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Applied Animal Behaviour Science 87 (2004) 131–149
APPLIED ANIMAL BEHAVIOUR SCIENCE
www.elsevier.com/locate/applanim

Effects of handling at weaning on manageability and reactivity of foals

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Received 11 February 2003; received in revised form 22 December 2003; accepted 22 December 2003

Abstract

The horse's temperament, including its manageability and reactivity and/or fearfulness, is of importance as it can result in problems and can render horses unsuitable for inexperienced riders.

Early experience, including handling during infancy, may influence the horse's adult behaviour and reduce its fear of humans and other potentially frigthening situations. In the various species studied, handling has generally been undertaken during the neonatal period. The aim of the present study was to test the effects of handling young horses around the time of weaning, a period which has been demonstrated to be effective in increasing ease of handling in cattle and goats.

Sixteen Anglo-Arab foals were handled for 12 days either immediately following weaning (early handled: EH) or 21 days later (late handled: LH); eight additional non-handled foals served as controls (C). Handling consisted of haltering, gently petting all parts of the body, picking up feet and leading the foal over 120 m. During handling sessions, EH were easier to handle than LH: time taken to fit them with a halter, to pick up feet, and "walk-ratio" (time walking under constraint/total time walking) were significantly lower for EH. During subsequent tests conducted over 2 days, 4, and 7 months, as well as 10 months and to some extent 18 months after the end of handling period, EH and LH were easier to handle and less reactive than controls, although differences diminished with time. The period following weaning can therefore be qualified as an "optimal period" for handling. Some of the effects persist for at least 18 months.

Keywords: Horse; Handling; Weaning; Manageability; Emotional reactivity; Fearfulness; Human–animal relationship

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doi:10.1016/j.applanim.2003.12.011

1. Introduction

These days horses are mainly used for sport or leisure activities. Selection is based almost exclusively on genetics, physical traits and on the animal's performances during competitions. Although of importance, the horse's temperament is almost always neglected. Consequently, some horses may be unsuitable due to their temperament, especially if intended for inexperienced riders or children. Temperament however is often neglected in horse breeding, in spite of its importance in determining the suitability of a horse for a particular rider.

Temperament is defined as a set of "biologically rooted individual differences in behaviour tendencies that are present early in life and are relatively stable across various kinds of situations and over the course of time" (Bates, 1989). Some of these characteristics are of importance when considering human–horses relationships, especially fearfulness.

Fearfulness is a characteristic of the individual that leads him to react with the same trend to a variety of frightening events. Aside from enhancing the risks of accidents, both for the animals themselves and for their handlers, a high fear level may affect various aspects of behaviour and productivity. Emotional reactivity, including fearfulness, has been demonstrated to strongly influence social rank in cattle (Bouissou, 1978; Bouissou and Gaudioso, 1982; Plusquellec et al., 2001), maternal behaviour (pig: Kilgour and Dalton, 1984; sheep: Putu, 1990; horse: Arnold, 1985), reproduction in pigs (Hemsworth et al., 1986, 1991), growth of calves and quality of veal meat (Lensink et al., 2000). High levels of fearfulness impair learning ability in mice (Chapillon and Debouzie, 2000) as well as in horses (Fiske and Potter, 1979). Finally, intense or chronic fear reactions can cause significant reductions in welfare (for review: Boissy, 1998; Hemsworth and Coleman, 1998).

Manageability is obviously another essential characteristic for the rider–horse relationship. Horses would also need to be managed if they were to be used for draft or transportation. Manageability, tractability, ease of handling or docility are often used interchangeably but without being clearly defined (Sato et al., 1981; Le Neindre et al., 1996; Simpson, 2002). We define manageability as the ease with which a person can impose a routine handling procedure on horses such as the fitting of a halter, picking up feet or leading. It reflects a combination of behavioural traits including fearfulness, aggressiveness, propensity to accept restraints, etc. and also reactivity to humans. One important question often asked to applied ethologists is how to improve such traits of temperament?

In comparison to routine handling, extra handling confers beneficial effects on various aspects of production, as well as reducing fear of human beings in a variety of farm animal species (for review: Rushen et al., 1999) including horses (Jezierski et al., 1999; Mac Cann et al., 1988; Simpson, 2002). The period during which the handling is applied is also of importance. Early experience is well known to strongly influence adult behaviour. Early handling reduces farm animals' fear of humans (for review: Krohn et al., 2001) and also often reduces more general reactivity and fearfulness in various species (for review: Denenberg, 1962; Mason, 2000), although results are sometimes contradictory. For example, some authors reported lower reactivity for handled animals (rat: Schaeffer, 1963; rabbit: Kersten et al., 1989; silver fox: Pedersen and Jeppesen, 1990) whereas others reported no differences between handled and non handled subjects (rat: Galef, 1970; hens: Jones and Faure, 1981).

Although it has become fashionable in the horse industry to use an early training procedure referred to as "imprint training" by Miller (1991), the question of the existence of an early sensitive period of contact in horses is still questionable (Williams et al., 2002). Moreover, the persistence of the effects of handling varies according to species (fox: Pedersen, 1994; cattle: Sato et al., 1981; Boissy and Bouissou, 1988; Boivin et al., 1992a; goat: Boivin and Braastad, 1996; horses: Lansade et al., 2002; Simpson, 2002).

Most studies have favoured the neonatal period as the ideal time for handling (for review: Boivin et al., 2001; Krohn et al., 2001). However this period, even if it seems efficient for the establishment of a good human–animal relationship, may also have some disadvantages in large farm animals. Simpson (2002) discussed some of the potential risks of handling of neonatal horses: the dam–young relationship could be broken, the handler may be subjected to aggression from a protective mother, later in life the animals might not 'respect' humans, instead considering them as conspecifics (play, dominance behaviour). In addition, early human contact is less effective for lambs reared in the presence of their dam than for those reared without their dam (Boivin et al., 2002).

Therefore other developmental periods, such as the time of weaning should also be considered. Artificial weaning, a common practice for farm animals, implies an abrupt separation between dam and young that is associated with changes in the social and physical environment. Such treatments are known to induce stress (pig: Dybkjaer, 1992; Weary and Fraser, 1997; Orgeur et al., 1998, 2001; cattle: Lefcourt and Elsasser, 1995; horse: Mac Call et al., 1985; Malinowski et al., 1990; Houpt et al., 1984). Bateson (1979) suggests that any period of reorganization, associated with stress, could be a period of special sensitivity to external stimuli. Thus, animals could be particularly susceptible to environmental influences at that time, and human contact during this period may result in a more efficient and friendly human–animal relationship (cattle: Boivin et al., 1992a; goats: Boivin and Braastad, 1996). Furthermore, Veissier et al. (1989) described better learning performance of calves just after weaning than 1 month later.

The purpose of this study was first to establish if the weaning period can be considered as a favourable period for handling horses, and second to assess the duration of the effects of handling on horse's manageability, fear of human beings and more general reactivity.

2. Animals, material and methods

2.1. Animals

Twenty-four Anglo-Arab foals (12 males and 12 females), born in April and May 2000 were used. They were individually identified with an electronic chip in the neck.

Each dam–foal dyad was individually penned for the first 2 weeks of life. They were then maintained at pasture and supplemented with a concentrate feed until approximately 6 months of age (±1 month), when they were abruptly weaned (complete and definitive separation from the mother). They were then housed indoors in groups of four foals in 6 m × 6 m pens surrounded by solid walls, for 2 months corresponding to the duration of handling treatment period (see below). From 8 to 12 months (winter period), they were housed individually in 6 m × 3 m pens, and then from 12 to 18 months of age, returned to pasture as a group until the end of the experiments. During this latter period, when they had to be tested, foals were placed again individually in the 6 m × 3 m pens 24 h before the tests.

Outside of the handling periods, all foals received similar limited human contact necessary for routine husbandry: feeding when indoors, change of pasture and emergency veterinary care when necessary.

2.2. Experimental groups and handling procedure

The animals were randomly allocated within sex, age and sire (two stallions) to one of three treatments: two experimental groups ("handled") and a control group.

Animals from the experimental groups were handled (see below) over a 12-day period, twice a day, for 10 min:

- early handled foals (EH, $N = 8$; 4 males, 4 females) were handled from 12 h to 12 days after weaning;
- late handled foals (LH, $N = 8$; 4 males, 4 females) were handled from 21 to 33 days after weaning;

Animals from the control group (C, $N = 8$; 4 males, 4 females) were not handled, and received minimal human contact necessary for routine management.

At the beginning of each handling session, each foal was extracted from its group and led to a 6 m × 6 m pen similar to its rearing pen, visually isolated from the other animals. As soon as the foal entered the pen, an experienced male person (always the same), referred to as the handler, quietly entered the pen and remained immobile for 1 min near the entrance.

Afterwards, the handler gently tried to fit the foal with a halter. In case, he did not succeed within 3 min, a second person (the helper) also entered the pen to help with the fitting the halter (even by force, as it was necessary to have the foal haltered for the remaining tests). When haltered, the foal was held by the helper, while the handler gently stroked all parts of its body: successively the head, shoulders, back, hindquarters and legs, for a total of 2 min. He then tried to pick up the forelegs within 2 min. From the fourth day onwards, foals were additionally taken out of the pen and led back and forth in a 25 m long corridor (total distance 50 m, maximum time allowed 3 min). Beginning on the seventh day and until the end of the handling period, the handler also attempted to pick up each of the forelegs and hindlegs (maximum time allowed 2 min).

During the handling sessions various parameters were recorded: the time to fix the halter and to pick up the four feet and the total time to walk the length of the corridor.

Furthermore, to assess the ease of handling during leading in the corridor, we measured the time during which the foal walked voluntarily (the rope was loose, the foal was willing to follow the handler) and the time during which foal walked under constraint (the rope was tight, the foal had to be coaxed, i.e. it was gently slapped on the rump when necessary) or refused to walk despite coaxing. A "walk-ratio", defined as time "walking under constraint"/total time, was calculated. If the subject did not reach the criterion (covering the corridor within 3 min), it was given a ratio of 1.

2.3. Behavioural tests and procedures

To assess the effects of handling (both short- and long-term) on reaction to humans, ease of handling and general reactivity or fearfulness, foals were submitted to various behavioural tests described below.

Animals were submitted to this series of tests, over a 3 day period, on five occasions: 2 days ("2-day test"), 4 months ("4-month test"), 7 months ("7-month test"), 10 months ("10-month test") after the end of the handling treatment. Eighteen months after the end of the handling treatment, only handling tests were repeated because no difference existed between groups in open-field tests, and because it was judged too dangerous to fit unbroken 2-year-old horses with the belt for heart rate monitoring.

During all tests, the experimenter was an unfamiliar experienced person, and was not aware of the animals' status (EH, LH or C). This person was different for the different test periods.

Animals were submitted consecutively on day 1 to the three situations detailed below (isolation, human presence, novel object) in the open-field. Handling tests were performed on day 2 and surprise tests on day 3. The testing order of the animals was random except that succession of two subjects from the same treatment condition was avoided.

2.3.1. Handling tests

To assess the ease of handling and the effect of the handling regimen on later reactivity to humans, the above-described handling procedure was repeated.

2.3.1.1. Capture. As during the "handling session", the foal was extracted from its group and driven to a 6 m × 6 m unknown pen, similar to that where handling was performed.

An unfamiliar handler attempted to fit the foal with a halter. If he did not succeed within 3 min, a second person (helper) entered the pen. Both then had 5 min to catch the foal. If the foal could not be caught in 5 min, the test was terminated and the foal was not exposed to the subsequent tests. Time to catch the foal was recorded and the foals that were not caught were given the maximum time allocated for the various tests (480 s).

2.3.1.2. Picking up feet. When haltered the foal was restrained by the helper. The handler attempted to pick up each foot beginning with the forefeet. The total time to lift the four feet was recorded. A maximum time of 120 s was allocated.

2.3.1.3. Leading. The test consisted of leading the foal along a 60 m corridor back and forth (total distance 120 m, maximum time allowed 5 min). Half of the distance covered was known to all the foals (the same corridor where handling previously took place) and the other half was unknown. As during the handling session, time during which the foal walked voluntarily or under constraint was recorded, and the ratio "walking under constraint"/time "walking voluntary" calculated. If the subject did not cover the distance during the allocated 5 min, it was assigned a ratio of 1.

Due to management constraints in the stable, this test could not be done 10 months after the handling period. In each of the handling tests, defensive reactions (rearing, kicking or attempts to bite) were also recorded.

2.3.2. Reactivity tests

These tests were designed to measure general reactivity of the animals in various situations.

2.3.2.1. Open-field tests. We used situations classically reported to induce fear: isolation from conspecifics, presence of a human and presence of a novel object. Similar tests have been designed and validated in sheep (Romeyer and Bouissou, 1992), in cattle (Boissy and Bouissou, 1995) and in adult horses (Viérin et al., 1998).

The open-field arena was a square pen (6 m × 6 m), divided into nine sectors of equal size by a grid painted on the floor. This pen had solid walls, and was separated from other pens containing animals by an empty pen on both sides, so that the foal tested could not see other animals.

Each foal was individually and successively subjected to the three of the following fear-inducing situations, which lasted 2 min each:

- Isolation test:
  The behaviour of the isolated animals was observed without any additional fear-inducing stimuli.
- Human test:
  The experimenter quietly entered and stood stationary in sector 8, opposite the door.
- Novel object test:
  An object, unknown to the foals, was introduced in sector 8 without human intervention (dropped from above one of the walls). A red and white traffic cone (1 m high) was used for the tests conducted 2 days after the end of the handling period, and two blue plastic bags, a cardboard box (80 cm × 80 cm × 80 cm) and a yellow stool (60 cm high) during tests performed respectively at 4, 7 and 10 months following the handling period. As the results showed no differences between groups 10 months after the end of the handling period, these tests were not repeated 18 months after.

The observations were made from a hidden platform, 2 m high. Eleven behavioural items, previously interpreted as indicative of the presence or the absence of fear (Viérin et al., 1998), were recorded using a tape recorder (Table 1). The data were subsequently transferred to a

Table 1
Parameters of behaviour and abbreviations

Items	Abbreviations	Items	Abbreviations
Time spent in squares 1, 2, 3 (far from the stimulus) (s)	T123	Defecations (nb)	DF
Time spent in squares 7, 8, 9 (nearest to the stimulus) (s)	T789	Glances at the stimulus[a] (nb)	GL
Squares entered (nb)	SE	Latency of sniffings to the stimulus[a] (s)	LS
Immobilisation time (s)	IT	Mean duration of sniffings[a] (s)	DS
Neighs (nb)	NN	Sniffings[a] (number)	SN
Latency to first neigh (s)	LN		

[a] Parameters of behaviour not recorded during the isolation test.

computer and analyzed using the software "The Observer" (Noldus, 1991) which calculated frequencies, latencies and durations of the behavioural acts.

2.3.2.2. Surprise tests. Foals, fitted with a halter, were equipped with a heart rate monitoring system (Polar Accurex Plus) set to record heart rate every 15 s. Five minutes after the beginning of the test (foal haltered and held), an experimenter suddenly opened an umbrella twice, 1 m in front of foal's head, then closed it. The total duration of this test was 8 min.

This test could not be done 18 months after the end of the handling period because of the danger of fitting unbroken 2-year-old horses with the equipment without a training procedure.

2.4. Statistical analysis

During the "handling period", we chose to use a synthetic measure which reflects the behaviour of the foals over the entire period, to compare the EH and LH groups. Therefore, for each handling session and for each behavioural item, each foal was given a rank. The sums of the ranks were calculated for the entire period. Then, those sums of ranks were compared using Mann–Whitney *U*-test (Siegel, 1956).

Data from the "open-field tests" were subjected to principal component analysis of variance (Frey and Pimentel, 1978) to determine the relationships between the various parameters measured. Loadings from the three groups (EH, LH, C) were compared.

During the surprise test, heart rate was measured at 15 s intervals. For statistical purposes, the 8 min test period was divided into three phases:

(1) the initial 5 min, before the umbrella was opened;
(2) a 15 s period beginning when the umbrella opened (reaction to stimulus);
(3) a period of 2 min and 45 s until the end of the test.

For each foal, the mean heart rate/interval was calculated for phases 1 and 3. These mean scores and the value for phase 2 were compared between groups. Furthermore, the increase in heart rate during phase 2 was calculated for each foal, i.e. the difference between the average heart rate during the minute preceding the opening of the umbrella (four samples) and the value measured at 5 min + 15 s (phase 2).

Due to the small number of subjects, and nature of measurements, non-parametric statistics were used. Groups were compared using Mann–Whitney *U*-tests. Bonferroni correction was used each time the three treatments were compared.

Values indicated in the text and figures are medians, inter-quartile range and critical value of *P*.

3. Results

3.1. Handling period

During the handling period, the sum of ranks of time to place halter, time to pick up feet and "walk-ratio" were significantly lower for EH than for LH foals ($P < 0.01$,

Table 2
Median ± inter-quartile of each group during handling tests

Items	Test period	Group		
		EH	LH	C
Time to fit halter (s)	2 days	16.5 [13; 21] a	22 [14; 26] a	88.5 [40; 184] b
	4 months	119 [34; 197] ab	52 [29; 152] a	224 [128; 420] b
	6 months	15.5 [12.5; 24.5] a	18 [14.5; 36] a	23 [17; 62] a
	10 months	11 [7.5; 21] a	19 [15; 31] a	18 [10; 25] a
	18 months	16.5[14.5; 25] a	59[33; 124] b	30[17; 480] b
Time to pick up feet (s)	2 days	32.5 [22; 34.5] x	28 [24; 35] x	120 [120; 120] y
	4 months	36 [33.5; 119] axy	62 [42.5; 114] ax	120 [120; 120] by
	6 months	55 [47; 58] a	57.5 [53; 60] a	118 [60; 120] b
	10 months	39.5 [35; 49] ax	49 [42; 51] axy	61 [53; 120] by
	18 months	23|15.5; 32] a	32[28; 42] b	34[22;120] b
Walk-ratio	2 days	0.59 [0.07; 0.79] x	0.31 [0.09; 0.49] x	0.94 [0.8; 1] y
	4 months	0.32 [0.17; 0.44] x	0.21 [0.09; 0.46] x	0.93 [0.86; 0.99] y
	6 months	0.12 [0.04; 0.23] a	0.22 [0.07; 0.35] a	0.57 [0.48; 0.69] b
	10 months	–	–	–
	18 months	0.5[0.16; 0.62] a	0.04[0; 0.65] a	0.37[0; 1] a
Defenses (nb)	2 days	5.5 [3; 6.5] x	1 [1; 4] x	17.5 [13; 23] y
	4 months	3.5 [0.5; 4] a	1 [1; 2] a	3 [0; 11.5] a
	6 months	0 [0; 0] a	0 [0; 0.5] ab	1 [0; 10] b
	10 months	0 [0; 0] ax	0 [0; 0] abxy	2 [0; 7] by

Medians on a same line with different letters (a, b, c: $P < 0.05$ and x, y, z: $P < 0.01$) differ significantly.

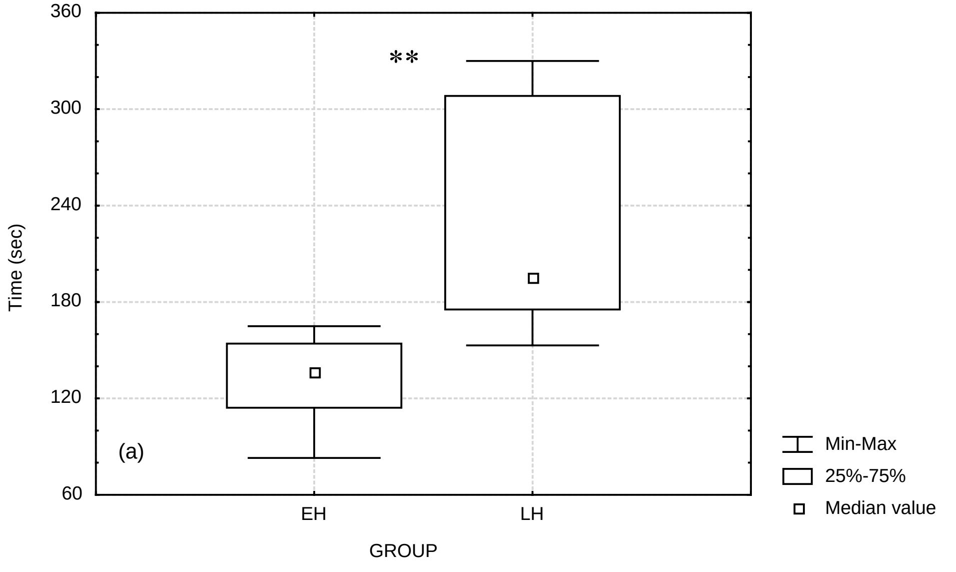
<0.05 and <0.01, respectively Fig. 1a–c), which implies that EH foals were easier to handle.

3.2. Handling tests

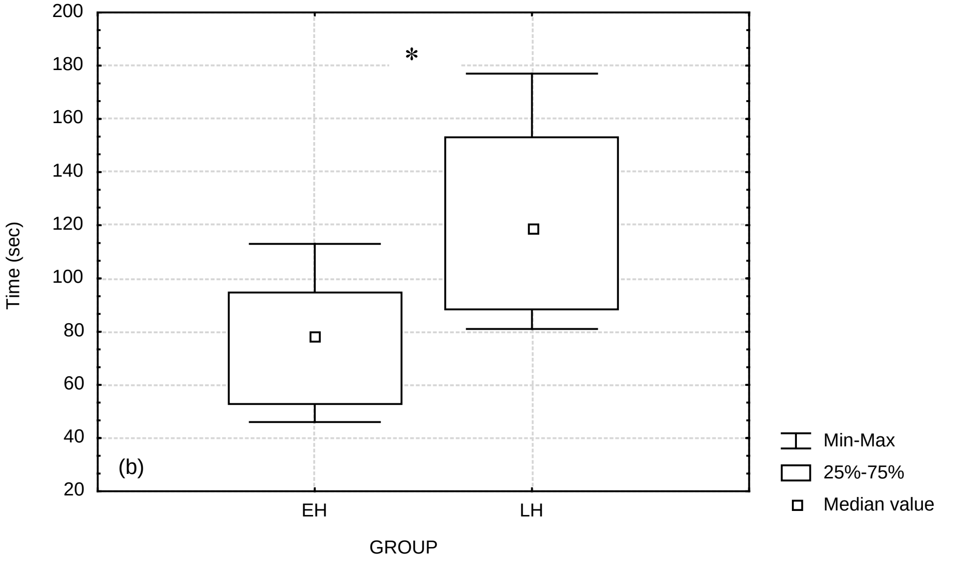
The results are shown in Table 2. Whatever the test period, none of the four variables (time to catch the foal, time to pick up the four feet, “walk-ratio”, defensive reactions) differed significantly between the two handled groups (EH and LH). By contrast, both EH and LH significantly differed from controls.

Two days after the end of the treatment period, the animals of both of the handled condition were easier to handle than controls. The time to fit them with a halter (EH versus C: $P < 0.05$; LH versus C: $P < 0.05$), time to pick up feet (EH versus C: $P < 0.01$; LH versus C: $P < 0.01$), “walk-ratio” (EH versus C: $P < 0.01$; LH versus C: $P < 0.01$), and number of defensive reactions (EH versus C: $P < 0.01$; LH versus C: $P < 0.01$) were significantly lower for both EH and LH foals than for the controls.

When tested 4 months post-treatment, time taken to pick up feet (EH versus C: $P < 0.05$; LH versus C: $P < 0.01$) and “walk-ratio” (EH versus C: $P < 0.01$; LH versus C: $P < 0.01$) were still significantly lower for the handled groups (EH and LH) than for controls. Time to fit the halter did not differ between EH and C foals, but was significantly ($P < 0.05$)

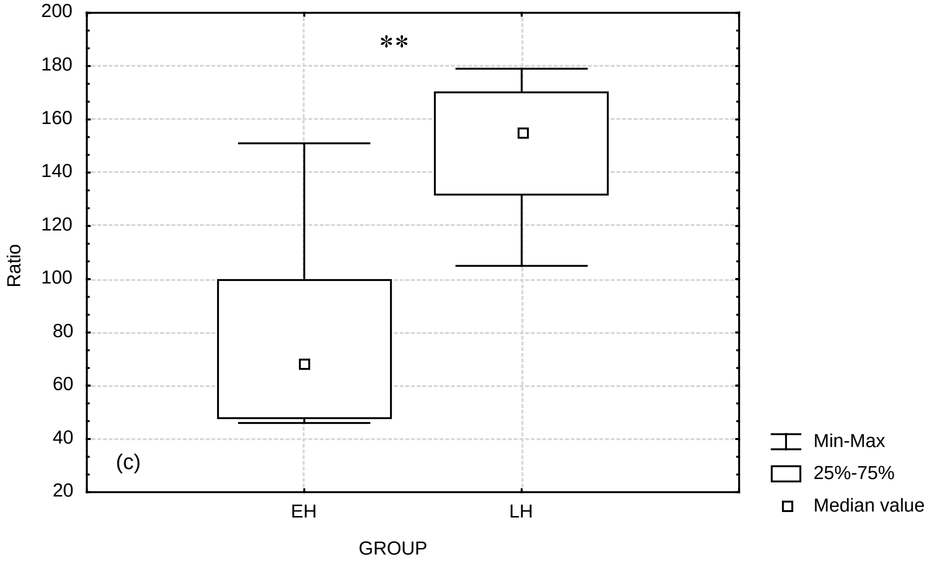


* p<0.05 ; **p<0.01 ; ***p<0.001



* p<0.05 ; **p<0.01 ; ***p<0.001

Fig. 1. Box plot by groups for items measured during handling period. (a) Sum of rank of time to place halter; (b) sum of rank of time to pick up feet; (c) sum of rank of ratio for walking test.



* p<0.05 ; **p<0.01 ; ***p<0.001

Fig. 1. (*Continued*).

lower for LH group than for C. There was no difference between groups for the number of defensive reactions.

During the tests conducted 7 months after the end of the treatment period, time to pick up feet (EH versus C: $P < 0.05$; LH versus C: $P < 0.05$) and "walk-ratio" (EH versus C: $P < 0.05$; LH versus C: $P < 0.05$) were still significantly lower for handled groups than for controls. There was no longer a difference between groups for the time to fit the halter. The number of defensive reactions was significantly ($P < 0.05$) lower for EH than for controls, but did not differ either between LH and C groups or between EH and LH groups.

At 10 months test post-treatment, the only significant differences were a shorter time to pick up the feet ($P < 0.01$) and a lower number of defensive reactions ($P < 0.01$) for EH foals as compared to C ($P < 0.01$) foals, and a shorter time to pick up feet for LH foals as compared to C ($P < 0.05$) foals.

One LH foal and one control foal died before the 18 months tests (colic and fracture). The time taken to fit a halter and to pick up feet were significantly lower for the EH group than for LH and C groups ($P < 0.01$ and <0.05, respectively). No other differences are significant. Moreover, all EH foals were caught in less than 1 min, whereas only 3 LH and 4 C foals were caught within that time ($X^2 = 6.19$; $P < 0.05$). Furthermore, it was impossible to catch 2 C foals within the 8 min allowed.

3.3. Open-field tests

3.3.1. Isolation

During the "2-day tests", the first factor of the principal component analysis explained 45% of the total variance. The number of squares entered (SE), and number of neighs (NN)

Table 3
PCA analysis, percentage of total variability explained by component 1 and selection of behavioural items according to their loading

Phase	Test period	Percentage of total variability	Contribution of items[a]
Isolation	2 days	45	SE: 0.25; IT: 0.2; NN: 0.2; LN: 0.2
	4 months	35	SE: 0.24; IT: 0.21; DF: 0.2
	7 months	38	SE: 0.27; IT: 0.26; NN: 0.16; DF: 0.14
	10 months	42	T123: 0.21; T789: 0.20; NN: 0.24; LN: 0.17
Human	2 days	33	T123: 0.12; SE: 0.17; IT: 0.15; SN: 0.12
	4 months	29	T789: 0.14; SE: 0.18; IT: 0.21; LN: 0.11; SN: 0.13
	7 months	34	T123: 0.12; T789: 0.2; SE: 0.11; IT: 0.17; GL: 0.1; DS: 0.11
	10 months	35	LS: 0.15; T123: 0.17; T789: 0.21; SN: 0.15; DS: 0.11
Object	2 days	34	LS: 0.14; T789: 0.18; GL: 0.11; SN: 0.19; DS: 0.14
	4 months	40	T789: 0.13; SE: 0.13; IT: 0.14; LN: 0.12; SN: 0.12
	7 months	43	T123: 0.1; T789: 0.16; SE: 0.11; IT: 0.14; NN: 0.12; SN: 0.11; DS: 0.1
	10 months	43	T123: 0.10; T789: 0.17; NN: 0.10; SN: 0.17; LS: 0.11; GL: 0.13

[a] Contribution of items which are higher than 1/7 for isolation test and than 1/11 for others.

had high positive loadings (0.88 and 0.79, respectively) on this factor. Immobilisation time (IT) and latency to the first neigh (LN) had high negative loadings (−0.79 and −0.78, respectively) on it (Table 3).

Score for animals in the EH group differed significantly from those of the control group. There were no other significant differences (Table 4).

During the "4-month tests", the first factor explained 35% of the total variance. The number of squares entered and number of defecations (DF) had high positive loadings (0.76 and 0.69, respectively) whereas immobilisation time had a high negative loading on this factor (−0.70).

Table 4
Median ± inter-quartile of each group on PC1 during open-field test

Phase	Test period	Group		
		EH	LH	C
Isolation	2 days	−1.12 [−2.3; 0.13] a	−0.14 [−1.93; 1.33] ab	1.38 [0.12; 3.01] b
	4 months	−0.24 [−2.09; 0.62] a	−0.23 [−0.93; 0.19] a	1.27 [−0.43; 1.5] a
	7 months	0.03 [−2.13; 0.72] a	1.15 [−1.14; 1.5] a	0.76 [−0.46; 2.04] a
	10 months	−0.12 [−1.03; 0.33] a	−0.65 [−1.11; 1.25] a	−0.19 [−0.85; 1.01] a
Human	2 days	−0.15 [−1.2; 0.78] axy	−1.74 [−2.41; −1.15] ax	2.14 [0.49; 2.65] by
	4 months	0.54 [−0.84; 2.9] a	0.10 [−0.97; 1.48] a	−1.06 [−1.97; −0.51] a
	7 months	0.42 [−2.13; 1.82] a	0.57 [−0.6; 1.44] a	0.22 [−1.23; 0.52] a
	10 months	−1.15 [−1.92; −0.18] a	−0.45 [−1.55; 2.36] ab	2.35 [−0.52; 2.78] b
Object	2 days	1.5 [−0.08; 2.22] axy	−1.08 [−1.6; −0.91] bx	0.16 [−0.25; 0.87] ay
	4 months	0.6 [−1.04; 2.04] a	−0.25 [−2.24; 1.27] a	0.78 [−0.12; 1.02] a
	7 months	0.94 [−2.46; 2.6] a	−0.31 [−1.73; 0.04] a	0.85 [−0.93; 1.7] a
	10 months	−0.64 [−1.97; 0.53] a	−0.84 [−3.08; 1.16] a	1.52 [−0.44; 3.02] a

At 7 months post-treatment, the first factor explained 38% of the total variance. The number of squares entered, number of defecations (DF) and number of neighs had high positive loadings (0.84, 0.61 and 0.65, respectively) whereas immobilisation time had a high negative loading (−0.83) on this factor.

During the “10-month tests” the first factor explained 42% of the total variance. Time spent in squares 7, 8, 9 (T789) and number of neighs had high positive loadings (0.77 and 0.85, respectively) whereas time spent in squares 1, 2, 3 (T123) and latency to the first neigh had high negative loadings on this factor (−0.79 and −0.71, respectively).

There were no significant differences between group scores on this first factor for any of these periods (“4-month test”, “7-month test” and “10-month tests”) (Table 4).

Whatever the period this first factor can be interpreted as a reaction to social isolation.

3.3.2. *Human presence*

During the “2-day tests”, the first factor explains 33% of the total variance. Time spent in squares 1, 2, 3 and number of squares entered had high positive loadings (0.67 and 0.78, respectively) on this factor, whereas immobilisation time, and number of sniffings (SN) had high negative loadings (−0.73 and −0.65, respectively) (Table 3).

Animals from group C are located at one end of factor 1. Their score significantly differs from those of the two other groups which did not differ from each other (Table 4).

During the “4-month tests”, factor 1 explained 29% of the total variance. Time spent in squares 7, 8, 9, immobilisation time latency to the first neigh and number of sniffings had high positive loadings on factor 1 (0.66, 0.81, 0.59 and 0.63, respectively) (Table 3). Number of squares entered had a high negative loading on this factor (−0.75). There were no significant differences between group scores (Table 4).

During the “7-month tests”, factor 1 explained 34% of the total variance. Time spent in squares 1, 2, 3, number of squares entered and number of glances at the stimulus (GL) had high positive loadings on factor 1 (0.68, 0.63 and 0.62, respectively). Time spent in squares 7, 8, 9 immobilisation time, and duration of sniffings (DS) had high negative loadings on this factor (−0.87, −0.81 and −0.64) (Table 3). There were no significant differences between group scores (Table 4).

During the “10-month tests”, factor 1 explained 35% of the total variance. Latency to sniff the stimulus (LS), and time spent in squares 1, 2, 3 had high positive loadings on factor 1 (0.77 and 0.81, respectively). Time spent in squares 7, 8, 9, number of sniffings, and duration of sniffings had high negative loadings on this factor (−0.89, −0.77 and −0.65, respectively) (Table 3). Neither group scores for LH and C foals or EH and LH foals differed significantly. The group score for EH foals, tended to differ ($P = 0.037$) from that of controls (Table 4).

Whatever the period, this first component can be interpreted as the motivation to interact with humans.

3.3.3. *Novel object*

During the “2-day tests”, the first factor explained 34% of the total variance. Latency to sniff the object (LS) and glances to the stimulus (GL) had high positive loadings (0.73 and 0.63, respectively) on factor 1. Time spent in squares 7, 8, 9, number of sniffings and duration of sniffings had high negative loadings (−0.82, −0.83 and –0.72, respectively) on factor 1 (Table 3).

Animals from group LH are located at one end of factor 1. They interacted significantly more with the object. Their score significantly differs from those of the two other groups, which did not differ within each other (Table 4).

During the “4-month tests”, factor 1 explained 40% of the total variance. The number of squares entered had high positive loadings on factor 1 (0.76). Time spent in squares 7, 8, 9, immobilisation time, latency of first neigh and number of sniffings had high negative loading on this factor (−0.75, −0.79, −0.71 and −0.72, respectively).

During the “7-month tests”, factor 1 explained 43% of the total variance. Time spent in squares 1,2, 3, number of squares entered and number of neighs had high positive loadings on factor 1 (0.70; 0.73; 0.75). Time spent in squares 7, 8, 9, immobilisation time, number of sniffings and duration of sniffings had high negative loadings on this factor (−0.86, −0.82, −0.73 and −0.70, respectively) (Table 3).

During the “10-month tests”, factor 1 explained 43% of the total variance. Latency to sniff the stimulus (LS), time spent in squares 1, 2, 3, and glances at the stimulus (GL) had high positive loadings on factor1 (0.71, 0.70 and 0.79, respectively). Time spent in squares 7, 8, 9, number of neighs and number of sniffings had high negative loadings on this factor (−0.90, −0.68 and −0.90, respectively) (Table 3).

From 4 to 10 months there were no significant differences between group scores (Table 4).

This factor can be interpreted as the motivation to interact with the object.

3.4. *Surprise test*

The results are shown in Table 5.

Before the opening of the umbrella (phase 1), the heart rate was recorded as the “initial level”, which reflects both the foals’ reaction to a human being and to restraint. During the second phase, the heart rate reflects the foals’ reaction to the surprising event, and during the third phase the return to initial level was measured.

Table 5
Cardiac frequency (median ± inter-quartile) of each group during the three phases of the surprise test

Test period	Group	Heart rate (bpm)			
		Phase 1	Peak	Phase 3	Variation
2 days	EH	51.5 [47.4; 58.6] ax	65.5 [56.5;78.5] ax	53.2 [46.1; 54.8] ax	11.5 [6.19; 87] ax
	LH	59.4 [53.6; 67.4] axy	112 [98;125] by	59.3 [53.1; 66.4] axy	46 [39.1; 72.1] by
	C	74 [67.1; 77.1] by	90.5 [83;104.5] by	73.6 [65.6; 78.5] by	21.1 [18.4; 28.5] ax
4 months	EH	68.1 [59.1; 68.8] a	104 [96;140.5] a	61.6 [59.6; 69.4] a	42.62 [33.5; 70.2] a
	LH	71.9 [62.2; 73.1] a	124 [90;157] a	71.4 [58; 79.4] a	64.7 [37.7; 79] a
	C	66.5 [60.6; 72.1] a	109 [86.5;125] a	66.1 [60.1; 73.6] a	42.6 [25.2; 64.4] a
7 months	EH	51.3 [46.3; 56.4] a	73.5 [61.5;86.5] a	49.4 [47.5; 53.4] a	24.6 [12.9; 35] a
	LH	53.3 [49; 60.1] a	97.5 [90.5;117] b	52.7 [50.1; 62.9] a	47 [39.2; 59] b
	C	58.7 [51.4; 66.2] a	111 [87;142] b	57.8 [50.4; 91.4] a	48 [18.5; 85.7] ab
10 months	EH	43.1 [37; 47.9] a	61 [46;72] a	43 [36.1; 50.1] a	16.1 [8.1; 25.4] a
	LH	45.2 [41.2; 47.5] a	82 [63;98] b	43.9 [40.9; 46.9] a	30.7 [22.2; 55.5] b
	C	49.7 [39.9; 57.1] a	86 [61;100] b	50 [42.7; 52.4] a	35 [12.2; 56.5] ab

bpm: beat per minute.

During phases 1 and 3 of the "2-day tests", there were no differences between groups EH and LH, but the heart rate of group C was significantly higher than that of the EH or LH group (phase 1: EH versus C: $P < 0.01$; LH versus C: $P < 0.05$; phase 3: EH versus C: $P < 0.01$; LH versus C: $P < 0.05$). During phase 2 (reaction to surprise), EH foals had significantly lower heart rates than LH or C foals (EH versus LH: $P < 0.01$; EH versus C: $P < 0.01$). The increase in heart rate was significantly higher in LH foals than EH and C foals ($P < 0.01$). EH and C foals did not differ significantly.

During the "4-months" test, no differences had been found between the three groups regardless of the period.

During the "7-months" and "10-months" tests, EH foals had lower heart rates than both LH and C foals in response to the surprise effect (phase 2: EH versus LH: $P < 0.05$; EH versus C: $P < 0.05$). There were no other differences between groups, regardless of the phase considered. With regard to the increase in heart rate, the EH group had significantly lower increase than the LH group ($P < 0.05$). No other differences were significant (Table 5).

4. Discussion

The aims of this study were two-fold:

- to determine whether the period following weaning is a favourable period for the early handling of horses, i.e. does handling at that time have a greater effect than the same treatment performed 3 weeks later;
- to assess the short and long-term effects of handling on the horse's later responses to humans, ease of handling and general reactivity.

The handling procedure used in this experiment (human presence and handling per se, as well as brief isolation from conspecifics) was effective with respect to manageability of the foals. Handled animals (early handled and late handled) were easier to handle than non-handled foals (C) during tests conducted 2 days to 10 months later, however the differences diminished progressively. When the animals were 2-year-old, EH horses were still easier to handle than LH and C.

When tested 2 days after the end of the handling period non-handled animals had higher heart rates than handled ones (EH and LH) during the first phase of the surprise tests (before the surprise event), which reflects reactions to a human and to restraint. However, this difference was not subsequently observed.

Thus, it can be concluded that handling after weaning is effective in facilitating manageability, and to a certain extend in reducing general reactivity, at least on a short-term basis. These results are in accordance with those reported by other authors for various species (Rushen et al., 1999; Simpson, 2002).

Another question addressed in this study was the possible existence of a sensitive period just following weaning for the effects of handling. It is generally known that an individual's characteristics may be more strongly influenced by a given event at one stage of development than at another stage (Bateson, 1979). Hess (1973) defined "sensitive" or "optimal" periods as: "periods of time during which the animal or child has the greatest sensitivity to certain aspects of the environment and thus can respond most readily to certain kinds of learning situations. This sensitivity remains in the animal or child for an extended period of time. It has also been observed that such learning can, of course, also take place at some other time, but less easily and less effectively or completely (Riesen, 1961). Furthermore, the effects of learning during the optimal period are not necessarily permanent."

During the handling period, EH foals were easier to handle: they were caught more quickly, it was easier to pick up their feet and they had a lower walk-ratio "C/T" than LH. From 2 days until 10 months after the handling period, there were no differences between EH and LH, however, two differences were observed when they were tested 18 months later.

Just after the handling period, EH foals were less agitated than LH foals when placed in social isolation (open-field test), and interacted less with the human and the novel object. In similar situations, sheep and cattle display increased locomotor activity which appears to reflect fear (Vandenheede et al., 1998; Romeyer and Bouissou, 1992; De Passillé et al., 1995): thus EH foals may have been less frightened by social isolation than LH foals. However, Romeyer and Bouissou (1992) and Vandenheede et al. (1998) concluded that sheep that have no interaction with the stimulus (balloon, umbrella or human), or those that stay away from the stimulus are more frightened. EH foals may therefore be considered as being more frightened than LH foals. This interpretation appears to contradict other results obtained during handling tests and before the surprise event of the "umbrella test", which indicate that EH foals were less frightened than controls by a human. Another explanation could be that the presence of a human or novel object might have been ignored by EH foals. Furthermore, Simpson (2002) found that handled foals approached humans more than control foals. Nevertheless, these two results are not necessarily contradictory; a foal that is not frightened by a human can either approach him or ignore him.

During the surprise test performed 2 days, 7 or 10 months after the handling period, EH foals displayed a lower increase in heart rate in response to the surprise effect than did LH foals. If higher heart rate is considered to be a possible indicator of fear (Clément and Barrey, 1995) EH foals appeared to be less frightened by suddenness than LH foals.

To summarize, EH foals were easier to handle than LH foals during the handling period. These results are consistent with those obtained in cattle (Boivin et al., 1992b) and goats (Boivin and Braastad, 1996), which were easier to handle just after weaning than 5–6 weeks later. EH foals were also less reactive to handling, isolation, presence of a human or an object, and to suddenness during subsequent tests. These data support the contention that the period immediately following weaning may be considered an "optimal period" in the sense of Hess (1973).

Several hypotheses may be offered to explain why weaning might be an optimal period for the effects of handling. The first hypothesis concerns the social reorganisation induced by weaning. In cattle, Veissier and Le Neindre (1989) established that weaning strengthened social relationships between heifers. Moreover, Boivin and Braastad (1996) suggested that the weaning period was favourable for establishing a positive relationship between goat kids and humans because "at this period kids could be more susceptible to form new social bonds, motivated either by the need for peers or the need for being adopted by a new mother". They proposed "that the human might serve also as a surrogate mother". In horses, a similar process might exist. Nevertheless, in the study by Boivin and Braastad (1996), kids were weaned at 1 week of age (natural weaning normally occurs around 6 months), whereas foals of this study were weaned at 6 months of age (natural weaning normally occurs around 6–9 months), when they were already relatively independent from their mothers. Thus, the hypothesis put forward for kids cannot totally explain our results. Moreover, it is possible that handling of earlier weaned foals would be even more effective.

The second hypothesis concerns learning ability, which is reported to be better just after weaning than 1 month later in cattle (Veissier and Le Neindre, 1989). To our knowledge no such study has been conducted with horses, however if the same phenomenon exists, it could partly explain the higher efficiency of handling at this period. However, EH foals were easier to handle from the very first days after weaning. Thus, differences in learning ability alone cannot completely explain the observed differences between EH and LH foals.

A final hypothesis is that the stress induced by weaning (Malinowski et al., 1990) is limited to 1 or 2 weeks (Houpt et al., 1984; Mac Call et al., 1985). Thus, only EH foals would have been under stress during handling. Bateson (1979) suggests that any period of reorganization, associated with stress, could be a period of special sensitivity to external stimuli.

Furthermore, this stress might have induced a state of "learned helplessness". According to Overmier and Seligman (1967), "learned helplessness might well result from receiving aversive stimuli in a situation in which all instrumental responses or attempts to respond occur in the presence of the aversive stimuli and are of no avail in eliminating or reducing the severity of the trauma". The trauma in our situation was induced by the separation from the mother, and the foal learns that resistance is ineffective in reducing stress factors. The foal no longer reacts to his environment and thus would become easier to handle.

The second purpose of this study was to assess the long-term effects of handling at weaning. Regardless of the test period (2 days, 4, 7 or 10 months and, to some extend, 18 months after the end of the handling period), the handled animals (EH and LH foals) were generally easier to handle than controls. Moreover, 10 months after the handling period, the general reactivity of EH foals was still lower than that of the controls as reflected by their reactions during the surprise test. The effects of handling just after weaning are thus relatively persistent.

For the entire study, it should be noted that some effects may not have been revealed statistically because of the relatively small number of subjects per group. Thus, the differences between groups and the duration of the effect might be even stronger than observed in our limited sample.

The results of other studies of handling just after birth (Lansade et al., 2002; Simpson, 2002; Williams et al., 2002) showed less persistent results: 3 months after the end of the treatment there were no, or almost no, differences between handled and control animals (Lansade et al., 2002; Williams et al., 2002). Although direct comparisons between the different studies is difficult because of breed differences and differences in environmental conditions, neonatal handling nevertheless seems to be less effective. The presence of the mother could be responsible for this effect in the case of newborn foals. It has been demonstrated in sheep that the presence of the mother may be a factor that limits the establishment of human–animal relationships (Boivin et al., 2001).

Finally, it should be pointed out that the repeated tests themselves represent an experience, so the controls become less naïve with each subsequent test. Nevertheless, in the present study, each test was less than 15 min long and was repeated only five times over 1 year, which is unlikely to affect fear of humans and manageability. However, it would be interesting to replicate this experiment, but comparing groups only once at 18 months after the handling period.

From a theoretical point of view, it would be interesting to study more precisely the respective roles of the different factors evoked above to explain why weaning, and especially the period immediately following mother–young separation, appears to be especially suitable for the effects of handling.

From a practical point of view, it could be recommended that breeders handle their animals just after weaning. At that time, handling is still reasonably easy to perform and the effects on manageability and general reactivity are relatively persistent.

Acknowledgements

The authors are grateful to Mrs. C. Trillaud-Geyl, director of the "Station expérimentale des Haras Nationaux", Chamberet (France) for allowing us to use the animals and facilities. We thank the staff of this Institute for their help during handling and tests. H. Dubroeucq (INRA, Theix) and C. de Monteil (MS student) are gratefully acknowledged for their help during handling and tests. We wish to thank Dr. R. Porter for this help with the English language usage. We are especially grateful to Andrew Mac Lean (Institute of Land and Food Resources, University of Melbourne, Australia) who kindly improved the final version of the manuscript.

References

Arnold, G.W., 1985. Parturient behaviour. In: Fraser, A.F. (Ed.), Ethology of Farm Animals. Elsevier, Amsterdam.

Bates, J.E., 1989. Concepts and measures of temperament. In: Kohnstamm, G.A., Bates, J.E., Klevjord Rothbart M. (Eds.), Temperament in Childhood. Wiley, NY, pp. 3–26.

Bateson, P., 1979. How do sensitive periods arise and what are they for? Anim. Behav. 27, 470–486.

Boissy, A., 1998. Fear and fearfulness in determining behaviour. In: Grandin, T. (Ed.), Genetics and the Behavior of Domestic Animals. Academic Press, pp. 67–111.

Boissy, A., Bouissou, M.F., 1988. Effects of early handling on heifers' subsequent reactivity to humans and to unfamiliar situations. Appl. Anim. Behav. Sci. 20, 259–273.

Boissy, A., Bouissou, M.F., 1995. Assessment of individual differences in behavioural reactions of heifers exposed to various fear-eliciting situations. Appl. Anim. Behav. Sci. 46, 17–31.

Boivin, X., Boissy, A., Nowak, R., Henry, C., Tournadre, H., Le Neindre, P., 2002. Maternal presence limits the effects of early bottle feeding and petting on lambs' socialisation to the stockperson. Appl. Anim. Behav. Sci. 77, 311–328.

Boivin, X., Braastad, B.O., 1996. Effects of handling during temporary isolation after early weaning on goat kids' later response to humans. Appl. Anim. Behav. Sci. 48, 61–71.

Boivin, X., Le Neindre, P., Chupin, J.M., 1992a. Establishment of cattle–human relationships. Appl. Anim. Behav. Sci. 32, 325–335.

Boivin, X., Le Neindre, P., Chupin, J.M., Garel, P., Trillat, G., 1992b. Influence of breed and early management on ease of handling and open-field behaviour of cattle. Appl. Anim. Behav. Sci. 32, 313–323.

Boivin, X., Nowak, R., Garcia, A.T., 2001. The presence of the dam affects the efficiency of gentling and feeding on the early establishment of the stockperson–lamb relationship. Appl. Anim. Behav. Sci. 72, 89–103.

Bouissou, M.F., 1978. Effect of injections of testosterone propionate on dominance relationships in a group of cows. Horm. Behav. 11, 388–400.

Bouissou, M.F., Gaudioso, V., 1982. Effect of early androgen treatment on subsequent social behavior in heifers. Horm. Behav. 16, 132–146.
Chapillon, P., Debouzie, A., 2000. BALB/c mice are not so bad in the Morris water maze. Behav. Brain Res. 117, 115–118.
Clément, F., Barrey, E., 1995. Fluctuations de fréquence cardiaque chez le cheval au repos: (1) investigation de la dynamique cardiaque par l'analyse spectrale. C. R. Acad. Sci. Paris, Sci. de la vie 318, 859–865.
De Passillé, A.M., Rushen, J., Martin, F., 1995. Interpreting the behaviour of calves in an open-field test: a factor analysis. Appl. Anim. Behav. Sci. 45, 201–213.
Denenberg, V.H., 1962. The effects of early experience. In: Hafez, E. (Ed.), The Behavioural of Domestic Animals. Baillière, Tindall & Cassell, London. pp. 95–130.
Dybkjaer, L., 1992. The identification of behavioural indicators of "stress" in early weaned piglets. Appl. Anim. Behav. Sci. 35, 135–147.
Fiske, J.C., Potter, G.D., 1979. Discrimination reversal learning in yearling horses. J. Anim. Sci. 49, 583–588.
Galef Jr., B.G., 1970. Aggression and timidity: responses to novelty in feral Norway rats. J. Comp. Physiol. Psychol. 70, 370–381.
Hemsworth, P.H., Coleman, G.J., 1998. Human–Livestock Interactions. CAB International, Wallingford.
Hemsworth, P.H., Barnett, J.L., Hansen, C., 1986. The influence of handling by humans on the behaviour, reproduction and corticosteroids of male and female pigs. Appl. Anim. Behav. Sci. 15, 303–314.
Hemsworth, P.H., Coleman, G.J., Barnett, J.L., 1991. Reproductive performance of pigs and the influence of human–animal interactions. Pig News Inf. 12, 563–566.
Hess, E.H., 1973. Imprinting, Early Experience and the Developmental Psychobiology of Attachment. Van Nostrand Reinhold, New York.
Houpt, K.A., Hintz, H.F., Butler, W.R., 1984. A preliminary study of two methods of weaning foals. Appl. Anim. Behav. Sci. 12, 177–181.
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. Appl. Anim. Behav. Sci. 62, 1–11.
Jones, R.B., Faure, J.M., 1981. The effects of regular handling on fear in the domestic chick. Behav. Process. 6, 135–143.
Kersten, A.M.P., Meijsser, F.M., Metz, J.H.M., 1989. Effects of early handling on later open-field behaviour in rabbits. Appl. Anim. Behav. Sci. 24, 157–167.
Kilgour, R., Dalton, C., 1984. Livestock Behaviour. A Practical Guide. Methuen, New Zealand, 320 pp.
Krohn, C.C., Jago, J.G., Boivin, X., 2001. The effect of early handling on the socialisation of young calves to humans. Appl. Anim. Behav. Sci. 74, 121–133.
Lansade, L., Bertrand, M., Boivin, X., Bouissou, M.F., 2002. Effects of early handling on the behaviour of young horses. In: Koene, P. (Ed.), Proceedings of the 36th International Congress of the ISAE, The Netherlands, 6–10 August 2002.
Le Neindre, P., Boivin, X., Boissy, A., 1996. Handling of extensively kept animals. Appl. Anim. Behav. Sci. 49, 73–81.
Lefcourt, A.M., Elsasser, T.H., 1995. Adrenal responses of Angus × Hereford cattle to the stress of weaning. J. Anim. Sci. 73, 2669–2676.
Lensink, B.J., Fernandez, X., Boivin, X., Pradel, P., Le Neindre, P., Veissier, I., 2000. The impact of gentle contacts on ease of handling, welfare and growth of calves and on quality of veal meat. J. Anim. Sci. 78, 1219–1226.
Mac Call, C.A., Potter, G.D., Kreider, J.L., 1985. Locomotor, vocal and other behavioral responses to varying methods of weaning foals. Appl. Anim. Behav. Sci. 14, 27–35.
Mac Cann, J.S., Heird, J.C., Bell, R.W., Lutherer, L.O., 1988. Normal and more highly reactive horses. II. The effects of handling and reserpine on the cardiac response to stimuli. Appl. Anim. Behav. Sci. 19, 215–226.
Malinowski, K., Hallquist, N.A., Helyar, L., Sherman, A.R., Scanes, C.G., 1990. Effect of different separation protocols between mares and foals on plasma cortisol and cell-mediated immune response. J. Equine Vet. Sci. 10, 363–368.
Mason, W.A., 2000. Early developmental influences of experience on behaviour, temperament and stress. In: Moberg, G.P., Mench, J.A. (Eds.), The Biology of Animal Stress. CABI Publishing, New York, USA.
Miller, R.M., 1991. Imprint training of the newborn foal. Western Horseman Inc., Colorado Springs, CO, pp. 44–87.
Noldus, L.P.J.J., 1991. The Observer: a software system for collection and analysis of observational data. Behav. Res. Meth. Instr. Comp. 23, 415–429.

Orgeur, P., Mavric, N., Yvoré, P., Bernard, S., Nowak, R., Schaal, B., Lévy, F., 1998. Artificial weaning in sheep: consequences on behavioural, hormonal and immuno-pathological indicators of welfare. Appl. Anim. Behav. Sci. 58, 87–103.

Orgeur, P., Hay, M., Mormède, P., Salmon, H., Le Dividich, J., Nowak, R., Schaal, B., Lévy, F., 2001. Behavioural, growth and immune consequences of early weaning in 1-week-old large-white piglets. Reprod. Nutr. Dev. 41, 321–332.

Overmier, J.B., Seligman, M.E.P., 1967. Effects of inescapable shock upon subsequent escape and avoidance responding. J. Comp. Physiol. Psychol. 63, 28–33.

Pedersen, V., 1994. Long-term effects of different handling procedures on behavioural, physiological and production-related parameters in silver foxes. Appl. Anim. Behav. Sci. 40, 285–296.

Pedersen, V., Jeppesen, L.L., 1990. Effects of early handling on later behaviour and stress responses in the silver fox (*Vulpes vulpes*). Appl. Anim. Behav. Sci. 26, 383–393.

Plusquellec, P., Bouissou, M.F., Le Pape, G., 2001. Early predictors of dominance ability in heifers (*Bos taurus*, L.) of the Hérens breed. Behaviour 138, 1009–1031.

Putu, I.G., 1990. Maternal behaviour in Merino ewes during the first two days after parturition and survival of lambs. Ph.D. Thesis, The University of Western Australia.

Riesen, A.H., 1961. Imprinting, Early Experience and the Developmental Psychobiology of Attachment. Van Nostrand Reinhold, New York (in Hess, E.H., 1973).

Romeyer, A., Bouissou, M.F., 1992. Assessment of fear reactions in domestic sheep, and influence of breed and rearing conditions. Appl. Anim. Behav. Sci. 34, 93–119.

Rushen, J., Taylor, A.A., de Passillé, A.M., 1999. Domestic animals' fear of humans and its effect on their welfare. Appl. Anim. Behav. Sci. 65, 285–303.

Sato, S., Shiki, H., Yamasaki, F., 1981. The effects of early caressing on later tractability of calves. Jpn. J. Zootech. Sci. 55, 332–338.

Schaeffer, T., 1963. Early experience and its effects on later behavioral processes in rats. II. A critical factor in the early handling phenomenon. In: Scott, J.P. (Ed.), Critical Periods. Dowden, Hutchinson, Ross, Stroudsburg, PA, pp. 272–290.

Siegel, S., 1956. Non Parametric Statistics for the Behavioral Sciences. McGraw-Hill, New York.

Simpson, B.S., 2002. Neonatal foal handling. Appl. Anim. Behav. Sci. 78, 303–317.

Vandenheede, M., Bouissou, M.F., Picard, M., 1998. Interpretation of behavioural reactions of sheep towards fear-eliciting situations. Appl. Anim. Behav. Sci. 58, 293–310.

Veissier, I., Le Neindre, P., 1989. Weaning in calves: its effects on social organization. Appl. Anim. Behav. Sci. 24, 43–54.

Veissier, I., Le Neindre, P., Trillat, G., 1989. Adaptability of calves during weaning. Biol. Behav. 14, 66–87.

Viérin, M., Bouissou, M.F., Vandenheede, M., Trillaud-Geyl, C., Arnaud, G., 1998. Développement d'une méthodologie destinée à mesurer les réactions de peur chez le cheval. 24ème journée d'étude de la recherche équine, Paris, pp. 171–183.

Weary, D.M., Fraser, D., 1997. Vocal response of piglets to weaning: effect of piglet age. Appl. Anim. Behav. Sci. 54, 153–160.

Williams, J.L., Friend, T.H., Toscano, M.J., Collins, M.N., Sisto-Burt, A., Nevill, C.H., 2002. The effects of early training sessions on the reactions of foals at 1, and 3 months of age. Appl. Anim. Behav. Sci. 77, 105–114.