EQUINE VETERINARY JOURNAL
Equine vet. J. (2008) **40** (1) 46-50
doi: 10.2746/042516408X245171

Effects of a calm companion on fear reactions in naïve test horses

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Keywords: horse; behaviour; fear; heart rate; social influence

Summary

Reasons for performing study: **In fear-eliciting situations, horses tend to show flight reactions that can be dangerous for both horse and man. Finding appropriate methods for reducing fearfulness in horses has important practical implications.**

Objectives: **To investigate whether the presence of a calm companion horse influences fear reactions in naıve subject horses.**

Hypotheses: **The presence of a habituated (calm) companion horse in a fear-eliciting situation can reduce fear reactions in naıve subject horses, compared to subject horses with a nonhabituated companion (control).**

Methods: **Minimally handled (n = 36), 2-year-old stallions were used, 18 as subjects and 18 as companions. Companion horses (n = 9) were habituated to an otherwise frightening, standardised test stimulus (calm companions), whereas the rest (n = 9) of the companion horses remained nonhabituated (control companions). During the test, unique pairs of companion and subject horses were exposed to the test stimulus while heart rate and behavioural responses were registered. Subsequently, subject horses were exposed to the stimulus on their own (post test).**

Results: **Subject horses, paired with a calm companion horse, showed less fear-related behaviour and lower heart rate responses compared to subject horses with control companions. Results from the post test suggest that the difference between treatment groups remained in the subsequent absence of companion horses.**

Conclusions and potential relevance: **It appears possible to reduce fear reactions in young, naıve horses by allowing them to interact with a calm companion horse in fear-eliciting situations.**

Introduction

Equids have coevolved with predators for millions of years in the wild and this association has directed the evolution of their morphology, habitat choice and behaviour. Like other prey species, equids have evolved antipredator responses both to actual encounters with predators and to generalised threatening stimuli, such as loud noises and sudden events (Frid and Dill 2002). During domestication, the threshold for experiencing fear has been elevated, but domestic animals still show the same type of responses as their wild ancestors once that threshold has been reached. Therefore, antipredator strategies that evolved in wild ungulates persist in domestic animals, even in the absence of natural predators (Byers 1997).

Accordingly, domestic horses tend to react with avoidance or flight to unfamiliar situations and potential dangers. Fear responses can be dangerous for a restricted horse as well as for human subjects who are in direct contact with the frightened horse. In addition, high levels of fear can have negative consequences on performance, health, reproduction and welfare (Boissy 1995), and 'freedom from fear' is included in the 5 freedoms that define ideal states for welfare (UK Farm Animal Welfare Council). It is, therefore, highly relevant to explore scientifically the possibilities for reducing fearfulness in horses, for instance through the development of appropriate habituation procedures (Christensen *et al*. 2006), and through provision of appropriate environments (e.g. access to social partners).

The social system of horses should offer opportunities for social transmission of behaviour between individuals (Nicol 2002, 2005) and, in practice, older and experienced horses are sometimes used as companions when young horses are introduced to traffic and other fear-eliciting situations. There are, however, very few scientific data investigating social influence on behaviour in horses; and previous studies did not find any evidence of social learning in discrimination learning tasks (Baer *et al*. 1983; Baker and Crawford 1986; Clarke *et al*. 1996). In these experiments, subject horses were allowed to observe a trained demonstrator horse choosing correctly between 2 differently coloured or marked food buckets, but the authors reported no significant effects of prior observation in their horses. Clarke *et al*. (1996) found, however, that observer horses were significantly faster to approach the goal in their first trial, indicating that the horses had learned something about the location of food. Lindberg *et al*. (1999) investigated whether the presence of a trained conspecific could facilitate the acquisition of an operant foot press response in order to obtain food. During demonstrations, observer animals were free to interact with a

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[Paper received for publication 01.06.07; Accepted 10.09.07]

similar device, as that successfully operated by the demonstrator, but the authors found no significant effects of the social observation. One possible explanation for this is that these experiments did not replicate situations in which social transmission of information and behaviour is most likely, for instance in more biologically relevant contexts, such as fear-eliciting situations. Indeed, Henry *et al*. (2005) demonstrated that foals learn by observation of their mother's reaction towards man and react accordingly when tested alone up to one year later.

In the present study, horses were offered an attractive food source for a limited period, during which they were exposed to a frightening, sudden stimulus. Instinctively, horses flee from sudden, unknown stimuli and they are therefore subjected to a motivational conflict between feeding and avoiding the stimulus. We hypothesised that the presence of a habituated (calm) companion horse would reduce fear reactions to the sudden stimulus in naıve subject horses, compared to subject horses with a nonhabituated companion (control). The aim was to investigate the effect of a calm companion horse on fear reactions in naıve subject horses and also how subject horses react in the subsequent absence of companion horses.

Materials and methods

The study conformed to the *'Guidelines for Ethical Treatment of Animals in Applied Animal Behaviour and Welfare Research'* by the Ethics Board of the International Society of Applied Ethology (www.applied-ethology.org).

Animals

Thirty-six 2-year-old Danish Warmblood stallions from a large studfarm were used. The colts had experienced similar rearing conditions (group housing) and had received a minimum of handling. One month prior to the experiment, the colts were pastured in a large enclosure (30 ha) and all habituated to wearing halters and heart rate monitoring equipment, and to being led by a human handler. In addition, all horses were habituated to social isolation and to feeding from a container inside a test arena. Horses were regarded as habituated and ready for the experiment when they entered the test arena voluntarily, walked directly to the food container and fed for at least 90 s of a total of 120 s. The required number of initial habituation sessions was noted for each horse. For the experiment, 18 horses acted as subjects and 18 as companions, and the groups were balanced according to reactivity, based upon the reactions of the horse during the initial handling (i.e. required number of initial habituation sessions) and also to sire. Nine companion horses were habituated to the test stimulus (see below; calm companions), whereas the other companion horses remained nonhabituated (n = 9; control companions). Therefore, 18 unique pairs were created, each containing a subject horse and either a calm or a control companion horse. Subject horses with calm companion horses are referred to as TEST horses and those with nonhabituated companion horses as CONTROL horses.

Test arena

Within the 30 ha summer enclosure, a smaller capture enclosure (1 ha) contained a fenced waiting area (50 m^2). Next to the waiting area a start box (2.5 m^2) and a circular test arena (10 m in diameter) of straw bales (1.2 x 1.2 x 2.4 m) in 2 layers was constructed, making the height of the walls of the arena 2.4 m (Fig 1). Two food containers, placed 2 m apart in the middle of the arena, contained a mixture of alfalfa and the horses' usual winter food (oat, barley, soybean oil meal, minerals and molasses).

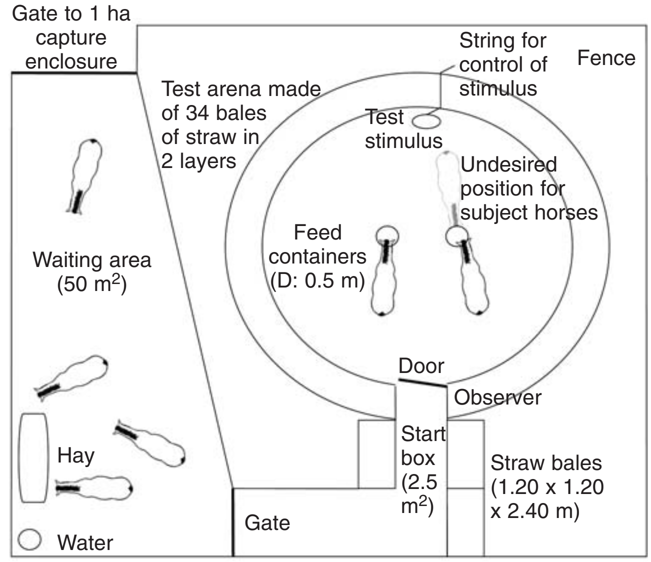

Fig 1: Test arena with 2 food containers and test stimulus. Unexpectedly, some pairs of horses consistently fed from the same container, and some subject horses took an inappropriate position in the semi-circle between the stimulus and the container.

Test stimulus and habituation of companions

The test stimulus was a black plastic bag (0.8 x 0.6 m), which was lifted 1 m from the ground at a speed of approximately 1 m/s by use of a string pulled from outside the arena (Fig 1). The bag contained 1 kg of sand to keep it steady. The nonmoving stimulus bag was present in the arena during the initial habituation to the test environment so that it did not act as a novel object during the tests.

The 9 horses that were to act as calm companions, were exposed to the test stimulus during 5 daily training sessions until they met a predefined habituation criterion. The criterion was met when the horse showed no, or only a minor behavioural reaction ('head up', Table 1), and an increase in heart rate by a maximum of 20 beats/min during the stimulus exposure. The horses needed 4–13 exposures before they met the habituation criterion. On test days, these companion horses were exposed to the test stimulus twice, immediately before the exposure with the naıve subject horse, ensuring that they remained habituated and calm.

Experimental procedure

The horses were tested in 2 blocks with an equal number of TEST and CONTROL horses (*Block 1*: 12 pairs, *Block 2*: 6 pairs), because not all 18 pairs were able to be tested in one day. On Day 1 (*Block 1*) and Day 2 (*Block 2*) horses were tested with companions (pair-test), and on Day 4 (*Block 1*) and Day 5 (*Block 2*) the subject horses were tested alone (post test). During the pair test, each pair of horses was exposed to 3 sessions of 3 min each. The stimulus was applied once during each session and horses allowed to feed for

TABLE 1: Ethogram of reactivity scores

Reaction	Description
1. None[1]	The horse does not react to the test stimulus and chewing is not interrupted.
2. Head up[1]	The horse raises its head from the food container and chewing may be briefly interrupted, but the horse is not alert (see below) and does not move away from the food container.
3. Alert	The horses stands vigilant with elevated neck, with or without tail elevation, head and ears oriented towards test stimulus, chewing is interrupted and the horse may move up to 2 steps away from the food container.
4. Away	The horse moves 3 or more steps backwards or sideways away from the food container in response to the test stimulus, typically followed by alertness.
5. Flight	The horse turns/jumps away from the food container in a sudden movement, typically followed by trotting/galloping, alertness and possibly snorting.

[1]Reaction allowed in habituation criterion for calm companion horses.

30 s before the stimulus was applied. The interval between sessions was 5 min, during which the pair was removed from the arena by 2 handlers and returned to visual contact with the other horses in the waiting area, while the arena was prepared for the next session. During the post test, the procedure was the same except that all subject horses were tested alone in 2 sessions of 3 min each, again with one stimulus exposure per session.

Recordings

Behavioural reaction to the test stimulus (Reactivity score; Table 1) and latency to resume feeding after each stimulus exposure were registered using a handheld computer (Workabout)[1]. The observer sat quietly on top of the straw wall next to the start box. All horses were habituated to the presence of the observer from the initial habituation to the test arena. Heart rate (HR) was recorded with Polar s810i[2], which consisted of an electrode belt with a built-in transmitter and a wristwatch receiver. Water and gel were used to optimise the contact between electrode and skin. The HR monitoring equipment was fitted on the horses in the waiting area prior to testing and the receiver stored data from the transmitter (R-R recordings). Subsequently, data were downloaded via a Polar Interface[2] to a PC, using the software Polar Precision Performance SW 4. The experiments were recorded on video for later analysis of the position of the horses prior to the stimulus exposure.

Data analysis

All horses, on both treatments, returned to the food within the test time and it was unnecessary to consider censored data in the analysis of latencies. Analysis of the heart rate data showed that there was no difference between the treatment groups in initial heart rates, which were measured for 5 min in the waiting area before the tests (mean ± s.e., TEST: 51.5 ± 1.66, CONTROL: 49.4 ± 1.53, ANOVA: F1,15 = 0.83, P = 0.376). Loss of contact with the electrodes of the heart rate monitors caused major disturbances in the recordings during flight reactions. Unfortunately, this inaccuracy made it impossible to use the R-R recordings for analysis of heart rate variability as was originally intended. However, maximum heart rate responses (HR_{max}) were obtained for most horses, reflecting the immediate response to the test stimulus.

Data were analysed using a Mixed Model Repeated Measures ANOVA in SAS 8.0 (www.sas.com) with session as repeated measure. The response variables were reactivity score, latency to resume feeding and maximum HR; fixed effects were treatment, session and their interaction. Block was fitted as a random factor in the model. The model was reduced by stepwise removal of insignificant terms. Data from the post test, when subject horses were exposed to the stimulus alone, were analysed separately in a similar model.

Correlations between reactivity score, latency and HR were also investigated; and correlations in responses between companion and test horses, using Spearman rank order correlations.

Methodological considerations

The set-up was chosen in order to allow horses to move around freely during the test and the use of food containers was aimed at controlling the position of the horses at the time of stimulus exposure. Unfortunately, some pairs of horses fed consistently from the same food container and some subject horses placed themselves closer to the test stimulus than the companion horse, i.e. the subject horse standing in the semi-circle closest to the stimulus and the companion horse standing in the semi-circle opposite the stimulus (Fig 1). Horses standing with their hindquarters directly in front of the test stimulus were more frightened when the stimulus moved suddenly, compared to horses standing on the other side of the food container. Through detailed analysis of the video recordings, it was possible to identify pairs of horses that were in an unintended position at the time of stimulus exposure and data on these pairs were omitted from that particular session.

The number of horses per session was subsequently: Session 1: TEST = 5 and CONTROL = 9, Session 2: TEST = 7 and CONTROL = 9, Session 3: TEST = 7 and CONTROL = 9. More TEST pairs placed themselves in an inappropriate position, compared to CONTROL pairs. This may have been a treatment effect, because the calm companion horses were aware that the stimulus bag could suddenly move and therefore placed themselves furthest away from the bag and as many of these pairs fed from the same container, the TEST horse was closer to the stimulus.

In the post test, data were likewise omitted for horses that stood in the semicircle closest to the stimulus at the time of the exposure, and the number of horses was subsequently: Session 1: TEST = 5 and CONTROL = 7, Session 2: TEST = 7 and CONTROL = 8.

Results

Pair test: with companions

Results showed that TEST horses received lower reactivity scores (Mixed Models: $F_{1,14}$ = 8.35, P = 0.011; Fig 2a), had shorter

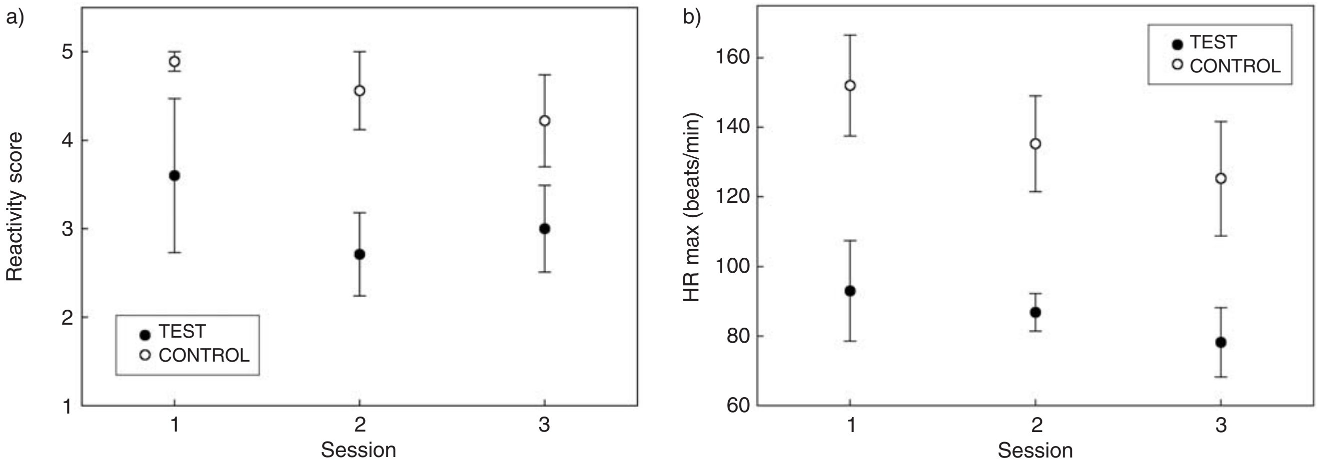

Fig 2: Reactivity score (a) and maximum heart rate responses (b) of subject horses (mean ± s.e.) during the 3 exposures with calm companions (TEST) or control companions (CONTROL) in the pair test.

latencies to return to the food (Mixed Models: mean ± s.e., TEST: 4.91 ± 4.36 s vs. CONTROL: 18.19 ± 3.51 s, $F_{1,14}$ = 5.91, P = 0.029), and lower heart rate responses (Mixed Models: $F_{1,13}$ = 7.54, P = 0.016; Fig 2b) compared to CONTROL horses. There were no significant effects of session and no significant interactions.

Post test: without companions

In the post test, where subject horses were tested without companions, TEST horses also received lower reactivity scores (Mixed Models: $F_{1,14}$ = 4.79, P = 0.045; Fig 3a) and had shorter latencies to return to the food (Mixed Models: mean ± s.e., TEST: 6.69 ± 5.03 s vs. CONTROL: 20.58 ± 4.36 s, $F_{1,14}$ = 4.74, P = 0.046). There were also significant effects of session because the horses reacted less during the second exposure (Mixed Models: Reactivity score: $F_{1,14}$ = 6.49, P = 0.031; Fig 3a; Latency: session 1: 17.21 ± 2.93 s vs. session 2: 10.06 ± 2.36 s, $F_{1,14}$ = 3.53, P = 0.093). For the heart rates, a significant interaction was found between treatment and session (Mixed Models: HR_{max}: $F_{1,14}$ = 8.26, P = 0.021, Fig 3b).

Correlations

Strong correlations were found between the reactivity score, latency to return to the food and HR of each test horse, indicating that all 3 measures reflect reactivity (Spearman rank correlation: Session 1: Latency and HR: rS = 0.70, n = 13, P = 0.007; latency and score: rS = 0.66, n = 14, P = 0.009; score and HR: rS = 0.57, n = 13, P = 0.041; Session 2: Latency and HR: rS = 0.78, n = 15, P<0.001; latency and score: rS = 0.82, n = 16, P<0.001; score and HR: rS = 0.76, n = 15, P<0.001; Session 3: Latency and HR: rS = 0.90, n = 14, P<0.001; latency and score: rS = 0.91, n = 16, P<0.001; score and HR: rS = 0.77, n = 14, P<0.001).

Responses of companion and test horses correlated significantly (Spearman rank correlation: Reactivity score: rS = 0.82, n = 14, P<0.001; Latency: rS = 0.90, n = 14, P<0.001; HR_{max}: rS = 0.89, n = 9, P<0.001).

Discussion

The findings of this study provide a first demonstration of social influence on horse reactions in a fear-eliciting situation. Horses

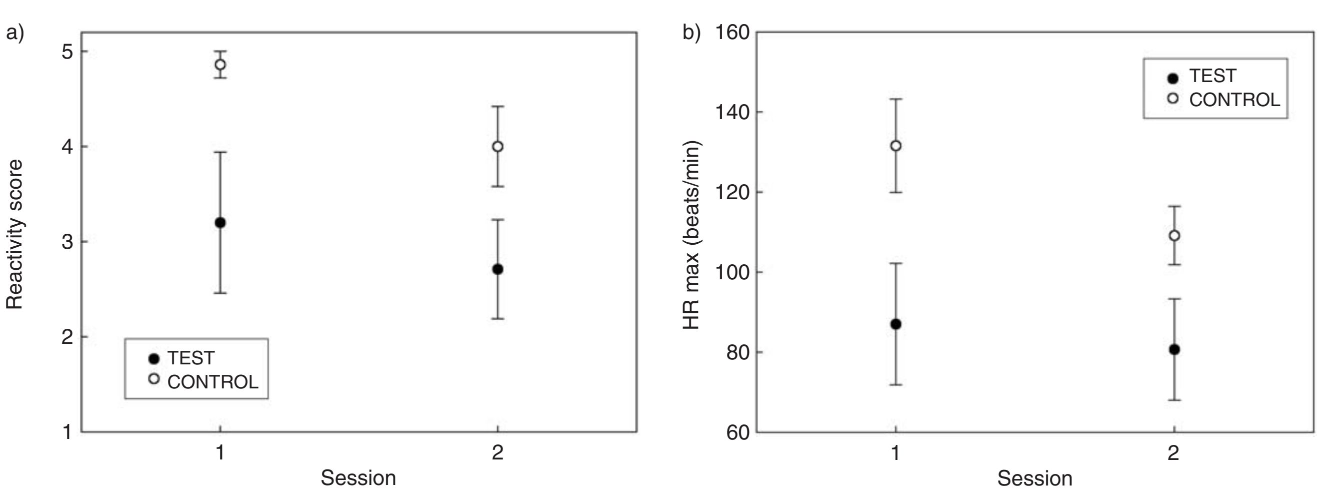

Fig 3: Reactivity score (a) and maximum heart rate responses (b) of subject horses when subsequently exposed to the stimulus alone (post test).

that were paired with calm companions reacted less to the test stimulus in terms of both behavioural and heart rate responses, compared to control horses paired with control companions. Similarly, responses of companion and test horses correlated significantly. The treatment groups were carefully balanced for reactivity during the initial handling and habituation to the test arena, and, therefore, accidental allocation of more fearful animals to the control group was avoided. The results confirm the benefits of using calm, experienced companions for habituation of naıve horses. The data also suggest that the subject horses reacted similarly as in the pair test in the subsequent absence of companion horses (post test). This result implies that the horses were able to retain information from the previous exposures in the subsequent absence of companion animals. This is in contrast to previous studies on social learning in horses (Baer *et al.* 1983; Baker and Crawford 1986; Clarke *et al.* 1996; Lindberg *et al.* 1999) but may be explained by the fact that we tested unconditioned responses in a simpler and, possibly, more biologically relevant context and that the subject horses were together with the companion during the demonstrations. Therefore they needed only to recall their own reaction to the stimulus during the previous exposure, rather than the reaction of the companion. It appears that, in a social context, horses tend to react like their conspecifics, but when isolated they may rely mainly on own experiences. Such a strategy would probably be adaptive in the wild because, in a social context, reacting like a conspecific can minimise antipredation costs, whereas an animal would have to rely on individual learning when alone (Kendal *et al.* 2005).

In the post test, a significant effect of session was also found, indicating that horses start to habituate to the test stimulus. A previous study has shown that horses generally cease reacting to a sudden but harmless stimulus after 5 exposures (Christensen *et al.* 2006), which is in accordance with results on other species (e.g. wallabies, Griffin and Evans 2003). In the present study, the subject horses received only 5 stimulus exposures in total (3x in the pair test and twice in the post test) to prevent that individual habituation would override the treatment effect. Strong correlations were found between reactivity scores, latency to resume feeding and heart rate, which indicates that all 3 measures of reactivity are linked. This is in accordance with Christensen *et al.* (2006) who applied the same reactivity scores in a habituation experiment.

In group-living species, social influence on responses is likely to be improved if the subject is allowed to interact with a demonstrator (Moscovice and Snowdon 2006). In order to provide the best opportunities for social transmission the horses were allowed to interact and move freely during the tests in the present study. This set-up, however, caused some methodological complications. In spite of the use of 2 food containers, aimed at controlling the position of the horses at the time of stimulus exposure, some pairs of horses consistently fed from the same container and some subject horses thereby placed themselves closer to the test stimulus than the calm companion horse, facing in the opposite direction. In future experiments it may be necessary to consider whether the benefits of allowing the animals to move freely during the test outweigh the risk of data loss caused by inappropriate positions during the stimulus exposure.

In conclusion, there appears to be substantial potential for exploiting the possibility of social influence on reduction of fear in horses. In the domestic environment mature horses are typically kept singly and young horses are kept in single age groups. However, the keeping of horses in social groups and inclusion of older, experienced horses into groups of young horses could facilitate habituation to various fear-eliciting stimuli. Likewise, it may be possible to use older, calm horses to habituate young naıve horses to various veterinary practices, reducing the need for calmatives. Further research is needed in this area in order to investigate in which situations or contexts social influence on behaviour is most apparent, as well as whether social influences are stronger between certain individuals, e.g. family members or socially dominant horses.

Acknowledgements

We greatly acknowledge Viegaard Horse Stud, Denmark, for kindly allowing us to use their stallions. Eric L. Decker, Gynther Nielsen and Dr Eva Søndergaard, Faculty of Agricultural Sciences, University of Aarhus, for help and comments. Kristina Olsson, Swedish University of Agricultural Sciences (SLU), for technical assistance. This study was funded by a SLU strategic horse research fund, Hippocampus, and the Danish Research Council for Technology and Production.

Manufacturers' addresses

[1]Psion PLC, London, UK.
[2]Polar Electro Oy, Kempele, Finland.

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Author contributions All authors contributed to the initiation, conception, planning and writing of this study. Its execution and statistics were by J.W.C. and J.M.