



## Readability and histological biocompatibility of microchip transponders in horses



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### ABSTRACT

Identification of horses by microchip transponder is mandatory within the European Union with only a few exceptions. In this study, the readability of such microchips in 428 horses with three different scanners (A, B and C) and the histological changes at the implantation site in 16 animals were assessed. Identification of microchips differed between scanners ( $P < 0.001$ ), and with 'side of neck' ( $P < 0.001$ ). Scanners A, B and C identified 93.5%, 89.7% and 100% of microchips, respectively, on the 'chip-bearing' side of the neck. From the contralateral side, scanners A, B and C identified 21.5%, 26.9% and 89.5% of transponders, respectively. Microchip readability was affected by age ( $P < 0.001$ ), but not by breed of horse.

At necropsy, transponders were found in the subcutaneous fat ( $n = 3$ ), inter- or peri-muscular connective tissue ( $n = 8$ ), or musculature ( $n = 5$ ), where they were surrounded by a fibrous capsule ranging in thickness from 12.7 to 289.5  $\mu\text{m}$  in 15 animals. In two animals, immature granulation tissue with attendant granulomatous inflammation, and a granulomatous myositis, surrounding the microchip were identified, respectively. Severe ( $n = 1$ ), moderate ( $n = 1$ ), and mild ( $n = 3$ ) lymphohistiocytic inflammation was noted within the fibrous capsule. Microchip transponders were found to be a highly reliable and biocompatible method of horse identification.

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### Introduction

Identification of horses is required for studbook recordings, to preclude animal substitution in competitions or sales, and to facilitate disease control. Microchip transponders are available for this purpose and, with a few exceptions, have been made mandatory for horses within the European Union. 'Hot-iron branding', traditionally used to identify horses, induces more adverse reactions than microchip implantation (Lindegård et al., 2009), and in foals is associated with a generalised increase in superficial body temperature (Erber et al., 2012). Branding causes immediate localised tissue necrosis consistent with 'third degree' thermal injury (Erber et al., 2012; Aurich et al., 2013). Such branding of horses has been prohibited in some European countries on welfare grounds, and in any case such marking often does not fully result in unambiguous visual identification (Aurich et al., 2013).

The traceability of microchip transponders in horses has been investigated in a limited number of small studies to date (Stein et al., 2003), but a large-scale assessment of this method of identification remains to be carried out using different scanners and

horses of differing sizes. Experiences with small companion animals has indicated that a high degree of microchip identification is possible (Lord et al., 2008, 2010), whereas in cattle, depending on implantation site, between 2.5% and 10% of transponders were undetectable 8 months after implantation (Løken et al., 2011). There is no clinical evidence of acute inflammation at the site of transponder implantation (Erber et al., 2012; Gerber et al., 2012), although controlled histopathological analysis of the tissue responses at the implantation site are lacking.

The objectives of the present study were to investigate the readability of microchip transponders in horses with different, commercially-available scanners, and to assess the extent of the tissue reaction elicited by the implant (i.e. its biocompatibility) by histological examination of the insertion site in horses submitted for necropsy unrelated to microchip implantation.

### Material and methods

#### Animal selection

For the first part of the study (experiment 1), we investigated the readability of microchip transponders with different scanners using 428 horses of 10 different breeds (223 Standardbred trotters, 64 Warmblood sport horses, 53 Friesians, 52 Icelandic Horses, 13 Draught horses, 12 ponies, 8 Haflingers, and 1 each of Lusitano, Quarter Horse, and Thoroughbred). Horses ranged in ages from <1 year (i.e. born

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in the year of evaluation) to 16 years (median 3.0 years). The horses belonged to different owners and were stabled at 24 locations. Of the 428 horses, 136 (32%) were intact males, 76 (18%) castrated males and 216 (50%) females.

In line with EU and German national regulations on equine identification (European Commission, 2008), all horses had been implanted with a microchip transponder that met international standards (ISO 11784 and 11785). Implantations were made in the neck, between the poll and withers into the nuchal ligament or musculature, either on the right (all trotters registered initially in Germany), or left (all other horses) sides. To meet current legal requirements, 424 horses had been implanted with a microchip during their first 6 months of life, while four animals had been 'microchipped' after this age (those born in 2004, 2006, 2009 and 2010 and those implanted in 2011 or 2012).

For the second part of the study (experiment 2), we assessed the extent of the tissue reaction elicited by the implant by histological examination, using 16 horses of 9 different breeds (4 Warmblood sport horses, 3 Friesians, 2 Arabians, 2 Pura Raza Española, and 1 each of Tinker, Standardbred trotter, Haflinger, Quarter horse, and Thoroughbred). These animals had been cases submitted for necropsy to the Department of Pathology, University of Veterinary Medicine, Hannover. Microchip transponders were detected using an Isomax III scanner (Virbac) and the age of animals selected ranged from 2 months to 25 years (in the case of one adult, the exact age could not be determined). Three of the 16 horses were intact males, 9 were castrated males, and 4 were females. Information as to when the transponders had been implanted was not available.

#### Experimental procedures

##### Experiment 1

Horses were available for the reading of their microchip implantation sites in an environment familiar to them (their home stables), and owner consent was obtained prior to study commencement. Because reading of microchips is not associated with pain, suffering or injury of animals, in accordance with national legislation, the study was not considered animal experimentation.

Three different, commercially available scanners were used to locate and read the microchip transponders on both sides of the neck of all animals. All scanners (A, Minimax II; B, i-Max plus; and C, Isomax V) were supplied by Virbac, and microchips were coded and structured according to ISO standards 11784 and 11785. The scanners differed with regard to diameter and field strength of the antenna. Scanner C was equipped with a digital signal processing function that filters interfering signals.

Scanning for the implanted microchip commenced at the poll on the left side of the neck, followed the 'crest line' to the withers, returned cranially approximately 5 cm below this line, and then coursed a 'meandering, back and forth' path until the microchip was detected or the ventral aspect of the neck was reached. All three scanners were applied in this fashion, initially on the left, and then on the right, sides of the neck of each horse. The order in which scanners were used was randomised, to ensure all scanners were used to the same extent for the first, second and third trial, respectively. 'Time until detection' of the microchip was recorded for each scanner for both sides of the neck. Where a transponder was identified, the obtained code number was compared with the information provided in that horse's passport. Since some of the horses were implanted with transponders on the left, and others on the right, both sides were scanned in all cases.

For each scanner and for each side of the neck, the percentage of 'readable' and 'non-readable' microchips was calculated and, for all readable microchips, the time until detection recorded. To determine the location of the microchip in the cranial, middle or caudal third of the neck, scanner A was used and the site evaluated through approaches from a cranial, caudal, dorsal and ventral direction. The putative site of implantation was determined from each horse's passport.

##### Experiment 2

Tissue surrounding the microchip transponders in cases submitted for necropsy was excised, examined macroscopically, fixed in 10% neutral buffered formalin, and processed into paraffin wax. Tissue sections, between 2 and 3 µm thick, were stained with haematoxylin and eosin, and evaluated microscopically. Selected sections were additionally stained with azan and elastica-van Gieson stains. Changes were 'scored' semi-quantitatively as 'mild', 'moderate' and 'severe'. An inflammatory infiltrate of <5 cells was considered 'mild', single small clusters of 5–15 cells as 'moderate', and numerous clusters or diffuse infiltration of >15 cells as 'severe'.

The thickness of any fibrous capsule present was measured at four different sites using imaging software (Cell-D, Soft Imaging Solutions), and the mean determined. For immunophenotyping inflammatory cells, serial sections were treated with murine monoclonal antibodies specific for CD79a (1/60, clone HM57, Dako), and myeloid/histiocyte antigen (1/200, clone MAC387, Dako), or a rabbit polyclonal antibody specific for CD3 (1/1000, Dako) as described by Kleinschmidt et al. (2012).

#### Statistical analysis

Statistical comparisons were made using statistical software (version 17.0, SPSS). Data were analysed using a generalised linear models procedure for binary data with microchip identification ('positive' or 'negative'), as dependent variables and 'scanner type', 'side of the neck', 'age', and 'breed group' as factors. To facilitate

breed comparisons, horses were allocated to one of three categories: group one ( $n = 293$ ) consisted of Warmbloods, Quarter horses, trotters, and Thoroughbreds; group two ( $n = 70$ ) comprised ponies, Haflingers, and Icelandic horses, and group three ( $n = 65$ ) were draught/carriage horses and Friesians. For age comparisons, horses were grouped as follows: born in year of study ( $n = 47$ ), born previous year ( $n = 99$ ), 2 years old ( $n = 49$ ), 3–4 years old ( $n = 68$ ), 5–7 years old ( $n = 80$ ), and 8–25 years old ( $n = 85$ ). 'Age x breed' interactions were included in the statistical model. In addition, 'time-until-microchip-detection' was analysed separately for the ipsilateral and contralateral neck by Kaplan–Meier survival analysis with 'type of scanner' as factor, and a generalised Wilcoxon test was used for pair-wise comparisons between scanners. For all statistical comparisons,  $P < 0.05$  was considered significant.

## Results

### Identification of microchip transponders

A microchip transponder was identified in the vast majority of horses but the correct reading of the microchip differed between the three scanners ( $P < 0.001$ ). In all 428 horses, scanner C correctly identified the transponder on the chip-bearing side: not all microchips could be read with scanners A and B. From the contralateral side, 89.5% of the microchips could be read with scanner C, in comparison with a detection rate of <30% for the other two scanners

**Table 1**

Percentage of correct microchip transponder readings on each side of the neck of 428 horses using three different scanners (see text for details).

Scanner	Side of neck	Microchips read correctly (%)
A	Ipsilateral	93.5
	Contralateral	21.5
B	Ipsilateral	89.7
	Contralateral	26.9
C	Ipsilateral	100.0
	Contralateral	89.5

**Table 2**

Factors associated with the readability of microchip transponders in horses. The total number of readings was 2568 (represents readings taken by three scanners from both sides of the neck of 428 animals).

Factor	Readings (n)	Correctly identified microchips (%)	Significance	
Scanner type	A	856	57.5	$P < 0.001$
	B	856	58.3	
	C	856	94.7	
Side of neck	Ipsilateral	1284	94.4	$P < 0.001$
	Contralateral	1284	46.0	
Age	Born year of study	282	77.7	$P < 0.001$
	Born previous year	594	72.1	
	2 years	294	63.6	
	3–4 years	408	62.7	
	5–7 years	480	74.7	
	8–25 years	510	69.4	
Breed <sup>a</sup>	Group 1	1758	69.0	Not significant
	Group 2	420	72.9	
	Group 3	390	72.6	
Age x breed	2568		$P < 0.001$	

<sup>a</sup> To facilitate breed comparisons, horses were allocated to one of three groups: group one consisted of Warmbloods, Quarter horses, trotters, and Thoroughbreds; group two of ponies, Haflingers, and Icelandic horses; and group three of draught/carriage horses and Friesians.

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