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Original Article

Comparison of Pregnancy Detection Methods in Live White-Tailed Deer

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ABSTRACT Assessing ungulate reproduction is important to biologists for managing populations and predicting trends. We compared efficacy of trans-abdominal ultrasound and pregnancy-specific protein B (PSPB) white-tailed deer (*Odocoileus virginianus*) pregnancy estimates, respectively, from January to mid-April 2009–2011 in the south-central Upper Peninsula of Michigan, USA. We observed a strong agreement ($K = 0.68$, SE = 0.13, 95% CI = 0.42–0.94) of PSPB and ultrasound in categorizing pregnant and nonpregnant deer. Five deer were determined to be pregnant by ultrasound but not by PSPB and 6 females were judged to be nonpregnant using either method. Total cost for PSPB testing of 101 deer was US$2,220, whereas ultrasound equipment cost US$14,150. Trans-abdominal ultrasound and PSPB provided accurate detection of pregnancy in live white-tailed deer. We recommend PSPB for studies testing comparatively small numbers (up to several hundred) of deer. However, we recommend ultrasonography if real-time pregnancy determination is needed (e.g., vaginal implant transmitter use), particularly for large numbers (i.e., several hundred to thousands) of deer. © 2012 The Wildlife Society.

KEY WORDS capture, condition, deer, *Odocoileus virginianus*, pregnancy, PSPB, ultrasound.

Assessing ungulate reproduction is important to wildlife biologists for understanding and predicting population trends (Andelt et al. 2004, Bishop et al. 2009). Reproductive rates are frequently estimated through lethal techniques (e.g., harvest; Jones et al. 2010), which often may not be acceptable due to public perception or harvest regulation. Alternatively, reproductive rates can be estimated using nonlethal methods, particularly for demographic studies where animals are captured, marked, and released (Ropstad et al. 1999). Nonlethal techniques, such as camera surveys (McKinley et al. 2006) or visual counts (Campbell et al. 2005) have been used to characterize reproduction rates, but are often limited to assessing realized fecundity or recruitment and cannot account for conceptus death (Rowell et al. 2000) or fetal abortion (Andelt et al. 2004, Barbknecht et al. 2009).

Determining pregnancy rates provides inference as to what percentages of females are bred, and to possible in utero mortality. Several nonlethal methods, including trans-rectal (Ropstad et al. 1999, Willard et al. 1999) or trans-abdominal (Drew et al. 2001, Carstensen et al. 2003, DelGiudice et al. 2005, Bishop et al. 2009) ultrasonography, have been used to determine in-utero pregnancy and conceptus mortality of ungulates. Ultrasonography provides a minimally invasive method to detect the products of conception (e.g., placentomes) and development (Ropstad et al. 1999); however, need of a trained observer (Willard et al. 1999) and costly equipment hinder common field use. Blood serum characteristics have also provided a minimally invasive method of pregnancy detection, but some blood characteristics (e.g., progesterone) require a subjective determination of the threshold concentration of pregnancy (Wood et al. 1986) and may be unreliable during early gestation (Ropstad et al. 1999).

Biologists have used pregnancy-specific protein B (PSPB) levels to determine pregnancy of several wild ungulate species (Wood et al. 1986, Rowell et al. 1989, Ropstad et al. 1999, Drew et al. 2001, Andelt et al. 2004). Pregnancy-specific protein B is a protein produced in the binucleate cells of the placenta and pregnancy is determined by analyzing PSPB concentrations in blood sera using a radioimmunoassay test (Sasser et al. 1986). Pregnancy can be confirmed using PSPB testing throughout gestation (Wood et al. 1986), including the first trimester (Sasser et al. 1986, Rowell et al. 1989), and ordinarily requires a single blood sample (Noyes et al. 1997). Similar to ultrasonography, PSPB has a high pregnancy detection rate (e.g., 99%; Wood et al. 1986), but there is limited research comparing these common methods (but see Ropstad et al. 1999).

White-tailed deer (*Odocoileus virginianus*) are an intensively managed species and pregnancy rate estimates are essential to population management (Demarais and Krausman 1999).

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Ultrasonography (DelGiudice et al. 2006) and PSPB (Wood et al. 1986) have been used to assess pregnancy in white-tailed deer, although efficacy has not been directly compared. Additionally, selection of method may depend on whether additional sampling is needed (e.g., rump fat depth; Stephenson et al. 1998) and sampling costs. Therefore, managers could benefit from knowledge of the efficacy and cost-benefit of these methods. Our objective was to compare pregnancy detection efficacy of trans-abdominal ultrasound and PSPB in white-tailed deer from their first through mid-third trimesters.

METHODS

We captured white-tailed deer in baited Clover traps (Clover 1956) from January to mid-April 2009–2011. We manually restrained captured deer and hand-injected them intramuscularly (IM) in the rump or shoulder using a 3.75-cm needle with a 3:1 (4 cubic cm total) or 4:1 (5 cubic cm total) combination of ketamine (100 mg/mL; Ketaset®; Fort Dodge Laboratories, Inc., Fort Dodge, IA) and xylazine (100 mg/mL; X-Ject E™; Butler Schein Animal Health, Dublin, OH). We applied ophthalmic ointment to the eyes and blindfolded deer before we removed them from traps. We recorded deer body weight using a spring scale and extracted a lower canine for age estimation (Nelson 2001).

We collected ≤39.5 mL of blood in BD Vacutainer® tubes (Becton Dickinson, Franklin Lakes, NJ) through jugular venipuncture. We allowed blood collected in serum separation tubes to clot and centrifuged samples before decanting and freezing sera. We submitted serum samples to BioTracking (Moscow, ID) to determine pregnancy using BioPRYN$_{WILD}$, a sandwich enzyme-linked immunosorbent assay (ELISA) using bovine (Sasser et al. 1986) and moose (*Alces alces*; Huang et al. 1999) PSPB antibodies. The ELISA detects pregnancy postbreeding and the precision of the assay using a moose PSPB standard was measured with intra-assay precision coefficient of variation (CV) = 3.2% and inter-assay precision CV = 5.9% (J. Branen, BioTracking, personal communication). Direct estimates of PSPB concentrations were not available from BioTracking.

We used trans-abdominal ultrasonography (SonoSite 180™; L38/10–5 MHz transducer; SonoSite, Inc., Bothell, WA) to determine pregnancy (Bishop et al. 2007) and estimate maximum rump fat depth (MXF; Cook et al. 2001) to the nearest 0.1 mm, as described by Stephenson et al. (1998). Generally, our ultrasound pregnancy observation time was ≤5 minutes. We determined mean body condition score (BCS; Gerhart et al. 1996; Cook et al. 2001, 2007; Stirling et al. 2008) for each deer from 2 trained independent observers' scores based on palpation of soft tissue at the withers, ribs, and rump, rated from 1 (emaciated) to 5 (obese). We affixed a radiocollar and implanted a vaginal implant transmitter (VIT; Advanced Telemetry Systems, Isanti, MN) if an animal was determined pregnant by ultrasonography. We administered 1.5 mL (10 mg/mL) or 2.2–7 mL (2 mg/mL) of yohimbine (Hospira©; Forest Lake, IL) intravenously or IM to antagonize effects of xylazine and released all deer at the capture site. All procedures were approved by the Mississippi State University Institutional Animal Care and Use Committee (no. 09-004).

We tested the strength of agreement between PSPB and ultrasound when used to classify pregnant and nonpregnant deer using Cohen's Kappa, which measures agreement between 2 raters (i.e., PSPB and ultrasound) assigning data to exclusive categories (*K*; Zar 1999). We used descriptive statistics to describe body weight, body condition score, and maximum rump fat depth of pregnant and nonpregnant females by age class. Data are presented with means and standard deviations (SD), standard error (SE), and 95% confidence intervals (95% CI). Statistical analyses were performed in Program R 2.12.1 (R Development Core Team 2010) and we used $\alpha = 0.05$.

We determined the total cost incurred to assess pregnancy via PSPB sampling by multiplying the number of PSPB samples by US$20 per sample (BioTracking) and adding the cost of blood-handling equipment (e.g., needles). Total cost for ultrasonography was determined by summing the cost of the ultrasound unit, transducer, and additional equipment (e.g., carry case and software). We did not determine costs of field personnel time, because methods were similar.

RESULTS

We collected 101 paired trans-abdominal ultrasound and PSPB deer pregnancy estimates. We captured most (93%) deer from January through mid-March across years. We judged 90 (83 of 85 ad, 7 of 10 yearlings, and 0 of 3 fawns) and 95 deer (87 of 88 ad, 8 of 10 yearlings, and 0 of 3 fawns) to be pregnant, by using PSPB and ultrasonography, respectively. We observed a strong agreement ($K = 0.68$, SE = 0.13, 95% CI = 0.42–0.94) in evaluation of pregnant and nonpregnant deer between PSPB and ultrasound; agreement was less ($K = 0.32$, SE = 0.24, 95% CI = −0.16–0.80) between pregnant and nonpregnant adults. Mean adult age was 8.3 years (range = 2.5–15.5 yr). We determined 5 deer (4 ad and 1 yearling) as pregnant by ultrasound but not by PSPB. Six females (1 ad, 2 yearlings, and 3 fawns) were determined to be nonpregnant using either method.

We identified singleton ($n = 36$) or twin fetuses ($n = 10$) or placentomes in females determined to be pregnant using ultrasonography. We observed twin fetuses from 21 January through 2 March, but positive identification of independent fetuses was difficult before mid-January and after about the first week of April. We did not test body condition and mass estimates between pregnant and nonpregnant deer due to limited sample size. Nonpregnant adult and yearling mean body masses were 72.0 kg ($n = 1$) and 49.5 kg (SD = 6.36, range = 45.0–54.0, $n = 2$), respectively. Mean adult and yearling BCS was 3.3 ($n = 1$) and 3.0 (SD = 0.4, range = 2.75–3.25, $n = 2$) and mean MXF was 1.1 ($n = 1$) and 1.1 (range = 1.06–1.07, $n = 2$), respectively (Table 1).

Total cost for PSPB testing of 101 deer was US$2,220, including BioTracking testing ($20/deer) and blood-handling equipment (approx. $200). Total cost for all

Table 1. Mean, standard deviation (SD), and range of body-condition characteristics of pregnant white-tailed deer (*Odocoileus virginianus*) by age class, in the Upper Peninsula of Michigan, USA, from January to mid-April, 2009–2011.[a]

	Adult				Yearling			
Characteristic	Mean	SD	Range	*n*	Mean	SD	Range	*n*
Body mass (kg)	71.0	11.1	43.0–93.0	83	58.0	9.8	43.0–73.8	7
Body condition score[b]	2.8	0.4	1.75–3.75	83	2.6	0.5	2.00–3.25	7
Max. rump-fat depth (cm)[c]	1.1	0.4	0.22–2.20	83	0.7	0.5	0.16–1.47	7

[a] Pregnancy confirmed by serum evaluation of pregnancy-specific protein B concentration and trans-abdominal ultrasonography.
[b] From 2 trained independent observers' scores, based on palpation of soft tissue at the withers, ribs, and rump, rated from 1 to 5 (emaciated–obese).
[c] Ultrasound estimate of rump-fat depth dorsal and anterior to the ischial tuberosity.

ultrasound equipment was US$14,150, including ultrasound ($5,800), transducer ($5,000), and additional equipment (e.g., carry case and software; $3,565).

DISCUSSION

Trans-abdominal ultrasound and PSPB provided efficient and minimally invasive detection of pregnancy in live white-tailed deer. We observed strong agreement of pregnancy prediction, especially in adults, between PSPB and ultrasonography, as observed with other ungulates (Ropstad et al. 1999, Willard et al. 1999, Drew et al. 2001, Andelt et al. 2004). Discrepancies between 5 deer determined pregnant by ultrasound but not PSPB may have resulted from ultrasound operator misinterpretation of conceptus presence. Also, a 13-year-old female determined nonpregnant by ultrasound had PSPB levels approaching the pregnancy optical density cutoff value (0.443), suggesting embryonic loss (G. Sasser, BioTracking, personal communication). Despite a moderate body condition score, this female weighed 27.6 kg less than the mean adult body weight, and possibly experienced embryonic loss due to nutritional stress (Rowell et al. 2000). However, this was unlikely evidence of senescence (DelGiudice et al. 2007) because both methods determined females up to 15 years old were pregnant and 36 of 37 late-aged (>7.5 yr; Verme 1969) females were pregnant. Therefore, we believe age did not influence either pregnancy detection method.

Although we could not test for differences between mean body condition and body mass estimates, pregnant deer appeared to have less subcutaneous fat than nonpregnant deer. In contrast, Gustine et al. (2007) found pregnant woodland caribou (*Rangifer tarandus caribou*) had greater rump-fat depth than nonpregnant caribou. Most deer in our study were captured during winter, when they experience negative energy balance (DelGiudice et al. 2000), primarily due to food restriction. Therefore, increased lipolysis (Mautz 1978) and muscle catabolism among deer during winter may have limited differences between pregnant and nonpregnant deer body condition and mass estimates.

Using ultrasonography, we were able to occasionally detect twins by increasing observation time (approx. 3 min) and transducer search area around the abdomen. However, twins became difficult to detect at the beginning of the last trimester (early Apr) due to increased fetus size, which reduced our ability to discern independent fetuses. Although we did not assess ultrasound twinning-estimate accuracy, we recommend conducting ultrasonography from mid-January until early March in northern latitudes with an experienced observer to improve estimates of number of fetuses. We were not able to assess twin versus singleton determination using PSPB because the ELISA test does not provide an estimate of PSPB concentration, only estimates of optical density relative to PSPB concentration. However, Andelt et al. (2004) stated only 7% of variation in PSPB concentration was related to number of fetuses and days since conception in mule deer (*O. hemionus*). Further investigation of this relationship is warranted because estimates of twinning rate would be useful to wildlife biologists for comparisons of in utero and realized fecundity rates.

MANAGEMENT IMPLICATIONS

Trans-abdominal ultrasound and PSPB have been successfully used by wildlife biologists to determine pregnancy of many ungulate species, and our results further support their use with white-tailed deer. We recommend PSPB for studies testing pregnancy in comparatively low (up to several-hundred) numbers of deer, potentially without need for chemical immobilization. However, if individual fetus detection or real-time pregnancy determination is needed (e.g., VIT use), we suggest using ultrasonography. Despite initial costs, ultrasonography may provide a comparatively less expensive method with larger numbers (i.e., several hundred to thousands) of deer, and over time because equipment can be reused. Further, less expensive ultrasound units can be used, reducing per-deer sampling costs. Ultrasonography may also be preferred if biologists want to collect other deer body-condition data (e.g., subcutaneous fat; Stephenson et al. 1998, Cook et al. 2001). Biologists should consider study objectives and per-deer sampling costs over the length of study when deciding which method to use.

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