spatial Planning who had funded my studies. My PhD project was part of a Nordic collaborative project called: Group housing horses under Nordic conditions: Strategies to improve horse welfare and human safety.

This work would not have been possible without the support from many people. In particular, I wish to express my sincere gratitude to:

My supervisor Linda Keeling who gave me the chance to start this PhD project. Thanks for your enthusiasm, inspiration and constructive criticism along the way, your immense positive attitude and patience to keep me on track. You have taught me so much about research!

Margareta Rundgren, my co-supervisor for being always ready to discuss applied horse questions and for participating in fruitful debates that have improved my experiments and written work.

Thanks to all members in the Nordic project (Eva Søndergaard, Seppo Hyyppä, Knut Bøe, Grete Jørgensen, Cecilie Mejdell, Jan Ladewig, Susanne Särkijärvi) for sharing so many good thoughts at our meetings in Finland, Norway and Denmark, and for staying active and keeping up the good work. Special thanks to Eva who has been around for my last study and for taking the time for commenting on the manuscript.

Janne Winther Christensen for her help with big parts of my studies, particularly for the hours we spent together in the stable shaping the set up of my experiments and for helping with the training of the experimental

subjects, our beloved horses, before we even could start testing. Thank you for all the nice time we spent together in Denmark and for your continuous support when I was trying to put everything on paper.

A big thank you to the Foulum' staff members Anton Steen Jensen and Henrik Krogh Andersen and the students who were brave enough to get in close contact with the four legged youngsters, making all the week long testing possible, and to Erik Luc Decker who was always on-site to fix technical problems. Thanks also to Karen Thodberg, Lene Munksgaard and Birthe Houbak for inviting me to their homes and in giving me the trust to take care of their houses and pets.

I wish to thank the personal at the Department of Clinical Sciences at SLU, especially Mari Wallbring, for allowing me to work with the horses and for the continuous helpfulness in all practicalities around purchasing and managing these horses.

Katharina Selling and Mikael Elenius for guiding me through the statistical challenges of my data and for answering lots of extra questions.

Therese Rehn who was around from the very start and who helped with many smaller and bigger matters arising during my work, for knocking on my office door and being there when I needed another opinion. My past and present colleagues Emma, Yezica, Birgitte and Ragen, Malin, Nadine, Anna, Sophie, Anette, Elin, Arnd and Harry for your inspiration and for making HMH in Uppsala a very nice place to work and all others that I shared fika breaks with.

My colleagues at the department and all PhD students who have given their support during my work and for making my years at HMH enjoyable.

Thanks to all members of the organising committee of ISES Sweden 2010 for being part of the odyssey of organising the 6th International Equitation Science Conference. I am grateful for all your positive feedback, your belief in my skills and all your hard work that contributed to make the conference a success. I will never regret to have taken up this enormous task during my PhD as it was an additional immense learning experience. I would not hesitate to do it all over again and I hope to catch up with you in the future. Vi ses i Nederländerna 2011!

My fantastic parents who have been fully supportive throughout my life, whatever choices I finally made. Thank you so much for giving me the opportunity to study in Edinburgh and for sending me around the globe which has opened doors for me and had ultimately affected me so positively in many ways. I am forever grateful for this! My sister for being a patient listener and advisor and her family for sharing funny times with me.

Fredrik for taking care of my emotional well being in the last four years and for helping me in all possible ways to make my life in Sweden enjoyable, and for being my very best friend.

Marianne Sundén and Helena Wiklund who have accompanied me on my journey of experiencing what it is like to be a horse owner. Special thanks to Helena, without your generous support I would have given up, and to Cassandra who took so good care of Curioso when I could not afford to do anything else but to work and for appreciating and enjoying his lovely Spanish personality. Paulo Bastos, for being patient with me and Curioso and for all your encouraging words and professional guidance through all training.