Final report

Implantable identification for cattle - field trials

Project code: V.RDA.0004

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Date published: October 2022

PUBLISHED BY
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NORTH SYDNEY NSW 2059

Meat & Livestock Australia acknowledges the matching funds provided by the Australian Government to support the research and development detailed in this publication.

Abstract

This project investigated the technical feasibility of polymeric Passive Integrated Transponder (PIT) devices in cattle as a potential solution to lifetime traceability in cattle. The work centred around two field trials that were carefully designed by consultation with producers, processors and feedlotters, experts from academia, and technology suppliers.

A 140-day pasture/feedlot trial conducted at an Australian university (Trial 1) and a 90-day commercial feedlot (Trial 2) on a total of 320 *Bos indicus* x *Bos Taurus* and *Bos Taurus* beasts were conducted. Two PIT device sizes (22mm and 32 mm) in bare polymer and a polymer coated with a tissue growth coating were tested, and their performance in relation to PIT device rejection, migration, and infection were compared, as indicators of the devices being retained within the beast.

Both field trials support the efficacy of implantable PIT devices in cattle to improve electronic device retention when applied to the middle-back of ear location. Devices were applied in a similar way to hormone growth promotants (HGPs) and do not require veterinary oversight during application. There is a low risk of device migration away from the implantation site, especially if the implantation site does not have pre-existing ear tags or infection.

Executive summary

Background

The National Livestock Identification System (NLIS) is Australia's system for the identification and traceability of cattle, sheep and goats [1]. In the case of cattle, a key component of that system is that individual beasts are identified by an NLIS-accredited electronic device (usually an ear tag), which is used to record individual animal movements from one property to another. Unfortunately, some producers have reported that these ear tags fall out, and therefore pose a threat to lifetime traceability. The causes of tag loss are varied and include, but are not limited to, being caught on fences, and fixture breakage due to UV degradation of polymers used in the tags. The annual cost to the Australian cattle industry to replace lost tags alone has been estimated at around $10 million per annum [2]. Previous Integrity System Company funded project "Assessing the feasibility of an implantable electronic RFID for cattle" (Project code: V.RDA.0002) highlighted a clear desire amongst some producers for alternative animal electronic identification tag options to address the problem of electronic tag loss in cattle. That report recommended that modern polymeric implantable tags (Passive Integrated Transponders or PIT device) could be field-tested to investigate primarily whether these devices could be adequately retained in the animal at a suitable implantation site without negative consequences on animal health. The PIT device is fitted with a transponder which is able to be read using the same infrastructure as existing NLIS devices.

This project focussed on assessing the feasibility of an implantable PIT tag in cattle in the paddock and in feedlots. Particular interest was to gather data on features of modern polymeric implantable PIT tag options (such as the device size and material alternatives) and compare how these devices performed in terms of being retained in the beast. Learnings about the methods of device implantation, applicator tools, and procedures were also made. This project provided the first practical, evidence-based approach to address whether implantable PIT devices could be a potential alternative option, if accredited for use by NLIS, to external electronic ear devices, for Australian cattle producers in certain circumstances.

Objectives

This project aimed to assess the suitability of a polymeric Passive Integrated Transponder (PIT) in cattle. The trials aimed to validate the remaining assumptions related to technical device challenges and adoption hurdles listed below.

1. Validate the middle back of the ear as a suitable implantation site for a PIT device.
2. Explore and measure the three factors of PIT device retention; drop-out, rejection/infection, and migration from the original implantation site.
3. Determine suitable PIT device design options (22mm vs. 32mm / coated [tissue growth coating on a polymer substrate] vs. uncoated (polymer substrate only]).
4. Capture any potential supply chain issues and animal welfare considerations that require further investigation.
5. Scope recommendations and next steps to advance the assessment of implantable PIT device options across the full supply chain.

This project was successful in achieving these objectives.

Methodology

A comprehensive program of two discrete field trials that leveraged the skills and experience of six delivery partners were conducted.

Trial 1: Pasture / feedlot trial on 120 animals for 140 days.

Trial 2: A commercial feedlot only trial on 200 animals for 90 days.

The in-field retention performance of the various device design options (22mm vs. 32mm length / coated (tissue growth coating on a polymer substrate) vs. uncoated (polymer substrate only)) were assessed at various time points. The key indicators of device retention were rejection, migration, and infection assessed throughout the trials predominantly by qualitative analysis and blood analysis. Postmortem assessment of 50 ears obtained from trial cattle that were implanted with PIT devices were subjected to closer interrogation of retention performance by laboratory histological quantification techniques.

Results/key findings

Trial data confirmed the following:

- The middle-back of the ear is an appropriate site for implantation to minimise food safety risks associated with PIT device migration.
- The 22mm length device was reported as being easier to implant.
- The condition of the ear (i.e. free of pre-existing damage and excessive tags) is important in achieving the desired retention.
- If PIT device migration occurred, the direction of migration was always towards the beast's head. Device migration is a parameter that should continue to be monitored and reported in future, longer-term trials.
- The application of a coating as an experimental coating to promote tissue growth led to superior retention and anti-infection performance than uncoated polymer PIT devices.
- The successful implantation of both 22mm and 32mm PIT devices was achieved. Further advancement in the implantation protocol as well as refinements to both the device design and applicator gun will make the task of implantation easier for producers.
- This trial confirmed that there is not a requirement for qualified vets or highly specialised knowledge to apply the devices.

Benefits to industry

Beyond biosecurity risks (estimated at a $2b annual loss in the event of an FMD or BSE outbreak), it is estimated that lost tags cost the industry approximately $10m in replacement costs annually [2]. The implantable PIT devices assessed through these trials have the potential to provide an alternative identification method for Australian cattle with particular interest coming from Australian cattle producers who currently suffer high tag loss, and/or have a high proportion of breed stock that could benefit from a “tag for life”. The development of any innovative electronic devices that have the potential to address known issues, such as tag loss, within Australia's traceability system by improving the retention and performance of identification technologies is desirable.

Future research and recommendations

Further work is recommended to continue investigating the potential of implantable PIT devices for use in the Australian cattle industry. Specifically, commercial supply chain trials are proposed to acquire longer-term performance data and test the needs of the entire supply chain. The commercial supply chain trials will progress the work detailed in this report.

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Background

The National Livestock Identification System (NLIS) is Australia's system for the identification and traceability of cattle, sheep and goats. NLIS reflects Australia's commitment to biosecurity and food safety and provides a competitive advantage in global markets. All cattle are identified with an NLIS accredited electronic radio frequency identification (RFID) tag that allows all property-to-property movement of beasts to be recorded on a central database [1]. Currently, two types of tags have been approved for use by the NLIS: a single electronic ear tag; and a ruminal bolus and external visual ear tag combination [3]. Option 1 is the most commonly employed solution.

Integrity Systems Company (ISC) has been working to improve tracking and tracing of Australian cattle. Implantable microchips or passive integrated transponders (referred to hereafter as "PIT devices") have been successfully used within the domestic companion animal industry for decades and have been adopted by some farming industries such as fish, pigs, goats, and sheep across the globe for individual animal identification. Such use cases have prompted stakeholders within the Australian red meat industry to question if PIT device technology could be applied to cattle, sheep or goats. Several field studies using PIT devices in cattle have been completed over the last twenty years, primarily using glass-based devices (see Appendix 13). Unfortunately, glass-based PIT devices are prone to breakage and present an unacceptable food safety risk. Although much of the previous research on implantable PIT devices contained scant experimental details and only general analysis, it was clear that readability failure, poor device retention and infection was a key barrier to their adoption.

The Project Coordinators, working with a range of delivery partners, reassessed the feasibility of modern polymeric (hard plastic) PIT devices as alternative devices for livestock identification, exploring the state-of-the-art technologies with the aims of finding a solution that:

- was non-glass to reduce food safety concerns;
- did not present a danger to beast welfare through infections caused by its application;
- attains robust tissue integration in a 'safe site' within the beast to ensure long term device retention and function.

More recent research-based studies confirmed the enhanced robustness of polymeric PIT devices (compared to originally tested glass-based devices) [5,6]. Furthermore, further surface modifications to these devices by applying a tissue growth coating [7,8], has also been explored to test the capacity of this material to enhance tissue adhesion and antibacterial properties of the device [8,9]. Such coated devices have been validated in a small animal model (rats) with good success [10].

Project "Assessing the feasibility of an implantable electronic RFID for cattle" (project code V.RDA.0002)) highlighted a clear market desire for alternative animal electronic identification tag options to address the problem of poor tag retention for some producer segments, and recommended that modern polymeric PIT devices be field-tested to investigate whether they could be adequately retained in the animal without unacceptable negative consequences on animal health.

This project provided first practical evidence within the Australian cattle supply chain about the potential technical feasibility of polymeric PIT devices in cattle. The field trials were carefully designed by consultation with producers, processors and feedlotters, as well as academia, and technology suppliers. A program that consisted of two discrete field trials that leveraged the skills and experience of six delivery partners.

1 Project Objectives

This project focussed on field trialling polymeric PIT devices to:

1. Validate the middle back of the ear as a suitable implantation site;
2. Explore and measure the three factors of PIT tag retention; rejection, migration and infection.
3. Determine essential PIT tag design requirements [22mm vs. 32mm / tissue growth coating on a polymer substrate) vs. uncoated (polymer substrate only)].
4. Capture any potential supply chain issues and beast welfare considerations that require further investigation.
5. Scope recommendations and further steps to advance the assessment of polymeric PIT tag options across the full supply chain.

2 Methodology

2.1 Project phases and general activities

Phase 1 - Project Launch: Jun-Oct 2021

- Identify and contract delivery partners
- Integrity Systems Company (ISC) confirmation of project intent.
- Supply chain stakeholder engagement protocol and project plan for Trial 1 & 2 (Appendix 1)
- Reporting guidelines for delivery partners (Appendix 3)
- 2 x ethics approvals (trials 1 and 2)
- Project initiation and planning
- Meetings with delivery partners (on-site and virtually)
- Device procurement & functionality testing
- Device tissue growth coatings conducted at the University
- PIT tag implantation procedure (Appendix 4) & data recording training protocol (Appendix 5)
- Risk identification and mitigation plan

Phase 2 - Monitor: Feb-July 2022

- Explore and measure the three factors of PIT device retention - rejection, migration and infection as per data collection procedure.
- Commencement of 2 trials

Phase 3 - Deliver: July-Sept 2022

- Final sample and data collection at trial facilities
- Feedback and assessment session with project delivery partners
- Histology assessment - ongoing at the time of this report publication
- Delivery partner data collection and final report synthesis.

Polymeric PIT devices, inclusive of both 22mm and 32mm lengths, were coated with a tissue growth coating using previously described protocols [11]. Representative digital images of uncoated and coated devices can be seen in Appendix 9.1. A total of 180 coated devices were supplied to the Project Coordinators for subsequent use across the cattle trials.

This study was conducted in strict accordance with the guidelines obtained from the respective parties under which the following ethics approvals were issued:

- Authority No.: ARA21-067 (University Animal Ethics Committee);
- CSB RVF21/1989 (NSW Government Department of Primary Industries ACEC); and,
- 2022/AE000324 (University Animal Ethics and Integrity)

2.2 PIT tag implantation procedure overview

The information below is an overview of the method and technique for implantation of all PIT devices (known hereafter as "devices"). All devices in this study were implanted on-site by the device manufacturer. Additional information can be found in Appendix 4.

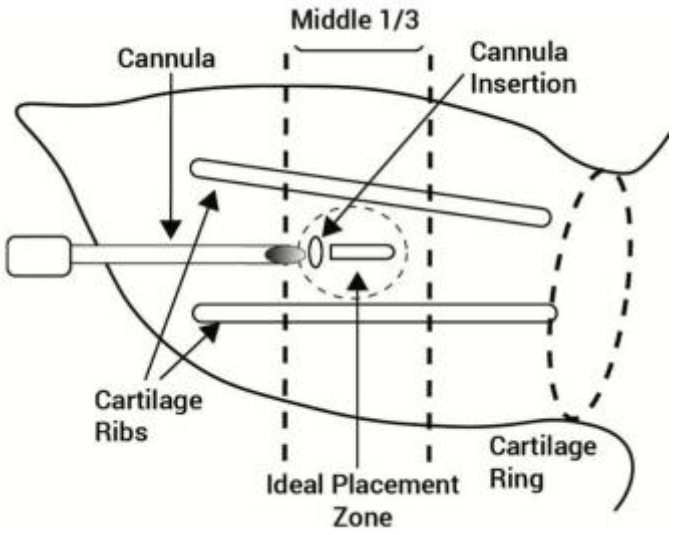
The method for device implantation:

- Based on the physical features of each beast, the project team determined the best ear for the implantation. While the research shows the offside ear as preferable, the ear selection of implantation was dependent on the existence and location of existing ear tag(s) and ear damage.
- Use a single shot injection syringe rather than a gun. The syringe was sterilised following each application.
- Position the device between the middle and upper vein on the mid-back of the beast's ear (see Figure 1).
- Insertion procedure was: inject, plunge fully and then retract. There is a clicking noise once the plunger is fully depressed which indicates the device has left the injection syringe.
- The injection device is very similar to a cannula.

Technique details:

- Ensure the beast's head is properly restrained in the crush.
- Do not penetrate any cartilage.
- While holding the point of the ear, slide the needle under the skin towards the base of the ear, being careful to remain above the cartilage; withdraw the applicator slowly while squeezing the trigger.
- Pinch the injection site closed and feel the implant to make sure of correct placement.
- The implant procedure will be conducted in consultation with the producer.

Figure 1: Device implantation location



3.3 Field Trials

Trial 1: Pasture / feedlot

The trial was led by an Australian University and conducted on their Smart farm and feedlot facility. The project was a 2x2x2 factorial design testing the device length (22mm and 32mm), with and without device coating (coated = tissue growth coating on a polymer substrate vs. uncoated polymer substrate only), and cattle type (Bos taurus and Bos indicus).

The trial commenced in early February 2022, with an initial health check completed 4 days prior (t= -4 days) on all the trial beasts by a qualified vet. A total of 120 beasts (280-350 kg) were successfully implanted with the 22mm and 32mm, coated and uncoated devices as described. Following the implantation of the devices, four equal groups of 30 beasts were formed (22mm coated, 22mm uncoated, 32mm coated, and 32mm uncoated). The trial duration and data collection occurred over a total of 140 days, with 56 days in paddock grazing naturalised pastures and 84 days in the feedlot (standard feedlot ration) to simulate realistic supply chain circumstances.

Across the 140-day trial period, a number of data collection events were scheduled at various time points that included observations of the device implantation procedure, visual inspections of the beast and device, infection monitoring via visual, thermal and blood sampling and biopsy.

Table 1 below details scheduled data collection events and Appendix 2 provides additional details on each activity.

Table 1: Timeline and type of In-field data collection

Day	-4	0	7	14	21	28	56	140

# beasts	**120**	**120**	**96**	**72**	**72**	**72**	**48**	**24**
Procedure	Allocate groups	Implant devices					Enter feedlot	Trial ends
	Vet Inspection							
		Photograph	Photograph	Photograph	Photograph	Photograph	Photograph	Photograph
	Thermal image	Thermal image	Thermal image	Thermal image			Thermal image	Thermal image
			Histology	Histology			Histology	Histology
	Blood sample					Blood sample		
			Inspection	Inspection	Inspection	Inspection	Inspection	Inspection
			PIT readability	PIT readability	PIT readability	PIT readability	PIT readability	PIT readability
	Weighing					Weighing	Weighing	

Data was collected to assess rejection, migration, and infection on day 7, 14, 21, 28, 56, and 140 of the trial period. Rejection was assessed as either 'yes' or 'no,' migration was assessed by measuring the PIT device's distance to the base and tip of the ear over time (comparing migration to previous measurements), and infection was initially assessed with the expertise of the Australian University team through a visual inspection and palpation. On day 7, 14, 56, and 140, biopsies were taken of 24 beasts (6 beasts from each group) to provide quantitative data on any localised inflammatory responses. Beasts subjected to biopsies were subsequently eliminated from the trial, reducing the number of beasts in the trial at these time points. Biopsies were analysed using a 4-tier scoring system, comparing the "treatment" ear (ear with device) to the opposite "control" ear. The 4 tiers were (additional details provided in Appendix 6):

0 - Absence of inflammation
I - Mild inflammation
II - Moderate inflammation
III - Marked inflammation

Inflammatory responses were assessed by a range of features including, but not limited to, the number of inflammatory cells (neutrophils, eosinophils, lymphocytes, plasma cells, macrophages), necrosis, vascular changes, oedema, fibroblasts, and fibrosis. Trial beasts were processed at a processing facility, with 25 ears (24 with devices, 1 control ear with no device) were collected and sent to the University for further analysis described in section 3.4 below.

Trial 2: Commercial Feedlot

A second trial was conducted under feedlot conditions to determine the effect that intensive feedlotting conditions may have on the implanted device retention. It was hypothesised that the close proximity of beasts and feedlot conditions could lead to poorer device retention and increased likelihood of infection. This trial was led by the Veterinary Experts. Trial protocol was modelled from work conducted in Trial 1 and executed via consultation with the feedlot management team and Device Supplier microchips, led on-site in Australia. The required ethics application and approval process was managed by the trial partners were coordinated by the Project Coordinators.

A total of 200 beasts (280-350 kg, Bos indicus x Bos Taurus and Bos Taurus) were successfully implanted with the 22mm and 32mm coated and uncoated devices. Following implantation, the

beasts were divided into four equal groups of 50 (22mm coated, 22mm uncoated, 32mm coated, and 32mm uncoated). Prior to implantation, an initial health check of the beasts was performed by a qualified vet, and the trial duration was 90 days.

A group of 6 from each group (24 in total) were identified for in-field data collection and monitoring on day 2, 7, 14, 28, 56, and 90 (trial completion). On these days, a visual inspection (including photos), palpation, blood work (day 28 only), and a visual record of device location was collected of a representative ear model. All field data was collected by qualified veterinary staff. In this trial, all beasts were destined to meet commercial customer requirements and therefore a modified, less invasive, trial protocol was devised to meet these needs. Further, all implanted devices in this trial were not electrically active, as requested by the commercial feedlot, to prevent potential interference with existing NLIS devices in the beast.

All beasts were sent to the processing facility in at the completion of the 90-day trial. A total of 24 ears from trial cattle were collected and sent to an Australian University for further analysis, described in section 3.4 below.

3.4 Device recovery and post mortem assessment

At the conclusion of each of the trials, cattle ears implanted with either uncoated or coated devices (of 22mm and 32mm length) were retrieved at the processing facility and sent to an Australian University for detailed laboratory analysis. Ears were stored in 10% neutral buffered formalin (NBF) and kept on ice during transit to the University. Images related to specimen storage can be seen in Appendix 9.2. The number of cattle samples provided to the University from the trials is detailed in Table 2 below.

Table 2: Samples send for post mortem assessment

Details	Plain ear	Uncoated device		Coated device		Total
		22mm	32mm	22mm	32mm	
Trial 1: Pasture/feedlot	1	6	6	6	6	25
Trial 2: Commercial feedlot	0	6	6	6	6 *(5 + 1 ear with "lost device")*	24
Total	1	12	12	12	12	49

Upon receipt of samples by the University, all tissue specimens were re-immersed in fresh 10% NBF. Gross imaging and observation of the tissue specimens was completed before the skin was shaved to remove hair around the implant region. The specimen was then trimmed to size using a scalpel blade, imaged and re-stored in 10% NBF for a further 12 hours. Following complete fixation, the samples were rinsed in running water before being sent for histological processing. Post-clinical quantifications, including the extent of device retention, evidence of infection and other observations, were measured as per the metrics outlined in Appendix 9.3.

The tissue specimens, including the plain ears and ears containing devices, underwent routine histological processing. Transverse cuts (6µm thickness) were collected at the 'base' end of the device

and were fixed to microscope slides for use in downstream staining analyses.

Hematoxylin and eosin (H&E) staining was used to provide a gross overview of the tissue, highlighting nuclei and parts of the cytoplasm that contain ribonucleic acid (RNA) in purple and the remainder of the cytoplasm and extracellular matrix (ECM) in pink.

Masson's Trichrome (MT) staining was used to visualise Type I collagen/fibres throughout the tissues, illustrating muscle regions, cytoplasm, and keratin in red, with collagen fibres staining blue and cell nuclei as black. This was coupled with Picrosirius Red (PSR) staining to selectively highlight the organisation of collagen networks throughout the tissue in red, and cell nuclei as black. Histological quantifications, including the degree of implant vascularisation, cell infiltration, collagen fibre alignment, capsule thickness and inflammatory cell (leukocyte) characterisation were completed as per the protocols outlined in Appendix 9.4.

3 Results

3.1 Trial 1: Pasture / feedlot

Device implantation and in-field data analysis

A total of 110 of the 120 beasts were implanted with devices according to the experimental procedure, hassle-free, as shown in Figure 2. On 8 occasions, with the 32mm coated devices only, the applicator gun jammed. This was due to the increased diameter due to the coating not being able to be accommodated by the applicator gun. This issue would not present a problem in a normal commercial setting as applicator guns for this sized device could be easily modified for the task. Generally, the 32mm devices were slightly more challenging to implant than the 22mm devices due to the larger physical size. Another factor of note was the presence of pre-existing ear damage in some beasts due to past notches, and various forms of tags, which in extreme cases, forced the location of the implantation of the device away from the ideal position in the mid-back of ear. Due to pre-existing ear damage as described, a total of 17 devices were implanted in the top of the ears. Any physical differences between the ears of Bos Indicus and Bos Taurus did not have an effect on the ease of device implantation.

Figure 2. Device implanted in the ear

A total of 19 beasts had a pre-existing infection and/or some type of pre-existing ear damage, with 7 being so severe that they were excluded from further trial participation. Five of the remaining 12 beasts with pre-existing conditions, recorded negative results at some stage in relation to measures of infection, retention and/or device migration as a direct result of this pre-existing condition. All 5

devices that recorded an issue relating to one of our retention measures were of the uncoated type. Table 3 shows a list of the 12 beast data sets subjected to further interrogation.

Table 3: Beast numbers that demonstrated signs of migration, infection, or poor retention according to in-field data collection.

Cattle ID #	Size	Coating	Decision & Rationale
4	22mm	uncoated	Included
13	22mm	uncoated	Included
27	22mm	uncoated	Included
85	32mm	uncoated	Included
76	32mm	uncoated	Included* - Subsequent migration measurements after Day 7 suggest measurement error.
25	22mm	uncoated	Excluded - Residual infection in the ear at application
17	22mm	uncoated	Excluded - Implantation failure after 2 attempts due to existing damage, resulting in further ear damage.
10	22mm	uncoated	Excluded - significant existing ear damage, application location unacceptable at the top of the ear
28	22mm	uncoated	Excluded - significant existing ear damage and poor ear quality. Application location unacceptable at the base of the ear. Migration measurement error
65	32mm	uncoated	Excluded -Implantation failure after 3 attempts due to existing damage, resulting in further ear damage.
53	32mm	coated	Excluded - significant existing ear damage and residual infection. Application location unacceptable at the bottom of the ear.
60	32mm	coated	Excluded - significant existing ear damage, application location unacceptable at the top of the ear.

Performance Criteria – 3 factors of device retention

Table 4 below is a summary of the data collected at each period through to day 140 for all beasts included for the trial duration. As per the trial objectives, the preliminary assessment of retention performance was as follows:

- Rejection - the percentage of devices NOT rejected from the ears at the time of assessment
- Migration - the percentage of devices that did NOT migrate (<20mm from implantation site)
- Infection - the percentage of ears NOT infected as judged visually

For any of the beasts that exhibited migration, a lack of device retention, or infection, the beast's trial number along with the corresponding 'issue' is aligned to the period and listed below the overall rates of retention, migration, and infection.

Table 4: Performance of trial beasts as assessed by in-field data collection for retention, migration, and infection over 140 days

Results (controlled)	**Day 7**	**Day 14**	**Day 21**	**Day 28**	**Day 56**	**Day 140**
Rejection - % **not** rejected	100.00%	100.00%	100.00%	100.00%	100.00%	97.83%
Migration - % did **not** migrate	99.12%	100.00%	100.00%	100.00%	100.00%	95.65%
Infection - % were **not** infected	100.00%	100.00%	100.00%	100.00%	98.51%	100.00%
Issue Devices	**Cattle ID Number**					
Rejection						27
Migration	76					4,13
Infection					85	

Only 5 beasts of 113 that were implanted with devices for the 140-day trial duration recorded notable negative data on the performance measures related to retention, migration, and infection.

- **Rejection:** 99.1% of all devices were retained, with one rejected on Day 140.
- **Migration:** 97.2% of all devices exhibited no signs of migration. Three devices migrated, 1 device on Day 7, and 2 devices on Day 140. Of all devices that migrated, all moved towards the beasts head but remained within the ear implantation zone, migrating no more than 30 mm from the original implantation site.
- **Infection:** 99.1% of all devices exhibited no visual signs of infection, with 1 device demonstrating visual signs of infection on Day 56, which subsequently cleared up and was no longer seen at day 140.

This information is summarised in the Table 5 below:

Table 5: Comparison of in-field data on retention, migration, or infection by device features.

Cattle ID Number	**Size**	**Coating**	**Rejection**	**Migration**	**Infection**
76	32mm	uncoated	N	Y	N
85	32mm	uncoated	N	N	Y
4	22mm	uncoated	N	Y	N
13	22mm	uncoated	N	Y	N
27	22mm	uncoated	Y	N	N

Device comparison

- For the 5 beasts with adverse performance data on key indicators, 3 were implanted with 22mm devices and 2 were implanted with 32mm devices.
- For the 5 beasts with adverse performance data on key indicators, all were of the uncoated (polymer substrate only).
- Specifically, regarding the performance data on key indicators;
    - **Rejection:** The only device rejected was a 22mm implant.
    - **Migration:** 2 out of 3 devices that migrated were 22mm implants, and 1 was a 32mm implant.
    - **Infection:** The only device demonstrating visual signs of persistent infection was

associated with a 32mm implant.

Beast health observations

- Pre-implantation bloods showed average values of neutrophils are on the border of normal range. A high neutrophil value can indicate the body has an infection. As these beasts were purchased through saleyards and were trucked to the trial site, it is usual that many beasts may have had a pre-existing infection, or may have picked up an infection during transport and changing environments, despite being acclimatised on the trial site for a week prior to trial commencement.
- Biopsy data at days 7, 14, 56, 140 revealed that the majority of beasts were classed as Tier II [Moderate Inflammation - refer to the University biopsy scoring system (Appendix 6)].
- Blood parameters by day 28 showed all blood parameters fell within a healthy range, indicating any infections were sufficiently resolved. Refer to Appendix 7 for further information.
- The average weight of beasts across all groups was 398 kg at day 110. There was no significant difference ($p>0.05$) in weight between any of the 4 groups.

Device readability

Readability was not a key focus of this trial, especially given that the 32mm device was experimental (not ICAR accredited) and the electronic e-rod transponder component of any device can be improved and tuned separately with a view to satisfying read range requirements set by regulatory authorities. However, a high-level assessment of readability was conducted during inspections on days 56 and 140. All active devices read at a 100% level on day 56 and 93% on day 140. The readability of the 32mm length implantable devices were less reliable with 20 of 23 devices recording a read on day 140 compared to a total of 23 of 23 of the 22mm length devices reading on day 140, as measured by the handheld wand reader. Device readability appeared unaffected by the presence or absence of the tissue growth coating.

Learnings for trial 2 & revised application protocol

Several learnings were made from the pasture/feedlot trial after completing device implantations, which were subsequently applied for use in the feedlot trial:

1. Quality, undamaged ears are essential to facilitate easy implantation of implantable devices, and are also critical to achieve the desired performance measures (high levels of retention, and low levels of infection and migration). These conditions facilitate the implantable device to be “locked” into the inserted position.
2. The small number of devices that have migrated or have not been retained, have done so due to trauma at the incision wound site during application. Further advancement in application hardware and consumables is required and ongoing, and it is anticipated that the resulting improvements will be available for future trials.
3. This is a world-first trial of polymeric implantable devices in cattle and has been an important step in fine-tuning a system that improves the ease of application for unskilled operators.

The learnings gained from the Australian University trial were incorporated into the commercial feedlot trial application protocol, as detailed below.

1. Each tag supplied is packaged in a sterile disposable needle kit.
2. The needle kit has its needle guide set with a 1.2mm gap between the underside of the guide

and the top surface of the needle; this holds the needle at the correct depth during insertion.

3. The thickness of the needle guide has been increased to 2mm to minimise flex during the needle's travel under the beast's skin layer, giving total control of the needle depth during the process.
4. The needle guides' upturn angle at the front end has been increased to set and control the initial insertion depth.
5. The distance of the needle protrusion past the upturned end of the needle guide was reduced to bring the guide into contact with the skin the moment after the needle tip punctured the skin, giving total depth control.
6. The middle-back of the ear is confirmed as the ideal location for the application. As a result of existing damage to the middle-back of the ear in some cattle, the devices were applied to the top, bottom, or base of the ear. While initially viewed as a positive opportunity given the ease of application, the final results of this trial have shown that an implantable device in the areas outside of the middle-back of the ear results in less retention and more migration and infection.

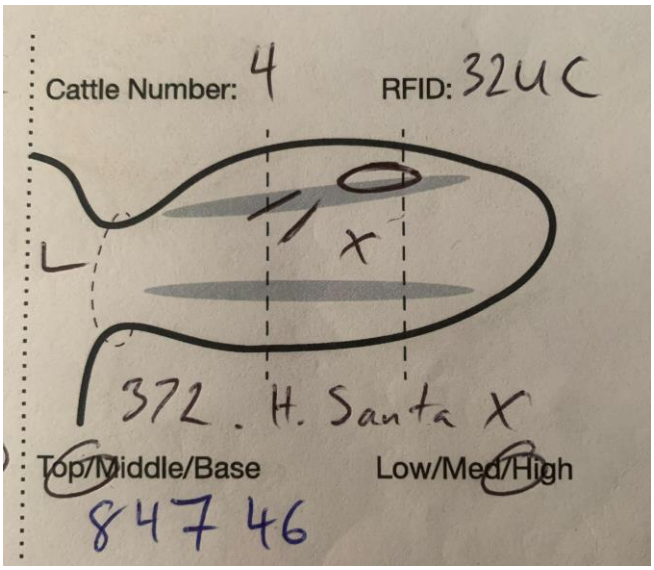
3.2 Trial 2: Commercial feedlot

Device implantation and in-field data analysis

The commercial feedlot trial commenced in late April, 2022, with 200, 90-day grain fed beasts at a commercial feedlot being implanted. This trial was not able to include an additional, smaller cohort of 140-150 day cattle due to start time delays and commercial requirements of the feedlot. Furthermore, there were processor concerns about the potential downstream impacts of using multiple RFIDs on the beast during transportation and processing, and as such, feedlot management opted to use "dumb" (inactive RFID) devices for the trial. The specific concern with potentially having two active RFIDs on a beast is the RFID-enabled auto draft systems at the processors could result in non or inaccurate scanning.

A standardised proforma to facilitate the assessment of ear quality at application and device migration over the trial was designed as shown in Figure 3. For each beast, the Cattle Number in the trial (top left), RFID number (top right), and visual identification number (bottom left) were collected in addition to weight and breed (middle left and right, respectively). The key difference between this information and the Australian University trial was the recording of ear quality, Low / Med / High (bottom right), and the markings on the ear. The ear was divided into three parts (base, middle, and tip), with the ear cartilage running through the centre. During application, the location of the HGPs and the existing ear tag were also captured (the two lines and the x, respectively). After device implantation, an oval was drawn on the ear to provide a reference point for the device position at each assessment point.

Figure 3: Commercial Feedlot Data Collection



Performance Criteria – 3 factors of device retention

Table 6 below is a summary of the data collected at each period through to Day 90 for the sample group and final results of all 200 beasts at the conclusion of the trial. As per the trial objectives, the preliminary assessment of performance is as follows:

- Rejection - the percentage of devices retained in the ears at the time of assessment.
- Migration - the percentage of devices that did NOT migrate (<20mm from implantation site)
- Infection - the percentage of ears NOT infected

Table 6: Performance of trial beasts as assessed for rejection, migration, and infection over 90 days

Results (controlled)	**Day 14** (24 cattle sample data)	**Day 28** (24 cattle sample data)	**Day 56** (24 cattle sample data)	**Day 90 (complete 200 cattle data)**
Rejection - % **not** rejected	100.00%	100.00%	96.00%	99.00%
Migration - % did **not** migrate	100.00%	100.00%	100.00%	100.00%
Infection - % were **not** infected	96.00%	100.00%	100.00%	95.50%
Issue Devices	**Cattle ID Number**			
Rejection			114	55
Migration				
Infection	7			109, 1, 137, 73, 31, 12, 93, 117, 24

A total of 2 beasts of 200 implanted with a range of devices monitored and measured over the first 56 days demonstrated some notable data on the performance measures related to rejection, migration, and infection.

- **Rejection:** 99% (198 of 200) of all devices were retained, with one rejected at day 56 and another at day 90.
- **Migration:** 100% of devices exhibited no signs of migration.
- **Infection:** 94.5% (191 of 200) of all devices exhibited no visual infection, with 1 device demonstrating infection on Day 14, which was resolved by Day 28, and 9 showing minor signs of thickening by day 90 (see Figure 8).

For the 11 beasts with adverse performance data on key indicators (i.e. rejection, migration, or infection), a summary of results is presented in Table 6 below. Notable mentions for cattle number 114, 117, and 55 are presented in detail. Note that cattle number 117 (Figure 9) is representative of the condition seen in all beasts listed recording an infection.

Table 7: Comparison of in-field data on retention, migration, or infection by device features.

Cattle Number	Size	Coating	Rejection	Migration	Infection
114	32mm	coated	Y	N	N
109	32mm	coated	N	N	Y
137	32mm	coated	N	N	Y
117 - Figure 7	32mm	coated	N	N	Y
1	32mm	uncoated	N	N	Y
31	32mm	uncoated	N	N	Y
12	32mm	uncoated	N	N	Y
24	32mm	uncoated	N	N	Y
73	22mm	uncoated	N	N	Y
93	22mm	uncoated	N	N	Y
55 - Figure 6	22mm	uncoated	Y	N	N

Device Comparison

A total of 64% of beasts with adverse performance data on key indicators were implanted with uncoated devices. None of the coated 22mm devices recorded adverse performance data.

It is likely that the device in cattle number 114 was not retained at Day 56 due to the proximity to the management tag, which had become infected at that site. In Figures 4-7, the recorded location of the device on Day 0 (the black circle is an existing hole in the ear), the ear condition, the existing tag infection on Day 28, and finally, the ear at Day 56 is shown. The findings and images on Day 56 for this beast show the absence of the device. If infection caused by the visual tag resulted in rubbing of the application site by the beast, it is possible that the device could have been rejected either through the hole created by the management tag or resulted in the device hole reopening.

Figure 4: Day 0

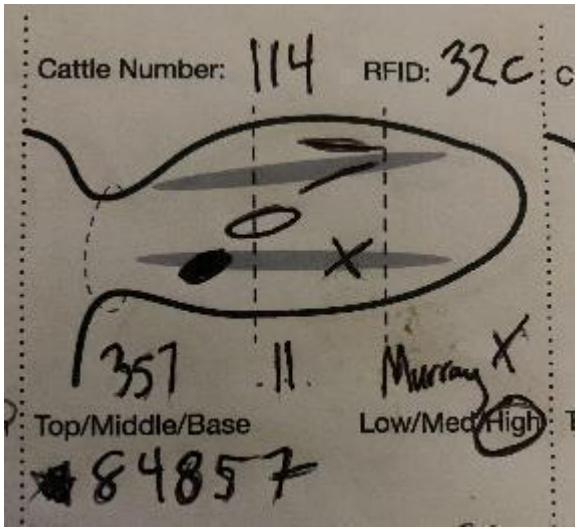


Figure 5: General Ear condition

Figure 6: Tag infection

Figure 7: Day 56

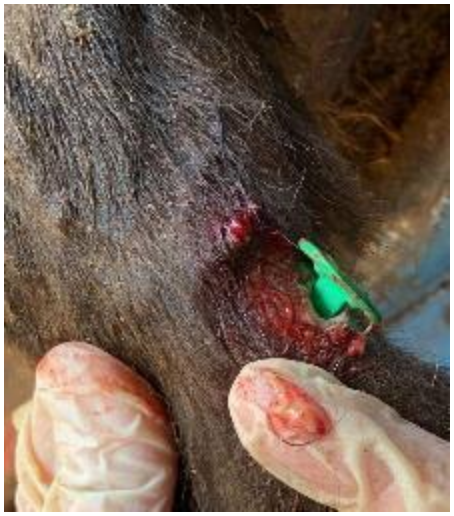
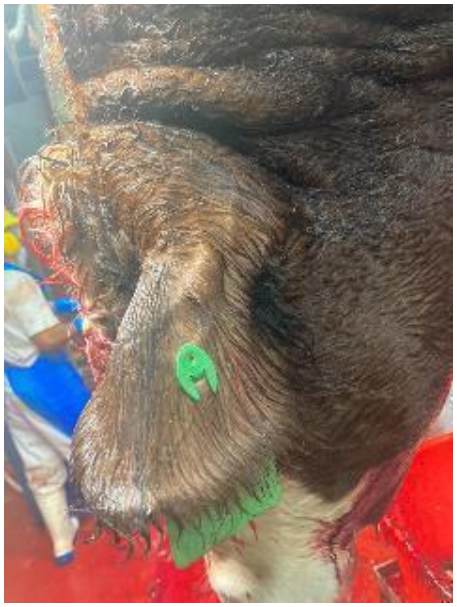


Figure 8 shows an open implantation wound for cattle number 55 that led to the device not being retained at Day 90. A possible reason for this is the proximity to the external tag potentially interfering with the beasts natural healing process.

Figure 8: Cattle no. 55 rejection

A total of 9 beasts recorded signs of a thickened/ abscessed implant at Day 90. A typical example of this is shown in Figure 9.

Figure 9: Tag infection Day 90



Beast Health

From visual inspections conducted by qualified veterinary professionals, none of the total 200 beasts demonstrated adverse responses as a result of the device implantation. The 24 sample group beasts on Day 28 received a full blood analysis (Appendix 8) performed by the NSW Department of Primary Industries, which also confirmed healthy beasts with no adverse, or systemic response noted. No additional blood work was taken on day 90 as the 24 cattle from the sample group showed no significant signs of infection.

Readability

Readability was not in scope for this trial. None of the devices were electrically active, as requested by the commercial feedlot to prevent potential interference with existing NLIS devices in the beast and cause potential compliance issues.

Learnings for future work & revised application protocol

The learnings and revised application protocol developed from the Australian University trial and applied to the commercial feedlot trial were successful in improving the application process. The same application protocol will be carried out with future trials and continuously be improved.

4.3 Post mortem assessments for Trials 1 and 2

Post mortem assessment of 50 ears implanted with devices from both trials were subjected to closer interrogation of retention performance by observation and laboratory histological quantification techniques at the University.

Observations from the received cattle ear specimens are detailed in Appendix 12.1 and 12.2, respectively. Digital images of the ear specimens and the extracted tissues can be seen in Appendix 10. This information supports in-field observations of minor cases of inflammation and infection related to the device implantation procedure. There may be some benefit of the tissue growth coating to reduce device movement, however, this was not statistically validated.

Histological assessment reports can be found in the following appendices;

Appendix 9.4.1: Vascularisation - vascular networks surrounding the devices.
Appendix 9.4.2: Cell infiltration/adhesion at the device interface.
Appendix 9.4.3: Capsule thickness of tissue surrounding the device.
Appendix 9.4.4: Collagen fibre alignment.
Appendix 9.4.5 : Inflammatory cells.

The information presented in the appendices above are discussed below.

Retention

Across both trials' specimens, when implanted in the middle ⅓ of the ear (or largely within this region), device retention was drastically increased. If devices were implanted in, or migrated towards the inner ⅓ of the ear, and in some cases, the cartilage ring, device migration could be possible due to the skin / cartilage / cervical auricular muscle environment, and the increased frequency of tissue movement in this region (i.e. the point where the ear meets the head).

During device recovery, it was noticed for both trials' specimens, but especially for those received from Trial 2, that the coated devices (both 22mm and 32mm lengths) were much more better adhered to the tissue, with a considerably tighter capsule being formed around the device. For the uncoated devices, although well retained, the surrounding capsule did not have a tight grip on the device. For those implanted in the middle ⅓ of the ear, where minimal movement is experienced, this is likely the result of the initial pocket created when the device was inserted, which has never healed completely. Implant locations towards the inner ⅓ of the ear, showed such pockets were slightly more obvious for the uncoated devices, due to the greater level of device movement experienced at the cartilage ring/muscle interface. The device in trial 1, beast 4 (recorded as migrated during the trial period) shifted proximally into the cartilage ring/muscle region of the ear and rotated 90°. During recovery, the device was moving freely within the tissue, showing no signs of adhesion whatsoever.

The benefits of the coating were notable when it came to the specimens from Trial 2. One third of the uncoated 32mm devices were classified under 'poor' retention, with the remaining falling into the 'good' category. By decreasing the length to 22mm, the uncoated devices were able to slightly improve their observational category, having one device in both the 'fair' and 'excellent' categories, with the majority (66%) meeting a 'good' standard of retention. The coated 22mm devices were unmatched on performance, with 100% of the devices meeting an 'excellent' level of retention, with the 32mm variant falling not far behind, with one-third of devices shifting down to a 'good' retention measure.

Based on these observations, it could be possible to link these variations to the conditions of the respective trial locations, however, this cannot be definitely linked due to the discrepancy in sample age (140 days exposure (Trial 1) vs. 90 days (Trial 2)). However, a clear and consistent indicator of achieving adequate device retention is the device must be implanted in the middle ⅓ of the ear. Furthermore, the retention of an uncoated device is improved by zero/small surrounding pocket size created during implantation, with larger pockets demonstrating not to heal as effectively as that seen for the coated devices. In perfect cases where the middle ⅓ of the ear is available as an implant site, and device implantation best practice is followed, the requirement for a coated device may not be as critical. However, for beasts that have a significant number of pre-existing ear tags, and thus limited implant sites available, the coating is desirable for increasing retention capacity following insertion within the inner ⅓ of the ear and beyond.

Inflammation and infection

Two-thirds of the Trial 1 coated devices were shown to have no signs of inflammation, with the remaining third only exhibiting minor levels at best. These results were matched for both the 22mm and 32mm lengths. The uncoated 32mm devices saw a 50:50 split between minor and zero levels of inflammation, with the uncoated 22mm devices showing a greater favour to the 'minor' inflammation category (66%).

For Trial 2 specimens, the coated 22mm devices performed the best, showing no signs of inflammation for all except one device. The 32mm equivalent did not perform quite as well, with 50% of devices showing minor signs of inflammation. For the uncoated devices, the 22mm length had two-thirds of devices showing zero inflammation, and the remaining third demonstrating only 'minor' signs. For the uncoated 32mm devices, 50% of devices demonstrated 'minor' inflammation and another third exhibiting 'mild' symptoms, while only one device showed no signs of inflammation. Across both trials', an increase in inflammation was observed for devices that were situated primarily within the inner ⅓ of the ear (where greater tissue movement is experienced).

When observing for infection, all devices from Trial 1 passed the test, with no symptoms present across all of the experimental groups. In Trial 2 specimens, two out of six 32mm uncoated devices exhibited mild infections.

Vascularisation of implant

Based on the counting of vascular systems surrounding the devices, it can be said that the quality of cell infiltration and subsequent tissue adhesion at the interface is slightly improved as the number of vascular networks increases. Blood vessels play an important role in tissue growth, with endothelial cells allowing the formation of new capillaries, ultimately increasing the vascular network within a tissue and thus the opportunities for regulation and signalling between the bloodstream and surrounding tissues to advance vascularisation and subsequent tissue growth and organisation. Analysis of H&E stained specimens showed a higher degree of tissue alignment surrounding the devices, across both the uncoated and coated groups, when implanted near pre-existing auricular arteries (see Appendix 11).

Use of the coating, in combination with native vascular structures, may be expected to increase the level of tissue growth and subsequent adhesion following implantation, however, results were variable. This improvement however was observed in specimens from Trial 2 in comparison to Trial 1, with some of the coated 22mm devices showing at least a two-fold increase in the number of vascular structures surrounding the implant. However, the overall increase in vascularisation of specimens received from Trial 2 could be due to more-informed implantation procedures, when compared to Trial 1.

Overall, in addition to implanting devices preferentially within the middle ⅓ of the ear, it also bodes well to further optimise device localisation to be near existing primary vascular structures to promote angiogenesis. In cases, such as for older beasts, where the middle of the ear may no longer be a viable implant site, the coating may help to minimise the possibility for migration of devices injected into these regions.

Cell infiltration/adhesion

Coupled with vascularisation, H&E staining revealed that the degree of cell infiltration and tissue adhesion at the device interface was greater for the coated devices than for the uncoated equivalents. From Trial 1, uncoated 22mm devices showing 'fair' (67%) to 'good' (17%) levels of cell infiltration, with one device showing rating 'poor'. This result was slightly improved with the 32mm variant, featuring two out of the six devices in the 'good' category. The 22mm coated devices were fairly consistent in their outputs, with 5 out of the 6 devices meeting 'good' levels of tissue adhesion, with the remaining device falling into the 'excellent' tier. The 32mm coated variants all achieved 'fair' to 'excellent' levels of cellular attraction, especially for those devices located within the inner ⅓ of the ear.

Trial 2 results were slightly more consistent within each of the experimental groups. Positively, there were no cases of 'poor' cell infiltration, with the worst performing of the bunch being the uncoated 32mm devices, all of which only met a 'fair' degree of tissue adhesion.

Capsule thickness

MT staining proved to be a useful tool for quantifying the thickness of the immediate capsule surrounding the devices.

For Trial 1 specimens, similar variations in capsule thickness were seen for the uncoated vs. coated devices. On average, the 22mm uncoated devices averaged 141µm of surrounding capsule, with the 32mm equivalent measuring 103µm mean thickness. The coated devices averaged approximately 267µm and 262µm for the 22mm and 32mm lengths respectively. Trial 2 results showed a similar trend in the capsule thicknesses between uncoated and coated devices, with a high degree of variability.

The variability in the overall thickness of the encapsulation tissue can be attributed to the niche environmental conditions for each device (i.e. middle ⅓ of ear vs. cartilage/muscle ring region), as well as histological processing; in some cases, the device may not adhere to the glass slide, and subsequently remove neighbouring tissue (i.e., some of the capsule) during washing stages. Although not an uncommon technical issue when working with implants, such impacts could possibly be offset with a larger sample pool, in future.

It is notable that the additional 50 days of implantation time offered to the Trial 1 beasts has shown no advantage to the uncoated devices in terms of capsule thickness. However, the additional 50 days for the 22mm coated group in Trial 1, however, was able to increase the capsule thickness by ~80µm.

Collagen fibre alignment

Interrogation of the PSR staining results allowed the degree of wound healing and subsequent encapsulation to be assessed. The extent of collagen fibre alignment surrounding a device is a key indicator as to the overall progression of tissue integration with the device. The majority of uncoated 22mm devices achieved 'good' fibre alignment at best (66%), with one of the devices only meeting 'fair' standards. The uncoated 32mm devices from Trial 1 revealed a majority (50%) of its set to have only a 'fair' degree of fibre alignment, however still achieving one third in the 'good' category and one device even meeting 'excellent' standard. The coated devices demonstrated 50% of devices could meet a 'good' level of collagen alignment surrounding the device, with one and two devices meeting 'excellent' standard for the 32mm and 22mm variants respectively.

Trial 2 saw similar results**,** with no devices rating 'poor' fibre alignment across any of the experimental groups. To see a greater number of devices overall from Trial 2 fall into the upper two echelons of the scale could once again be linked to more informed, quality implantation protocols for the devices

retrieved from this group.

Inflammatory cells

H&E stained sections were used to identify inflammatory cells/leukocytes (based on cell morphology) for granulocytes (neutrophils, eosinophils and basophils), monocytes, and lymphocytes. Although possible to identify with H&E, it is recommended that additional staining for inflammatory cell markers be completed, as well, to guarantee correct identification of the cell types. Furthermore, as some of the cell interface has been washed away as a result of histological processing, these results are not to be taken as fully representative of the specimen condition. For Trial 1 specimens, the uncoated 22mm devices showed the highest rates of granulocytes and monocytes present near the cell/device interface out of all the experimental groups, with 33% of devices affected. One device within this group also featured some lymphocytes, the same of which can be said for the uncoated and coated 32mm devices. One each of the 22mm and 32mm coated devices also showed very minor levels of monocytes near the implant. For Trial 2 specimens, one of the uncoated 22mm specimens showed signs of granulocyte infiltration, as well as 2 further cases of monocytes near the device interface. 50% of the uncoated 32mm devices were positive for both granulocytes and monocytes, with a third also exhibiting lymphocytes in the surrounding tissue. This was to be expected, as two of the specimens from this experimental group demonstrated mild signs of infection upon visual inspection.

Compared to the control tissues, the uncoated devices showed moderate levels of leukocytes within the surrounding tissues, though none of these cases would be considered as evidence of severe or chronic inflammation.

4 Conclusions

This report details the findings of two separate trials that explored the retention performance of implantable devices in Bos indicus x Bos Taurus and Bos Taurus beasts in the middle-back of ear location.

Trial data confirmed the following:

- The condition of the ear is important in achieving the desired result, and will strongly influence the fate of the injectable in terms of infection and tissue integration thereafter. The middle-back of the ear, if no other devices/infections are pre-existing, is a suitable location for an injectable device – uncoated or coated, 22mm or 32mm length. Ears that were damaged, infected or had a high number of other existing tags made it difficult to find an appropriate implantation target site and were more likely to have poor retention performance. This information is important to identify and educate particular producers who may find particular value in this type of tagging alternative if approved. Implanting devices into young beasts at weaning and marking or identifying beasts that have ears unaffected by other devices are ideal use cases.

- Devices implanted outside the designated middle-back ear location would require a coated device or similar to reduce the likelihood of migration from the implant site. Coated devices showed superior tissue adhesion for both cartilage/skin interfaces, as well as those dense with muscle. In such instances, a 32mm device may be favoured to better handle increased movements in this region.

- In-field data collection indicated that the 22mm length device provided slightly superior performance in terms of retention measures and was reported that this smaller device size was easier to implant.

- All device options (22mm, 32mm, coated and uncoated) remain a technically feasible solution as an implantable device alternative to external ear tags. The coated devices performed marginally better in terms of tissue integration and infection prevention, capsule formation and demonstrating more advanced wound healing with respect to capsule fibre alignment. This was supported by the lack of considerable inflammatory cells at the device interface and within the surrounding tissue.

- The results from this study included 13% of available specimens as part of a detailed analysis. The extracted data cannot be considered as definitive nor representative of the performance for each of the device groups. Despite this, however, the results in this study post-mortem, appear to support the in-field data collection and observations that occurred during both trials.

- Device migration was observed only in the Pasture/feedlot trial only, and in 3 beasts (2x 22mm, 1x 32mm, all uncoated), which did not exceed 30mm. The direction of migration was always towards the beast's head. Device migration is a parameter that should continue to be monitored and reported in future longer term trials.

- Device rejection occurred in only 3 beasts (2x 22mm uncoated, 1x 32mm coated) and was attributed to contributions from infection or poor wound healing/no wound closure. These circumstances may have been caused by suboptimal application of the device due to operator error or a challenging beast.

- Implantation of devices did cause infection in a small number of beasts (10/320). One of these infections were resolved during the course of the trial naturally by the beast, and nine showed visual signs of minor infection at the trial completion. None of these were deemed to require veterinary intervention. Infection was more likely in beasts with larger 32mm devices (8/10) and 7/10 devices were uncoated.

- The trial team believes that uncoated devices are adequate for the task, despite coated devices appearing to perform better than uncoated devices in terms of the factors that affect device retention. The addition of a coating entails an additional step in the manufacturing of the device and would certainly incur additional costs, raising the price of the devices for the consumer. It is advised that when evaluating their market offering, device manufacturers take into account the numerous commercially available or experimental coatings that have a proven record of reducing infection and/or accelerating tissue growth. The choice of materials and the functionality of the device may offer fertile ground for competition among device manufacturers.

- Intensive feedlot conditions do not provide an environment that would cause implantable device technologies to fail. Other important factors, such as ear condition, location of implant and implant procedure are more deterministic in the success of the technology.

- The successful implantation of both 22mm and 32mm devices was achieved. Further advancement in the implantation protocol as well as refinements to both the device design and applicator gun will make the task of implantation easier. This trial confirmed that qualified vets or highly specialised knowledge on how to apply the devices is not required.

4.1 Benefit to Industry

The Australian cattle industry is a major part of the Australian economy. Australia's reputation for producing clean and safe red meat products relies on lifetime traceability of its cattle. The implantable technology assessed through these trials has the potential to provide an alternative identification method for Australian cattle. Particular interest in this type of device may come from particular segments of Australian cattle producers who may suffer high tag loss and who have a high proportion of breed stock that they desire to "tag for life". The results in this paper show that industry concerns around food safety risks and device retention were not prevalent. Further improvement of the technology is possible through innovation in materials, designs and application procedures. Implantable devices are unlikely to be the best option for all Australian cattle producers, but offer a potential alternative option for some Australian producers. The development of any device options that have the potential to address known issues within Australia's traceability system by improving the retention and performance of identification technologies are required.

5 Future research and recommendations

The results from the completed field trials show that various implantable devices (22 and 32mm, tissue growth coated and uncoated) offer a high retention rate at the implantation site, and minimal adverse effects on beast welfare (infection). Based on these results, further work is recommended to continue developing implantable devices as a potential commercial identification option for Australian producers. Specifically, commercial supply chain trials are proposed to acquire longer-term performance data and test the supply chain needs. The purpose of these supply chain trials will be to expand on the work completed under the scientific field trials, to identify and address the remaining adoption barriers. Activities will include;

1. application of implantable RFID devices to younger cattle (~6-month-old, at weaning and marking or similar). This producer cohort is targeted as they have a high interest in an implantable solution as an alternative to current external RFID ear tag.
2. device readability measures in different on-farm scenarios, with different brands and types of readers (i.e. stick v panel readers).
3. investigate any potential supply chain operations issues, adoption barriers and tooling requirements with stakeholders. This will include identifying potential solutions to overcome these.

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7 Appendix

Appendix 1: Project protocol developed and finalised project plan

Trial 1: Pasture Field Trial

Partners | Australian Universities, technology providers, researchers

Role | Conduct a 140 day, combined feedlot-pasture trial on 280-350kg beasts purchased at saleyards. An Australian University will be used to perform non-invasive histology assessments during the trial duration, with end-of-trial samples collected and assessed by the University.

Where | NSW

Scale | 120 Head (dependent on available supply at saleyard)

Trial 2: Commercial Feedlot Trial

Partners | Australian Universities, technology providers, veterinary experts

Role | Conduct a 90 day commercial feedlot trial to test injectable RFID device retention. Invasive histological assessment of tissue integration will occur at interim timepoints of 14, 28, 56 days as well at the trial end. These will be measured by ears procured at 'end of life' in processing facilities, with trial samples collected and assessed by the University

Where | NSW

Scale | 200 head

Project Phases

Phase 1 - Launch: Jun-Oct 2021

Contracting Delivery Partners | ISC/MLA Confirmation of Intent | Research Protocol and Detailed Project Plan for Trial 1 & 2 | Reporting Guidelines for delivery partners | 2 x Ethics Approvals (Trial 1 and 2) | Project Initiation and Planning Meetings with delivery partners (on-site) | Device procurement & test | Device coating | Device Implantation & Data Recording Training Protocol | Risk Identification and Mitigation Plan

Phase 2 - Monitor: Feb-July 2022

This will include exploring and measuring the three factors of device retention;

- Device Drop-out - The device can "back out" of the injection wound within 24-48hrs of application. The trial focus will be within the first 48hrs from application to assess device retention.
- Rejection (infection related expulsion) - Infections can lead to devices being rejected by the beast. Data collection will include images of swelling, infection and measures of rejection over time (pre and post removal).
- Migration - The tendency of the devices to move from the original injection site. Data collection will include non invasive distance measurements over time and post-slaughter analysis to assess tissue integration and device movement.

Phase 3 - Deliver: Jul-Sept 2022

Final sample and data collection at trial facilities | Feedback and assessment session with project delivery partners| Histology assessment | Delivery partner report synthesis | Draft Final Report

including recommendations and next steps | Final Presentation with MLA/ISC | | Final written report in MLA format

● Appendix 2: Reporting activity breakdown

Allocate groups

Approximately 120 beasts comprising approximately 60 Bos indicus x Bos taurus and 60 Bos Taurus beasts (typical age of 350-380 days but anywhere up to 22 months) which will be randomly stratified on breed and weight into 4 treatment groups (exact makeup of breed and composition is dependent on breed availability at time of the trial)

Vet Inspection

Inspection will consist of visual examination for inflammation, infection, measurement of device migration (visual appraisal of device movement through the ear notionally divided into upper, mid and lower regions) and lesion development. Device location will be measured using a ruler measuring from the base of the ear.

Photograph

Any signs of ears with inflammation, infection, or device migration will be photographed by the assessor. A digital image will be taken of the implanted sites on each inspection day.

Thermal image

At these time periods a thermo image will be taken using on-site thermal imaging cameras to assess inflammatory response and preliminary tissues adhesion

Histology

Samples will be sent to the lab and undergo a histology assessment to provide scientific evidence of tissue adhesion and performance differences between the polymer/tissue growth coating and 22mm/32mm devices. Biopsy samples for histology measurements will be taken from 24 beasts (6 per group) on days 7, 14, 56 and 140 (prior to being sent to slaughter). These beasts will then effectively be removed from the trial, however, will remain with the cohort. They will be examined 10 days after the biopsies have been taken to ensure they are healing correctly.

- Prior to the biopsy being taken, the device will be removed. To remove the implant a small incision (1cm) will be made with a scalpel at one end of the device implantation site and the device should easily be pushed out. This incision will happen after the area has been disinfected and local anaesthetic has been applied as listed below in the biopsy procedure.
- After careful removal of the device, a 10mm biopsy punch will be conducted at the exact implant site.

The biopsy procedure will follow a 'Skin biopsy technique – Cattle' SOP. The procedure is as follows:

- The biopsy and device removal will be conducted by a veterinary professional who has previous experience in conducting skin biopsies.
- Digital images at biopsy sites to document tissue adhesion will be taken.
- We will also take another biopsy from the opposite ear to act as an internal control.
- Biopsy tissue samples will be. examined for indicator cell (e.g. neutrophils, monocytes,

lymphocytes) counts and proportions.

Blood sample

Blood samples will be analysed through the CELL-DYN haematology analyser at a delivery partner facilities. The blood samples will be collected via the jugular venipuncture. The procedure is as follows:

- Utilise a 18g x 25mm needle.
- Restrain beasts in head bail of crush.
- Restrain the beast by holding the beast by the head and neck and pulling the head to the side away from where the blood sample is being taken.
- Swab the site with alcohol wipes.
- Locate jugular vein and apply thumb pressure below the intended site of needle insertion.
- Insert needle up into distended vein and push the tube to the end of the holder, puncturing the rubber stopper. The vein must be clearly located before any blood is collected and the puncture carried out positively.
- Maintain thumb pressure until the tube is full.
- Remove the tube once the sample has been collected.
- Immediately apply pressure with the thumb to the needle insertion point to lessen the risk of haematoma and bleeding from the puncture site.
- Release the beast.

Device readability

Confirmation of the readability of the device will occur on days 1, 7, 14, 28, 56 and 140 and be noted.

Weighing

Body weights will be taken at regular intervals throughout the trial period.

Appendix 3: Reporting guidelines for partners

Reporting guidelines have been established for all project partners. These guidelines include the content description of formal written reports, details on the contents and scope of data collection, and the timeframes for delivery. To ensure detailed and effective reporting at key project junctures, the Project coordinators will attend/participate in trial activities including, but not limited to: 1. Application of devices; 2. Biopsy and histological assessments at the Australian University trial; 3. Transfer to feedlot at the Australian University trial; and 4. End of trial for collection of ears.

Supporting this in-person reporting and communication will be weekly ‘stand-ups’ with each of the project partners. These stand-ups will follow a consistent structure:

1. What have we done in the last week?
2. What are we planning to do next week?
3. What barriers/risks could we encounter?
4. What is our plan to address or mitigate these barriers/risks?

During weeks in the trial where data is collected, the Project Coordinators will convene two meetings with each of the trial teams. The first, to occur before data is collected on the cattle, will confirm the activity steps, process, data to be collected, and a final confirmation of potential risks and the team’s approach to mitigating these risks. The second meeting will provide an opportunity to collect a high-level summary of the results which will be compiled into progress reports for ISC/MLA, and collectively

identify learnings from the process which can be applied in the following stages of the trial to ensure ongoing performance improvements.

For both trials, if at any point there are adverse findings with the beasts, these will be documented through either photos, thermal imaging, and/or blood samples. For the Australian University, all unexpected adverse events will be reported to the Animal Ethics Committee (AEC) in writing, via the Animal Ethics Officer, within 24 hours and a formal unexpected adverse event report form submitted within 72 hours of the event.

Overall, the range of reporting guidelines will ensure we have adequate levels of data and content for the Milestone 4 (28/2/22) and Milestone 5 (1/6/2022) progress reports as well as the Milestone 6 Final Report (28/06/22). A summary of specific, formal reporting guidelines/timeframes and details with the project partners can be found below.

Table 8: Trial 1 Pasture/feedlot milestones

Report	Description	Date Due
Milestone 1	Execution of agreement Ethics approval obtained Cattle acquired	January 14, 2022
Milestone 2	Devices implanted Trial progressing as planned up until day 56 beasts have entered the feedlot **Midpoint status report**	March 27, 2022
Final Report	Trial completed **Final report submitted**	June 17, 2022

Table 9: Trial 2 Commercial feedlot milestones

Report	Description	Date Due
Milestone 1	Trial Commencement The trial will be conducted at a commercial feedlot and will be facilitated by Veterinary Experts during the trial.	January 18, 2022
Milestone 2 *(ongoing)*	Data collection and regular written status updates to the Project Coordinators. (including pictures, video, spreadsheet data, observations etc). Report on animal welfare, trial results and future recommendations	January 19 - June 17, 2022

	3-4 status reports	
Project Conclusion	Trial completed Arrange for collection, sample identification, and transportation of beast ears (including devices) for histological analysis **Final status report**	June 18 - June 28, 2022 (*at the latest)*

Table 10: University agreed milestones

Report	Description	Date Due
Milestone 1	Validate translation of coating procedure from 22mm Fofia implantable devices to both 22mm and 32mm implantable devices, focusing on replication of tissue growth presence and coating morphology (as seen in previous studies conducted by the University).	January 10, 2021
Milestone 2	Apply tissue growth coating to up to 250 devices (inclusive of both 22mm and 32mm lengths) to be used in scientific cattle trials performed by the Project Coordinators.	January 17, 2021 Continency (commercial feedlot trial only) - February 21, 2021
Project Conclusion	Assess the performance of 50 ear tissue samples (in total) from the cattle trial(s) performed by the Project Coordinators, with emphasis on comparing the uncoated and tissue growth coated devices. All tissue samples will be collected by the Project Coordinators at the absolute end points of the respective Trials. A maximum of 50 ear tissue samples (comprising an	Pending delivery of 50 ears post slaughter Project updates to be supplied monthly

	even distribution of ears containing 22mm uncoated/tissue growth coated devices and 32mm uncoated/tissue growth coated devices) will be required for assessing the modified devices.	

Appendix 4: Method details, including the implantation procedure

Device Application Guidelines

Applicator and Device Manufacturer Procedure

The applicator used for this trial was an 'individual device loading system', which means only one device can be loaded into the loading slot each time. The project personnel performing the application have been instructed to load no more than one device tag at a time to avoid damaging the applicator or jamming the procedure.
Figure 10 & 11: Applicator components

Following a sterilization of 100% alcohol (must be performed before each application), the needle nut is placed on the body of the applicator. The needle nut then slides over the needle and is screwed tightly onto the body. Next, the user of the applicator pulls the 'push rod & push grip' assembly back to load the device.
Figure 12 & 13: Device loading and release from the applicator

The device will be placed into the loading slot. The domed end of the device will be pointed towards the needle to ease the injection into the beast. The device will discharge from the applicator needle when the 'push rod and push grip' assembly is depressed. Once all the devices have been implanted, it is advisable to wash the applicator in warm soapy water and then rinse with clean water before drying and storing in a clean place. To ensure safe use and effectiveness, the tip of the applicator should be kept sharp to a V point, removed, and preferably spiked on a cork or foam when not in use to prevent injury.

Procurement of coated and non-coated devices

In total, 560 devices have been procured for this trial - 200 x 32mm and 400 x 22mm. Due to global silicon chip shortages as a result of Covid-19, 200 x 32mm devices was the maximum quantity that could be obtained for this trial. The total number of devices is sufficient to deliver 120 total devices to the Australian University trial and up to 240 devices for the commercial feedlot trial. These quantities ensure there are adequate numbers of 'back-up' or 'replacement' devices in the event of application or device issues at trial commencement. Given the unproven robustness or half-life of the University tissue growth coating in storage, the project team (based on consultation with the University) will not be coating the devices until just before trial commencement. To mitigate against challenges coating the devices in a more condensed time frame, samples of both the 22mm and 32mm have been sent to the University in advance to validate and streamline the process. The requisite number of devices must be coated by the University on or before January 10, 2022, or if the contingency is enacted, 120 devices by February 21, 2021 (please see Section 4.3 for more details on the Australian University).

Device manifest

Trial 1: 120

- 32mm coated - 30
- 32mm uncoated - 30
- 22mm coated - 30
- 22mm uncoated - 30

Trial 2: 200

- 32mm coated - 50
- 32mm uncoated - 50
- 22mm coated - 50
- 22mm uncoated - 50

22mm and 32mm devices

Images of 22mm and 32mm device comparison as well as bulk quantities of devices below.

Figure 14: 32mm device (top) and 22mm device (bottom)

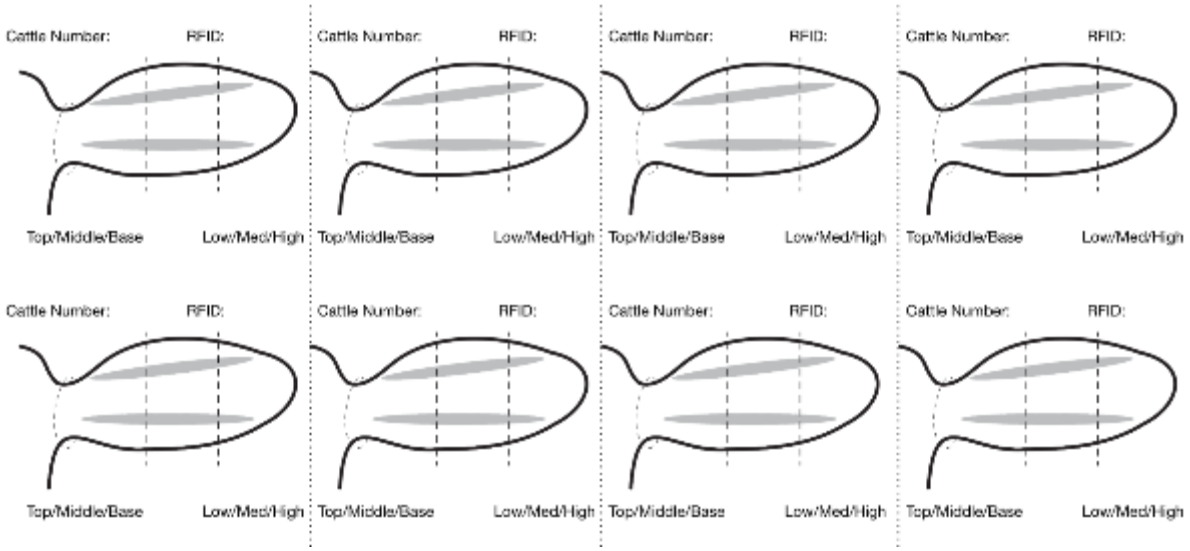
Figure 15 & 16: Full supply of 400 22mm devices (left) and 200 32mm devices (right)

Figure 17 & 18: 32mm applicator (left) and 22mm applicator (right)

Appendix 5: Data Recording Training Protocol

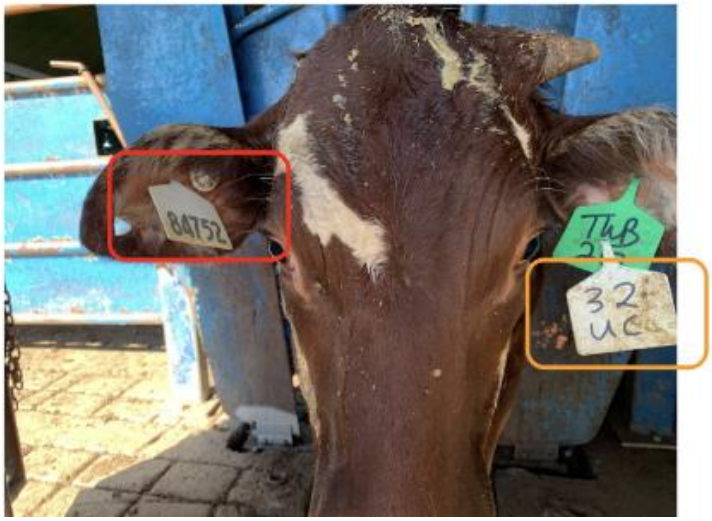
A standardised proforma to facilitate the assessment of ear quality at application and device migration over the trial was designed. A data collection sheet was developed and was provided to the trial partners (figure 19).

Figure 19: Data recording printout



Next the cattle identification number and device type being inserted is to be noted (Figure 20).

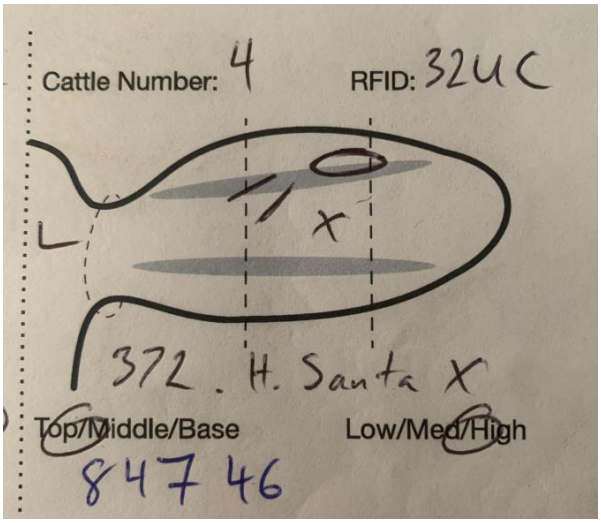
Figure 20: Cattle ID in red, device type and size in yellow.



The number of the beast within each of the trial is (1-200) recorded under “Cattle Number” and location of the device to be drawn on the recording sheet (example shown in Figure 21).

The ear was divided into three parts (base, middle, and tip), with the ear cartilage running through the centre. During application, the location of the HGPs and the existing ear tag were also captured (the two lines and the x, respectively). After implanting a device, an oval was drawn on the ear to provide a reference point for the device position at each assessment point. Example of recorded data below.

Figure 21: Example of completed data recording for 1 ear



This data was then transferred into a digital format for further analysis and documentation.

Appendix 6: Biopsy scoring system

Biopsy samples for histology measurements were taken from 24 beasts (6 per group, balanced by ear type) on days 7, 14, 56 and 140. These beasts were then effectively removed from the trial however they remained with the cohort.

Local anaesthetic was administered at the base of the ear by injecting 3 x 1.0mL of 2% lignocaine subcutaneously with a 21-gauge needle, forming a “blister” (bleb) in the skin. Prior to the biopsy being taken, the device was removed. To remove the implant a small incision (1cm) was made with a scalpel at one end of the device implantation site and the device was easily pushed out. After careful removal of the device a 10mm trephine (biopsy punch) was conducted at the exact implant site. The same procedure was performed on the beast’s other ear to act as an internal control. Biopsies were placed in 5ml of formalin and shipped to be assessed.

Biopsies were analysed using a 4-tier scoring system, comparing the “treatment” ear (ear with device) to the opposite “control” ear. The 4 tiers were:

0 - Absence of inflammation
I - Mild inflammation
II - Moderate inflammation
III - Marked inflammation

This approach, common in projects involving pathology, considers all features of the inflammatory process. “Inflammation” encompasses a range of features including, but not limited to, numbers of inflammatory cells (neutrophils, eosinophils, lymphocytes, plasma cells, macrophages), necrosis, vascular changes, oedema, fibroblasts and fibrosis.

Table 11: Description of the 4-tier scoring system for analysing the ear biopsies

Tier	Properties
0	- Indistinguishable from the control or - No to minimal inflammation
I	- Few inflammatory cells present - No haemorrhage to mild haemorrhage - Absent to small amounts of oedema - Absent to rare fibrin and/or necrosis
II	- Moderate numbers of inflammatory cells present - Moderate amounts of necrosis, oedema, fibrin, haemorrhage and/or fibrosis
III	- Moderate to abundant amounts of inflammatory cells present - Marked amounts of necrosis, oedema, fibrin, haemorrhage and/or fibrosis

Appendix 7: Trial 1 Blood work

All remaining beasts in the trial had blood collected on day 28.

White Cell Count (WCC)
Statistical analysis showed no significant difference in white cell count (WCC) between day -4 and day 28 for any of the treatment groups.

Figure 22:. Boxplot of WCC for each group on day -4 and day 28.

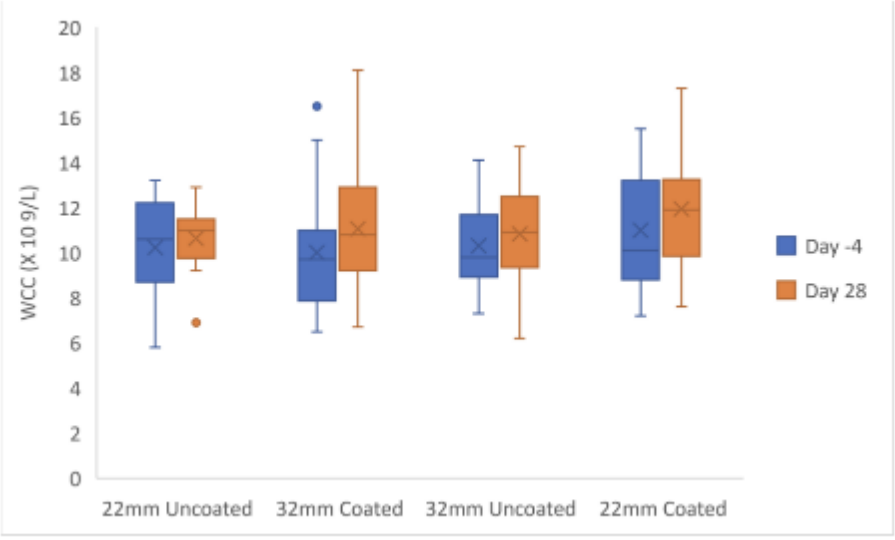
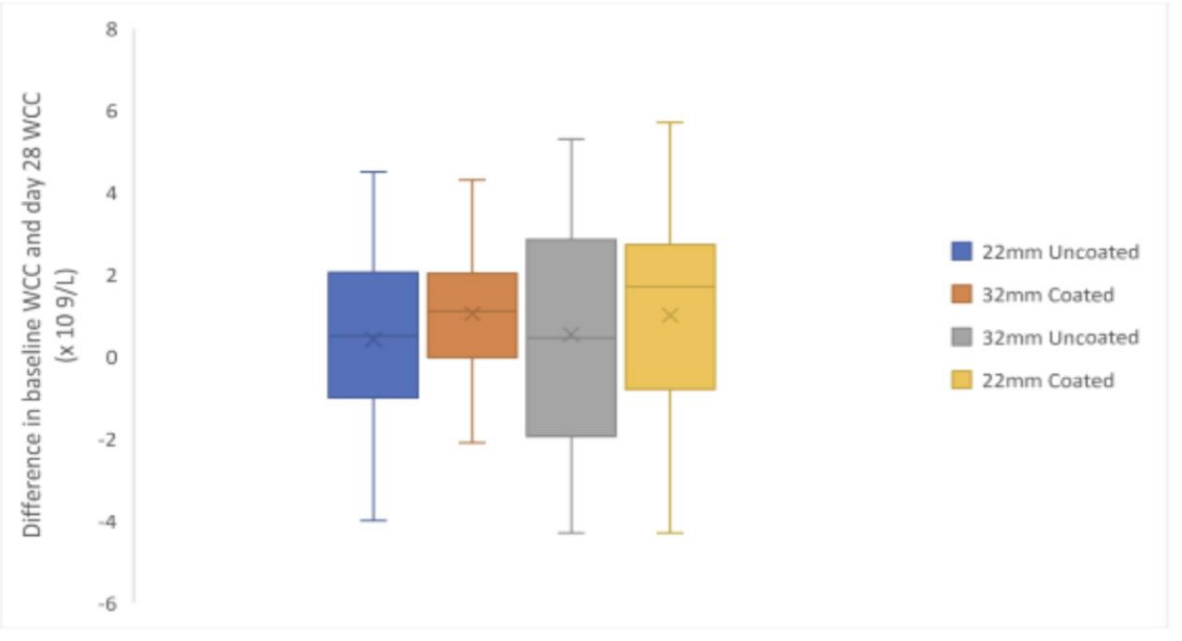


Figure 23: Boxplot of the difference in WCC from baseline (day -4) and day 28



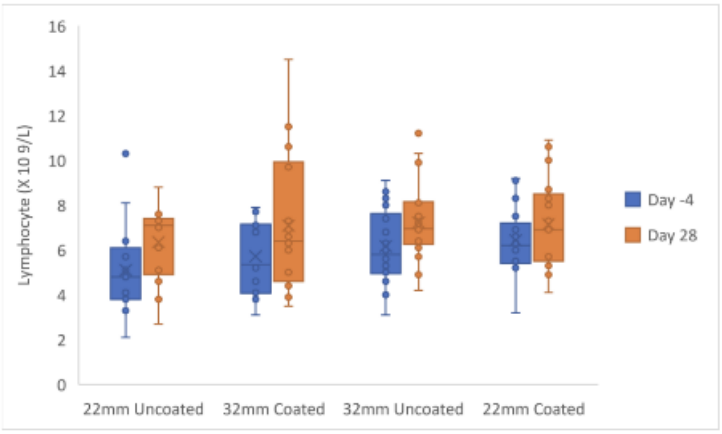
Beast #5 showed signs of infection on day 14 and the WCC at day 28 is slightly elevated in comparison to day-3 bloods (12.9 vs 10.6). This may indicate an immune response to an infection.

Lymphocytes

Statistical analysis showed no significant difference in lymphocytes between day -4 and day 28 for any of the treatment groups.

The 22mm uncoated and 32mm uncoated groups were approaching a significant difference (p=0.053 and p=0.077 respectively) between day -4 and day 28. The average values for both of these groups were still within the acceptable bovine range for healthy beasts.

Figure 24: Boxplot of lymphocyte values for each group on day -4 and day 28

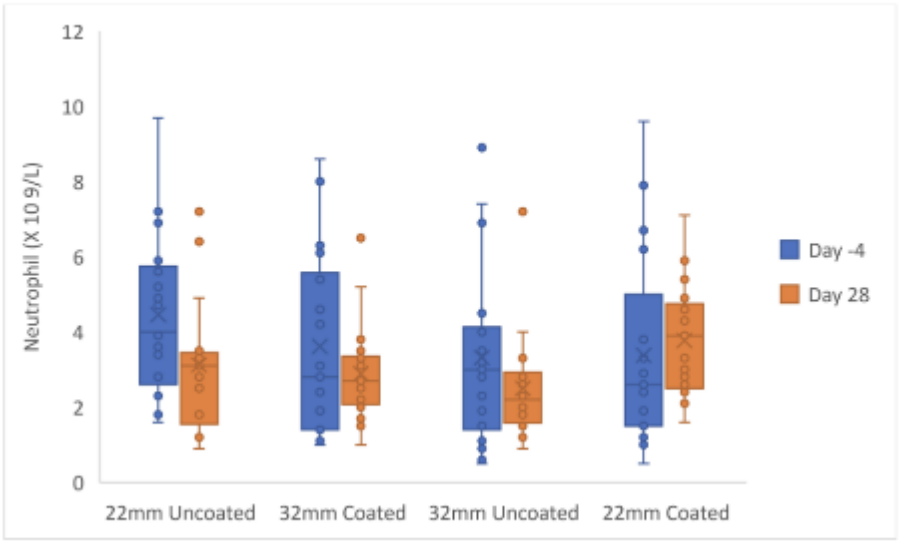


Neutrophil

Statistical analysis showed no significant difference in neutrophils between day -4 and day 28 for the 22mm coated, 32mm coated or 32mm uncoated groups. The 22mm uncoated group was approaching

a significant difference (p=0.055) between day -4 and day 28. The average values for this group were still within the acceptable bovine range for healthy beasts.

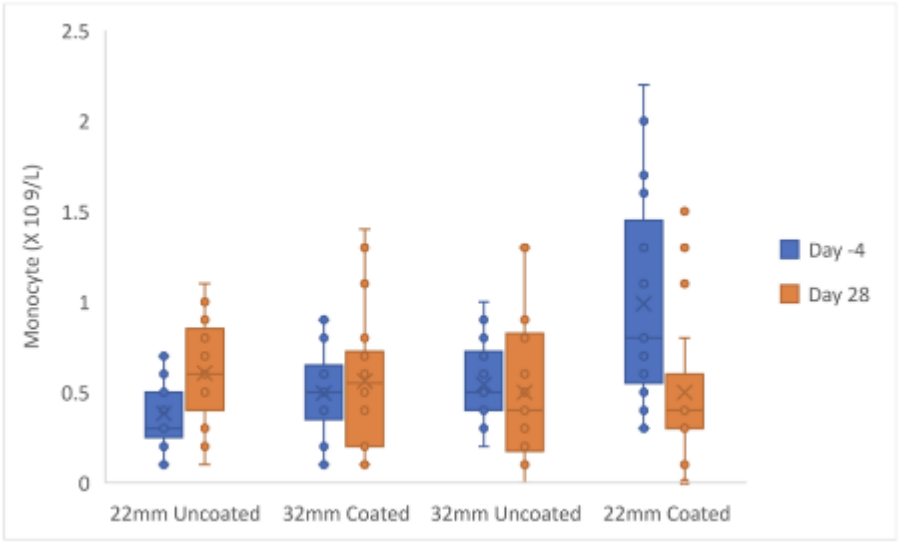
Figure 25: Boxplot of neutrophil values for each group on day -4 and day 28



Monocyte

Statistical analysis showed a significant difference ($p<0.05$) in monocyte values between day -4 and day 28 for the 22mm uncoated and 22mm coated groups. No significant difference was observed in the 32mm coated or 32mm uncoated groups. The average monocyte values for all groups within the acceptable reference range. However, 13 beasts were above this range on day 28. Of these 13 beasts, all had either the same value monocyte count or increased in comparison day 0-4 values.

Figure 26: Boxplot of monocyte values for each group on day -4 and day 28

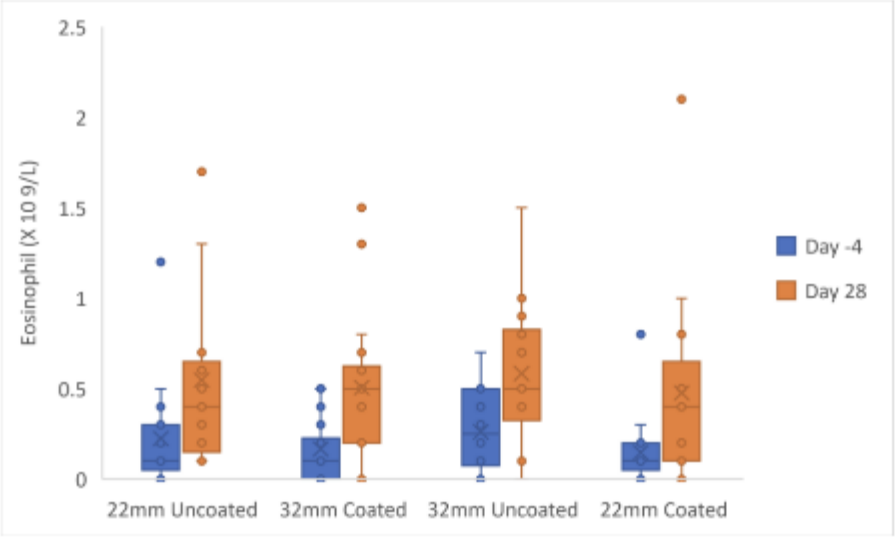


Eosinophil

Statistical analysis showed a significant difference ($p<0.05$) in eosinophil between day -4 and day 28 for all of the treatment groups. The average eosinophil value for all

groups was well within the acceptable reference range with only 1 beast (#116) being above 2 (x 10 9/L).

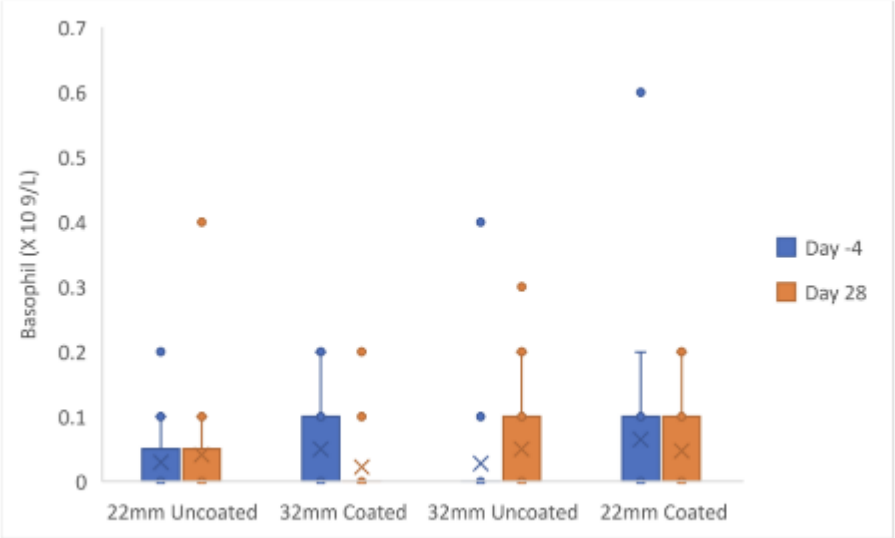
Figure 27: Boxplot of eosinophil values for each group on day -4 and day 28



Basophil

Statistical analysis showed no significant difference in basophil between day -4 and day 28 for any of the treatment groups.

Figure 28: Boxplot of basophil values for each group on day -4 and day 28.



Summary

All groups' mean values fell within the healthy range for each blood parameter. Statistical analysis showed significant differences in some parameters between day -4 and day 28 however as the group

means are within the normal range, these differences are likely driven by extreme individual measurements on day 28, exacerbating the differences between these sample points.

Table 12: Mean blood parameters for day 28

Table 10. Mean blood parameters for day 28 measurements.

Group	RBC ($x10^{12}$/L)	Haemoglobin (g/L)	Haematocrit (L/L)	MCV (fL)	MCH (g/L)	MCHC (g/L)	PLT ($x10^9$/L)	WCC ($x10^9$/L)	Neutrophil ($x10^9$/L)	Lymphocyte ($x10^9$/L)	Monocyte ($x10^9$/L)	Eosinophil ($x10^9$/L)	Basophil ($x10^9$/L)
22mm uncoated	9.0	121	0.38	43	14	318	306	11	3	6.3	0.61	0.5	0.04
32mm coated	9.1	126	0.40	44	14	319	376	11	3	7.1	0.56	0.5	0.02
32mm uncoated	9.1	123	0.39	43	14	319	369	11	3	7.2	0.50	0.6	0.05
22mm coated	8.6	117	0.37	44	14	316	321	12	4	7.1	0.49	0.5	0.04
Normal range	5-10	80-150	0.24-0.46	40-60	11-17	300-360	160-650	4-12	0.6-4	2-7.5	0-0.8	0-2.4	0-0.2

Appendix 8: Commercial feedlot Blood work - Day 28 of Trial

Table 14: Feedlot Trial Blood Work results for day 28 part 1

Cattle Number	PROTEIN	FIBRINOGEN	PR/FI	PCV	RBC	HAEMOGLOBIN	MCV	MCHC	MCH
70	75	7	10 L	35	7.2	11.6	49	33	16
57	81	7	11 L	37	7.35	13	50	35	18
69	70	6	11 L	32	6.57	11.2	49	35	17
64	77	5	14 L	37	6.93	12.4	53	34	18
153	80	7	10 L	34	7.18	11.8	47	35	16
158	75	6	12 L	38	8.85 H	12.7	43 L	33	14
170	70	7	9 L	37	7.18	12.1	52	33	17
156	74	6	11 L	33	7.62	11.4	43 L	35	15
5	73	8 H	8 L	34	6.1	11.4	56	34	19
13	71	5	13 L	36	6.7	11.3	54	31	17
19	74	6	11 L	31	5.83	9.8	53	32	17
8	80	7	10 L	36	6.58	11.6	55	32	18
10	76	6	12 L	35	6.92	11.3	51	32	16
102	71	4	17	37	6.49	11.7	57	32	18
112	72	5	13 L	37	6.76	11.8	55	32	17
105	79	6	12 L	35	6.63	10.3	53	29 L	16
114	75	5	14 L	35	6.29	10.6	56	30	17
Normals	65-85 g/L	3-7 g/L	15-100	23-44 %	5.00-8.00 10^12/L	8.0-15.0 g/dL	44-62 fL	30-35 g/dL	14-20 pg

Table 14: Feedlot Trial Blood Work results for day 28 part 2

Cattle Number	WBC	BAND NEUT.	NEUTROPHILS	BANDS/NEUT.	LYMPHOCYTES	MONOCYTES	EOSINOPHILS	BASOPHILS	PLATELETS

70	8.5	0	4.59 H	0	2.98	0.43	0.51	0	*
57	6.6	0	1.65	0	4.49	0.33	0.13	0	*
69	9	0	2.79	0	5.49	0.18	0.54	0	*
64	11.3	0	4.52 H	0	5.99	0.11	0.68	0	*
153	13.5 H	0	6.89 H	0	6.35	0.27	0	0	692
158	9.4	0	5.17 H	0	3.76	0.47	0	0	*
170	8.4	0	3.02	0	5.12	0.08	0.17	0	318
156	13.5 H	0	9.72 H	0	3.24	0.41	0.14	0	*
5	8.8	0	4.14 H	0	4.58	0.00 L	0.09	0	508
13	10.5	0	5.78 H	0	4.1	0.32	0.32	0	275
19	17.4 H	0	10.79 H	0	5.74	0.87 H	0	0	402
8	14.7 H	0	5.73 H	0	8.67 H	0.29	0	0	*
10	10.7	0	5.99 H	0	2.78	0.64	1.28	0	325
102	12.9 H	0	6.71 H	0	4.64	0.77	0.77	0	232
112	8.6	0	4.64 H	0	3.78	0.09	0.09	0	417
105	14.1 H	0	7.76 H	0	5.92	0.28	0.14	0	534
114	14.4 H	0	10.66 H	0	3.6	0.14	0	0	492
Normals	4.0-12.0 10^9 /L	0.00-0.12 10^9 /L	0.60-4.00 10^9 /L	0.00-0.20	2.50-7.50 10^9 /L	0.03-0.84 10^9 /L	0.00-2.40 10^9 /L	0.00-0.20 10^9/L	100-800 10^9 /L

Palatets :* Platelet clumping observed; platelets appear adequate on blood film

Appendix 9: University Report additional details

Appendix 9.1: Digital images of implantable devices

Figure 29: Uncoated and tissue growth coated devices (22mm and 32mm lengths)

Appendix 9.2: Transit conditions for cattle ear specimens

The recommended transport conditions, as outlined by the University, to best ensure the retention of specimen quality during transit was as follows:

Samples to be immersed in sufficient 10% NBF within 2 hours following tissue harvesting;
Samples to be kept at 4ºC until received by the University; and,
Delivery to be made to the University within 8 hours following tissue harvesting.

9.2.1 Trial 1: Pasture/feedlot trial

Ear samples were collected at the processor and immediately placed in containers with 10% NBF. Samples were kept on ice and transported via road (using a courier company) to the University. Delivery was received by the University 24 hours following tissue harvesting. On arrival, samples were found to be stored in an insufficient volume of fixative that was heavily compromised by bodily fluids. This can be seen in Figure 30 below. The containers were refrigerated at 4°C, nonetheless. Samples were thus treated as “fresh”, and the fixation process was re-commenced.

Figure 30: Samples from Trial 1 on arrival at the University

9.2.2 Trial 2: Commercial feedlot

Ear samples were collected at the processor and immediately placed in containers with 10% NBF. Samples were kept on ice and transported via road (by the project coordinators) to the University. Delivery was received by the University at 12 hours following tissue harvesting. On arrival, samples were found to be stored in a more appropriate volume of fixative that was mildly compromised of bodily fluids. This can be seen in Figure 31 below. The containers were still at refrigerated conditions (the ice had 95% melted). Samples were re-immersed in fresh 10% NBF on arrival and left for a further 12 hours.

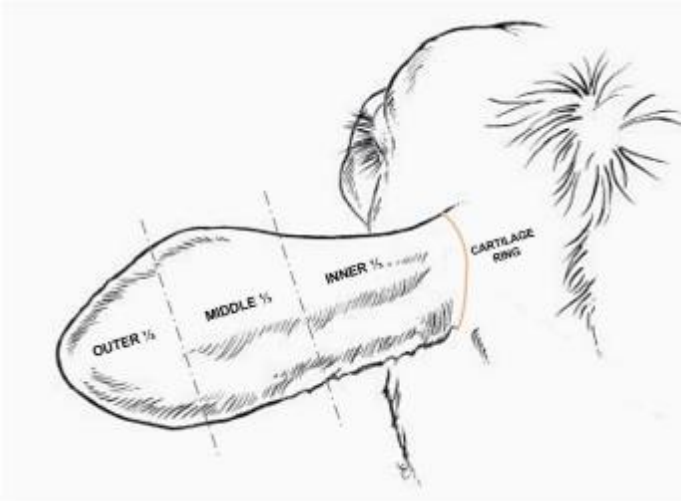
Figure 31: Samples from Trial 2 on arrival at the University

Appendix 9.3: Post-mortem assessment metrics

9.3.1 Device Location

Final device location was recorded in line with the diagram below in Figure 32. For devices located on the thresholds of the regions, the primary location was noted first in the description (e.g., inner/middle ⅓ refers to a device that is primarily located in the inner ⅓ of the ear, but not completely).

Figure 32: Cattle ear segmentation - middle-back of the ear was the target



9.3.2 Retention Quality

Retention quality was assessed by observing the overall 'tightness' of the capsule surrounding the device.

Table 15: Retention quality

Metric	Explanation	Example
Poor	Capsule/pocket very large/obvious.	
Fair	Capsule/pocket obvious.	
Good	Capsule/pocket not present.	
Excellent	Capsule pocket not present. Tight grip on device.	

9.3.3 Inflammation & Infection

Inflammation and infection were assessed by observing the overall swelling of the device region, as well as for the presence of any infected regions (e.g. pus or cysts etc.).

Table 17: Inflammation & Infection

Metric	Explanation	Example
None	No inflammation observed.	
Minor	Slight inflammation observed.	
Mild	Device moderately inflamed and infected (abscessed).	
Severe	Device heavily inflamed and infected (abscessed/bleeding open wound).	Image not available.

Appendix 9.4: Histological data collection

9.4.1 Vascularisation - vascular networks surrounding the devices

Counts for vascular networks (e.g. blood vessels, arteries etc.) were completed using Aperio ImageScope Software on H&E stained images. See example below.

Figure 33: Capsule thickness using Aperio ImageScope Software on H&E stained images

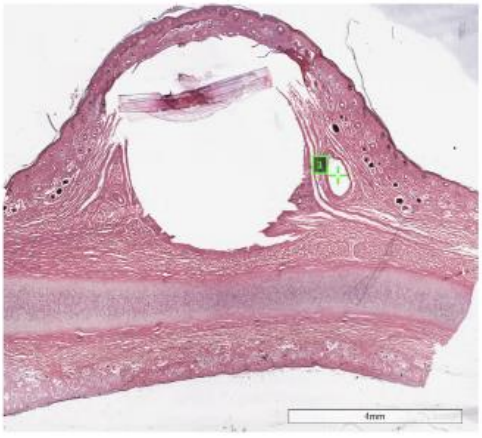
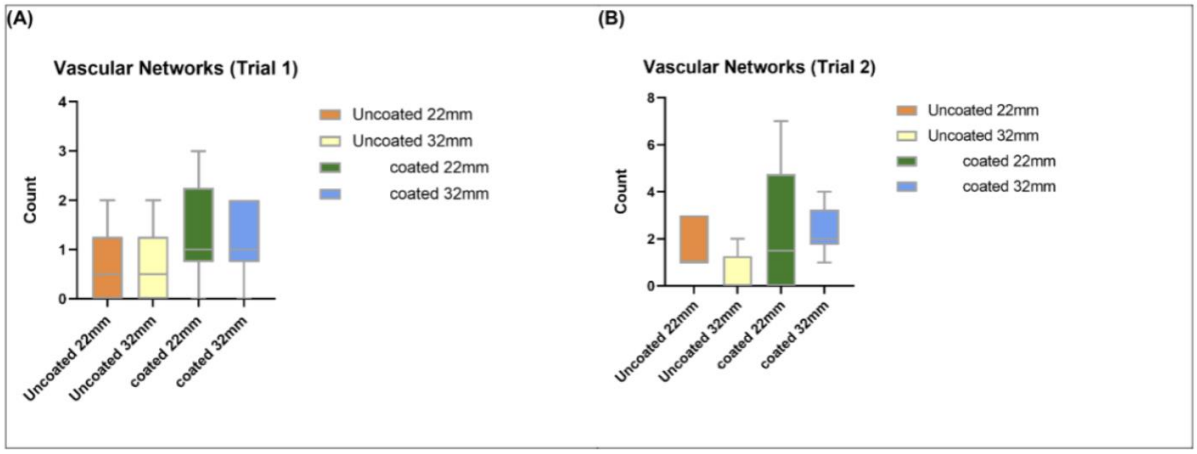


Figure 34 highlights the distribution within and between the four experimental groups.

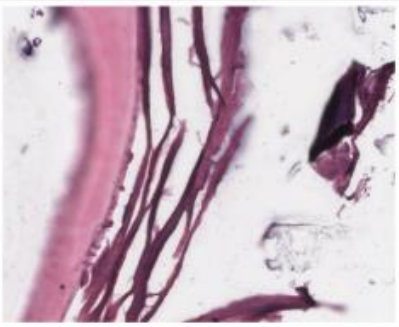
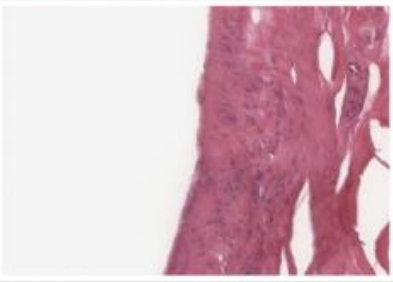
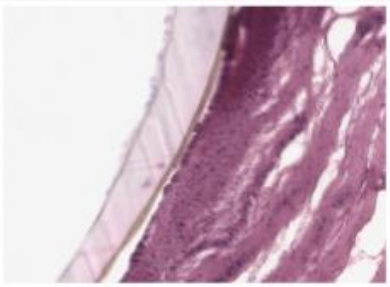
Figure 34: Vascular network counts surrounding the devices. (A) Trial 1 specimens; (B) Trial 2 specimens



9.4.2 Cell infiltration/adhesion at the device interface

Cell infiltration/adhesion was assessed by observing the tissue interface surrounding the device as depicted in the H&E stained images.

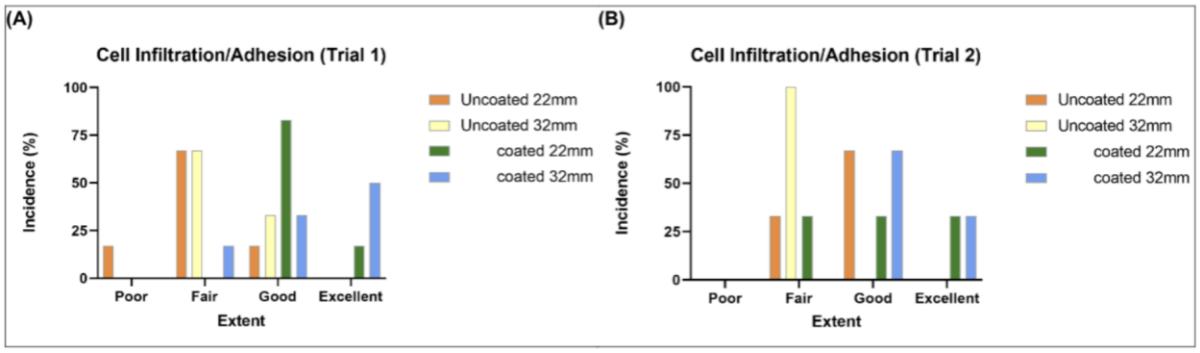
Table 17: Cell infiltration

Metric	Explanation	Example
Poor	Minimal cell nuclei present around the device.	
Fair	Minor level of cell nuclei present around the device.	
Good	Moderate level of cell nuclei present around the device.	

Excellent	Excellent level of cell nuclei present around the device.	

Figure 35 highlighting the distribution of the degree of cell infiltration/adhesion at the device interface within and between the four experimental groups.

Figure 35: Level of cell infiltration/adhesion at the device interface. (A) Trial 1 specimens; (B) Trial 2 specimens



9.4.3 Capsule thickness of tissue surrounding the device

Capsule thickness measurements were completed using Aperio ImageScope Software on MT-stained images. See example below.

Figure 36: Capsule thickness measurements were completed using Aperio ImageScope Software on MT-stained images

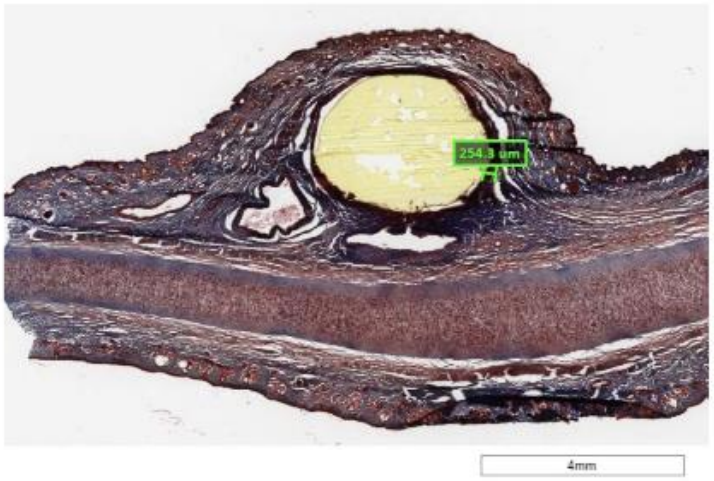
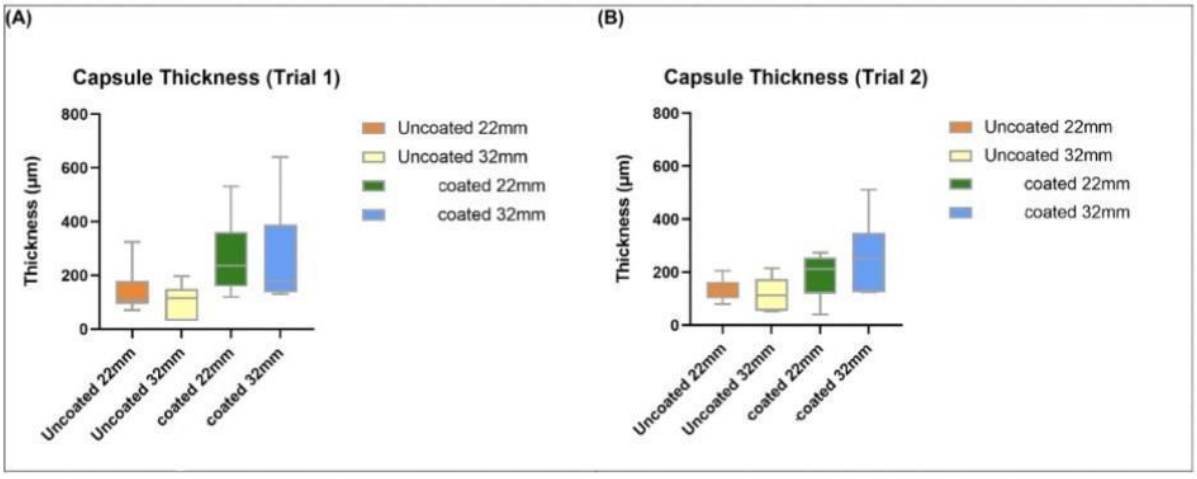


Figure 37 below shows the thickness measurements of the tissue capsules surrounding the devices, highlighting the distribution within and between the experimental groups.

Figure 37: Level of cell infiltration/adhesion at the device interface. (A) Trial 1 specimens; (B) Trial 2 specimens



9.4.4 Collagen fibre alignment

Collagen fibre alignment (i.e. degree of healing) was assessed by observing the tissue interface surrounding the device as depicted in the PSR stained images.

Table 18: Collagen fibre alignment

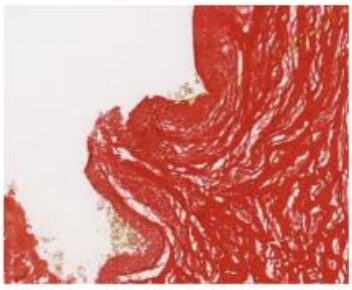
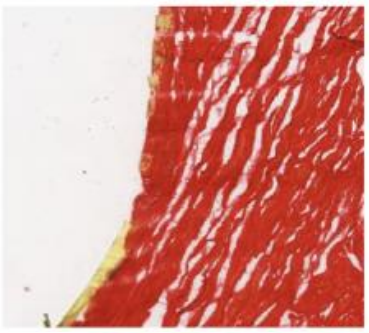
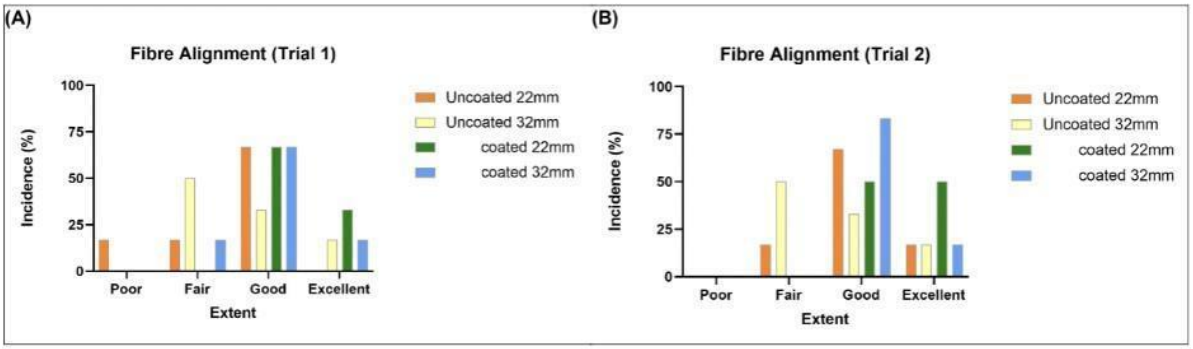
Metric	Explanation	Example
Poor	Minimal aligned fibres present around the device.	
Fair	Minor degree of fibre alignment surrounding the device.	
Good	Moderate level of fibre alignment surrounding the device.	
Excellent	High level of fibre alignment surrounding the device.	

Figure 38 below shows the degree of collagen fibre alignment around the device.

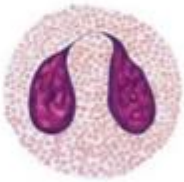
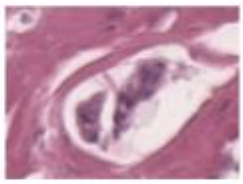
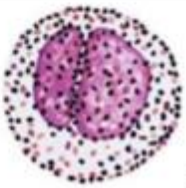
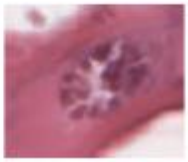
Figure 38: Degree of collagen fibre alignment around the device. (A) Trial 1 specimens; (B) Trial 2 specimens

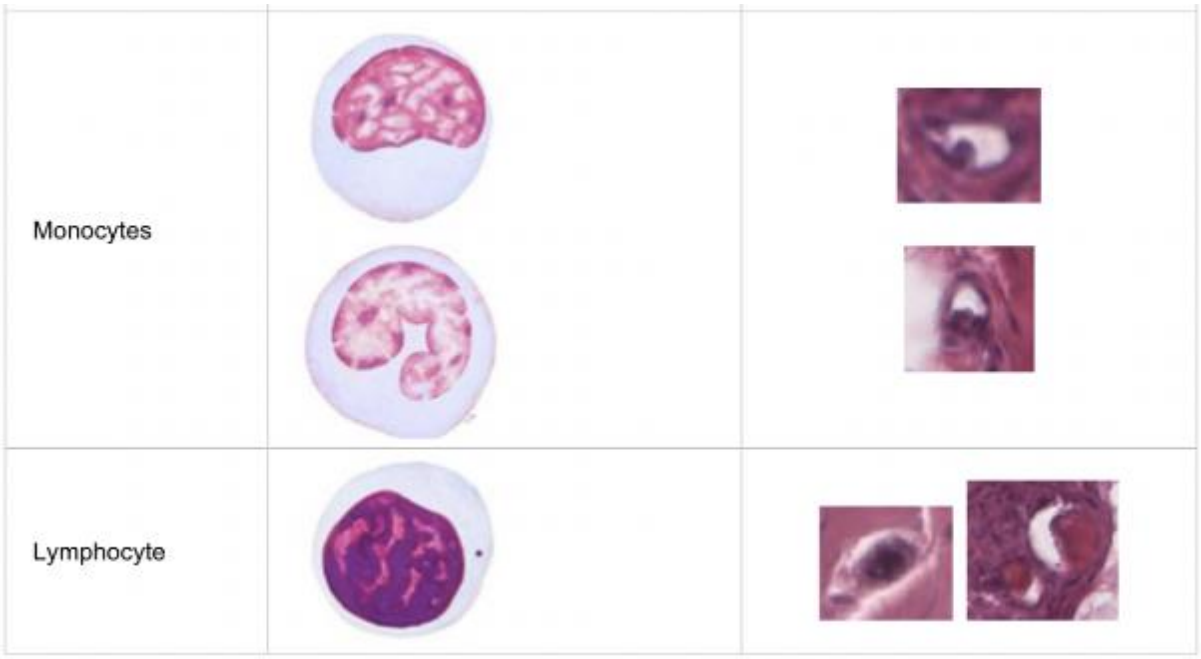


9.4.5 Inflammatory cell characterisation

The presence of inflammatory cells (leukocytes) was completed on H&E stained tissue sections. Identification of these cells, specifically, granulocytes, monocytes, and lymphocytes, was completed based on cellular morphology.

Table 19: Inflammatory cell characterisation

Cell Type	Graphic[15]	Example in H&E
Granulocytes	 neutrophil	
	 eosinophil	
	 basophil	



Observations for inflammatory cells within the cattle ear specimens received from Trials 1 and 2 are detailed below in Figures 39 & 40, respectively.

Figure 39: Percentage of devices showing inflammatory cells near device interface (Trial 1 specimens)

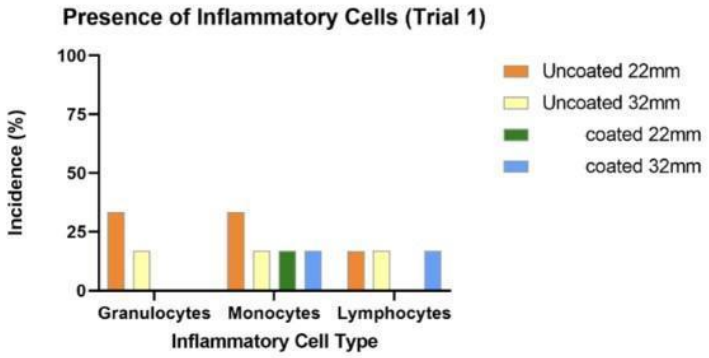
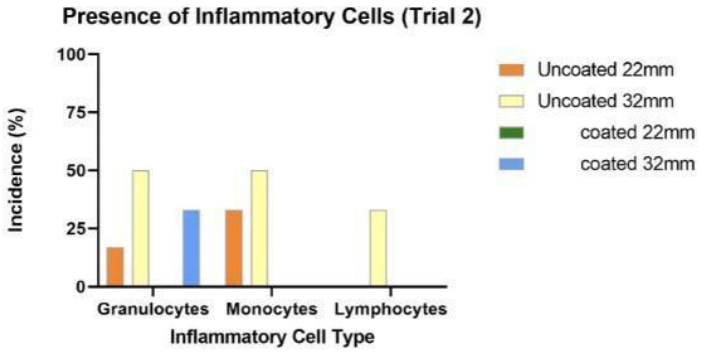


Figure 40: Percentage of devices showing inflammatory cells near device interface (Trial 2 specimens)

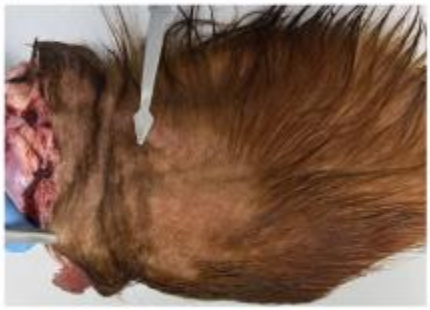


Appendix 10: University Post-Mortem Gross Specimen Imaging

Appendix 10.1: Trial 1: pasture/feedlot

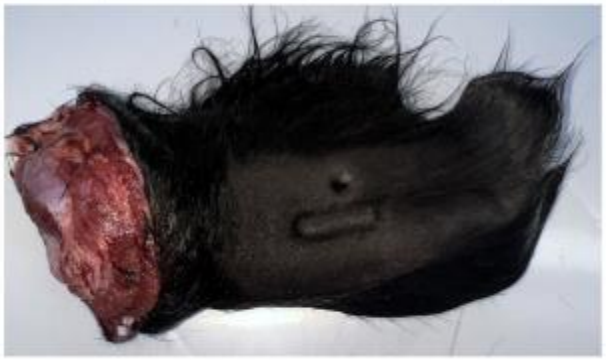
Cattle ID #	27	Device Size	N/A	Group	N/A

Cattle ID #	4	Device Size	22mm	Group	Uncoated



Cattle ID #	11	Device Size	22mm	Group	Uncoated

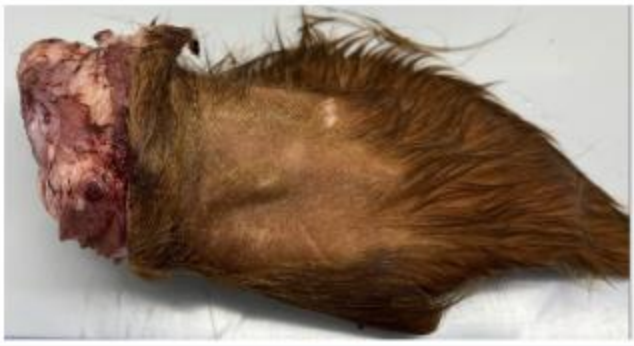
Cattle ID #	13	Device Size	22mm	Group	Uncoated



Cattle ID #	20	Device Size	22mm	Group	Uncoated

Cattle ID #	21	Device Size	22mm	Group	Uncoated

Cattle ID #	28	Device Size	22mm	Group	Uncoated



Cattle ID #	71	Device Size	32mm	Group	Uncoated

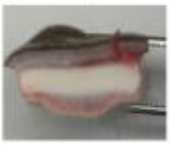
Cattle ID #	62	Device Size	32mm	Group	Uncoated

Cattle ID #	72	Device Size	32mm	Group	Uncoated

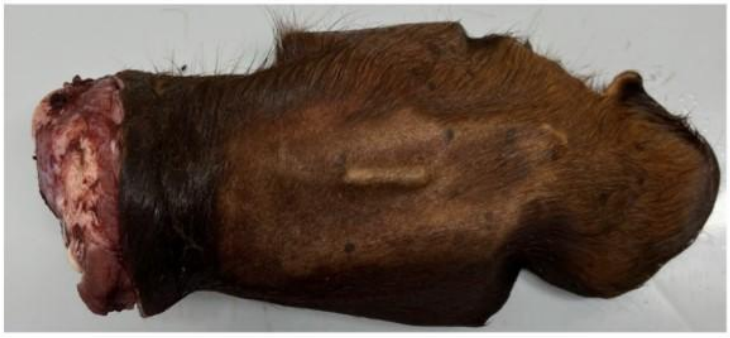
Cattle ID #	77	Device Size	32mm	Group	Uncoated

Cattle ID #	81	Device Size	32mm	Group	Uncoated

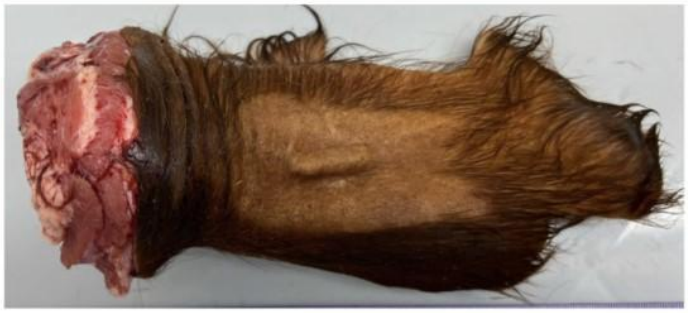
Cattle ID #	90	Device Size	32mm	Group	Uncoated



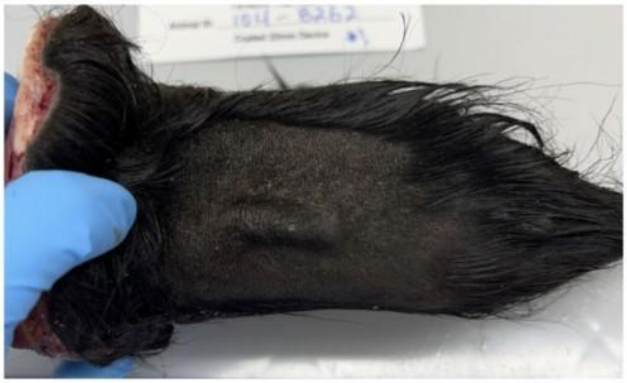
Cattle ID #	100	Device Size	22mm	Group	coated



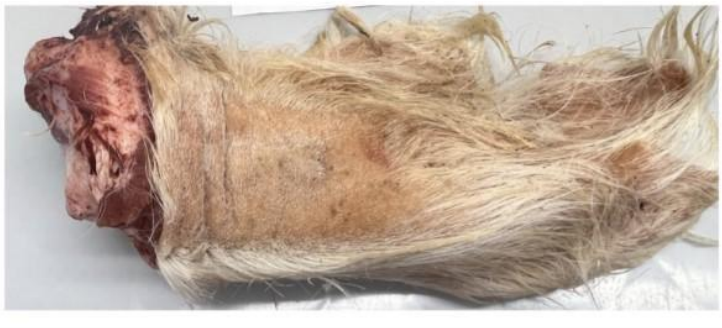
Cattle ID #	103	Device Size	22mm	Group	coated



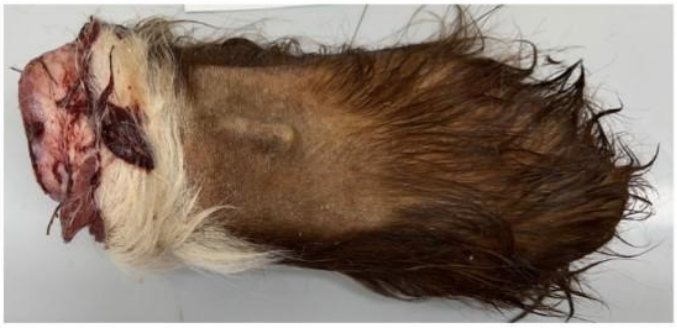
Cattle ID #	104	Device Size	22mm	Group	coated



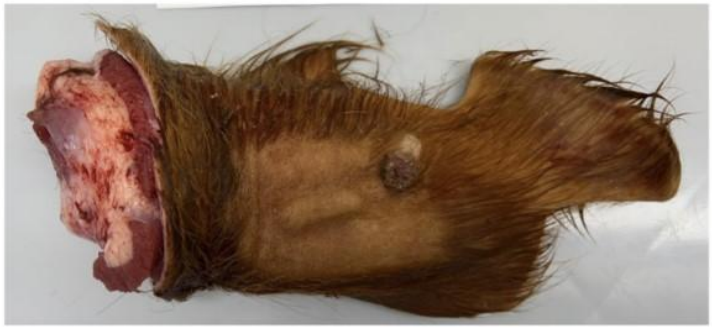
Cattle ID #	111	Device Size	22mm	Group	coated

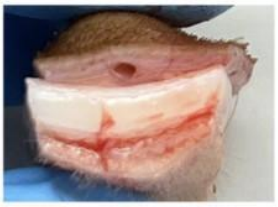


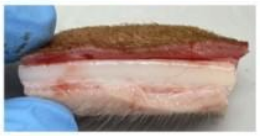
Cattle ID #	117	Device Size	22mm	Group	coated



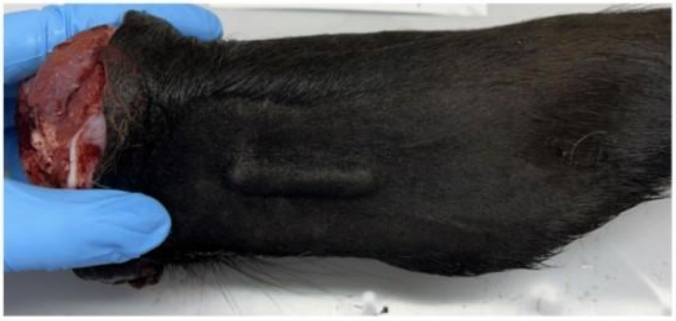
Cattle ID #	118	Device Size	22mm	Group	coated



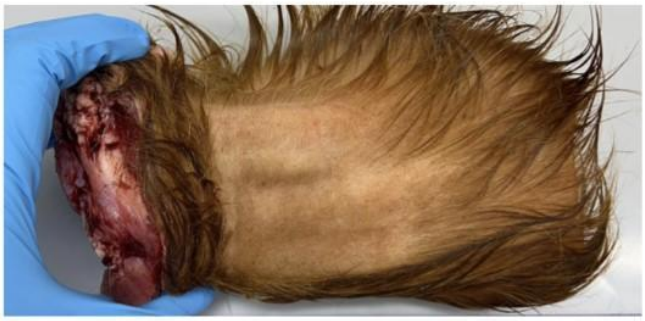


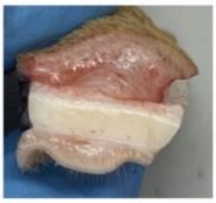


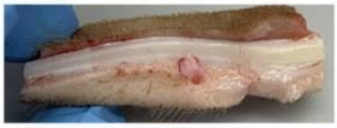
Cattle ID #	31	Device Size	32mm	Group	coated



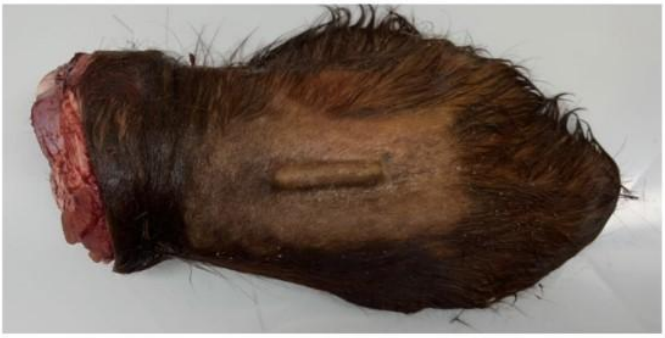
Cattle ID #	33	Device Size	32mm	Group	coated

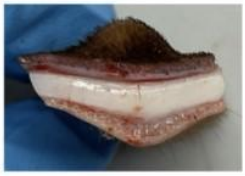




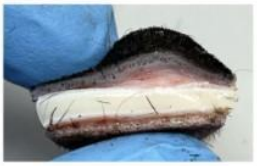


Cattle ID #	43	Device Size	32mm	Group	coated

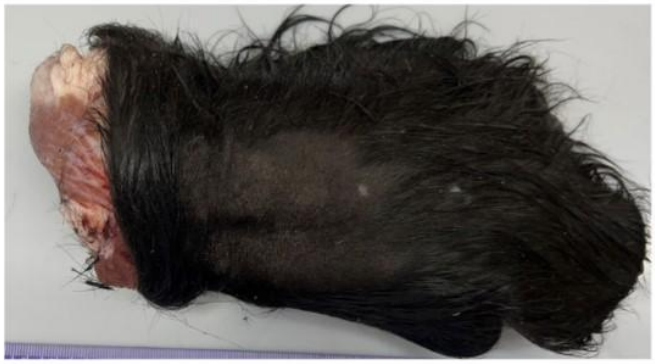


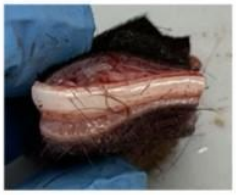


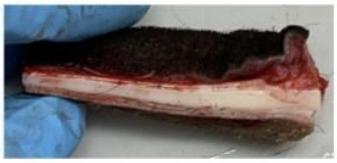
Cattle ID #	52	Device Size	32mm	Group	coated



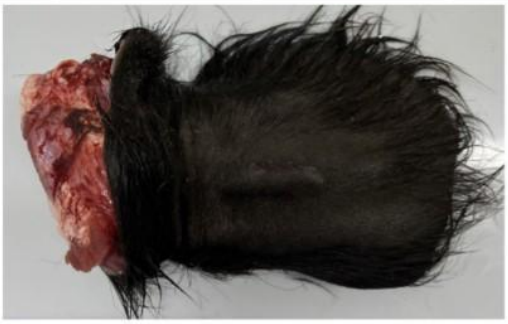
Cattle ID #	54	Device Size	32mm	Group	coated





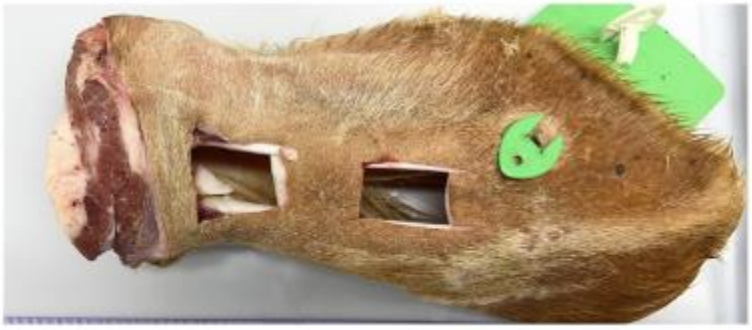


Cattle ID #	59	Device Size	32mm	Group	coated

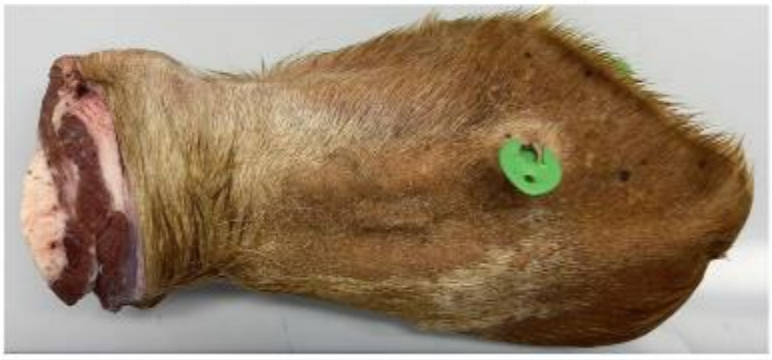


Appendix 10.2: Trial 2 :commercial feedlot

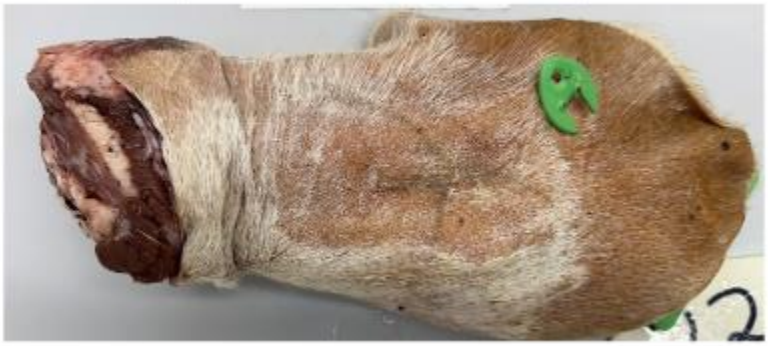
Cattle ID #	674	Device Size	N/A	Group	N/A



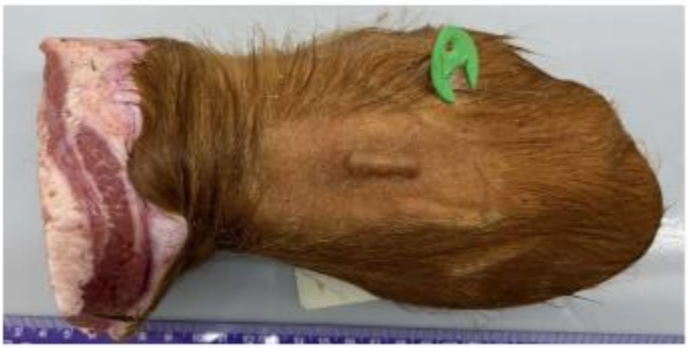
Cattle ID #	674	Device Size	22mm	Group	Uncoated



Cattle ID #	675	Device Size	22mm	Group	Uncoated



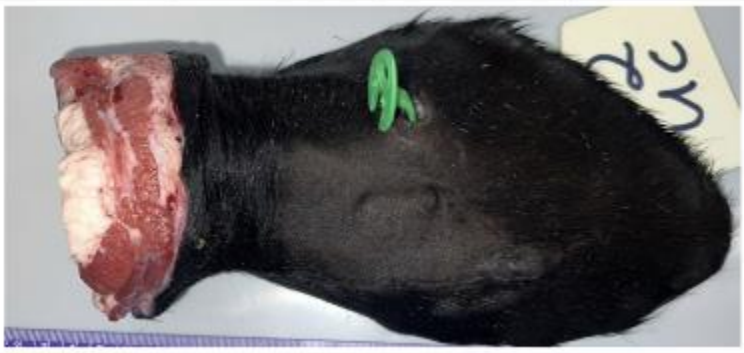
Cattle ID #	692	Device Size	22mm	Group	Uncoated



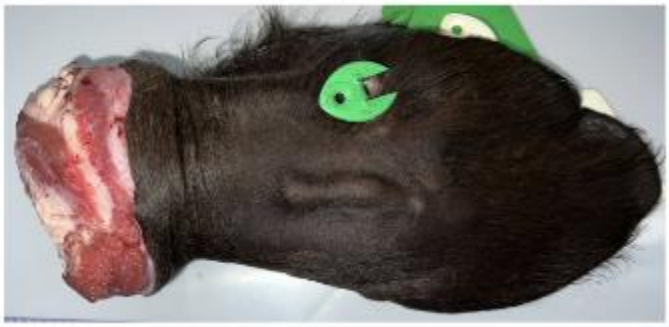
Cattle ID #	678	Device Size	22mm	Group	Uncoated

Cattle ID #	689	Device Size	22mm	Group	Uncoated

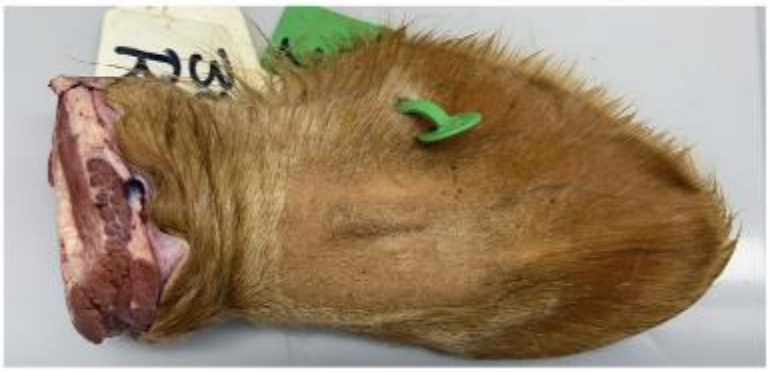
Cattle ID #	680	Device Size	22mm	Group	Uncoated



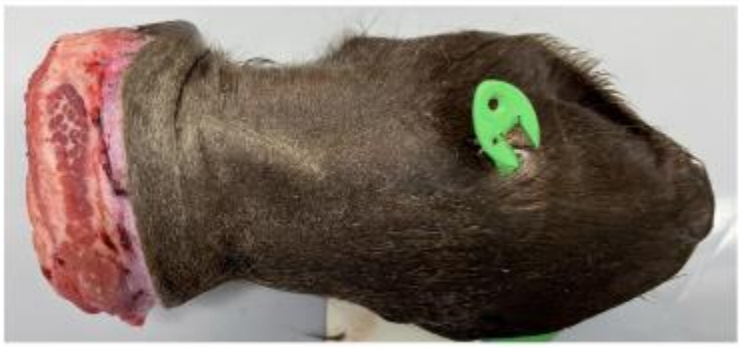
Cattle ID #	677	Device Size	32mm	Group	Uncoated



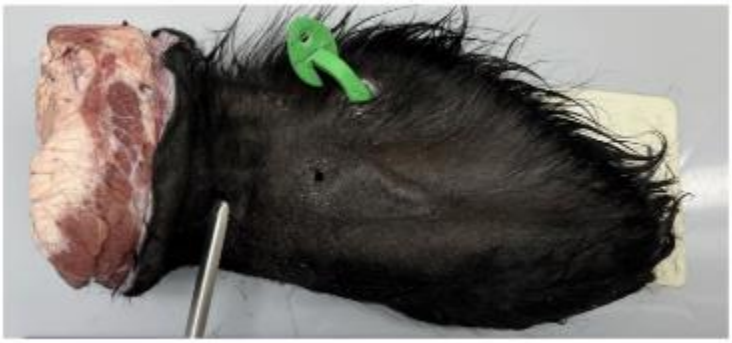
Cattle ID #	671	Device Size	32mm	Group	Uncoated



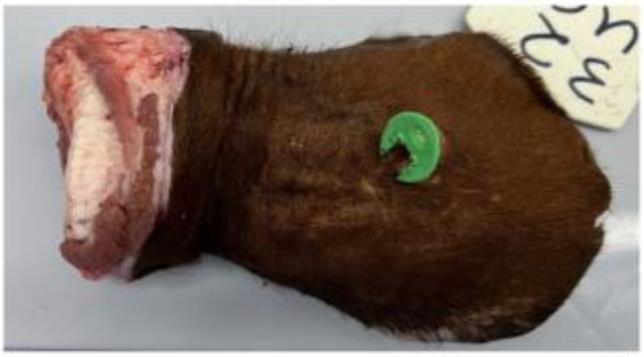
Cattle ID #	681	Device Size	32mm	Group	Uncoated



Cattle ID #	693	Device Size	32mm	Group	Uncoated



Cattle ID #	690	Device Size	32mm	Group	Uncoated



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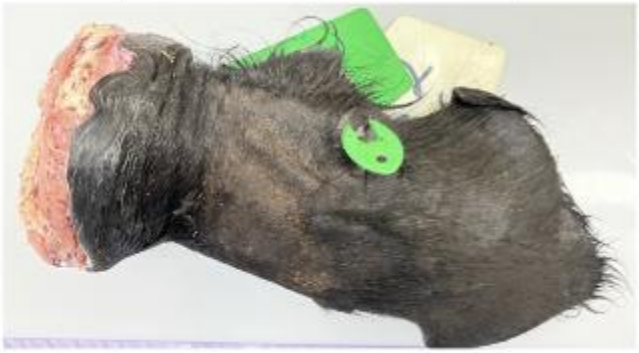
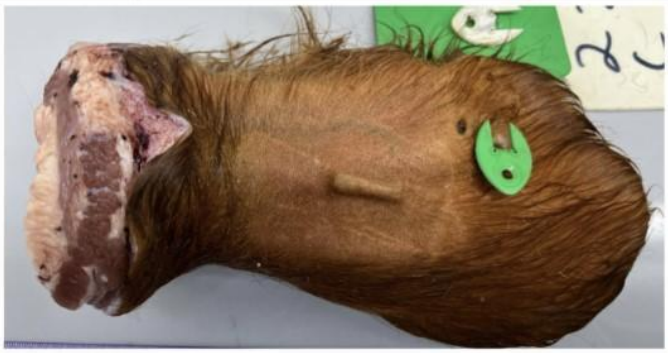


Cattle ID #	694	Device Size	22mm	Group	coated

Cattle ID #	672	Device Size	22mm	Group	coated



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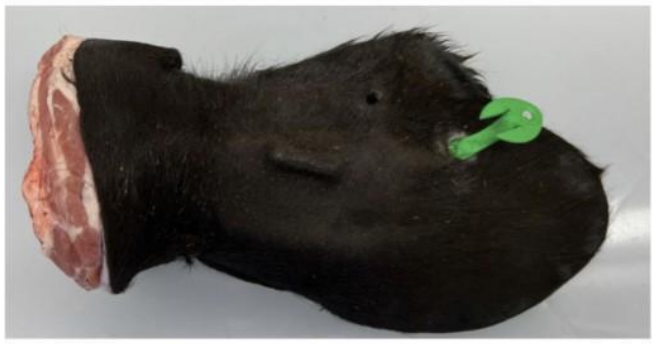

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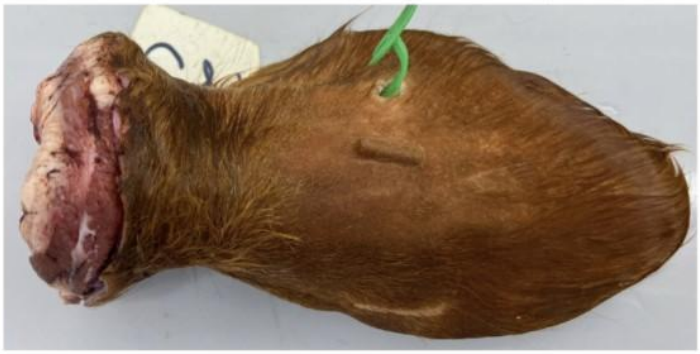

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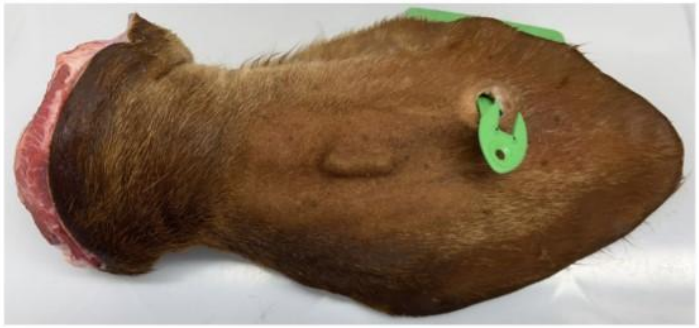
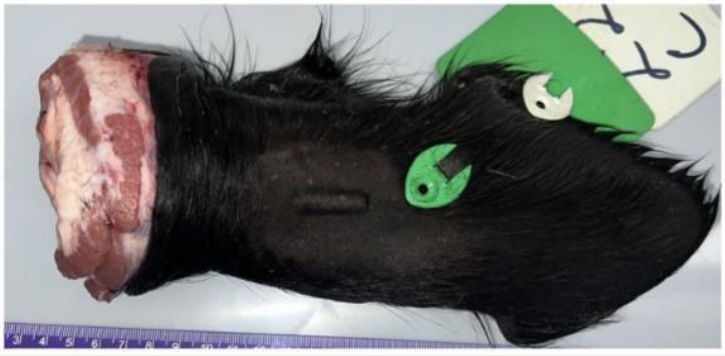
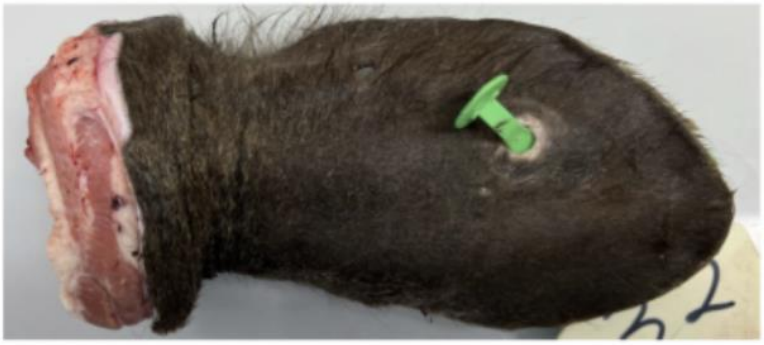


Cattle ID #	682	Device Size	22mm	Group	coated



Cattle ID #	686	Device Size	32mm	Group	coated



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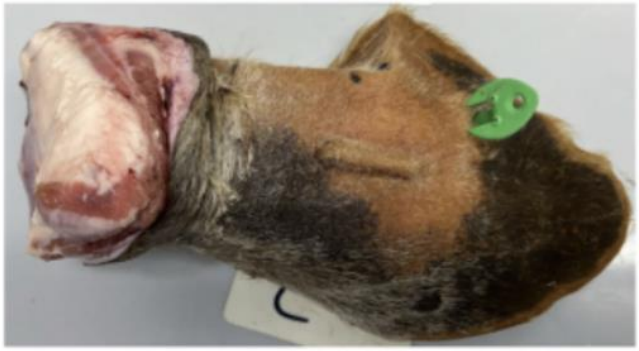

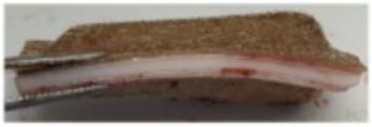

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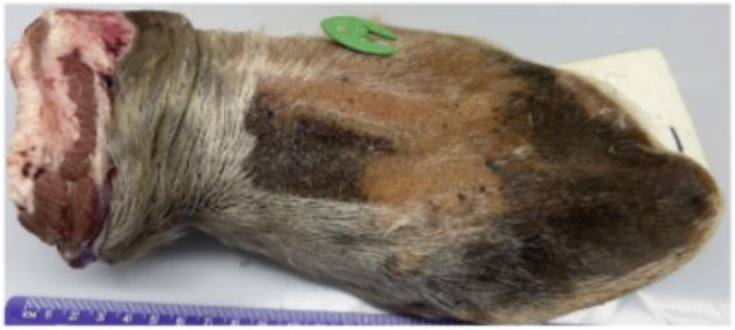
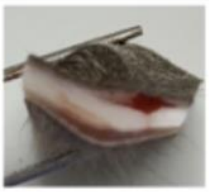
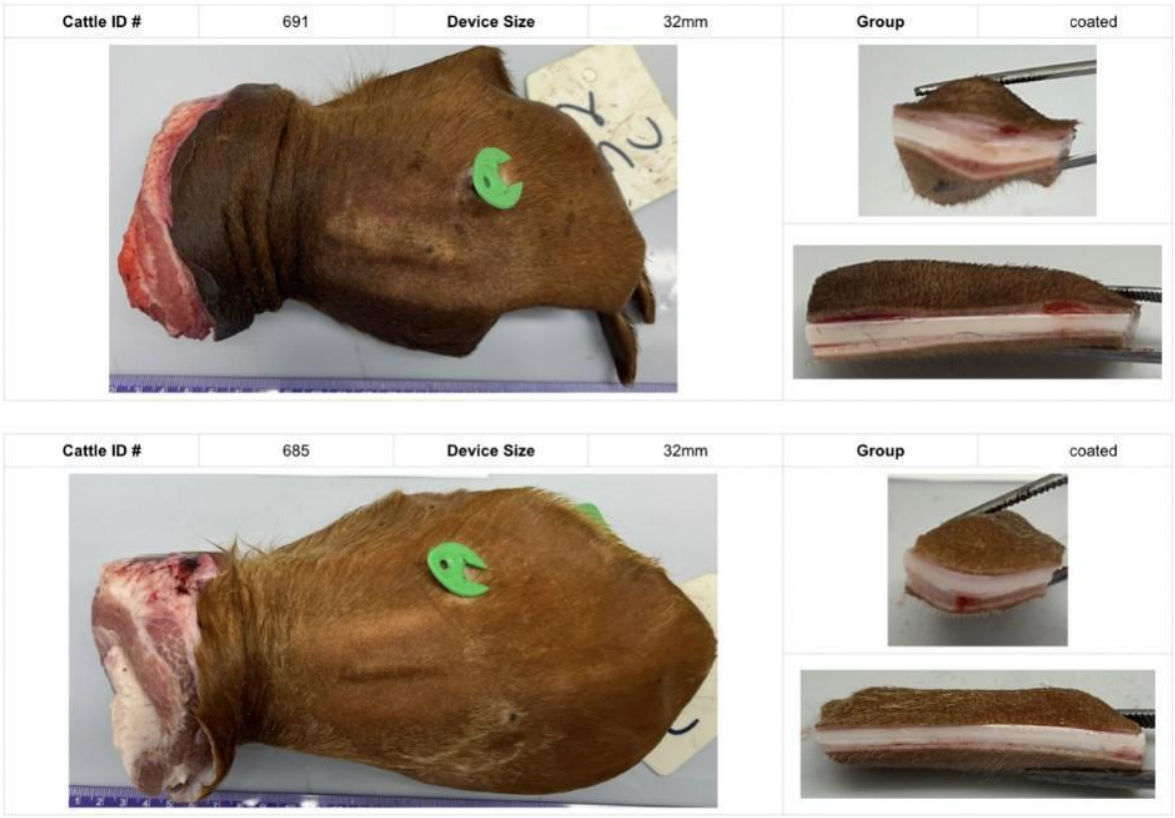


Cattle ID #	683	Device Size	32mm	Group	coated





Appendix 11: Arterial vessels within cattle ear

Figure 41: Anatomical specimen

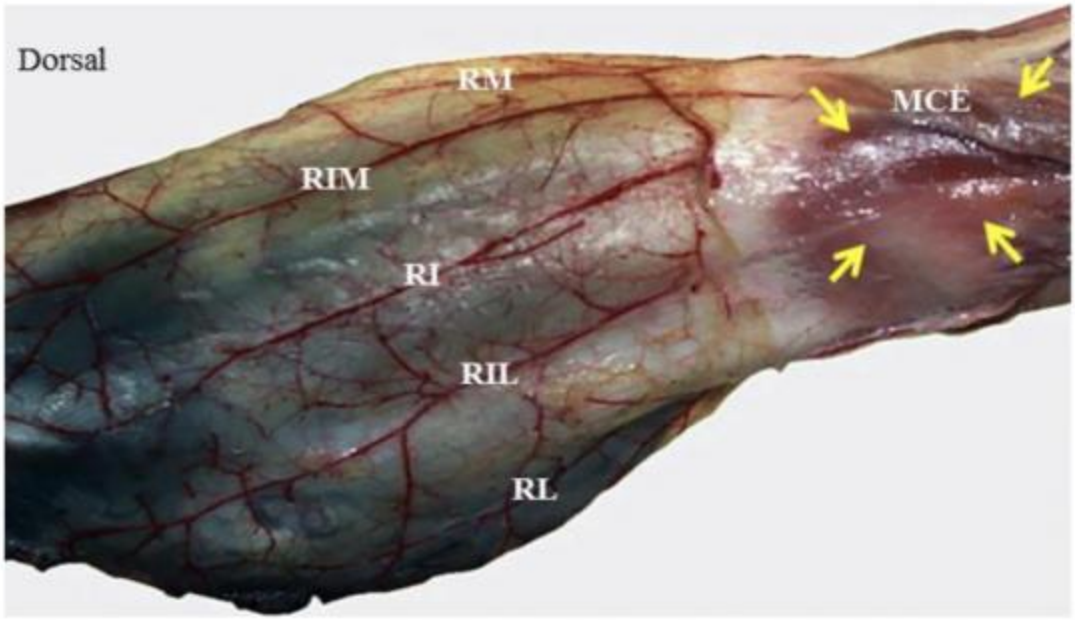


Figure 41: Anatomical specimen showing the arterial vessels of the left ear of a 1-year-old crossbred bovine (Bos taurus x Bos indicus), weighing nearly 200 kg Cervicoauricular muscle (MCE); Auricular branch of the caudal auricular artery (RL); lateral intermediate branch (RIL); intermediate branch (RI); medial intermediate branch (RIM) and medial branch of the rostral auricular artery (RM).

Appendix 12: Post-Mortem Gross Assessment

Appendix 12.1: Post-mortem analysis of Trial 1 specimens

Table 20: Post-mortem analysis of Trial 1 specimens

Cattle ID #	Device Size	Group	Tag Location	Retention Quality	Inflammation	Infection	Comments
27	None	None	N/A	N/A	None	None	Plain ear with no injectable devices. Control specimen for middle ⅓ region.
4	22mm	Uncoated	Inner ⅓; cartilage ring	Poor	Minor	None	Device moved into cartilage ring zone. Device orientation changed by 90°.
11	22mm	Uncoated	Inner/Middle ⅓	Excellent	None	None	Excellent retention – pocket not present.
13	22mm	Uncoated	Middle ⅓	Excellent	Minor	None	Pocket slightly inflamed, likely due to surrounding damage.
20	22mm	Uncoated	Inner/Middle ⅓	Fair	Minor	None	Pocket slightly inflamed.
21	22mm	Uncoated	Inner/Middle ⅓	Excellent	None	None	Excellent retention – pocket not present.
28	22mm	Uncoated	Inner ⅓	Poor	Minor	None	Device retained, but cartilage ring has prevented fixed location.
71	32mm	Uncoated	Inner/Middle ⅓	Excellent	None	None	Excellent retention – pocket not present.
62	32mm	Uncoated	Inner/Middle ⅓	Fair	Minor	None	Pocket slightly inflamed.
72	32mm	Uncoated	Middle/Inner ⅓	Excellent	None	None	Excellent retention – pocket not present.
77	32mm	Uncoated	Inner/Middle ⅓	Fair	Minor	None	Pocket slightly inflamed.
81	32mm	Uncoated	Inner/Middle ⅓	Good	None	None	Good retention – pocket not present.
90	32mm	Uncoated	Middle/Inner ⅓	Fair	Minor	None	Pocket slightly inflamed.
100	22mm	coated	Middle ⅓	Excellent	None	None	Excellent retention – pocket not present.
103	22mm	coated	Middle ⅓	Excellent	None	None	Excellent retention – pocket not present.
104	22mm	coated	Middle/Inner ⅓	Good	None	None	Pocket slightly loose.
111	22mm	coated	Inner ⅓	Fair	Minor	None	Pocket slightly inflamed.
117	22mm	coated	Inner/Middle ⅓	Good	None	None	Good retention – pocket not present.
118	22mm	coated	Inner/Middle ⅓	Good	Minor	None	Good retention – pocket not present. Damage in surrounding area.
31	32mm	coated	Middle/Inner ⅓	Excellent	None	None	Excellent retention – pocket not present.
33	32mm	coated	Inner ⅓	Good	Minor	None	Good retention – pocket slightly present.
43	32mm	coated	Middle ⅓	Excellent	None	None	Excellent retention – pocket not present.
52	32mm	coated	Inner/Middle ⅓	Good	Minor	None	Good retention – pocket slightly inflamed.
54	32mm	coated	Inner ⅓	Good	None	None	Good retention – pocket slightly inflamed.
59	32mm	coated	Middle/Inner ⅓	Excellent	None	None	Excellent retention – pocket not present.

Appendix 12.2: Post-mortem analysis of Trial 2 specimens

Table 21: Post-mortem analysis of Trial 2 specimens

Cattle ID #	Device Size	Group	Tag Location	Retention Quality	Inflammation	Infection	Comments
674	None	None	N/A	N/A	None	None	Plain ear with no injectable devices. Control specimen for inner ⅓ region.
674	22mm	Uncoated	Middle ⅓	Good	None	None	Good retention – pocket not present.
675	22mm	Uncoated	Middle ⅓	Good	None	None	Good retention – pocket not present.
692	22mm	Uncoated	Middle ⅓	Excellent	None	None	Excellent retention – pocket not present.
678	22mm	Uncoated	Inner/Middle ⅓	Fair	Minor	None	Pocket slightly inflamed.
689	22mm	Uncoated	Inner/Middle ⅓	Good	None	None	Good retention – pocket not present.
680	22mm	Uncoated	Middle ⅓	Good	Minor	None	Good retention – pocket slightly present.
677	32mm	Uncoated	Middle/Inner ⅓	Good	Mild	Mild	Retention is OK – but infected towards outer ⅓ (implant insertion area).
671	32mm	Uncoated	Middle/Inner ⅓	Good	Mild	Mild	Retention is OK – but infected towards outer ⅓ (near HGP implant).
681	32mm	Uncoated	Inner ⅓	Poor	Minor	None	Poor retention at base of the ear.
693	32mm	Uncoated	Inner ⅓	Poor	Minor	None	Poor retention at base of ear moving into cartilage ring.
690	32mm	Uncoated	Middle/Inner ⅓	Good	Minor	None	Pocket slightly inflamed.
673	32mm	Uncoated	Middle/Inner ⅓	Good	None	None	Good retention – device orientation changed slightly but is secured.
694	22mm	coated	Middle/Inner ⅓	Excellent	None	None	Excellent retention – pocket not present.
672	22mm	coated	Middle ⅓	Excellent	None	None	Excellent retention – pocket not present.
676	22mm	coated	Middle/Inner ⅓	Excellent	None	None	Excellent retention – pocket not present.
687	22mm	coated	Middle ⅓	Excellent	None	None	Excellent retention – pocket not present.
688	22mm	coated	Middle ⅓	Excellent	Minor	None	Excellent retention – pocket slightly present.
682	22mm	coated	Middle/Inner ⅓	Excellent	None	None	Excellent retention – pocket not present.
686	32mm	coated	Middle ⅓	Lost Device	Minor	None	Pocket slightly inflamed. Device lost due to pre-existing infection.
679	32mm	coated	Middle ⅓	Excellent	None	None	Excellent retention – pocket not present.
684	32mm	coated	Middle ⅓	Excellent	None	None	Excellent retention – pocket not present.
683	32mm	coated	Middle ⅓	Excellent	None	None	Excellent retention – pocket not present.
691	32mm	coated	Middle/Inner ⅓	Good	Minor	None	Pocket slightly inflamed.
685	32mm	coated	Middle/Inner ⅓	Good	Minor	None	Pocket slightly inflamed.

Appendix 13: Summary of previous cattle field trials with injectable devices

Table 22: Summary of previous cattle field trials with injectable devices

Document Reference	Injectable Devices Covered	Performance Testing?	Relevant Data	Other Notes
Klindtworth, M., Wendl, G., Klindtworth, K. and Pirkelmann, H. (1999). Electronic identification of cattle with injectable transponders. Computers and Electronics in Agriculture, 24(1-2), pp.65-79.	23 mm and 32 mm glass capsule transponders.	N/A	Failures were mostly causes by breakage of the glass capsule. It was "assumed" that these breakages were due to impacts of external forces.	This is a review paper of field studies carried out on cattle until 1998.
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(12), p.3001.	Glass encapsulated passive injectable transponders (23 mm and 32 mm sizes). The biocompatible glass was 0.33 mm thick.	No testing of transponder durability prior to implantation.	Broken transponders upon device recovery ~2% (it is unknown what caused these devices to break).	N/A
R.J. Fallon, P.A.M. Rogers, B. Earley. (2002) Electronic animal identification. Teagasc End of Project Report - Beef Production Series No. 46, pp.6-19.	Plastic capsule transponder (3.6 x 28 mm) and glass capsule transponders (3.6 x 28 mm & 2.8 x 19 mm)	No testing of transponder durability prior to implantation.	Failure rates of transponders ranged between 10 and 30% when recovered due to weakness of the transponder capsule.	Capsule fragility was apparent when the head often impacts with the floor post-stunning and within groups of bulls that experienced aggressive head-butting activities.
Loken, T., Vatn, G. and Kummen, E. (2011). Subcutaneous electronic identification in cattle: a field study. Veterinary Record, 169(10), pp.250-250.	Datamars polymer encapsulated transponders (2.16 x 13.9 mm) and glass encapsulated transponders (2.2 x 13.5 mm).	No testing of transponder durability prior to implantation.	Defective transponders at 20 months post-injection were 7.5% and 14.3% for polymer and glass devices respectively.	Post-mortem analysis found that a majority of the glass transponders were crushed/ physically damaged due to the feed barriers/pens the cattle were housed in.
Ribo, O., Korn, C., Maloni, U., Cropper, M., De Winne, P. and Cuypers, M. (2001). IDEA: a large-scale project on electronic identification of livestock. Revue Scientifique et Technique de l'OIE, 20(2), pp.427-436.	5 different injectable transponders tested (detailed list of devices is not publicly available, although it is known that some were glass-based). Devices IT1 and IT2 were 23 mm and 32mm respectively.	Tested devices for thermal, humidity, shock, vibration, free-drop and immersion stability prior to implantation. Only devices that passed these tests were implanted.	45% of the recovered tags that were tested and it was found that 0% were broken although on 52% of these were successfully read (no indication if the failed readings were due to broken devices).	True numbers of broken transponders are not known.