Tropical Animal Health and Production (2020) 52:3725–3733
https://doi.org/10.1007/s11250-020-02410-7

REGULAR ARTICLES

Innovative use and efficiency test of subcutaneous transponders for electronic identification of water buffaloes

Alexandre Rossetto Garcia[1] · Daniel Vale Barros[2] · Moisés Cordeiro Mourão de Oliveira Junior[3] · Waldomiro Barioni Junior[1] · Jamile Andréa Rodrigues da Silva[4] · José de Brito Lourenço Junior[2] · Jakeline dos Santos Pessoa[4]

Received: 26 May 2020 / Accepted: 24 September 2020 / Published online: 3 October 2020

Abstract

The objective of this study was to evaluate the viability of using transponders for the electronic identification of water buffaloes and compare their efficiency when used in animals of different age groups. Electronic transponders with RFID technology (2.1 × 12.2 mm) were implanted subcutaneously (D0) in the scutiform cartilage. The animals consisted of four groups: CLF-I (17 calves; 2.1 ± 1.9 months), CLF-II (20 calves; 5.1 ± 3.2 months), HFR (20 heifers; 22 ± 4.7 months) and STR (19 steers; 26.6 ± 6.7 months). The animals were kept under pasture grazing, a part of the year in the dryland and a part in the floodplain, and were monitored for up to 350 days. The average time required for individual transponder implant was 49.46 s, while the time required for reading the code was 3.76 s. The older calves required higher time for individual implant ($P = 0.0001$) and closer approximation of the reader in the D150 ($P = 0.0001$). The mean read distance was 2.98 cm in D0 and 1.94 cm in D150. The magnitude of the subcutaneous transponder migration was minimal, and was within an area of 17.2 mm^2. A slight bleeding was observed in 15.79% of the animals during the implant. A decreasing incidence of edema was observed until D21, with the heifers being more sensitive until that time ($P = 0.0099$). Considering the results, it is preferred to implant electronic transponders in calves up to two months of age. The physical rate of transponder loss was 1.3% and the loss of functionality was 9.2%. High reading rate was achieved when animals were raised both in dryland (93.9%) and floodplain (97.2%). Thus, the electronic identification of water buffaloes is a technique capable of replacing traditional and rudimentary methods to identify buffaloes and can provide safe identification of animals.

Keywords RFID · Microchip · Food safety · Traceability · *Bubalus bubalis*

Introduction

The water buffalo (*Bubalus bubalis*) has been regarded as an excellent alternative for both milk and meat production (Caria et al. 2014; Joele et al. 2017; Becskei et al., 2020) due to its rusticity, adaptability, high reproductive efficiency and feed conversion capacity from a wide variety of fodder. It is currently estimated that there are approximately 187 million animals raised in different countries. The largest herds are located in India, Pakistan and China, which account for 58%, 18%, and 13% of the water buffalo population in the world, respectively (Deb et al., 2016). In Europe, significant numbers of buffaloes (over 100.000) are currently found in Italy and Romania (Borghese and Moioli, 2016). In Africa,

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s11250-020-02410-7) contains supplementary material, which is available to authorized users.

✉ Alexandre Rossetto Garcia
alexandre.garcia@embrapa.br

[1] Laboratory of Biotechnology and Animal Reproduction, Brazilian Agricultural Research Corporation, Embrapa Southeast Livestock, São Carlos, SP, Brazil

[2] Institute of Veterinary Medicine, Federal University of Pará, UFPA, Castanhal, PA, Brazil

[3] Brazilian Agricultural Research Corporation, Embrapa Eastern Amazon, Belém, PA, Brazil

[4] Institute of Animal Health and Production, Federal Rural University of the Amazon, UFRA, Belém, PA, Brazil

water buffalo production is concentrated in Egypt, country where buffaloes are more numerous than cattle, while Brazil is the largest producer in the Americas (Lourenço Júnior and Garcia, 2008).

Although the nutritional properties of buffalo milk, dairy products and meat are valued and appreciated in many countries (Zhang et al. 2016), to effectively achieve confidence in the global market depends on guaranteeing food safety to consumers, which is currently considered as a key instrument for safeguarding public health (Murdoch and French, 2020). To this end, one of the most important premises is the product traceability system, which depends on the precise identification of each animal (Crandall et al. 2013). Traceability prevents data collection errors during the production process, and also prevents animal registration fraud in the food production intended for human consumption (Liang et al. 2015).

Over time, various techniques have been developed to identify the water buffalos, such as hot iron branding, freeze branding, tattooing, ear notching and plastic ear-tagging. However, there are some disadvantages to these techniques, such as causing pain, altering animal esthetics, causing skin lesions, reducing animal welfare (Garcia and Nahúm, 2006) and these techniques devalue the leather (Jacinto et al. 2009). In this context, electronic identification via radio-frequency identification (RFID) is a promising alternative to individualize the animals, and is considered essential for accurate feed management (Hristov et al. 2015), livestock health (Smith et al. 2015), reproduction and breeding.

RFID equipment is a system that uses radio frequency electromagnetic fields to transfer data from a device to a reader for identification and tracking purposes (Ryan et al. 2010; Liang et al. 2015). Several countries that produce and export meat and milk make use of RFID technology transponders as accepted animal identification devices, using specific criteria. It is the case of the United States, Brazil, New Zealand, Canada, Australia, and other countries (list of some countries' regulatory documents available in Supplementary Material S1). The use of this technology can benefit and optimize the traceability system, integrating all the links in the production chain such as farms, inspection service, dairy industries and slaughterhouses.

However, there are no reports in the scientific literature on the use of transponders with RFID technology in water buffaloes, whether it is about technical viability or possible biological reactions to the device. Because this is a pioneering study, there is also no previously published information on the use of microchips implanted subcutaneously in this animal species. Thus, the objective of this study was to evaluate the technical feasibility, from the instrumental and biological points of view, of using transponders to electronically identify water buffaloes and compare its efficiency when used in animals of different age groups.

Materials and methods

Location and animal management

The experiment was carried out at Embrapa Eastern Amazon, in Belém, Brazil (01°26′35"S and 48°24′27"W). The local climate is humid tropical climate, average rainfall of 2.876 mm/year, average annual temperature of 26.8 °C and relative humidity of 83%. The total pasture area comprised 120 ha and was divided into dryland areas with cultivated grasses and flooded areas with native forages. The animals were kept under permanent pastures, using a rotating pasture system, part of the year in the dryland and the other part in the floodplain. All animals had ad libitum access to water, provided in automatic waterers, and mineral salt made available in covered troughs.

Animals and experimental groups

Seventy-six water buffaloes were used, subdivided into four experimental groups: CLF-I ($n = 17$ calves; 2.1 ± 1.9 months; 102.5 ± 57.8 kg), CLF-II ($n = 20$ calves; 5.1 ± 3.2 months; 162.9 ± 73.3 kg), HFR ($n = 20$ heifers; 22.0 ± 4.7 months; 310.1 ± 125 5 kg) and STR ($n = 19$ steers; 26.6 ± 6.7 months; 391.4 ± 61.8 kg). The moment when the transponders were implanted marked the beginning of the experiment (D0, day zero) and the animals were evaluated up to 350 days, depending on the response variable considered.

Electronic identification and reading of transponders

The animals entered the experiment using plastic ear-tags in the right ear and ink tattooing at the base of the tail to ensure their permanent identification. Experimentally, each animal was implanted with a transponder in the left ear in the region of the scutiform cartilage, based on recommendations for cattle (Finkenzeller, 2003). After prior asepsis and local desensitization, each transponder ($n = 76$; ISO FDX-B, 134.2 KHz) was injected subcutaneously using a single-shot injector coupled to single-use sterile needle (Fig. 1), always by the same operator. The transponders had passive power source, were encapsulated in sterile bioglass 8625, overall dimensions of 2.1 × 12.2 mm (KT34/4, AnimallTAG, São Carlos, Brazil), compatible with the ISO Guidelines (ISO 11784 and ISO 11785), and ABNT Standards (NBR 14766:2012 and NBR 15006:2016). The standards references are given in Supplementary Material S2.

The numerical code reading of the transponders was performed before and immediately after being implanted to verify their correct functioning. In the first reading, the plastic ear tags and tattooed numbers, and the complementary animal data were automatically associated to the transponder code and recorded in electronic spreadsheets. The reading of the transponders was performed with a handheld RFID reader (185 mm length × 100 mm width × 40 mm; 295 g weight), coupled to the rechargeable battery (KT34/13, AnimallTAG, São Carlos, Brazil). The reader was held close to the head of the restrained animal and the reading was considered effective when the reader emitted a sound signal and the transponder number was displayed on the screen. The technical description, FDX-B Protocol and assurance quality tests are given in Supplementary Material S3.

Behavior of the binomial transponder-reader

The time required for individual implant (TRI, sec) was measured in D0. The TRI ($n = 76$) consisted of the time spent for inserting the transponder in the restrained animal and checking the correct positioning of the device (Conill et al. 2000). The time required for reading the code (TRR, sec) corresponded to the time spent immobilizing the animal in the chute for the actual reading of the transponder (Løken et al. 2011). The TRR was measured daily between D0 and D7, every three days between D8 and D21, and every 30 days until the animals reached a maximum of 350 days ($n = 1259$).

The read distance evaluation (RD, cm) was measured immediately after the transponder was implanted (D0) and 150 days after (D150) executing one or two reads per animal ($n = 132$). The RD was performed with a millimeter-scale metallic caliper juxtaposed to the reader. The minimum distance required between the transponder and the reader at the time of the actual reading was accepted, confirmed after the sound signal and the correct display of the transponder code on the screen. The read rate (RR) was determined as the number of successfully performed reads divided by the total number of attempts performed to read the field in the transceiver (Ryan et al. 2010). For calculating the RR, 1305 read attempts were performed ($n = 1305$).

Migration and clinical reactions

The measurement of subcutaneous transponder migration (MIG, mm) was carried out comparatively by direct palpation of the transponder and by marking its location immediately after being injected and also the days of subsequent evaluations. Permanent markings with India ink were used in the caudal face of the ear in D0, as anatomical references for measuring the mediolateral displacement (X axis) and ventrodorsal displacement (Y axis) using a metallic caliper at the implant site. The migrations were followed using a Cartesian plane (X, Y), where the implant coordinates in D0 were denominated as (0,0). Migration evaluations ($n = 773$) were performed from D1 to D21. Its recording considered the module, direction, and migration route. Only in the HFR Group the migration from D8 to D21 was not measured because on these dates the heifers were involved in reproductive management procedures.

Concurrently with the migration, we evaluated the clinical reactions to the transponder, which were classified as bleeding and edema formation. The bleeding ($n = 76$) was recorded when there was blood dripping immediately after removing the applicator. The edema formation ($n = 304$) was characterized as occurring when there was retention of extravasated fluid from the auricular microvessels, possibly injured by implanting the transponder. The clinical reactions were evaluated by clinical inspection of the animals, restrained in the chute, daily between D0 and D7, and every three days from D8 to D21. The rates of bleeding and edema formation were calculated by the ratio between the number of individuals

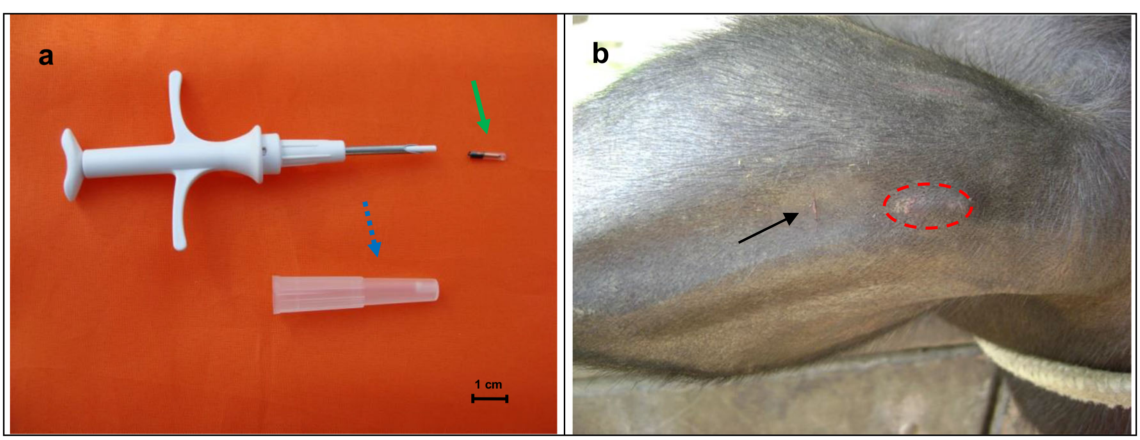

Fig. 1 Single-use sterile plastic injector, cap (dotted blue arrow) and transponder encapsulated in bioglass (2.1 × 12.2 mm, continuous green arrow) (a). Auricular region of water buffalo identified with subcutaneous RFID transponder (b); note a minimal scar at the applicator insertion location (continuous black arrow) and the location of the implanted transponder (dashed ellipse)

with any of the adverse signs and the total number of animals implanted and expressed as a percentage (%).

Statistical analysis

The General Linear Model (GLM) procedure of Statistical Analysis System (SAS, 2010) was used for the variables Time Required for Individual Implant (TRI, sec), Time Required for Reading (TRR, sec) and Read Rate (RR, %). The statistical model used in these analyses was $Y_{ij} = \mu + G_i + \varepsilon_{ij}$. where Y_{ij} = response variable, μ = general mean, G_i = effect of i-th Group (i = 1,2,3 and 4) and ε_{ij} is the random error NID$(0,\sigma^2)$. The data for Read Distance (RD, cm) were also submitted to analysis of variance using the General Linear Model, but considering the effects of day (D: D0 and D150), Group (G: CLF-I, CLF-II, HRF and STR) and the interaction day * group, following the model: $Y_{ijk} = \mu + D_i + G_j + (D*G)_{ij} + \varepsilon_{ijk}$, where Y_{ijk}: response variable, μ: general mean, D_i: effect of i-th Day (i = 1,2), G_j: effect of j-th Group (j = 1,2,3 and 4), $(D*G)_{ij}$: effect of interaction i-th Day and j-th Group; and ε_{ij} is the random error NID$(0,\sigma^2)$. The averages were compared by the Tukey test except for the bleeding and edema formation, which were compared by the Chi-square test.

Chi-square test was used for determination of statistical significance for comparison of incidence of clinical reactions, considering a contingency table for four Groups (CLF-I, CLF-II, HRF and STR), and the absence or presence of bleeding or edema. Regarding the edema formation, a Chi-square test was carried out for each considered time (D0, D1 to D7, D8 to D14 e D15 to D21). The average percentage of success in reading transponders on animals kept in different areas (dryland or floodplain) by group was compared by Student's t test at a 95% confidence level. The pattern of subcutaneous migration (MIG, mm) was evaluated graphically in scatter plot through a plane formed by the mediolateral (X) and ventrodorsal (Y) migration directions in D1 to D7, D8 to D14, and D15 to D21 per group. The residual term of ANOVA was represented by the root of means square for error (RMSE: root of MSE). The level of significance was previously set at 5% for all statistical procedures.

Results

The responses of transponder functionality are shown in the Table 1. The lower age animals, belonging to the CLF-II, required higher time for individual implant, with a significant increase according to the progressing age of the calves (P = 0.0001). There was a small delay on the time required for reading in the CLF-II animals (P = 0.0001).

Table 1 Time required for individual implant (TRI, sec), time required for reading (TRR, sec) and read rate (RR, %), for the use of transponders in the auricular region for electronic identification of water buffaloes of different ages

	CLF-I	CLF-II	HFR	STR	Average	RMSE	P value
TRI†	31.00[a]	75.00[c]	49.29[b]	40.11[ab]	49.46	23.40	0.0001
TRR‡	2.18[a]	7.08[c]	2.63[b]	2.08[a]	3.76	2.74	0.0001
RR§ [Ω]	91.91[a]	94.17[ab]	98.82[b]	99.38[b]	96.16	10.43	0.0047

† 76 observations; ‡ 1259 observations; § 1305 observations

CLF-I = 17 calves, 2 months old; CLF-II = 20 calves, 5 months old; HFR = 20 heifers, 22 months old; STR = 19 steers, 26 months old

RMSE = Root MSE (Means Square for Error of Anova)

[Ω] RR data was transformed by arsin(sqrt(RR/100))

[a, b, c] Columns marked with different letters differ significantly between groups

The read distance was not influenced by age in D0 ($P >$ 0.05). However, in the D150 the reader had to be held closer to the animals (Table 2) to conduct the electronic identification of the CLF-II (P = 0.0001). The read rate, which measures the overall reading efficiency of electronic devices, was 96.15% (n = 1250/1305), with a maximum value for the adult animals and a slight lower rate for the animals in the CLF-I (P = 0.0047). The success in reading the electronic transponders did not decrease when animals were maintained in the floodplain areas (Table 3).

The migration of the transponders in the X-dimension (mediolateral) varied from −3.0 to 2.2 mm and − 2.3 to 1.0 mm in the Y-dimension (ventrodorsal), independently of the group (Fig. 2). The transponders moved in the ear of the buffaloes in an approximate area of 17.2 mm^2, restricted to a rectangle of 5.2 × 3.3 mm.

Regarding the clinical reactions, 15.79% of the animals presented modest bleeding (Table 4) and 43.42% presented local edema formation after the implantation (Table 5).

Despite the age of animals, the edema formation reached 48.68% between D1 and D7. Nevertheless, in the period ranging from D8 to D14, the animals of the CLF-I and STR no longer had any edemas. Notwithstanding the progressive decreasing incidence of edema observed until D21, a higher percentage of animals in the HFR still exhibited this kind of local reaction between D15 and D21 (P = 0.0099).

The loss rate of transponder functionality was 9.2%, while the percentage of physical loss was 1.3%.

Discussion

In our study, due to the absence of specific references for buffaloes, the injection of the transponders in the region of the scutiform cartilage was based on allometric extrapolation,

Table 2 Read distance evaluation (RD, cm) for the use of transponders in the auricular region for electronic identification of water buffaloes of different ages, measured immediately after the transponder implantation (D0) and 150 days after (D150)

	CLF-I	CLF-II	HFR	STR	Average
RD at D0†	2.98[aA]	2.99[aA]	2.93[aA]	3.01[aA]	2.98
RD at D150‡	2.27[bB]	0.76[aB]	2.21[bB]	2.50[bB]	1.94

† 74 observations; ‡ 132 observations

CLF-I = 17 calves, 2 months old; CLF-II = 20 calves, 5 months old; HFR = 20 heifers, 22 months old; STR = 19 steers, 26 months old

RMSE = Root MSE (Means Square for Error of Anova) = 0.652; *P* value = 0.0001; Interaction (Day*Group): P value = 0.0001

[a, b] Columns marked with different letters differ significantly between groups

[A, B] Rows marked with different letters differ significantly between times

as recommended for cattle (Giro et al. 2019). Even so, it proved to be a suitable choice because this anatomical region is little vascularized and easily accessible when the animals are restrained. The strategy of approaching the animal by its left antimer was used because of its easy management access due to the architectural configuration of the zootechnical facilities employed. However, in principle, the use of the right ear could also be indicated due to its anatomical and vascular similarity with the left ear.

Although it is a static reading system and requires restraining the animal, using the handheld reader has the advantage of eliminating transcription errors commonly observed in non-automatic animal identification systems. For the rural producer, the time spent in executing the tasks is decisive for using or abandoning a technology (Vecchio et al. 2020). Regardless of animals age, the average time required for individual implant was lower than that observed by Klindtworth et al. (1999), which reported 60 s to perform the implant procedure in cattle. It is

Table 3 Average percentage of success (%) in reading transponders for electronic identification of water buffaloes of different ages, according to the production system

	CLF-I	CLF-II	HFR	STR	Average [Ω]
Dryland†	82.35[B]	92.70[B]	100.00[A]	99.38	93.97 ± 2.27[B]
Floodplain‡	94.12[A]	100.00[A]	97.50[B]	–	97.22 ± 1.47[A]

† 76 animals (2 to 17 observations/animal); ‡ 54 animals (4 to 13 observations/animal); [Ω] mean ± standard error

CLF-I = 17 calves, 2 months old; CLF-II = 20 calves, 5 months old; HFR = 20 heifers, 22 months old; STR = 19 steers, 26 months old

- steers were not maintained in floodplain during the experiment

[A, B] Rows marked with different letters differ significantly between systems of production

possible that the higher time for individual implant in the older calves (CLF-II) was due to these animals' larger size in relation to their younger counterparts, in addition to the greater care and sensitivity required for handling and restraining them inside the chute. The time required for implant in adult water buffaloes ranged from 40.1 to 49.2 s, on average, a value considered very convenient even when projecting its use in large-scale herds.

Another determining factor for the possible use of the technology is the time to read the codes, which presented an average lower than the 12 s required for digital identification of the bovines (Klindtworth et al. 1999). The mean time required for reading the electronic codes of calves (CLF-II) can be attributed to a greater reactivity to the physical restraint procedures observed in young animals as the experimental period progressed. However, the read distance may be related to the size of the transponder, which is directly proportional to the size of its internal coil (Barge et al. 2013). The minimal distance required to read an electronic code may also be influenced by the type of transponder technology, by the physical and transceiver characteristics, the strength of the electromagnetic field, and by environmental interferences (Klindtworth et al. 1999).

The overall reading efficiency of the transponder, regardless of the age of the animals, was higher than the 94.8% reported for subcutaneous auricular implants in cattle (Conill et al. 2000). The read rate achieved in this experiment for adult animals (≥98.82%) is noteworthy, and it is greater than 95%, a minimum value considered as acceptable, for example, for plastic ear tags with RFID technology (Wallace et al. 2008). Although considered to be quite adequate, the slight lower reading rate for the younger calves was possibly due to the increased restlessness of these young animals during the evaluations, associated to a greater probability of physical damage to the transponder. Thus, the average reading efficiency observed (96.16%) can be considered as high. This highlights the technology of RFID identification in relation to the use of plastic ear tags, which are susceptible to loss, deterioration due to sun and rain (Fosgate et al. 2006), as well as the use of skin branding, which is cruel (Garcia and Nahúm, 2006; Wulf et al. 2013) and become illegible due to the intense pigmentation of the water buffaloes. The achieved reading rate can be considered even more favorable when taking into account that the buffaloes were raised under pasture grazing and with permanent access to harsh environments with excessive dirt, dust and moisture. Where water buffaloes are maintained in flooded areas, animals are currently wet and muddy for posterior management. This is a challenging situation in which the RFID system is expected to function in a limited mode (Ruiz-Garcia and Lunadei, 2011). Nonetheless, in spite of the age, the success of reading electronic transponders achieved a high rate of efficiency whether animals had been maintained in dryland or in floodplain.

Day	Group	Axis (X)	Axis (Y)
D1 to D7	CLF-I	1,3	-1,2
	CLF-II	-0,8	0,3
	HFR	-0,1	0,5
	STR	2,2	0,9
D8 to D14	CLF-I	0,4	-1,6
	CLF-II	-2,5	-1,6
	HFR		
	STR	-0,6	1,0
D15 to D21	CLF-I	-0,4	-1,1
	CLF-II	-3,0	-2,3
	HFR		
	STR	2,1	1,0

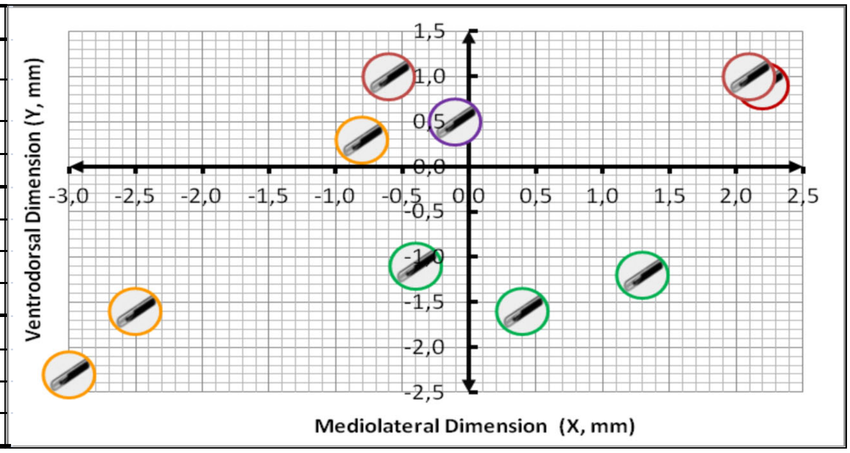

Fig. 2 Graphical representation of migration (mm) in the mediolateral (X) and ventrodorsal (Y) planes of the transponders implanted in the scutiform cartilage of the water buffalos, describing the area and average movement positions. D = Days after implant. CLF-I = calves, 2 months old; CLF-II = calves 5 months old; HFR = heifers, 22 months old; STR = steers, 26 months old

Migration is another critical aspect when using subcutaneous transponders, since their possible displacement may pose a risk to the animal health (Nakamura et al. 2019), as well as during the post slaughter industrialization of the carcass, compromising the traceability process and safety of the food production. Transponders migration had an absolute mean value lower than 3.0 mm, which is below that observed in bovines (Klindtworth et al. 1999). So, even with the largest migrations observed in calves in the second and third weeks after the implant, the migration showed a smaller magnitude. As the transponders used were 11.2 mm in length, it was found that the migration movements were relative to the size of only one transponder, in the X or Y direction. This result can be attributed to the static stability of the transponder and the implant technique employed.

It is possible that the older calves and steers exhibited bleeding because they were the first groups to undergo the experimental procedures. Therefore, the operator's ability may have improved, reducing the micro-hemorrhages in the other groups. Thus, specific prior training is recommended in order to standardize the operator's skill to inject the transponder, which may also influence the retention and subsequent functionality of the devices. The animals showed an increase in the percentage of edema formation up to seven days after the implant. This finding coincides with the first phase of the cicatricial process, which is characterized by increased capillary permeability, with edema and clot formation (Silva et al. 2014). This demonstrates that accurate monitoring of the animals up to seven days after implantation is recommended in order to observe the local inflammatory response.

Over the course of three weeks, animals from all groups showed a tendency to reverse localized edema. It is assumed that the higher levels of edema observed in the heifers could be due to the fact that they are females of reproductive age and therefore with high estrogen levels, a hormone with vasodilation and hypotension action capable of increasing vascular permeability (White, 2002). Although slightly uncomfortable to the animals, a small local inflammatory process could possibly contribute positively to securing the transponder, since the cicatricial granulation tissue formed reduces its possible migration (Nakamura et al. 2019).

The functionality loss rate observed is corroborated by Andreoni et al. (1994), who reported loss of functionality between 2 and 9% for electronic devices injected into the

Table 4 Bleeding incidence (% and frequency) during the procedure for auricular subcutaneous implantation of electronic transponders in water buffaloes of different ages

	CLF-I	CLF-II	HFR	STR	Average	P value*
Bleeding† (%)	0.00[a]	25.00[b]	0.00[a]	36.84[b]	15.79	0.0022
Bleeding (frequency)	0/17	5/20	0/20	7/19	12/76	–

† 76 observations

CLF-I = 17 calves, 2 months old; CLF-II = 20 calves, 5 months old; HFR = 20 heifers, 22 months old; STR = 19 steers, 26 months old

[a, b] Columns marked with different letters differ significantly between groups by Chi-Square Test

* P value associated with global Chi-Square Test, Group vs Bleeding (+,-)

Table 5 Edema incidence (% and frequency) for auricular subcutaneous implantation of electronic transponders in water buffaloes of different ages

Days after Implantation†	CLF-I	CLF-II	HFR	STR	Average	P value*
D0	23.53[a] (4/17)	15.00[a] (3/20)	70.00[b] (14/20)	63.16[b] (12/19)	43.42 (33/76)	0.0004
D1 to D7	52.94[a] (9/17)	35.00[a] (7/20)	60.00[a] (12/20)	47.37[a] (9/19)	48.68 (37/76)	0.4470
D8 to D14	0.0[a] (0/17)	5.0[a] (1/20)	35.0[b] (7/20)	0.0[a] (0/19)	10.53 (8/76)	0.0005
D15 to D21	0.0[a] (0/17)	5.0[a] (1/20)	25.0[b] (5/20)	0.0[a] (0/19)	7.89 (6/76)	0.0099

† 304 observations

CLF-I = 17 calves, 2 months old; CLF-II = 20 calves, 5 months old; HFR = 20 heifers, 22 months old; STR = 19 steers, 26 months old

[a, b] Columns marked with different letters differ significantly between groups by Chi-Square Test

* P value associated with global Chi-Square Test, Group vs Edema (+,-), by times

scutiform cartilage of cattle. It is possible that the loss of functionality was due to the mechanical compression in the physical restraining of the animals in the chute during routine management procedures, or due to the fact that in buffaloes the ear is a relatively exposed organ, which could predispose to mechanical trauma or the occurrence of myiasis (Barioni Junior et al. 2016). The observed functionality loss rate could initially be considered a disadvantage. But this loss does not seem to be so impressive, as it also occurs in other animal identification methods, whether permanent or temporary (Awad, 2016). For example, the rate of tag loss in adult cows is 11% (Seroussi et al. 2001; CCIA, 2016), but may range from 3.8 to 35.3%, depending on the handling and environment conditions (CCIA, 2016). In goats, the loss rate of flag-button plastic ear tags is 8.3% (Carné et al. 2009).

The physical loss of the transponder may have occurred due to ear movement, which may have forced the retrograde movement of the device. Even so, the observed value was lower than the 2% loss reported for cattle (Conill et al. 2000). Therefore, if the correct procedures are implemented for injecting the transponders, in addition to preventing contaminations, it is possible to prevent losses (Finkenzeller, 2003). One possibility not investigated in this study would be to consider other anatomical points of interest to execute subcutaneous implants, such as the caudal fold, the lateral neck, the upper scapula region or the lower scapula (Lee et al. 2016). In fact, the position to place electronic devices for animal monitoring is a critical point in standardizing the identification and auxiliary diagnostic techniques using automated systems (McCorkell et al. 2014). All the same, RFID electronic identification can contribute to the entire supply chain, and the stakeholders could take advantage of data availability in real time, in a collaborative network of shared platforms (Barge et al. 2013).

Considering the low incidence of bleeding during implantation, the rapid return to a normal tissue condition after transponder injection, and the lower time required for individual implant and for reading the codes, it is recommended to adopt the electronic identification of water buffaloes at the earliest possible age. With a uniform and early electronic identification of buffalo calves, the permanent and individual recognition is guaranteed. Moreover, sanitary recordings and future food traceability are facilitated. It must also be considered that the progress in using this type of technology depends on the convenience related to implementation, access and operation, in addition to assuring welfare to the animals. Last but not least, the results of this study may also contribute to research advances on the use of subcutaneous thermochips with RFID technology, which have recently been used to predict the internal body temperature of animals (Lee et al. 2016; Giro et al. 2019).

Conclusion

The use of transponders for electronic identification in water buffaloes is a high efficiency technique that requires little implanting and reading time, in addition to presenting low subcutaneous migration rates, both for young animals and for adult animals. The implant procedure is very successful if the correct restraining and injection techniques in the scutiform cartilage are used. The migration of transponders in the ventrodorsal or mediolateral directions is minimal, with an absolute movement smaller than its own length, and is considered almost negligible in relation to the size of the animal, independently of the age. However, the reading distance of the transponder is a factor that still requires further gains. Additionally, it is recommended that the operator should be trained to implant the transponders and that greater attention be given when working with calves and reproductive-age females, in order to reduce the formation of local edema. Considering the biological and technical results, it is preferred to implant electronic transponders in water buffaloes up to two months of age. Therefore, the identification of water buffaloes with subcutaneous transponders is a technique that can replace traditional methods, ensuring a reliable identification of animals, reduction in stress management and constituting the first step for incorporating buffalo populations to modern precision livestock farming.

Acknowledgments The authors would like to thank Benjamim Nahum and Talmir Neto for their contributions to the farming procedures.

Funding This study was funded by the Brazilian Agricultural Research Corporation - Embrapa (Precision Agriculture Network, grant#

11.14.09.001.03.03) and the Foundation for Research Support of the State of Pará - FAPESPA. ARG is CNPq - National Council for Scientific and Technological Development fellow.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval The experiment complies with the international and national guidelines for the care and use of animals. All experimental procedures were approved by the Technical Committee of Brazilian Agricultural Research Corporation-Embrapa Eastern Amazon, in view of legal and ethical aspects. The research was reported according to The ARRIVE Guidelines: Animal Research Reporting In Vivo Experiments (doi:https://doi.org/10.1371/journal.pbio.1000412).

References

Andreoni, D., Luini, M., Belloli, A., Brugola, L., and Casartelli, A., 1994. Application of electronic identification by implantable transponders in beef cattle: a field trial. Final Report of EU Research-Project: Electronic identification of farm animals using implantable transponders, Brussels, Belgium.

Awad, A.I., 2016. From classical methods to animal biometrics: A review on cattle identification and tracking. Computers and Electronics in Agriculture 123, 423-435. doi:https://doi.org/10.1016/j.compag.2016.03.014.

Barge, P., Gay, P., Merlino, V., and Tortia, C., 2013. Radio frequency identification technologies for livestock management and meat supply chain traceability. Canadian Journal of Animal Science 93, 23-33. doi:https://doi.org/10.4141/CJAS2012-029.

Barioni Júnior, W., Machado, C.G.C.F., Garcia, A.R., Esteves, S.N., and Paranhos, N.E., 2016. Avaliação da performance de identificador eletrônico por radiofrequência para bovinos de corte, produzido no Brasil. In Proceedings of the 1st Congress on Precision Livestock Farming, Florianópolis, Brazil, 2016, (Gessulli Agribusiness, São Paulo; Avesui 2016), 1-5.

Becskei, Z., Savić, M., Ćirković, D., Rašeta, M., Puvača, N., Pajić, M., Đorđević, S., Paskaš, S. Assessment of water buffalo milk and traditional milk products in a sustainable production system. Sustainability 2020, 12, 6616. doi:https://doi.org/10.3390/su12166616.

Borghese, A., Moioli, B., 2016. Buffalo: Mediterranean Region. reference Module in Food Science, doi:https://doi.org/10.1016/B978-0-08-100596-5.21232-8 2016.

Canadian Cattle Identification Agency (CCIA)., 2016. Tag Retention Project - mature cow preliminary findings. CCIA. 2016. https://www.canadaid.com/documents/CCIA_Releases_Tag_Retention_Project_Mature_Cow_Preliminary_Findings_2016-01-08.pdf.

Caria, M., Tangorra, F.M., Leonardi, S., Bronzo, V., Murgia, L., and Pazzona, A., 2014. Evaluation of the performance of the first automatic milking system for buffaloes. Journal of Dairy Science 97, 1491-1498. doi:https://doi.org/10.3168/jds.2013-7385.

Carné, S., Gipson, T.A., Rovai, M., Merkel, R.C., and Caja, G., 2009. Extended field test on the use of visual ear tags and electronic boluses for the identification of different goat breeds in the United States. Journal of Animal Science 87, 2419-2427. doi:https://doi.org/10.2527/jas.2008-1670.

Conill, C., Caja, G., Nehring, R., and Ribó, O., 2000. Effects of injection position and transponder size on the performances of passive injectable transponders used for the electronic identification of cattle. Journal of Animal Science 78, 3001-3009. doi:https://doi.org/10.2527/2000.78123001x.

Crandall, P.G., O'Bryan, C.A., Babu, D., Jarvis, N., Davis, M.L., Buser, M., Adam, B., Marcy, J., and Ricke, S.C., 2013. Whole-chain traceability, is it possible to trace your hamburger to a particular steer, a U.S. perspective. Meat Science 95,137-144. doi:https://doi.org/10.1016/j.meatsci.2013.04.022.

Deb, G.K., Nahar, T.N., Duran, P.G., Presicce, G.A., 2016. Safe and sustainable traditional production: The water buffalo in Asia. Frontiers in Environmental Science. doi:https://doi.org/10.3389/fenvs.2016.00038.

Finkenzeller, K., 2003. RFID Handbook: fundamentals and applications in contactless smart cards and identification, 2nd edition, (John Wiley & Sons Ltd, West Sussex).

Fosgate, G.T., Adesiyun, A.A., and Hird, D.W., 2006. Ear-tag retention and identification methods for extensively managed water buffalo (*Bubalus bubalis*) in Trinidad. Preventive Veterinary Medicine 73, 287-296. doi:https://doi.org/10.1016/j.prevetmed.2005.09.006.

Garcia, A.R., and Nahum, B.S., 2008 Diagnóstico sanitário e reprodutivo do rebanho bubalino do Campo Experimental do Baixo Amazonas (CEBA). Embrapa Amazônia Oriental. 2008. http://ainfo.cnptia.embrapa.br/digital/bitstream/item/27909/1/Doc337.pdf.

Giro, A., Bernardi, A.C.C., Barioni Junior, W., Lemes, A.P., Botta, D., Romanello, N., Barreto, A.D.N., Garcia, A.R., 2019. Application of microchip and infrared thermography for monitoring body temperature of beef cattle kept on pasture. Journal of Thermal Biology 84, 121-128. doi:https://doi.org/10.1016/j.jtherbio.2019.06.009.

Hristov, A.N., Oh, J., Giallongo, F., Frederick, T., Weeks, H., Zimmerman, P.R., Harper, M.T., Hristova, R.A., Zimmerman, R.S., and Branco, A.F., 2015. The use of an automated system (GreenFeed) to monitor enteric methane and carbon dioxide emissions from ruminant animals. Journal of Visualized Experiments 103, 52904. doi:https://doi.org/10.3791/52904.

Jacinto, M.A.C., Oliveira, A.R., and Andreolla, D.L., 2009. Avaliação técnica e operacional do sistema nacional de classificação da pele bovina. Embrapa Pecuária Sudeste. 2009. https://www.embrapa.br/busca-de-publicacoes/-/publicacao/578810/avaliacao-tecnica-e-operacional-do-sistema-nacional-de-classificacao-da-pele-bovina.

Joele, M.R., Lourenço, L.F., Lourenço Júnior, J.B., Araújo, G.S., Budel, J.C., and Garcia, A.R., 2017. Meat quality of buffaloes finished in traditional or silvopastoral system in the Brazilian Eastern Amazon. Journal of the Science of Food and Agriculture 97, 1740-1745. doi:https://doi.org/10.1002/jsfa.7922.

Klindtworth, M., Wendel, G., Klindtworth, K., and Pirkelmann, H., 1999. Electronic identification of cattle with injectable transponders. Computers and Electronics in Agriculture 24, 65-79. doi:https://doi.org/10.1016/s0168-1699(99)00037-x.

Lee, Y., Bok, J.D., Lee, H.J., Lee, H.G., Kim, D., Lee, I., Kang, S.K., and Choi, Y.J., 2016. Body temperature monitoring using subcutaneously implanted thermo-loggers from Holstein steers. Asian-Australasian Journal of Animal Science 29(2), 299-306. doi:https://doi.org/10.5713/ajas.15.0353.

Liang, W., Cao, J., Fan, Y., Zhu, K., and Dai, Q., 2015. Modeling and implementation of cattle/beef supply chain traceability using a distributed RFID-based framework in China. PLoS One. 2;10(10): e0139558. doi:https://doi.org/10.1371/journal.pone.0139558.

Løken, T., Vatn, G., and Kummen, E., 2011. Subcutaneous electronic identification in cattle: a field study. Veterinary Record 169, 250. doi:https://doi.org/10.1136/vr.d2789.

Lourenço Júnior, J.B., Garcia, A.R. Panorama da bubalinocultura na Amazônia. In: Encontro Internacional da Pecuária da Amazônia, 1., 2008, Belém, PA. Meio ambiente e Pecuária: [anais]. Belém, PA: FAEPA; Instituto Frutal; SEBRAE-PA, 2008. https://www.alice.cnptia.embrapa.br/alice/bitstream/doc/409969/1/LOURENCO2008AmazonpecPanorama.pdf.

McCorkell, R., Wynne-Edwards, K., Windeyer, C., and Schaefer, A., 2014. Limited efficacy of Fever Tag(®) temperature sensing ear tags in calves with naturally occurring bovine respiratory disease or induced bovine viral diarrhea virus infection. Canadian Veterinary Journal 55(7), 688-690.

Murdoch, D.R., and French, N.P., 2020. COVID-19: another infectious disease emerging at the animal-human interface. The New Zealand Medical Journal 133, 12-15.

Nakamura, S., Sakaoka, A., Ikuno, E., Asou, R., Shimizu, D., and Hagiwara, H., 2019. Optimal implantation site of transponders for identification of experimental swine. Experimental Animals 68(1), 13-23. doi:https://doi.org/10.1538/expanim.18-0052.

Ruiz-Garcia, L., and Lunadei, L., 2011. The role of RFID in agriculture: Applications, limitations and challenges. Computers and Electronics in Agriculture 79, 42-50. doi:https://doi.org/10.1016/j.compag.2011.08.010.

Ryan, S.E., Blasi, D.A., Anglin, C.O., Bryant, A.M., Rickard, B.A., Anderson, M.P., and Fike, K.E. 2010. Read distance performance and variation of 5 low-frequency radio frequency identification panel transceiver manufacturers. Journal of Animal Science 88, 2514-2522. doi:https://doi.org/10.2527/jas.2009-2070.

Seroussi, E., Yakobson, E., Garazi, S., Oved, Z., and Halachmi, I., 2011. Short communication: Long-term survival of flag eartags on an Israeli dairy farm. Journal of Dairy Science 94(11), 5533-5535. doi:https://doi.org/10.3168/jds.2011-4330.

Silva, C.D.C., Nascimento Junior, R.S., Paredes, L.J., Roos, T.B., and Almeida, M.B., 2014. Caracterização do processo de cicatrização cutânea por segunda intenção em búfalos (*Bubalus bubalis*). Revista Brasileira de Medicina Veterinária 36, 301-306.

Smith, J.L., Vanzant, E.S., Carter, C.N., and Jackson, C.B., 2015. Discrimination of healthy versus sick steers by means of continuous remote monitoring of animal activity. American Journal of Veterinary Research 76, 739-744. doi:https://doi.org/10.2460/ajvr.76.8.739.

Statistical Analyses System Institute (SAS) 2010. The SAS-System for Windows. User's Guide. Version 9.1.3, Cary, NC, USA.

Vecchio, Y., Agnusdei, G.P., Miglietta, P.P., and Capitanio, F., 2020. Adoption of precision farming tools: the case of Italian farmers. International Journal of Environmental Research and Public Health 17(3), pii:E869. doi:https://doi.org/10.3390/ijerph17030869.

Wallace, L.E., Paterson, J.A., Clark, R., Harbac, M., and Kellom, A., 2008. Readability of thirteen different radio frequency identification ear tags by three different multi-panel reader systems for use in beef cattle. The Professional Animal Scientist 24,384-391. doi:https://doi.org/10.15232/S1080-7446(15)30888-3.

White, R.E., 2002. Estrogen and vascular function. Vascular Pharmacology 38, 73-80. doi:https://doi.org/10.1016/S0306-3623(02)00129-5.

Wulf, M., Wohlsein, P., Aurich, J.E., Nees, M., Baumgärtner, W., and Aurich, C., 2013. Readability and histological biocompatibility of microchip transponders in horses. Veterinary Journal 198, 103-108. doi:https://doi.org/10.1016/j.tvjl.2013.04.028.

Zhang, Y., Wang, H., Gui, L., Wang, H., Mei, C., Zhang, Y., Xu, H., Jia, C., and Zan, L., 2016. Profile of muscle tissue gene expression specific to water buffalo: Comparison with domestic cattle by genome array. Gene. 577, 24-31. doi:https://doi.org/10.1016/j.gene.2015.11.015.

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.