Wildlife Society Bulletin; DOI: 10.1002/wsb.857

Tools and Technology

Vaginal Implant Transmitters for Continuous Body Temperature Measurement in Moose

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ABSTRACT Measuring body temperature in free-ranging ungulates is challenging. We evaluated a vaginal implant transmitter (TVIT) modified to collect continuous body temperature of captive and wild female moose (*Alces alces*) in Alaska, USA. We deployed TVITs in 18 moose between 2014 and 2016. We manually removed the TVIT after 51–338 days of deployment and sampled vaginal bacterial flora to assess negative effects of TVIT retention. For comparison, we also sampled vaginal flora from moose that did not have a TVIT. Mean bacterial growth scores were greater for moose with a TVIT than representative vaginal swabs from moose without a TVIT. The TVIT adequately collected body temperature measurements; however, the TVIT design could be improved to fit young, nulliparous moose. TVITs can be easily deployed and removed, but are limited by battery life, can only be deployed in adult female moose, and may increase vaginal bacterial concentrations. © 2018 The Wildlife Society.

KEY WORDS Alaska, *Alces alces*, body temperature, moose, vaginal implant transmitter.

Wildlife can respond to changes in daily and seasonal temperature through a combination of physiological, behavioral, and biochemical responses (Cain et al. 2006, Barboza et al. 2009). Physiological responses such as sweating, changes in respiration rate, or changes in deep body temperature can be used to assess when ungulates become heat- or cold-stressed (Parker and Robbins 1984, Renecker and Hudson 1986, Ostrowski and Williams 2006, Lust et al. 2007, Hetem et al. 2012). Measuring body temperature of wild and captive ungulates is challenging and previously used methods have their limitations. Rectal temperature has been recorded while animals are anesthetized or restrained, but cannot be recorded after release (Franzmann 1972, Parker and Robbins 1984, Renecker and Hudson 1986, Rostal et al. 2012, Brivio et al. 2015). Surgical implants into the abdominal cavity have gathered valuable data on body temperature; however, data must be transmitted constantly via radio transmission to a receiver (Sargeant et al. 1994), or data stored within the temperature logger must be retrieved by surgery, which can pose risks to animals' health and is difficult to do on large wild ungulates, or by euthanasia (Fuller et al. 2005, Lust et al. 2007, Hébert et al. 2008, Hetem et al. 2010, Shrestha et al. 2012). Ruminal transmitter units can record continuous body temperature and do not require surgery (Signer et al. 2010, Turbill et al. 2011), but diet-induced thermogenesis and drinking events influence rumen temperature (Lawler and White 2003, Crater and Barboza 2007). More recently, a device engineered for controlled internal drug release (CIDR) has been modified as a vaginal implant that can record short-term continuous body temperature in domestic cattle (*Bos taurus*; Kendall and Webster 2009, Vickers et al. 2010, Burfeind et al. 2011, Burdick et al. 2012, de Andrade Ferrazza et al. 2017) and domestic dogs (*Canis lupus familiaris*; Maeder et al. 2012, Geiser et al. 2014).

The CIDR platform, modified as a vaginal implant transmitter (VIT; Bowman and Jacobson 1998), has been used in wild ungulates to determine time of parturition and parturition site location, and aid in capturing neonates (Carstensen et al. 2003, Bishop et al. 2007, Barbknecht et al. 2011, Gilbert et al. 2014, Kaze et al. 2016). Typically, VITs are deployed in late winter or early spring and retained in

Received: 21 August 2017; Accepted: 18 November 2017

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the animal for 2–4 months until expelled at parturition (Carstensen et al. 2003, Barbknecht et al. 2009, Bishop et al. 2011, Gilbert et al. 2014). However, VITs deployed in nonpregnant animals are generally not expelled and must be manually removed at recapture, or they will be expelled at parturition if the animal is successfully bred (Barbknecht et al. 2009, Patterson et al. 2013).

We modified a VIT by adding a temperature logger and assessed its effectiveness at successfully collecting long-term, continuous body-temperature measurements in both wild and captive moose (*Alces alces*). We validated data collected by temperature loggers, and identified limitations of body temperature collected on a VIT platform. Lastly, we compared the suite of vaginal bacterial found in moose carrying a VIT for >51 days (min. length of VIT deployment) with vaginal bacteria of moose without a VIT for ≥28 days (to allow for vaginal flora concentrations to adjust without the presence of a VIT) to assess negative effects of VIT retention.

STUDY AREA

We studied both wild and captive moose on the western Kenai Peninsula, Alaska, USA, within Alaska Department of Fish and Game (ADFG) Game Management Unit 15. Captive moose were held at the Kenai Moose Research Center (MRC), operated by ADFG on the Kenai National Wildlife Refuge. The 10.4-km^2 MRC facility was established in the 1960s to study interrelationships between moose and their environment (Hundertmark et al. 2000). Captive moose remained in outdoor enclosures of approximately 2.6 km^2 in size that contained a mix of successional states of boreal forest, bogs, and lakes, similar to wild moose habitat in this study.

METHODS

Animal Handling

We immobilized captive, female moose (≥2 yr old; $n = 12$) by intramuscular hand-injection with a mixture of 0.45 mg Carfentanil citrate (3 mg/mL; ZooPharm, Windsor, CO, USA) and 25 mg Xylazine HCl (100 mg/mL; Lloyd Laboratories, Shenandoah, IA, USA). We reversed captive moose with 400 mg Tolazoline HCl (100 mg intravenous, 300 mg intramuscular; 200 mg/mL; ZooPharm) and 100 mg Naltrexone HCl (intramuscular; 50 mg/mL; ZooPharm). We immobilized wild female moose (≥2 yr old; $n = 41$) with a mixture of 4.2 mg Carfentanil citrate and 100 mg Xylazine HCl administered via a 3-cc dart fired from a rifle (Palmer Cap-Chur^{TM}, Douglasville, GA, USA) out of a helicopter (Robinson R-44 [Robinson Helicopter Company, Torrance, CA, USA] or Hughes 500 [Bell Helicopter, Fort Worth, TX, USA]). We reversed wild moose with 400 mg Tolazoline HCl (100 mg intravenous, 300 mg intramuscular) and 450 mg Naltrexone HCl (intramuscular). All procedures for care, handling, and experimentation were approved by the Animal Care and Use Committee, ADFG, Division of Wildlife Conservation (protocol #09-29, protocol #2013-21, and protocol #2014-17) and Texas A&M AgriLife Research Agricultural Animal Care and Use Committee (AUP# 2016-008A).

Temperature Vaginal Implant Transmitters

We modified a VIT (Model M3970; Advanced Telemetry Systems, Isanti, MN, USA) by incorporating a temperature and activity logger (ARChive ARC400; accuracy 0.5° C, resolution 0.25° C; Advanced Telemetry Systems) to record vaginal temperature at 5-min increments. The temperature vaginal implant transmitter (TVIT) also had a very-high-frequency (VHF) radio signal to allow us to determine whether an animal had retained the TVIT after deployment; the pulse rate of the VHF beacon doubled if the TVIT was exposed to ambient temperatures below normal body temperature. The ARChive tag within the TVIT could store 300,000 data points; however, it was limited by battery life to approximately 370 days when collecting temperature readings at 5-min intervals. We cold-sterilized all TVITs in 2% chlorhexidine diacetate solution (Nolvasan Solution; Fort Dodge Animal Health, Fort Dodge, IA, USA), and inserted them into the vagina up to the cervix with a lubricated (OB Lube; Jorgensen Laboratories Inc., Loveland, CO, USA), sterilized speculum (Sterile Disposable Vaginal Speculum; Jorgensen Laboratories Inc.; Patterson et al. 2013). Temperature data were stored onboard the TVIT, and downloaded via a wireless infrared interface after TVIT retrieval.

We deployed 7 TVITs in wild moose for 65–338 days. Sample size of TVITs for wild, nonpregnant moose was restricted due to a high pregnancy rate in our wild population, resulting in low numbers of nonpregnant moose that retained a TVIT from spring through autumn, which allowed us to manually remove the TVIT and collect samples during autumn captures. We deployed 6 TVITs in late November and December 2014. We manually removed 2 TVITs in March 2015 when we recaptured 2 moose. The remaining 4 wild moose were nonpregnant and retained their TVIT until we manually removed the TVIT following capture in November 2015. We deployed one additional TVIT in a nonpregnant wild moose in March 2016 (the same individual had a TVIT deployed in 2015), and it remained in the moose until manual removal in November 2016. We deployed TVITs in 12 captive moose over a 2-year span between 2014 and 2016, with some animals having TVITs deployed more than one time. We manually removed TVITs after varying lengths of deployment ranging from 51 to 245 days, with ≥28 days between deployments in any one animal. We measured the length of the TVIT antenna extending past the vulva in immobilized captive moose of known age and reproductive history to determine the depth of TVIT insertion into the vaginal canal (Table 1). We used depth of TVIT insertion to evaluate if young animals had a shallower vaginal cavity that may influence temperature recorded on the TVIT.

Temperature Validation

We used a water bath to validate the temperature logger on the TVIT (Signer et al. 2010, Vickers et al. 2010). We placed 14 TVITs, collecting temperature at 5-min intervals, into a

Table 1. Depth of insertion (cm) into the vaginal canal of a modified vaginal implant transmitter, and depth to the tip (cm) of the modified vaginal implant transmitter body from the vulva. Modified vaginal implant transmitters were deployed in April, 2015 to record continuous body temperature in captive moose of known age and reproductive status at the Kenai Moose Research Center, Alaska, USA.

Animal	Age (yr)	Depth of insertion (cm)	Depth to transmitter (cm)	Reproductive status
4014	3	14.5	3	N[a]
4001	3	15.5	4	N[a]
4003	3	15.5	4	N[a]
4012	3	16.5	5	N[a]
4011	3	17.5	6	N[a]
4007	7	17.5	6	P[b]
4010	6	18.5	7	P[b]
4006	12	19.5	8	M[c]
4008	13	19.5	8	M[c]
4013	6	20.5	9	P[b]

[a] Nulliparous.
[b] Primiparous.
[c] Multiparous.

water bath (Model WB28; VWR International, LLC, Radnor, PA, USA) with 18 L water preheated to 37° C. We measured the water bath temperature with National Institute of Standards and Technology–certified thermometer (Traceable TM Platinum Ultra-Accurate Digital Thermometer; accuracy ± 0.05° C, resolution ± 0.001° C; Fisher Scientific, LLC, Pittsburgh, PA, USA). Once the water temperature was stable, we recorded water temperature at 5-min intervals for 30 min, at which time we increased the water temperature by 0.5° C. We followed this procedure from 37° C to 40° C.

Vaginal Bacteria Sample

We collected vaginal bacterial flora with a sterile 15.2-cm-long swab, placed into an Amies gel with or without charcoal after sampling (BBL™ CultureSwab™ Plus, Becton, Dickinson and Company, Sparks, MD, USA; Starplex™ Scientific Starswab II™, Cleveland, TN, USA). We collected vaginal swabs from wild moose during March and November captures and in captive moose in March, April, August, October, and December. Number of moose sampled (n_m) and number of swabs collected (n_s) were different because some moose were sampled more than once at different times of the year. For moose that did not have a TVIT or VIT prior to immobilization (wild ≥142 days without VIT; captive ≥28 days without VIT), we sampled vaginal bacterial flora by opening the vulva with a gloved hand, and inserted the swab into the vagina up to the base of the swab cap (wild $n_m = 34$, $n_s = 40$; captive $n_m = 12$, $n_s = 52$). The inserted swab stem was then rotated several times, then we removed the swab and placed it into the gel medium. In moose that had a TVIT manually removed (during immobilization or without sedation in captive moose), we carefully removed the TVIT from the moose and then swabbed the base and wings of the TVIT (wild $n_m = 10$, $n_s = 11$; captive $n_m = 12$, $n_s = 29$). Captive moose were not sedated when we manually removed the TVIT; therefore, we could not safely swab the vaginal canal, and relied upon swabbing the TVIT body and wings. To be consistent, we also swabbed the TVIT after it was manually removed from sedated, wild moose. Sample size of swabbed TVITs also includes opportunistic swab samples taken from 4 non-temperature VITs that were deployed in wild moose in March and manually removed in November from another project on the same moose population (T. J. McDonough, unpublished data). When we manually removed a TVIT, we noted any vaginal discharge and assigned a vaginal mucus score based on discharge color and volume adapted from Williams et al. (2005). We kept vaginal swabs samples cool and froze them within 8 hr of collection. We shipped frozen samples to Colorado State University, Veterinary Diagnostic Laboratories (Fort Collins, CO, USA) for aerobic bacterial culture and identification. After 48 hr of incubation, lab staff assigned bacterial scores based on 5 levels: no growth (0), very light growth 1), light growth 2), moderate growth 3), and heavy growth 4). They identified bacteria to a minimum of genus, with some samples identified to species. We assigned bacteria species into 3 pathogenic potential categories based on the association with endometritis in domestic cattle (Category 1—bacteria that cause endometritis; Category 2—bacteria that rarely cause endometritis; Category 3—bacteria that do not cause endometritis; Sheldon et al. 2002, Williams et al. 2005); we assigned any bacteria not previously identified in domestic cattle vaginal flora to Category 3 (not recognized as a uterine pathogen).

Data Analyses

Depth of insertion of the TVIT to the anterior vagina was determined by subtracting the length of the exposed antenna from the vulva from the overall TVIT length. Depth to the distal end of the transmitter body was the difference between total and exposed antenna lengths. We analyzed bacterial and water bath data using programs in STATA version 14.0 (StataCorp LP, College Station, TX, USA). To validate the TVIT temperature in the water bath for biologically relevant temperatures between 37° C and 40° C, we used a mixed model with TVIT temperature as the response variable and water bath temperature as the explanatory variable, including individual TVIT as a random effect. We used mixed-effects linear regression to assess the effect of bacterial growth scores between vaginal swabs and swabs of vaginal temperature loggers. The model incorporated the covariates swab type (bacterial swab of the vagina from moose without a TVIT; bacteria swab of a manually removed TVIT), swab collection Julian day, and days without a TVIT, with individual as a random factor to account for repeated measures within individuals, assessing by each pathogenic potential category. We also assessed the effect of bacterial growth scores among differing vaginal mucus scores for manually removed TVITs. This model incorporated the covariates vaginal mucus score, swab collection Julian date, and number of days the TVIT was deployed, with individual as a random factor to account for repeated measures within individuals. We used the robust Huber–White sandwich estimator to relax assumptions of normal distribution and homogeneity of variance for regression (Rabe-Hesketh and Skrondal 2010). We compared model coefficients with zero using a z-test at $P < 0.05$.

If model coefficients were not significant, we dropped the variable with the highest *P*-value and reran the model.

RESULTS

Temperature Vaginal Implant Transmitters

We collected >1.5 million vaginal-temperature data points in 6 wild moose and 12 captive moose over a 2-year span. All TVITs were retained until manually removed. One TVIT stopped transmitting a VHF signal after 4 months of deployment, but was still collecting temperature data until it was removed. The battery failed on a second TVIT after it was deployed for 233 days; however, data were logged and recovered from the TVIT up to date of battery failure. The temperature logger incorporated into the VIT documented fluctuations in body temperature in moose both weekly (Fig. 1A) and daily (Fig. 1B). Overall, 95.2% of the body temperature data were >37° C, with a maximum record of 41.25° C. Four individuals comprised 99% of the temperature data below 37° C, ranging from 14° C to 36.75° C. Depth of insertion and depth to the distal end of the transmitting body of the TVIT increased by age and reproductive status (Table 1).

Temperature Validation

The model evaluating the TVIT temperature in the water bath, against the water bath temperature, was significant (Wald $\chi^2 = 30{,}079.33$, $P < 0.01$) and there was a significant difference between TVIT temperature and water bath temperature ($z = 175.44$, $P < 0.01$). The predicted TVIT temperature from the mixed model regression was 0.38° C ± 0.1 (SE) warmer than the water bath; however, this is within the accuracy of the temperature logger (±0.5° C). Furthermore, the 95% confidence intervals for the slope include 1 (0.999, 1.021), and the 95% confidence intervals for the constant include 0 (−0.502, 0.466).

Vaginal Bacteria Samples

We collected 132 bacterial swab samples from 53 individual moose. Bacterial growth was not detectable on 9 swabs after culture (8 vaginal swabs and 1 TVIT swab), while the remaining 123 swabs produced 12 different genera of bacteria and 1 fungus (*Candida* spp.; Table 2). Eight bacteria species occurred on both non-VIT moose vaginal swabs and swabs from TVITs deployed in moose (Table 2). For pathogenic potential Category 1, the model assessing bacteria growth score was significant (Wald $\chi^2 = 21.06$, $P < 0.01$), with greater bacterial growth scores from swabs collected from TVITs (Fig. 2A; $z = 2.77$, $P < 0.01$) and bacterial growth increased with Julian day into autumn ($z = 2.66$, $P < 0.01$), but not by days without a TVIT ($z = 1.15$, $P = 0.25$). The full model assessing bacteria growth score for pathogenic potential Category 2 (Wald $\chi^2 = 7.35$, $P = 0.06$) was not statistically significant; however, a simpler model with only the variable swab type ($z = 2.57$, $P = 0.01$) was significant, with greater bacteria growth scores from TVIT swabs (Fig. 2A; Wald $\chi^2 = 6.60$, $P = 0.01$). The model assessing bacteria growth for pathogenic Category 3 (Wald $\chi^2 = 2.13$, $P = 0.55$) and all variables were not significant (Fig. 2A). Of the 40 swabs collected from TVITs, vaginal mucus scores were predominantly in Category 0 (25%), and 1 (57.5%), with the remaining swabs in Category 2 (10%) and 3 (7.5%). The model assessing vaginal mucus score, swab collection date, and number of days the TVIT was deployed on bacterial growth (Fig. 2B; Wald $\chi^2 = 3.66$, $P = 0.60$), including all the variables, was not significant.

DISCUSSION

Our modified vaginal implant transmitters (VITs) were successful in collecting continuous body-temperature measurement in adult female moose. These continuous temperature loggers revealed changes in body temperature in moose similar to surgical implants observed in other ungulates (Fuller et al. 2005, Hetem et al. 2010, Shrestha et al. 2012). In 4 moose (3 captive and 1 wild), TVITs collected data that were improbable, likely because of shallow depth of insertion measured in the captive moose (<16 cm).

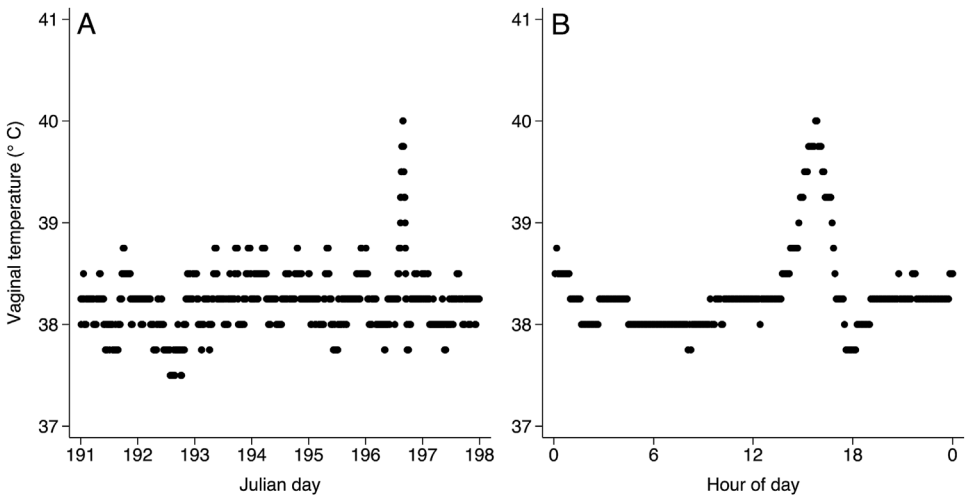

Figure 1. Example of continuous body temperature (° C) collected at 5-min intervals with a modified vaginal implant transmitter in an individual moose for (A) a week, 10 July to 16 July 2015 (Julian days 191–197) and (B) one day, 15 July 2015 (Julian Day 196) on the Kenai Peninsula, Alaska, USA.

Table 2. Number of isolates and occurrence (%) of aerobic bacteria and fungus from moose vaginal swabs ($n = 92$) and swabs of manually removed vaginal implant transmitters ($n = 40$) from moose on the Kenai Peninsula, Alaska, USA, during 2015–2017.

	Vaginal swab		Vaginal implant swab	
Species of isolated bacteria	**No. isolates**	**Occurrence (%)**	**No. isolates**	**Occurrence (%)**
Pathogenic potential category 1[a]				
Escherichia coli	9	6.72	8	11.11
Trueperella pyogenes	1	0.75	3	4.17
Pathogenic potential category 2[a]				
Bacillus spp.	2	1.49	1	1.39
Enterococcus spp.	2	1.49	3	4.17
Staphylococcus aureus	3	2.24	14	19.44
Streptococcus spp.[b]	28	20.90	16	22.22
Pathogenic potential category 3[a]				
Actinomyces spp.	0	0	1	1.39
Candida spp.[c]	1	0.75	0	0
Corynebacterium spp.	76	56.72	20	27.78
Klebsiella pneumoniae	0	0	1	1.39
Moraxella spp.	4	2.99	0	0
Proteus mirabilis	0	0	3	4.17
Pseudomonas spp.	3	2.24	1	1.39
Staphylococcus spp.[d]	4	2.99	1	1.39
Staphylococcus epidermidis	1	0.75	0	0
Total (all categories)	134	100	72	100

[a] Pathogenic potential category based on bacteria that can cause endometritis in domestic cows (Sheldon et al. 2002, Williams et al. 2005). Category 1—bacteria that cause endometritis. Category 2—bacteria that rarely cause endometritis. Category 3—bacteria that do not cause endometritis.
[b] Non-haemoltyic.
[c] Fungus.
[d] Coagulase negative.

When the TVIT was removed from these moose, the distal end of the TVIT was urine-stained, indicating that the transmitter body of the TVIT was posterior of the ureter opening into the vagina. In young, nulliparous moose, the transmitter body of the TVIT was closer to the vulva, and in certain positions may have been exposed, which would contribute to the lower temperature recordings (Vickers et al. 2010). A shorter transmitter body length TVIT may improve the temperature data collected with a TVIT by this method in young or smaller bodied moose.

Bacteria isolated in moose from vaginal swabs and swabs of TVITs were similar to bacteria found in both the vagina and postpartum uterus of domestic cattle (Noakes et al. 1991, Sheldon et al. 2002, Williams et al. 2005, Zambrano-Nava et al. 2011), with the exception of *Moraxella* spp. (4 isolates), which has only been documented in vaginal bacteria flora of one other wildlife species, the California sea lion (*Zalophus californianus;* Johnson et al. 2006*b*). Higher bacteria growth scores associated with TVITs may be a result of the shape of the TVIT, with the base and wings potentially trapping

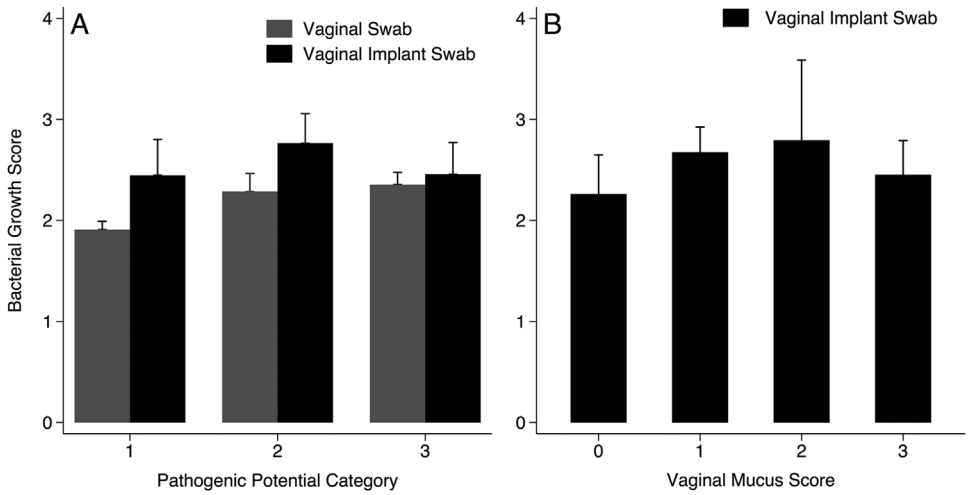

Figure 2. Bacterial growth score for (A) vaginal swabs and swabs of manually removed vaginal implant transmitters by pathogenic potential category, and (B) swabs of manually removed vaginal implant transmitters by vaginal mucus score, collected from 2015 to 2017 from captive and wild moose on the Kenai Peninsula, Alaska, USA. Pathogenic potential based on bacteria that can cause endometritis in domestic cows (Sheldon et al. 2002, Williams et al. 2005). Vaginal mucus score adapted from Williams et al. (2005); 95% confidence intervals at each predicted *x*-value.

mucus in the vaginal cavity. Time without a TVIT did not affect bacteria growth; indicating that after 4 weeks following removal of TVITs, there was no difference in bacteria growth compared with swabs collected up to 1 year after removal of a TVIT. The greater bacterial scores for pathogenic potential Category 1 for TVIT swabs was driven by the 3 isolates of *Trueperella pyogenes,* which provided the only heavy growth scores for this pathogenic category. *T. pyogenes* has also been associated with increased vaginal mucus in domestic cattle (Williams et al. 2005). Both *Escherichia coli* and *T. pyogenes* have been isolated in the vagina of clinically healthy domestic cattle (Zambrano-Nava et al. 2011), and are potential causes of endometritis in cattle after calving (Sheldon et al. 2002, Williams et al. 2005). The increased bacterial growth scores for *E. coli* and *T. pyogenes* associated with the presence of a TVIT in moose were collected between August and November, and may increase the likelihood that these bacteria could enter the uterus during estrus; however, no studies have assessed the effect of these bacteria on future reproductive success in moose if these bacteria are present in the uterus from conception.

The use of the VITs to record body temperature is a useful tool for physiological studies of free-ranging wildlife. Although TVITs are limited to females, the VIT platform has been widely used and evaluated for parturition studies (Johnson et al. 2006*a*, Bishop et al. 2011); incorporating a temperature logger can provide additional data to these studies. For example, use of TVITs paired with GPS collars could offer a valuable covariate when assessing habitat selection of moose at times of perceived heat stress (Lowe et al. 2010, Broders et al. 2012, Rice 2016). Continuous records of body temperature with other physiological metrics such as heart rate, respiration rate, activity, and surface temperature may provide valuable insights into thermoregulation studies as seen in domestic animals (Marai et al. 2007, Cardoso et al. 2015, Alfonzo et al. 2016). Additionally, TVITs can be used to assess capture-related hyperthermia and associated capture myopathy, allowing wildlife researchers to evaluate risks of capture and revise capture protocols to minimize negative effects on the animal (DelGiudice et al. 2001, Meyer et al. 2008, Jacques et al. 2009, Brivio et al. 2015). This application of a VIT to collect continuous measurements of body temperature will allow wildlife researchers to gain further knowledge into thermal physiology of wildlife.

ACKNOWLEDGMENTS

We thank A. Herberg, M. King, and J. Strait for assisting with captive moose; and T. Cambier (Chena River Aviation), J. de Creeft (Northwind Aviation), J. Fieldman (Soloy Helicopters), and N. Olson (Kenai National Wildlife Refuge) for assistance with wild moose captures and TVIT retrieval. This project was funded under Alaska Department of Fish and Game (ADFG) Federal Wildlife Restoration Grant AKW-4 Project #1.63 and #1.72, and ADFG Federal Wildlife Restoration Grant AKW-5. D. Thompson worked on developing the modified vaginal implant transmitter used in the study but has no financial interest in the product. J. Adsem was an employee of Advanced Telemetry Systems during the development process of the vaginal implant transmitter. Finally, we thank S. Farley, D. Hewitt, Associate Editor M. Hewison, and 2 anonymous reviewers for providing constructive feedback for this manuscript.

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Associate Editor: Hewison.