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Implantation of Temperature Loggers in 100 Danish Dairy Calves: Surgical Procedure and Follow-up

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Alban L, Chriél M, Tegtmeier C, Tjørnehøj K: Implantation of temperature loggers in 100 Danish dairy calves: surgical procedure and follow-up. Acta vet. Scand. 1999, 40, 75-83. – One hundred Danish dairy calves had temperature loggers implanted subcutaneously on the neck. Post-operatively, the calves were given a single antibiotic treatment, and tissue reactions were assessed on 6 post-operative visits. After approximately 5 months, the loggers were removed and material submitted for histologic examination. This paper presents 1) the surgical procedure, 2) the prevalence of tissue reaction at the post-operative visits, 3) the degree of implant recovery, 4) the results of histopathologic examinations, 5) an evaluation of age at implantation or veterinary practitioner as risk factors for tissue reaction and missing implant recovery 5 months after implantation, and 6) evaluation of tissue reaction as a risk factor for lack of recovery 5 months after implantation. The implant was rejected on 7 calves (7%). Additionally, 5 calves (5%) had the temperature logger removed because of presence of an abcess. No migration of the temperature loggers were observed. The results of a repeated measures analysis and the histopathological findings indicate that contamination during the surgery resulted in inflammation and abcess formation. It is recommended that in the presence of an abcess, the temperature logger should be removed.

telemetry; monitoring; histopathology; repeated measures.

Introduction

Implanted temperature loggers have been used for estrous detection (*Clapper et al.* 1990a, 1990b), and hereby, one day temperature loggers may be of value in the proces of the farmer's decision making. Today, temperature loggers are available for a low price (200$ a piece) which makes them economical applicable to use in observational studies, e.g. to monitor disease spreading. The tissue effect is usually low and the implant recovery high after implantation with small, injectable, electronic devices for identification, see e.g. *Dorn* (1987), *Lambooy & Merks* (1989), *Pirkelmann et al.* (1991), *Wade et al.* (1991), *Janssens et al.* (1996), but less is known of the effect of implantation with the larger temperature loggers.

In a prospective, longitudinal study on enzootic pneumonia in calves, it was intented to implant temperature loggers to monitor variations in body temperature. The StowAway® TidBit™ temperature logger (*Onset Computer Corporation* 1996) was chosen, since it has the ability to measure and store information without additional equipment. It consists of a battery, a memory, and a thermistor. The size is 30 mm×41mm×17mm, it weighs 23 g and is embedded in non-reactive epoxy (Fig. 1). Since little was known about the sequelae to implanta-

Figure 1. The StowAway® TidBit™ temperature logger, sized 30 mm×41 mm×17 mm, weight 23g.

tion, carefull post-operative monitorings were planned. The anticipated problems were: breakage or migration of the logger, difficulties with recovery or expulsion because of foreign body reaction either due to the logger per se or to contamination during surgery. The aim of this part of the study was to investigate the host response following implantation as well as the degree of recovery 5 months after.

This paper presents: 1) the surgical procedure, 2) the prevalence of tissue reaction at the post-operative visits, 3) the degree of implant recovery, 4) the results of histopathologic examinations, 5) evaluation of age at implantation or veterinary practitioner as risk factors for tissue reaction and missing implant recovery 5 months after implantation, and 6) an evaluation of tissue reaction as a risk factor for lack of recovery 5 months after implantation.

Materials and methods

Sterilisation procedure

Each temperature logger was packed seperately and formaldehyde autoclavated involving a 20 min 40% formaldehyde vapour bath followed by 18 rinses in water vapour, all at one atmospheric pressure and 80 °C (*Ellebæk* pers. comm. 1996).

Implantation location and surgical procedure

The temperature logger was placed subcutaneously on the center of the right side of the calf's neck. The operation was carried out in the calf's own box, where the calf was sedated by use of xylazin hydrochloride 2% (Rompun Vet.®, Bayer – 0.25-1.0 ml per 100 kg, intramuscular). The skin was shaved, and analgesia was obtained by infiltration of 10 ml of 2% lidokain (Skanderborg Pharmacy, Denmark), followed by desinfection by use of iodine and alcohol. A 6 cm long incision was made, and the subcutaneous tissue was split by use of a blunt pair of scissors. The temperature logger was inserted, and the skin was closed by 2 or 3 Wolf's interrupted mattress sutures (*Berge & Westhues* 1984). Finally, each calf was given a single injection with penethamate hydroiodide (Leocillin vet.®, Leo – 1 million IU per 50 kg), in the muscle.

Selection of animals

Two veterinary practitioners participated in the study, each with 5 selected dairy farmers. In December 1996, ten calves from each herd between 5 and 66 days old (mean = 34, SD = 19), had a temperature logger implanted. Four herds had less than 10 calves of the desired age, therefore, as calves were born into the herds, they were included in the study.

Post-operative visits and recovery

Six post-operative visits were carried out. The farms were visited on day 2-3 (visit 1), 5-6 (visit 2), 7-10 (visit 3), 14-19 (visit 4), 20-33 (visit 5), 38-92 (visit 6). On each occasion, all calves were individually inspected and palpated for symptoms of inflammation.

Preparation for histopathology

The temperature loggers were removed 5 months after the implantation. At this time, a biopsy (approximately 15 mm×5 mm×10-20 mm) was extirpated from the implant site of all calves with macroscopic lesions (tissue reaction >40mm) as well as from 10 randomly selected calves with apparently normal implant sites. The biopsy consisted of tissue from the skin surface down to and including the wall of the cyst surrounding the implant.

After fixation for one week in 10% buffered, neutral formalin, each block of tissue was trimmed into one longitudinally and 2 transversally orientated specimens. Each specimen was embedded in paraffin wax, cut at 3 to 4 μm and mounted on Super Frost slides (Hounisen, Denmark) according to standard laboratory procedures. All sections were stained with haematoxylin and eosin (HE) for a routine histopathological evaluation. Selected slides were stained with Van Gieson-Hansen's stain for connective tissue, and Luna's stain for eosinophils.

Statistical treatment of data

To evaluate 1) age at implantation and 2) veterinary practitioner as risk factors for missing implant recovery, unconditional maximum likelihood logistic regression analysis (*Hosmer & Lemeshow* 1989) was performed by use of PROC CATMOD in SAS (*SAS Institute Inc.* 1989), based on $p<0.05$. Because of a limited number of observations, only simple models were run. To account for overdispersion due to the potential clustering effect of herd, the models were run as random-effects logistic regression models by use of EGRET (*Statistics and Epidemiology Research Corporation* 1993).

The repeated structure of the data made it possible to model the tissue reaction as a result of time (0-6) for each calf by use of PROC REG in SAS (*SAS Institute Inc.* 1989). Tissue reaction at time zero was set to 40, since an implanted logger measured 40 mm in itself. Equidistant time intervals were used. One hundred models were estimated, explaining the individual course of tissue reaction. Since there was only one explanatory variable included (time), the models had an intercept and a slope only. It was hypothesized that these models – based on differences in slope – could be divided into 4 groups: 1) calves without tissue reactions, 2) calves which died during the investigation, 3) calves where the loggers had to be taken out because of fear of loosing the logger, and 4) calves which lost the logger.

This was tested in a model where intercept and slope were the responses and group the explanatory variable. The statistical model was formulated as:

$$\text{intercept slope} = \mu + \text{group}_{(1,2,3,4)} + \varepsilon_{ij}$$

where

$$\varepsilon_{ij} \sim N_2 (0,\Sigma)$$

A MANOVA-test with canonical correlations was used (*Sharma* 1996). This was supplemented by a univariate analysis of intercept and slope (PROC GLM in SAS). Finally, least squares means were calculated and compared statistically.

Results

Post-operative tissue reaction

The prevalence of tissue reaction at each of the post-operative visits is presented in Table 1. The prevalence was highest (42%) in the first

Table 1. Tissue reactions at 6 post-operative visits in 100 Danish dairy calves which had a temperature logger implanted.

Number of days after implantation (AI)	Number of calves examined	Prevalence of tissue reactions (%)	Days AI calf died	Days AI logger lost	Days AI logger removed	Average tissue reaction* (mm)	Tissue reaction* min-max (mm)
2- 3 (visit 1)	99	42				48.2	41-70
5- 6 (visit 2)	93	24				48.2	41-80
7-10 (visit 3)	100	7	14			54.1	42-90
14-18 (visit 4)	98	9	15,19,			58.0	41-90
20-33 (visit 5)	97	10	32			59.3	41-80
35-92 (visit 6)	92	10	46	37,43,52, 80,80,80, 115	66,75,97 103,106	54.4	45-80

AI: After implantation. *: only measured on calves with tissue reaction (>40mm).

Table 2. Tissue reactions in 12 calves which either lost their implant or had it taken out due to the presence of an abcess.

Calf	Farmer	Visit 1 (mm)	Visit 2 (mm)	Visit 3 (mm)	Visit 4 (mm)	Visit 5 (mm)	Visit 6 (mm)	Event and days after implantation (a.i.)
1	1	50	48	40	40	40	50	Observed lost 80 days a.i.
2	1	40	38	39	46	40	50	Observed lost 80 days a.i.
3	2	45	37	35	38	37	40	Observed lost 115 days a.i.
4	2	55	47	42	41	41	60	Observed lost 52 days a.i.
5	3	45	38	40	45	50	lost	Observed lost 80 days a.i.
6	4	40	40	40	40	60	80	Removed 97 days a.i. due to risk of bursted abcess
7	4	40	40	40	90	60	lost	Observed lost 43 days a.i.
8	5	40	40	40	50	60	lost	Observed lost 37 days a.i.
9	6	70	80	55	60	80	45	Removed 66 days a.i. due to risk of bursting abcess
10	7	45	60	90	80	80	50	Removed 106 days a.i. due to risk of bursting abcess
11	7	35	40	40	50	60	50	Removed 103 days a.i. due to risk of bursting abcess
12	7	46	*	40	40	40	60	Abcess bursted, logger saved, at 6th visit, 75 days a. i.

*: Visit was not performed. The correspondence between days after implantation and number of visit can be seen in Table 1.

days after implantation and lowest (7%) at the third visit, which was carried out on day 7-10. The number of calves which were inspected on the 6 visits varied from 92 to 100. The variation occurred partly because some calves died or lost their temperature logger during the first 3 months (Table 1), and partly because a few of the post-operative visits to calves which did not have the temperature logger implanted at the beginning of the study were not carried out.

Mortality

Five calves, originating from 2 herds, died during the study, all with clinical signs of diarrhoea or pneumonia.

Implants lost or removed

Seven calves lost their implant within the first 3 months, and on 5 calves the loggers were removed between day 66-106 to avoid that the abcesses would rupture. Material from one

abcess was cultivated and Actinomyces pyogenes was isolated. All lesions healed up completely, only leaving an area of indured, fibrous tissue, sized 40 mm×10 mm.

Calves with tissue reaction

Among the calves with tissue reaction, the reaction varied from 41 mm to 90 mm (Table 1). The average reaction was largest (59.3 mm) at the fifth visit (day 20-33), and smallest (48.2 mm) at the 2 first visits (day 2-3 and day 5-6). The degree of tissue reaction at each of the 6 post-operative visits is presented for the 12 calves which either lost their temperature logger or had it removed before the end of the observation period (Table 2). Tissue reaction at the first visit may have been a reaction to the operation more than an infectious reaction, hence, focus should be on the later visits. Thus, it is noted that on 6 calves (No. 2, 5, 6, 7, 8, 11), the tissue reaction began after the third visit, whereas only 3 calves (No. 4, 9, 10) presented a tissue reaction at all 6 visits.

Recovery and histopathology

At recovery, a thin capsule of fibrous connective tissue was observed, surrounding the logger in all calves. No migration of loggers was observed at all. The histopathologic results are presented in a general description, since the similarities between specimens were larger than the differences between specimens.

A rim of fibrous connective tissue of variable thickness was present above the cyst wall. The cyst wall consisted of a narrrow zone of mononuclear cells primarily, but neutrophils and eosinophils were occasionally observed as well. The zone of cells was continuous in most cases. However, in some cases the distinct cyst wall was not detectable occasionally, instead a sero-haemorrhagic exudate was seen. Some of the specimens contained a few granulomas, which in most cases contained a foreign material visible at either traditionally light microscopy, or observable in polarized light. The foreign material consisted of hair particles or small remnants of suture material. In the majority of cases, a slight eosinophilia, mainly perivascularlar, was observed. Fig. 2 presents the microscopic section of the tissue reaction of a typical calf.

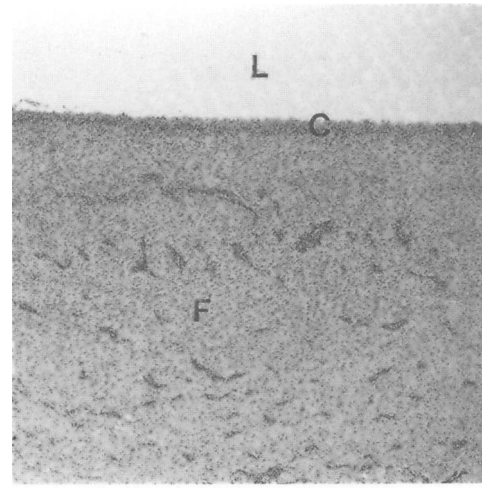

Figure 2. Microscopic section of tissue reaction associated with 5 months of implantation with a temperature logger embedded in a non-reactive epoxy (stained with haematoxylin and eosin; obj×2.5). C = cyst wall, F = fibrous connective tissue, L = cyst cavity containing the im plant in vivo.

Risk factors for missing recovery and repeated measures analysis

Age in days at implantation (5-66 days) was not a risk factor for missing implant recovery, there was no variation between surgeons, and there was no herd effect.

The results of the repeated measures analysis indicated that there was no difference in intercept between the 4 groups ($p = 0.17$), but a substantial difference in slopes ($p<0.001$). This parsimonous model described 51% of the variation in the data. The model fit can be inspected

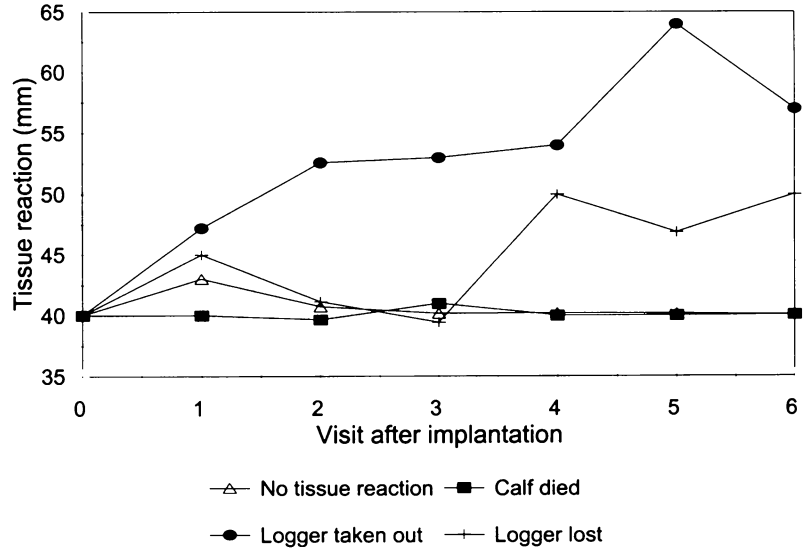

Figure 3. Average tissue reaction at the day of surgery and on six postoperative visits for 100 Danish dairy calves who had a temperature logger implanted for 5 months. The calves were divided in 4 groups depending on the course of implantation.

by looking at Fig. 3, which presents the average tissue reaction for each group at each time interval. The hypothesis of no overall difference in intercept and slope between groups was rejected (Wilk's Lambda $p<0.001$). The first canonical variate described 97% of the variance attributed to the eigenvalues, and the majority of the loading came from the slope and only to a minor degree from the intercept. The second canonical variate was not significant ($p = 0.09$). Hence, the results can be presented by a line. The least square means were calculated for the intercepts and slopes for each of the 4 groups and compared statistically (Table 3). The least square means of the intercept varied from 39.23 to 43.33 and there was no difference between the 4 groups, apart from a minor difference between group 3 and four. Calves which had the logger taken out (group 3) had the highest slope (3.07) and differed significantly from the 3 other groups. The calves which lost the logger (group 4) had the second highest slope (2.02) and differed significantly from the other 3 groups. Finally, tissue reaction from group one (calves without reaction) and group 2 (calves which died) could be described by one line with no slope.

Discussion

Implantation location

The aim of the logger study was to measure the temperature of the calf hourly. Hence, it was important that the logger was placed in such a way that there was a reasonable correlation between the registrered temperature and the body temperature. It was believed that this would occur by placing the temperature logger subcutaneously on the mid section of the right side of the neck. Furthermore, this place was considered to have the smallest impact on the calf's welfare, and the logger would be easy to implant and recover. This is in accordance with *Dorn* (1987) who recommends to place an implant in calves, 10 cm proximal to the scapula.

Table 3. Least squares means (lsmean) from a repeated measures analysis of course of implantation and tissue reaction at the six post-operative visits on 100 dairy calves.

Course (group)	Intercept (lsmean)	Statistical difference: Pr<|T|, H_0: $lsmean_i = lsmean_j$				
		i\j	1	2	3	4
(1) No tissue reaction	41.27	1	–	0.45	0.18	0.12
(2) Calf died	40.10	2	–	–	0.13	0.66
(3) Logger taken out	43.33	3	–	–	–	0.04
(4) Logger lost	39.23	4	–	–	–	–
Course (group)	Slope (lsmean)	Statistical difference: Pr<|T|, H_0: $lsmean_i = lsmean_j$				
		i\j	1	2	3	4
(1) No tissue reaction	–0.22	1	–	0.56	0.0001	0.0001
(2) Calf died	0.02	2	–	–	0.0001	0.0002
(3) Logger taken out	3.07	3	–	–	–	0.0465
(4) Logger lost	2.02	4	–	–	–	–

Contrary to this, *Fallon & Rogers* (1991) reported that they had problems at slaugther in recovering electronic transponders implanted at this position. The implants used for the present study are larger than electronic transponders. Therefore, it was believed that they would be easily found. In pigs, it is recommended to use the base of the ear when injecting an implant sized 29 mm×3.6 mm (*Lambooy & Merks* 1989, *Lambooij et al.* 1992). For the present study, the ear base was not found as a suitable place, as it was feared, that the calf would damage itself because of the construction of the pen.

Risk factors for missing implant recovery and repeated measures analysis

Age at implantation was no risk factor for missing implant recovery. This implies, that it is safe to implant even on very young calves.

The overall recovery was 83/95 = 87%. In a similar study by *Janssens et al.* (1996) 90% of the implants were recovered. The higher recovery in the latter study might be attributed to the implantation procedure in question, since Jannsen et al. were able to inject the smaller implants – a method involving less contamination of the wound than the method needed for the larger loggers.

The same kind of temperature logger as used in this study was used in a previous study on 8 crocodiles (*Anon.* 1994). In that study, there was no abcess formation. According to the researcher this was probably due to better aseptic conditions (*Lang* pers. comm. 1997). It can be speculated whether repeated antibiotic treatments given during the first days after implantation could have reduced the incidence of inflammation.

There was no migration of the loggers. Probably, this is related to the temperature logger's large size and round shape. The thin capsule of connective tissue which was noted in all animals at recovery, may explain the lack of migration. The thin capsule was also seen by *Janssens et al.* (1996).

Tissue reaction as a risk factor for loosing the implant

Five temperature loggers were removed after the sixth visit. Ideally, they should have been left in, but since the loggers contained valuable

information for the next part of the study, it was judged necessary to take them out. In one case (Table 2, calf No. 4) it was decided to remove the logger. However, when the veterinary practitioner came to the herd a couple of days later, he discovered that the logger had been expelled. The results of the repeated measures analysis showed that the tissue reaction of the calves from group 3 (which had the logger taken out) and group 4 (the calves which lost the logger) could be modelled as a line with a positive slope, while the tissue reaction of the calves from group 1 (no tissue reaction) and 2 (calves which died during the investigation) could be modelled with a line with no slope. A possible explanation is that if there was a lack of aseptic conditions at the time of surgery, an infection could occur which would result in a continued inflammatory process which would stop only when the implanted material was expelled from the body.

The effect of tissue reaction on missing implant recovery was also seen by *Jannsens et al.* (1996). In their study on 299 pigs it was found that when severe tissue reaction had occurred, the chance of non-retention was 51 times higher than the chance of retention.

Histopathology

The histopathological findings in the present study, i.e. the formation of connective tissue around the implant, the inflammatory response at the implant site, and the occasional exudation and haemorrhage into the cystic cavity containing the implant, are interpreted as evidence of a reparatory process, and are in accordance with observations in a former study performed in pigs (*Lambooij et al.* 1992). The occasional granulomas are presumably due to small remnants of suture material or contamination during surgery with hair particles.

Conclusions

There was no migration or breakage of the temperature loggers. Because of this and the relatively large size of the loggers, they were easily detected. There was a high overall recovery. The risk of missing implant recovery was not associated with the age of the calf at implantation or the veterinary practitioner.

The results of a repeated measures analysis and the histopathological findings indicate that contamination during the surgery resulted in abcess formation in some cases. It is recommended that in the presence of an abcess, the temperature logger should be removed.

Epilogue

When data was read out from the temperature loggers, it was noted, that they had functioned properly for one to 2 months only. Our hypothesis is that the temperature logger gradually gets destroyed by severe bumps, arising when the growing calf moves around in the box.

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Sammendrag

Implantation af temperaturloggere i 100 danske kalve: Operativ procedure og opfølgning.

Artiklen omhandler implantation af temperaturloggere subkutant på halsen af 100 danske kalve. Postoperativt fik kalvene en enkelt antibiotikum-behandling. Vævsreaktioner blev vurderet på 6 efterfølgende besøg. Efter cirka 5 måneder blev loggerne fjernet, og materiale blev udtaget til histologisk undersøgelse. Artiklen præsenterer 1) den operative procedure, 2) prævalensen af vævsreaktion ved de postoperative besøg, 3) den procentuelle genfinding af loggerne, 4) resultater af histopatologiske undersøgelser, 5) evaluering af alder ved implantation og den praktiserende dyrlæge som risikofaktorer for vævsreaktion og manglende genfinding fem måneder efter implantation, og 6) evaluering af vævsreaktion som risikofaktor for manglende genfinding 5 måneder efter implantation. Loggeren blev afstødt på 7 kalve (7%) og på grund af en abcess fjernet fra 5 kalve (5%). Migration af loggerne blev ikke observeret. Resultatet af en repeated measures analyse samt de histopatologiske resultater indikerer, at kontaminering under implantationen formentlig resulterede i inflammation og abcessdannelse. Det anbefales at fjerne loggeren i tilfælde af en abcess.

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