Paper

# Reduced-size microchips for identification of horses: response to implantation and readability during a six-month period

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**In this study, readability of reduced-size microchips in horses and the response to implantation were analysed. It was hypothesised that small microchips can be implanted stress-free but are less readable than larger microchips. Adult mares (n=40) were implanted with a reduced-size microchip (10.9×1.6 mm) at the left side of the neck (size of conventional microchips 11.4×2.2 mm). Microchips were identified with three different scanners (A, B, C) immediately, and at 6, 12 and 28 weeks after implantation. Twelve out of the 40 mares were submitted to microchip implantation and control treatments and cortisol, heart rate and heart rate variability (HRV) were determined. From the chip-bearing side of the neck, microchips were identified with all scanners in all horses at all times. From the contralateral side, correct readings were always 100 per cent with scanner C and with scanners A and B ranged between 60 and 100 per cent. Heart rate and HRV variable sd of beat-to-beat interval increased slightly (P<0.01) at microchip implantation and control treatment, but cortisol concentration did not increase. In conclusion, reduced-size microchips are highly reliable for identification of horses. Compared with conventional microchips, the reduction in size did not impair readability. Microchip implantation is no pronounced stressor for horses.**

## Introduction

Identification of horses is required for studbook recordings, disease control and to preclude substitution in competitions or sales. Identification has traditionally been effectuated by hot-iron branding with symbols specific for a breed or stud. As an alternative method of identification, microchip transponders are recommended and with few exceptions have been made mandatory for horses in the European Union.

By contrast with hot-iron branding (Lindegaard and others 2009, Aurich and others 2012), with high-quality scanners, microchips allow identification of 100 per cent of horses (Stein and others 2003, Wulf and others 2013). Microchip implantation in foals (Erber and others 2012) and adult horses (Lindegaard and others 2009, Lindegaard and Andersen 2012) is a largely stress-free procedure, and an inflammatory response to the implanted transponders is extremely rare (Erber and others 2012, Gerber and others 2012, Wulf and others 2013). Microchips are seen critical by many breed registries. Horse breeders often claim that size of the microchips does not allow stress-free implantation and assume long-term inflammatory responses at the implantation site (German Equestrian Federation 2013).

Stressful stimuli increase cortisol release from the adrenal cortex. Non-protein-bound cortisol rapidly diffuses into saliva, and salivary cortisol mirrors changes of free cortisol in blood plasma (Peeters and others 2011). Acute stressors also elicit an immediate response of the autonomic nervous system with release of epinephrine and increase in heart rate. Besides heart rate, heart rate variability (HRV) can be used as an indicator for the response of the animal to stress. HRV, that is, short-term fluctuations in heart rate, is based on the antagonistic oscillatory influences of the sympathetic and parasympathetic branch of the autonomic nervous system on the sinus node of the heart. In general, decreases in the HRV variables sd of beat-to-beat (RR) interval (SDRR) and root mean square of successive RR differences (RMSSD) reflect sympathetic dominance, while increased values indicate parasympathetic dominance (von Borell and others 2007).

A reduction in size of microchips may increase their acceptance by horse owners. However, it may also reduce the signal induced by the scanner at microchip readings and thus reduce reliability for identification of horses. In this study, we have investigated the acute stress response of adult horses to implantation of a reduced-size microchip transponder. Readability of the transponders was followed until six months after implantation. We hypothesised that small transponders can be implanted stress-free but, due to small size may be less readable compared to larger-size microchips.

## Material and methods

### Animals

A total of 40 Warmblood brood mares of the Brandenburg State Stud at Neustadt (Dosse), Germany, were available for the study. Mares were between 3 years and 18 years of age (7.5±4.1,±sd). They were kept on pasture with access to a group stable in summer and autumn

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and in straw-bedded group stables with daily access to an outdoor paddock in winter. During pasture time, mares were fed additional concentrates and oats. In winter, mares were fed oats and concentrates three times daily and hay twice daily. Water and mineral supplements were available at all times.

### Microchip implantation

All mares were implanted with a reduced-size microchip (BioTec slim, Virbac; transponder length 10.9 mm, transponder diameter 1.6 mm, outer diameter of implantation cannula 2.0 mm). Microchips were implanted at the left side of the neck, half-way between the poll and the withers and half way between the crest of the mane and the ventral line of the neck. The length of the implantation cannula was 2.7 cm (versus 2.9 cm for conventional, larger-size microchips) and the location is similar for conventional microchips (Erber and others 2012). All microchip implantations were performed by the same experienced person. Mares were held by another person and the microchip implantation site was disinfected but not clipped before implantation. Microchips were implanted to meet legal requirements on identification of horses, and all observations were made in agreement with German animal welfare legislation.

### Reading of microchips

Three different, commercially available scanners were used to locate and read the microchip in all horses and on both sides of the neck. All microchips were checked before implantation with all three scanners and were found to be functional. The three scanners Minimax II (A), i-Max plus (B) and Isomax V (C; all by Virbac), and the microchips were coded and structured according to ISO standards 11784 and 11785. For scanners A, B and C, size of the antenna was 43×14×4 mm, 90 mm in diameter and 120 mm in diameter, respectively, maximal reading distance was 10, 15 and 25 cm, respectively, induction was 700, 400 and 200 µH, respectively, and field strength was 21.7, 33.7 and 41.6 dBuA/m, respectively. Scanner C was equipped with a digital signal processing function that filters interfering signals.

Microchips were read immediately after implantation, and at 6, 12 and 28 weeks thereafter. Microchip reading started at the poll on the left side of the neck, following the crest line to the withers, back towards the head about 5 cm below the crest line and then in a meandering pattern back and forth until the microchip was found or the ventral aspect of the neck had been reached. All three scanners were used on the left side of the neck first and thereafter in the same way on the right side of the neck. Both sides of the neck were analysed with the same order of the scanners per horse. The order of scanners was randomised, so that all scanners were used to the same extent for the first, second and third trials, respectively. For each scanner and each side of the neck, the percentage of readable and non-readable microchips was calculated. For all readable microchips, the time until detection was recorded. For determination of the microchip location, scanner A was used, and location of the microchip was evaluated through an approach from a cranial, caudal, dorsal and ventral position as described (Stein and others 2003). The implantation site was checked visually and by palpation for pathological findings, such as swelling and increased sensitivity, but no attempt was made to score these findings.

### Response to microchip implantation

In a subgroup of 12 mares (4–8 years old, 5.5±1.5 years) cortisol release and changes in heart rate and HRV were determined. Mares received a microchip and were submitted to control treatment (pressure applied with a cannula at the implantation site without penetrating the skin), thus serving as their own controls. Microchip implantation and control treatment were performed on the left side of the horse's neck. Time between microchip implantations and control treatments was 14 days. The order of treatments was randomised with six mares receiving the control treatment first and implantation of a microchip thereafter, and the other six mares treated in opposite order.

### Heart rate and HRV

The cardiac beat-to-beat (RR) interval was recorded with a mobile recording system (S810i, Polar, Kempele, Finland) set to RR interval. Recordings were made continuously from one hour before to one hour after microchip implantation, as described (Schmidt and others 2010a, d). The recording time was then divided into one-minute intervals. For data analysis, baseline values were determined for one-minute intervals starting at 1, 15, 30 and 60 minutes before microchip implantation and control treatment, respectively. Further one-minute intervals were analysed starting at 1, 15, 30 and 60 minutes after microchip implantation and control treatments. From the recorded RR intervals, heart rate and the two HRV variables SDRR (sd of RR interval) and RMSSD were calculated. The HRV was calculated with the Kubios HRV software (Biomedical Signal Analysis Group, Department of Applied Physics, University of Kuopio, Finland). To remove trend components, data were detrended and an artefact correction was made as described (Tarvainen and others 2002, Schmidt and others 2010a, d).

### Cortisol

Saliva for determination of basal cortisol concentrations was taken at 60 and 30 minutes before and immediately (time 0), and at 15, 30, 60, 90 and 120 minutes after microchip implantation and control treatment. Saliva was collected as described (Schmidt and others 2010d) with cotton rolls (Salivette; Sarstedt) placed loosely onto the tongue of the horse for one minute with the help of a surgical arterial clamp until the swab was well soaked. The salivettes were then centrifuged for 10 minutes at 1000 g and saliva was aspirated and frozen at –20°C until cortisol analysis. Concentrations of cortisol were determined by enzyme immunoassay without extraction (Palme and Möstl 1997, Schmidt and others 2010d). Since the antiserum cross-reacts with cortisone and some cortisone metabolites, values were interpreted as cortisol immunoreactivity. The intra-assay coefficient of variation was 5.0 per cent, the inter-assay variation 6.7 per cent, and the minimal detectable concentration 0.3 pg/well.

### Statistical analysis

Statistical comparisons were made with the SPSS statistics package (V.17.0; SPSS). The numbers of identified and non-identified microchips, as well as time until microchip detection for each side of the neck, and times 0, 6, 12 and 28 weeks were compared by Kaplan-Meier survival analysis with type of scanner as factor, and generalised Wilcoxon test for pairwise comparisons between scanners. Changes in heart rate, HRV and salivary cortisol concentrations were analysed by analysis of variance using a general linear model for repeated measures with comparison between treatments (microchip implantation or control). All data given are mean±sem. For all statistical comparisons, a P value below 0.05 was considered significant.

## Results

### Identification of microchip transponders

From the chip-bearing (ipsilateral) side of the neck, microchip transponders were identified with all three scanners in all 40 horses immediately after microchip implantation, as well as 6, 12 and 28 weeks thereafter within maximally three seconds. From the contralateral side, correct readings with scanner C were 100 per cent at all times, while correct readings with scanners A and B were lower at 6, 12 and 28 weeks (Table 1). Maximal time until microchip detection from the contralateral side was six seconds. Kaplan-Meier survival analysis which takes into account the percentage of detected microchips as well as time until detection, revealed significant differences between scanners when used from the contralateral side at all times (day 0 $P<0.05$, 6, 12 and 28 weeks $P<0.001$; Table 1). All microchips were always identified at the original implantation site, and at no time swelling or increased sensitivity at the microchip implantation site was detected.

### Heart rate and HRV

Heart rate showed a small increase ($P<0.01$) at disinfection for microchip implantations and control treatments, and on average was slightly lower already before control treatments compared to microchip implantations ($P<0.01$, interactions time×experimental day $P<0.001$; Fig 1a). The HRV variable SDRR increased slightly at microchip and at sham implantations ($P<0.05$ over time, differences between

TABLE 1: Percentage of correctly identified microchip transponders and time until microchip detection with 3 different scanners in 40 horses directly and at 6, 12 and 28 weeks after microchip implantation

| | | Day 0 | | 6 weeks | | 12 weeks | | 28 weeks | |
|---|---|---|---|---|---|---|---|---|---|
| Side | Scanner | % read | Time until detection (seconds) mean±SD (range) | % read | Time until detection (seconds) mean±SD (range) | % read | Time until detection (seconds) mean±SD (range) | % read | Time until detection (seconds) mean±SD (range) |
| Ipsilateral | A | 100.0 | 1.0±0.0 – | 100.0 | 1.0±0.0 – | 100.0 | 1.0±0.0 – | 100.0 | 1.1±0.1 (1-3) |
| | B | 100.0 | 1.0±0.0 – | 100.0 | 1.0±0.0 – | 100.0 | 1.0±0.0 – | 100.0 | 1.0±0.1 (1-2) |
| | C | 100.0 | 1.0±0.0 – | 100.0 | 1.0±0.0 – | 100.0 | 1.0±0.0 – | 100.0 | 1.0±0.0 – |
| Contralateral | A | 100.0 | 1.3±0.8 a (1-4) | 85.0 | 1.4±0.9 a (1-4) | 92.5 | 1.3±0.9 a (1-6) | 60.0 | 1.9±1.3 a (1-5) |
| | B | 100.0 | 1.2±0.4 b (1-2) | 97.5 | 1.0±0.2 b (1-2) | 90.0 | 1.7±1.3 a (1-5) | 90.0 | 1.5±0.8 b (1-4) |
| | C | 100.0 | 1.0±0.0 c – | 100.0 | 1.0±0.0 b – | 100.0 | 1.0±0.0 b – | 100.0 | 1.0±0.0 c – |

a,b,c: differences between scanners (percentage of microchip detection and time until detection, Kaplan-Meier analysis)

treatments and interactions time×experimental day n.s.), while for RMSSD, significant differences were not found over time or between treatments (Fig 1b and c).

## Cortisol

No significant differences in salivary cortisol concentrations of horses were found at any time after microchip implantations and control treatments. Cortisol concentrations in saliva were initially higher in mares on the day of control treatments versus the day of microchip implantation, and decreased in the former and increased slightly in the latter (changes over time P<0.01, interactions time×experimental day P<0.001, differences between experimental days n.s.; Fig 2).

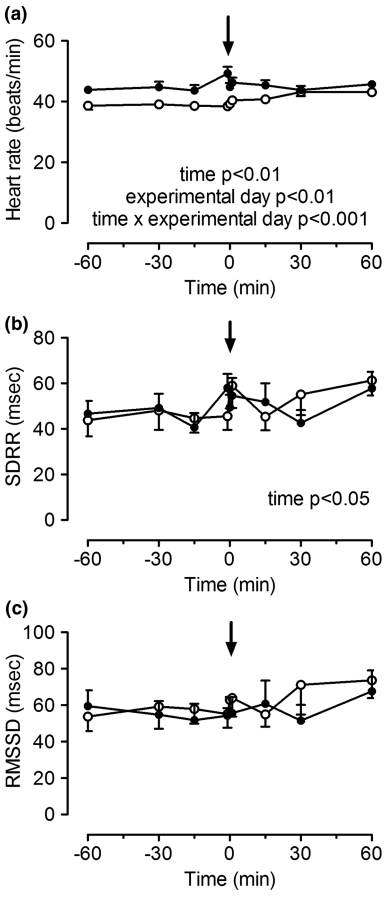


FIG 1: (a) Heart rate and heart rate variability variables (b) SDRR and (c) RMSSD in horses (n=12) before and after microchip implantation (●) and control treatment (○; arrow), all horses received both treatments in alternating order, significant differences indicated in the figures

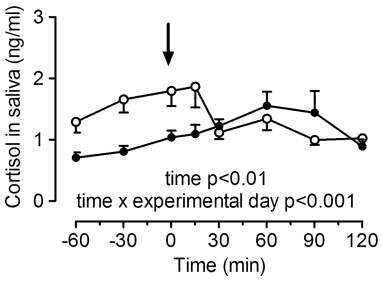


FIG 2: Cortisol concentration in saliva of horses (n=12) before and after microchip implantation (●) and control treatment (○; arrow), all horses received both treatments in alternating order, significant differences indicated in the figure

## Discussion

The results of this study show that reduced-size microchip transponders are highly reliable for the identification of horses. Conventional, larger microchips have been read correctly without exception in over 400 horses (Wulf and others 2013). The reduced-size microchips appear to be similar, and the marked reduction in size thus does not impair microchip readability. Most conventional microchip transponders measure 11.4 mm in length and 2.2 mm in diameter with an outer diameter of the injection cannula of 2.6 mm (Wulf and others 2013).

With all three scanners used, microchips were correctly identified from the chip-bearing side within seconds at four times within 28 weeks after microchip implantations. This is in agreement with a previous study on conventional microchips (Stein and others 2003) and a slightly better result than recent data (Wulf and others 2013) where only one out of three scanners was able to detect 100 per cent of conventional microchips. In the present experiment and the study by Stein and others (2003), microchips were implanted by the investigators, while Wulf and others (2013) analysed microchips implanted under field conditions by breed registry representatives. In the current study, microchips thus might have been placed in a more uniform way and were always identified at, or at least very close to, the original implantation side.

Only when microchips were read from the contralateral, non chip-bearing side of the neck, the percentage of correctly read microchips differed between scanners. With the most sophisticated device (scanner C), all microchips were read correctly also from the contralateral side of the horses` necks. This confirms previous studies on conventional microchips which were read correctly with a multimode extended range scanner in 53 horses, donkeys and mules from the chip-bearing and the non-chip-bearing side of the neck (Stein and others 2003). With scanners A and B, in our study 40 per cent and 10 per cent of microchips, respectively, were not detected from the contralateral side at 28 weeks after microchip implantation. Results compare favourably with conventional microchips in a previous study (Wulf and others 2013) which were not readable in 89 and 73 per cent with

scanners A and B, respectively, when checked from the contralateral side, that is, 'wrong' side of the horses` necks. With a restricted reading distance of the scanners, failure of microchip detection from the contralateral side is a consequence of increased distance between the microchip and the scanner. Correct microchip readings of approximately 90 per cent with scanners A and B indicate that only in individual horses the microchip was outside the limited reading range of these scanners when used from the contralateral side. Reading at 28 weeks after microchip implantation was made in January when horses were wearing a thick winter fur. Thus, the distance to the scanner was further increased, leading to a microchip detection rate as low as 60 per cent from the contralateral side with the scanner with the shortest reading distance (scanner A).

Some sport horse registries oppose the use of microchips in horses and claim a non-acceptable rate of identification failures (eg, German Equestrian Federation 2013) although this claim is not backed by scientific studies. With all scanners in our study, all microchips could be identified repeatedly from the chip-bearing side, and with the most advanced scanner microchips could always be read also from the contralateral side. With all scanners tested, identification results were also better than readability of branding signs, that is, the traditional method to mark horses in several countries. In over 200 German sport horses, the breed-specific branding symbol was consistently identified by three investigators in only 84 per cent of the animals and the individual, double-digit branding number was read correctly in less than 40 per cent (Aurich and others 2012).

Implantation of reduced-size microchips in adult horses did not elicit any stress response. By inference, this may indicate that the procedure was also not perceived as painful, however, a direct pain response was not evaluated. Fluctuations in salivary cortisol concentrations throughout the sampling period were extremely small compared to other situations to which domestic horses are frequently exposed, such as riding (Schmidt and others 2010a, Becker-Birck and others 2013, von Lewinski and others 2013), transport (Schmidt and others 2010b, c, d) or even the small cortisol release in response to branding and implantation of conventional-size microchips in foals (Erber and others 2012). Mean cortisol concentrations ranged from 0.7 to 1.6 ng/ml and 1.0 to 1.8 ng/ml on the days of microchip implantation and control treatment, respectively. This compares to immediate increases in salivary cortisol concentrations of nearly 2 ng/ml in response to riding of young horses (Schmidt and others 2010a) and more than 3 ng/ml in response to road transport (Schmidt and others 2010c). The difference in basal cortisol concentrations before microchip implantation and control treatment in the current study, although significant, has thus to be considered small. It may have been caused by one mare showing uncooperative behaviour before the start of experimental procedures on one day. Apparently more mares receiving the control treatment on that day were in close vicinity to that horse than mares receiving a microchip. While heart rate had returned to basal values before the experiment, cortisol concentrations may have still been slightly elevated.

Changes in heart rate and the HRV variable SDRR, although statistically significant, were only transient and extremely small, and no changes in the HRV variable RMSSD were found. These findings are in agreement with a lack of changes in plasma cortisol concentrations and heart rate in adult horses in response to implantation of conventional-size microchips (Lindegaard and others 2009). All microchips were always found at the original implantation site. Although with the number of mares in our study, the general possibility of microchip migration cannot totally be excluded, the results indicate that it is at least extremely unlikely.

Local responses, such as swelling or increased sensitivity at the implantation site, were not found in any of the horses. Because such responses were not quantified, only pronounced alterations might have been noticed. However, local alterations at 6–28 weeks after microchip implantation are unlikely. Acute responses within a few days after microchip implantation have been excluded for conventional microchips in other studies (Lindegaard and others 2009, Erber and others 2012).

In conclusion, reduced-size microchip transponders are highly reliable for the identification of horses, and 100 per cent of microchips could be read from the chip-bearing side with all scanners. Implantation of reduced-size microchips did not evoke a stress response in adult horses.

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