Technique

An innovative approach for combining marking of phocid seals with biopsy sampling using a new type of livestock ear tags.

Abbo van Neer[1*], Ana Rubio-Garcia[2], Stephanie Gross[1,2], Anna Salazar-Casals[2], Alberto Arriba-Garcia[2]
Peter Wohlsein[3], and Ursula Siebert[1]

[1]Institute for Terrestrial & Aquatic Wildlife Research (ITAW), University of Veterinary Medicine Hannover, Foundation, Germany. [2]Sealcentre Pieterburen, Netherlands. [3]Department of Pathology, University of Veterinary Medicine Hannover, Foundation, Germany.

Abstract

Marking of seals is done for different purposes such as re-identification of single individuals within a rehabilitation centre, or to conduct capture-mark-recapture studies assessing ecological parameters on a population scale. Until today most studies use conventional livestock ear tags applied to the interdigital tissue of the flippers of seals. Here we present a different type of tag which combines the marking of an animal with the acquisition of a biopsy sample. This combination enables researchers to gain more data within the same handling time, following the three Rs principle (Replacement, Reduction, Refinement) used in animal experiments to optimise the ratio between animal suffering versus knowledge gain. This study describes a first trial within a seal rehabilitation centre, with the aim to assess the application process of this new tag as well as the rate of bleeding and healing following the application. Results show that the new tag type is suitable even in places with unrestricted public access and enables the researcher to gain additional data further supporting ethical justification.
[JMATE 2020;12(1):10-16]

Keywords: phocid seal, flipper tag, biopsy sample.

Introduction

For several decades, seals have been tagged using conventional livestock ear tags (from here on referred to as 'conventional ear tags') during rehabilitation or in the wild for identification and/or research purposes. Currently used techniques have been assessed in the light of animal welfare and practicability (6,8,10,17). The primary reason for marking individuals is to identify and distinguish each animal in the wild or during rehabilitation based on the imprinted number and colour of the tag. The use of conventional ear tags has allowed for an alternative method of marking seals for long-term recognition. This contrasts with other marking techniques like freeze or hot branding, in which there is a greater stress to the animal (19,20). Even though issues concerning resighting rates of conventional ear tags on pinnipeds have been reported, such as readability of imprinted numbers or loss of tags, it still is considered to be a method that can potentially produce valuable data (1,4,17,21,22). When working with live animals, especially when using invasive methods, the main goal should be to reduce the impact of these methods on the study animal to the lowest possible level. This facilitates gaining a maximum of information as described in the three Rs principle with regards to animal experiments (2,16). The use of skin biopsy samples has been continuously increasing over the years, with the aim of collecting information on genetics, infectious diseases, immune status, pollution levels, fatty acids or stable isotopes from these samples (5,7,9,11,12,18). Specifically for cetaceans, several sampling techniques have been proposed and reviewed over the years (13). Whereas for pinnipeds, biopsy samples are usually gathered using a standard biopsy punch, a method that has not changed considerably. To our knowledge, the use of flipper tags in seals for marking in combination with biopsy sampling as a means of reducing handling time has not been described in the scientific literature.

Methods

Here we present a method utilising the "LabTag Boss 3" cattle ear tag (from here on referred to as "biopsy tags") distributed by "GEPE Geimuplast GmbH" which is commonly used in farm animal husbandry. The LabTag Boss 3 combines conventional ear tags (GEPE Q-flex®) with the "Geno Tissue Sampling System" by "Caisly Eartag Limited", which allows taking a biopsy sample while marking an animal (Figures 1a,b).

The biopsy tags are distributed with a clearly legible and durable laser imprint which is thought to increase the chance of resighting in comparison to hot foil stamping which is also used for conventional ear tags and where imprinted numbers have been reported to fade after the years (17).

The aim of this study was to assess the application procedure in light of applicability and veterinary considerations (especially wound healing and rate of bleeding after application) as well as discuss scientific benefits of this tag type for future use in the area of marine mammal research.

Submitted June 5, 2020; Accepted July 28, 2020.
Correspondence: Abbo van Neer
Telephone: +49 511 8568162
Email: abbo.van.neer@tiho-hannover.de

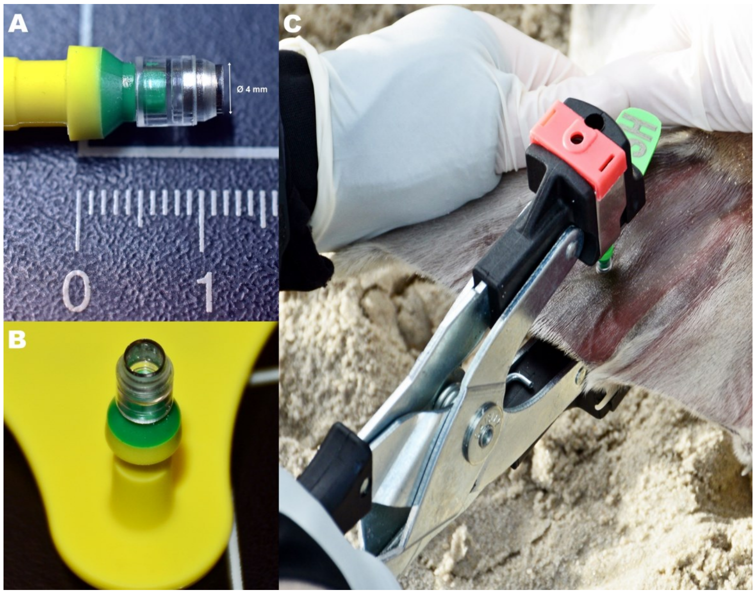

Figure 1: *The LabTag Boss 3. (a) Closeup of the Geno Tissue Sampling System ; (b) Further away view of the sampling system; (c) Demonstrating the application of this tag to the interdigital webbing of a juvenile grey seal.*

The biopsy tags are applied using the standard ear tag applicator recommended by the manufacturer, combined with a commercially available modification kit needed for the Geno Tissue Sampling System. The application procedure follows the standard methods recommended for phocid seals by applying the tag in the interdigital webbing of the hind flippers of the seal (6) (Figure 1c). The conventionally used ear tags such as the Dalton Jumbo Plastic Rototag™ utilise a thorn to penetrate the tissue (17). In contrast, the biopsy tags used in this study are equipped with a round stainless steel blade which cuts through the tissue resulting in a punch biopsy with a diameter of 4 mm (Figures 1a-c). Within the process of applying the biopsy tag, the tissue is cut, the sample transferred into a tissue container and instantaneously fully sealed with the lid which is part of the blade (Figure 1a). This has the advantage that the sample is directly transferred into a sealed container marked with the ID of the applied tag preventing contamination and confusion of samples. Additionally, the blade is safely stored within the container, decreasing the risk of injuries. The tubes are available with different preservative substances depending on the aim of the study (e.g. DNAgard® Tissue for the preservation of genetic samples). By integrating the taking of a biopsy sample within the processes of marking, the adverse impact on the animal is likely to be reduced. For example, by efficiently using the animal handling time to both mark and collect a tissue sample resulting in an increased gain of scientific output. In one process the animal can readily be used for further research such as for genetics or epidemiology data (3). In contrast to older models of conventional ear tags, these biopsy tags are produced out of very durable but yet flexible and smooth polyurethane to prevent abrasion of the surrounding skin. Also, in contrast to the prominent thorn of conventional tags, the counter part of the biopsy tag is surrounded by polyurethane to also prevent abrasion (Figure 2b).

To assess the suitability of the use of biopsy tags in pinnipeds, 20 harbour seals (*Phoca vitulina*) were tagged with the LabTag Boss 3 at arrival at the Sealcentre Pieterburen (the Netherlands) during their routine veterinary admission exam. The focus was to

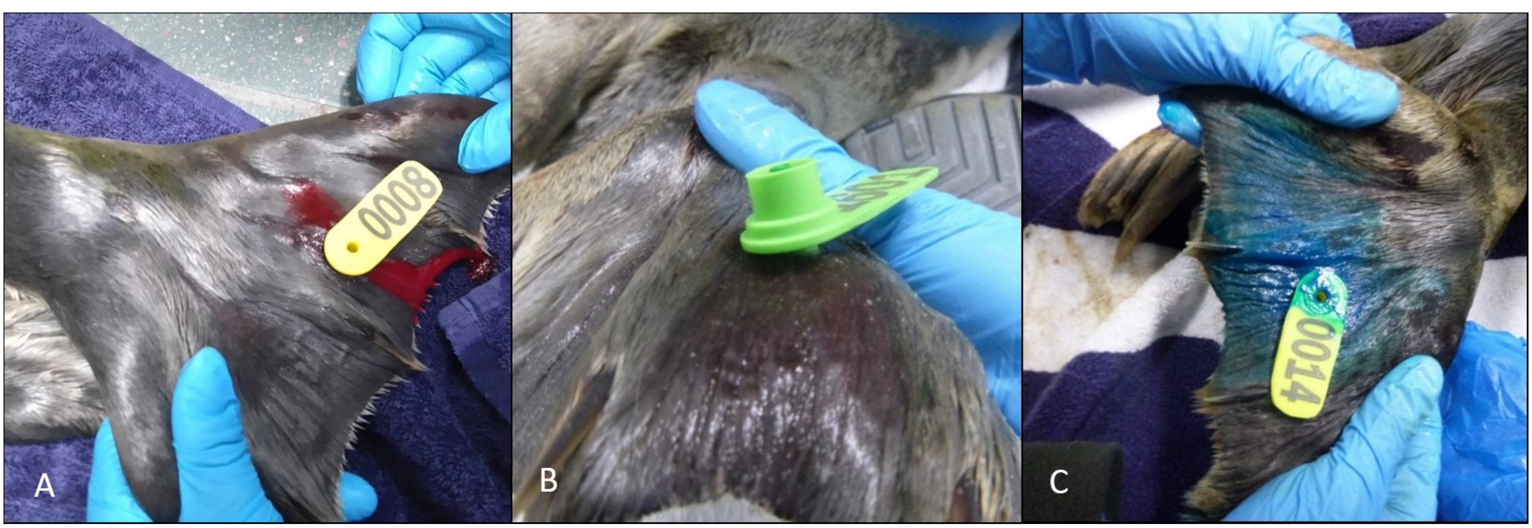

Figure 2: *Photographs after tagging day 0 using 3 different disinfection protocols: (a) Group 1 (G1); (b) Group 2 (G2); (c) Group 3 (G3).*

evaluate the practicability of this tag application, the rate of bleeding as well as the healing process. These are all critical issues, not only for the animal's welfare but also public perceptions. Specifically since these tags are also to be used in areas of unrestricted public access with the potential of raising public concern over the wellbeing of the animals. All tags were placed at the same position in the right hind flipper, in the interdigital tissue between toe 2 and 3 using the recommended "Universal Applicator" produced by "Caisly Eartag Limited". In one animal the tag was placed in the left hind flipper because of small wounds at the right hind flipper. Tags were applied by experienced staff.

In order to evaluate differing disinfection techniques prior to or just after tag application, wound bleeding and healing was assessed by dividing the seals into three groups: (G1, G2, G3) as follows. In group 1 (G1), seals (n=6) were tagged after disinfection of the interdigital web with alcohol 70%[a]. In group 2 (G2) seals (n=7) the interdigital area was disinfected using alcohol 70%[a] combined with the application of a Lotagen® solution[b] using a gauze prior to tagging. In group 3 (G3) seals (n=7) received the tag after disinfection of the area with alcohol 70%[a] and this was followed after tagging by application of CTC spray[c] on the tag wound.

In all three groups, photographs were taken from both sides of the hind flipper before the tag was applied and then again after application. In order to monitor the development of the tag wounds, with focus on wound healing, the presence of blood, formation of granulation tissue and signs of infection were recorded. Photographs were taken regularly up until release of the animal or, in some cases, until the animal died. Assessment of the tag wounds by a veterinarian occurred with every picture taken and any abnormalities (e.g. signs of infection, bleeding) were noted on the animal's individual records. Specifically, photographs were taken 3, 7, 15, 30, and 60 days after tagging in the first group (G1), whereas for the other two groups (G2,G3) these were done 3, 7, 15, and 20 days.

Health data collected during admission of the seals as well as days spend in rehabilitation or cause of death are summarized in the supplementary materials. Body condition scoring is adapted from Pugliares *et al.* (15). For details on the animals used in this study refer to the supplementary materials.

Results

Day 0, tag application on arrival - Bleeding (Figure 2):
G1: Moderate bleeding was observed in five of six seals directly after the application (Figure 2a), which stopped within 2 to 4 minutes. No bleeding was observed in one seal.
G2: Minimal bleeding was observed in two out of seven seals. (Figure 2b) Blood was visible in the wound of two out of the remaining five seals. There was no blood visible in the wound of three seals.
G3: Moderate bleeding was observed in four seals out of seven directly after application. This stopped when the CTC spray was applied. No bleeding was observed in three seals. (Figure 2c).

Post-tagging monitoring: Macroscopically, none of the tag wounds presented abnormalities such as inflammation or grossly visible granulation tissue during the post-tagging monitoring period. Only one seal (from G1) showed bleeding on the 12th day after tagging which stopped after rinsing the wound with cold water. Three seals (one from each group) died during rehabilitation due to lungworm related pneumonia.

During post mortem examination, no gross abnormalities were detected in and around the tag wound. Tissue samples from the tag wounds were collected and kept in 10% formaldehyde solution for histological analyses from one seal each from G2 and G3. The wound tissue from the seal of G2 (died 8 days after tag application) histologically showed a severe, suppurative inflammation at the cutting margin, which extended into the surrounding tissue. This indicated most likely a bacterial infection or other irritating agent complicating the healing process. The sample from the G3 seal (died 1 day after tag application) histologically showed a multifocal, mild to moderate, suppurative dermatitis in superficial and deeper parts of the dermis. In both cases, bacteria were not detected histologically in slides stained with Hematoxylin & Eosin, Gram, Giemsa and Warthin-Starry-silver impregnation. Nevertheless, a secondary bacterial infection cannot be completely excluded, as a cultural microbiological investigation was not performed.

As a comparison, tag wound tissue from three seals (died 1, 3 and 8 days after tag application) with conventional flipper tags was collected for histopathological analysis during necropsy. Also here, the samples had mild to severe, multifocal, suppurative

Footnote: [a]alcohol diluted 70% methylated; Eurovet Animal Health BV, 5531 AE Bladel, the Netherlands. [b]Lotagen® active component: 36% policresulen [metacresolsulphonic acid and formaldehyde condensation product]; Schering Plough Animal Care, 49500 Segré, France. [c]CTC spray active component: chlortetracycline hydrochloride 2,45% (w/w); Eurovet Animal Health BV, 5531 AE Bladel, the Netherlands.

inflammation. No bacteria were noted histologically using the staining as mentioned previously, but again secondary bacterial infection could not be completely excluded. All seals in this study were not healthy when admitted to the centre. Poor body condition and wounds of varying severity were the main findings in animals of up to one month of age (6/6). The animals 4 months or age or older, all showed signs of pneumonia and were mainly in poor body condition (10/14).

Discussion

The formerly used conventional ear tags squeeze a hole into the tissue while the biopsy tag is producing a straight cut. From a medical point of view, a cut with short bleeding is expected to have less impact on the surrounding tissue than squeezing, resulting in a higher potential for quick healing (14). The staff of the Sealcentre has utilized conventional ear tags during routine admission of animals and compared it to the application of the biopsy tags. In their experience, the latter more often lead to bleeding than the conventional tag application. The bleeding varied from none or mild to moderate and stopped after a short period of time. The bleeding could be reduced or even prevented by applying a policresulen solution on the skin before inserting the tag or by spraying the tag wound with CTC spray or other similar compound directly after tag insertion.

The process of taking a biopsy sample while applying the tags proved to be reliable. Specifically, all samples were correctly transferred and sealed in their respective sample containers. Further studies should be conducted in order to evaluate the full scientific potential of the biopsy samples, with regard to ecological as well as veterinary research.

Following the test with seals in human care, using the gained knowledge, a field trial was conducted by marking five juvenile grey seals on the island of Helgoland, Germany (Figure 3). Prior to tag application, the interdigital web was disinfected and a policresulen solution (18%) was applied on and around the intended tagging site. None of the tagged animals showed any bleeding either immediately or 30 - 60 minutes after the application of the tag.

It needs to be stated though, that the rate of bleeding also depends on the exact place of tagging and also on the exact course of the blood vessels. In this study, all tags were applied to roughly the same position,

Figure 3: *Wild juvenile grey seal wearing a custom shaped LabTag Boss 3, 160 days after application of the tag. Picture courtesy of Katrin Wiese.*

but the potentially slightly variable course of blood vessels might have influenced the results.

One seal tagged in the wild with a biopsy tag in February 2018 was admitted to rehabilitation around four and a half months later and no inflammation, abrasion or other signs of wounds were detected in the area during the clinical check-up of the animal. This further indicates the suitability of this tag type for scientific studies.

Conclusion

The presented study indicates that the use of biopsy flipper tags in seals has no obvious adverse medical impact on the animals in comparison to formerly used tag types. Also there is the advantage that the tissue is cut rather than separated by a thorn. If tagging is conducted in areas with unrestricted public access, visible bleeding, which the public commonly associate with pain, could negatively impact onlooker's perceptions of the procedure. This can be reduced by applying products such as Lotagen® or CTC spray.

In this study we have clearly demonstrated the benefits of using a tag with biopsy function over the conventional tag (e.g. reduced handling time and the gained tissue sample), both for use at rehabilitation centres and in field studies.

Acknowledgements

Abbo van Neer and Ana Rubio-Garcia contributed equally to the preparation of this manuscript. All authors wish to thank the staff and volunteers of the Seal Centre and the team from ITAW for their support. Further we would like to thank the staff and volunteers of the municipality of Helgoland as well as the "Verein Jordsand e.V." for aiding us during the fieldwork and gathering re-sightings of the tags.

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Supplemental material:

Group - Case	Age	Weight (kg)	BC	Diagnostic at admission	Reh-BR	Reh-D	Cause of death
1 - 1	>10 days	10.7	3/9	deep skin wound right lower jaw; right upper lip swollen	89		
1 - 2	>10 days	10.3	2/9	small wound LFF; RFF osteomyelitis (surgery); ulcer left eye	157		
1 - 3	1 month	10.7	3/9	Inflammated, wounded lips		8	Parasitic pneumonia and acute fibrino-purulent pleuritis
1 - 4	1 month	10.6	3/9	deep cut on right lower lip, superficial wound around left ear, mandibular symphysis not fused	72		
1 - 5	1 month	11.2	3/9	2 deep wounds on upper left lip, stomatitis and palatal ulcer	71		
1 - 6	1 month	11.9	3/9	swollen toe 1 RHF with small wound, deep wound between toe 1&2 RHF, flippertag placed in the LHF, superficial wounds over whole back, soft swelling under the chin with brown-redish liquid oozing	74		
2 - 1	4 months	14.9	2/9	Pneumonia suspicion		8	Parasitic pneumonia
3 - 1	4 months	13.7	2/9	Pneumonia suspicion	76		
3 - 2	4 months	15.2	2/9	Pneumonia suspicion	93		
3 - 3	4 months	13.6	2/9	Pneumonia suspicion	85		
2 - 2	6 months	15.2	2/9	Pneumonia suspicion	63		
2 - 3	6 months	15.6	3/9	Pneumonia suspicion	49		
3 - 4	6 months	18	3/9	Pneumonia suspicion	41		
2 - 4	6 months	18.3	4/9	Pneumonia suspicion	31		
3 - 5	6 months	20.2	4/9	Pneumonia suspicion	39		
2 - 5	6 months	25.5	5/9	Pneumonia suspicion	35		
3 - 6	6 months	13.4	2/9	Pneumonia suspicion	49		
2 - 6	6 months	19.8	4/9	Pneumonia suspicion	35		
3 - 7	6 months	16.8	3/9	Pneumonia suspicion		1	Parasitic pneumonia
2 - 7	6 months	15.2	2/9	Pneumonia suspicion	48		

Table 1: *Health data of the seals at admission to the centre as well as number of days spent in rehabilitation, before release (Reh-BR or at death (Reh-D) . The cause of death of the three deceased animals was determined macroscopically during dissection; BC= body condition; LFF = left front flipper, RFF = right front flipper, LHF = left hint flipper, RHF = right hind flipper.*

Code	Status
1	emaciated
2	very underconditioned
3	moderately underconditioned
4	slightly underconditioned
5	ideal condition
6	slightly overconditioned
7	moderately overconditioned
8	very overconditioned
9	obese

Table 2: *Body condition estimation scale following the recommendation of Pugliares et al. (15).*