Using iophenoxic acid injections of prey to identify mammals that feed on them

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Abstract Identifying species or individuals that feed upon other species of animals is an important aspect of some predation studies. We evaluated the effectiveness with which the biomark associated with iophenoxic acid (IA) injections was transferred from domestic goats to coyotes (*Canis latrans*) that fed on them. We injected doses of 100, 300, or 1,000 mg of IA into goats to raise serum iodine levels, fed meat from the injected goats to coyotes, and monitored serum iodine levels in both species for about 120 days. Within 3 days, mean serum iodine levels in goats increased from 5.33 mcg/100 ml to over 2,847, 10,233, and 11,567 mcg/100 ml, respectively, for the 100-, 300-, and 1,000-mg IA treatments. A gradual dissipation of serum iodine concentrations in the goats ensued, approaching mean levels of 943, 3,213, and 6,310 mcg/100 ml of serum by day 120. When we fed coyotes (2/treatment) 500 g of meat from IA-treated goats, mean serum iodine levels among the coyotes increased within 2 days from 8 mcg/100 ml to 194, 410, and 645 mcg/100 ml of serum respectively for the 3 treatments. Mean serum iodine concentrations among these coyotes then declined systematically to 30, 45, and 82 mcg/100 ml of serum 112 days after ingestion. When we fed coyotes 500 g of meat from goats slaughtered 120 days after they had been injected with IA, mean serum iodine levels increased from base levels (8 mcg/100 ml of serum) to 69, 242, and 526 mcg/100 ml respectively for the 100-, 300-, and 1,000-mg treatments. We concluded that we were able to detect coyotes that fed on marked goats any time during a 120-day period after the goats were treated. Nonlinear regression analysis suggested a relation between levels of serum iodine achieved and IA dose rate (mg/kg) received by the goats, with iodine levels reaching saturation with intramuscular injections of 25-30 mg/kg IA.

Key words biomarker, *Canis latrans*, coyote, goat, iophenoxic acid, physiologic marker, predation

For some studies of predation, it is important to identify species, or individuals of a species, that feed on a prey species of interest. In such cases, a long-term systemic marker of soft tissues that can be transferred from one species to another, preferably via a single feeding, is desirable (Windberg et al. 1997). Several long-term physiologic marking agents are available for wild species, including radioisotopes (Pelton and Marcum 1975, Knowlton et al. 1989, Chamberlain et al. 1997), tetracycline (Linhart and Kennelly 1967, Taylor and Lee 1994, Van Brackle et al 1994), and rhodamine B (Lindsey 1983, Knowlton et al. 1988, Fisher 1999). Most of these, however, do not meet all essential characteristics sometimes required. In contrast, iophenoxic acid (IA), which has been used in human medicine as an x-ray diagnostic material (Shapiro and Man 1960) and causes a long-term elevation of serum

iodine levels in most carnivores (Larson et al. 1981, Baer et al. 1985, Saunders et al. 1993, White et al. 1995), is a likely candidate because it is effective when used orally, creates a persistent mark, is distributed throughout vascularized body tissues, can be assayed in serum samples, and is benign in living animals (Eason and Batchelor 1991). In this study, we assessed persistence of elevated blood iodine levels in goats injected with IA, evaluated transfer of this mark from goats to coyotes (*Canis latrans*) fed a single meal of marked goat flesh, and quantified persistence of the mark (elevated levels of serum iodine) in coyotes.

Methods

We conducted this study between August 1989 and February 1990 at the United States Department of Agriculture's Predator Research Facility near Millville, Utah. We used 15 captive coyotes from that facility and 12 Angora goats acquired from commercial sources. Throughout the study, goats were pastured as a single flock and maintained by grazing supplemented with alfalfa pellets. Coyotes were housed individually in outdoor kennels (1.2 × 3.7 × 1.8 m) and maintained on a commercial diet prepared for the local fur industry (Furbreeders Agricultural Cooperative, Logan, Utah). Water was available *ad libitum*.

On the first day of the experiment, we weighed all goats, took pre-treatment blood samples from the jugular vein, stratified goats into groups of 4 by weight, and randomly assigned goats within each weight group to one of 3 treatments. We dissolved the IA in absolute ethanol and diluted it with propylene glycol so each ml contained a quarter of the appropriate dose of IA. We gave each goat within the respective treatments 2 2-ml intramuscular injections containing a total of 100, 300, or 1,000 mg of IA. We subsequently took blood samples from goats on days 3, 8, 12, 22, 29, 36, 64, 92, and 120. On days 8 and 120, we selected one goat at random from within each group that had received IA and slaughtered it with minimal loss of body fluid. After carcasses had cooled, they were skinned, the digestive tracts removed, the carcasses boned, and the flesh ground, homogenized, packaged, labeled, and refrigerated.

On day 9 we weighed 15 coyotes, took pre-treatment blood samples from the cephalic or brachial veins, stratified coyotes by sex, and assigned each to one of 9 treatments. On that day, we fed each coy-

ote in the first 3 groups, composed of one male and one female, one 500-gm feeding of meat from a goat that had been treated with 100, 300, or 1,000 mg IA, respectively. On day 121, we fed each coyote in 3 other groups (one male and one female each) 500 g of meat from goats that had been treated with 100, 300, or 1,000 mg IA, respectively, on day 1 and killed on day 120. We fed 3 additional coyotes 2 500-g meals, one each on days 9 and 16, of meat from goats treated with 100, 300, or 1,000 mg IA on day 1. We took blood samples from all coyotes on days 11, 16, 18, 23, 30, 37, 65, 93, and 121. We also took blood samples on days 123 and 128 from the coyotes fed treated goat meat on day 121.

We allowed blood samples to clot at room temperature for 3-4 hours. We then centrifuged the samples and aspirated the serum into individual vials, which were labeled and refrigerated. When sampling was complete for all goats and coyotes for each specific period, we refrigerated samples with cool packs and shipped them in insulated containers to Smith-Kline Biological Sciences Laboratories in Van Nuys, California, to be assayed for iodine concentrations in the sera. Herein we graphically depict the changes noted in serum iodine concentrations over time, as means within the respective treatments for each sampling period, and in the text as the means±SE (standard error of the mean). We assessed dissipation of the elevated serum iodine concentrations by regressing logarithmic values of the individual measurements within each treatment against days post-treatment. To assess serum iodine saturation, we used a nonlinear least squares regression (CurveExpert 1.3, Hyams 1997). We fit the Michaelis-Mentin resource uptake model (Tilman 1982), $Y = (aX)/(b + X)$, to 3-day post-treatment serum iodine levels in goats resulting from intramuscular injections of IA.

Results

Serum iodine levels in goats

Mean serum iodine levels among goats ($n = 12$) before treatment with IA was 5.33±0.42 mcg/100 ml of serum. Within 2 days following treatment, mean serum iodine levels increased dramatically to 2,847 ± 504, 10,233 ± 853, and 11,567 ± 1,093 mcg/100 ml for the 100-mg, 300-mg, and 1,000-mg treatments, respectively (Figure 1a). Thereafter, serum iodine levels declined slowly in a typical pattern of biological decay (Figure 1b). After 120 days, serum iodine levels were still notably elevated in all

Figure 2. Serum iodine concentration in Angora goats 3 days after receiving an injection of iophenoxic acid, expressed as a function of the dose rate (mg/kg) and an associated least squares regression equation.
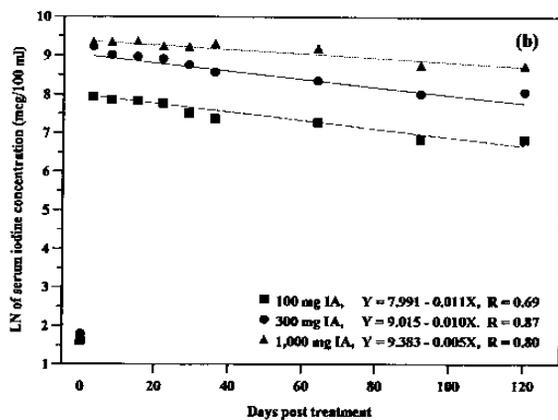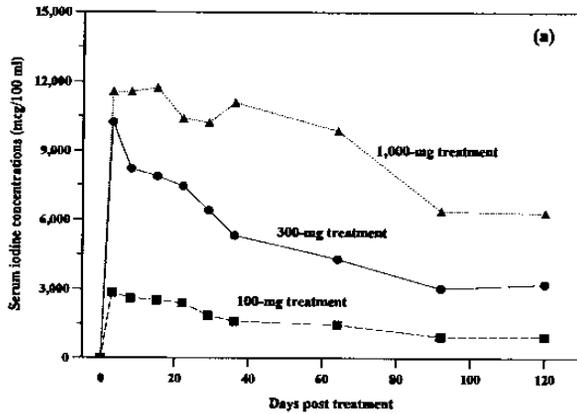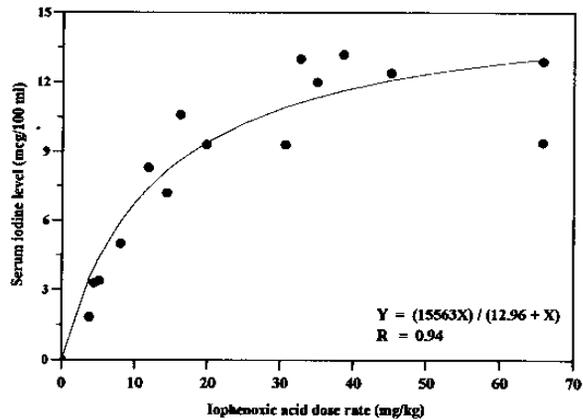

Figure 1. Mean serum iodine levels in Angora goats over a 120-day period following an intramuscular injection of iophenoxic acid (IA) at 3 dose levels, expressed in terms of (a) the serum concentration and (b) the natural logarithm of the serum concentration. The regression equations associated with dissipation of the elevated serum iodine concentration are based upon individual measurements.

treatments, 943 ± 190, $3,213 \pm 240$, and $6,310 \pm 857$ mcg/100 ml of serum, respectively, for the 100-, 300-, and 1,000-mg IA treatments.

At the beginning of the study, individual goats weighed between 8.6 and 26.0 kg. Assuming variations in weight represent differential dilutions of IA among the goats, we compared 3-day post-treatment serum iodine concentrations against the calculated IA dose rate (mg/kg). We noted a nonlinear pattern $(Y = 15,563X/(12.96 + X)$ of serum iodine levels associated with increased dose rates (Figure 2), with a calculated saturation level $(2 \times b$, Tilman 1982) of 26 mg/kg.

Serum iodine levels in coyotes

Base serum iodine levels among coyotes $(n = 15)$ before feeding on IA-treated goat meat was $8.00 \pm$

0.42 mcg/100ml. Two days after eating a single 500-g meal of IA-treated goat meat, mean serum iodine levels increased to 194 ± 8.0, 410 ± 35.0, and 645 ± 45 mcg/100 ml for 100-mg, 300-mg, and 1,000-mg treatments (Figure 3a). Serum iodine levels then dissipated systematically (Figure 3b), with mean levels declining to 30 ± 12, 45 ± 5, and 82 ± 4 mcg/100 ml, respectively, for the 100-mg, 300-mg, and 1,000-mg treatments on day 121 of the experiment (112 days after coyotes ingested the IA-treated goat meat).

The 9 coyotes not fed IA-treated goat meat until day 121 also served as a control treatment until day 121. On days 30, 37, and 65, we noted serum iodine levels among these animals were elevated to 20–30 mcg/100ml even though they did not have access to IA-treated materials (Figure 4). Serum iodine concentrations among these animals returned to base levels by the following sample period (day 93). The mean serum iodine concentrations for coyotes on these treatments were elevated on days 123 and 128 after ingesting a single 500-g meal of meat from goats treated with IA on day 1 and slaughtered on day 120. We noted means of 69 ± 16, 242 ± 14, and 526 ± 2 mcg/100ml, respectively, on day 123 for treatments involving 100, 300 and 1,000 mg IA (Figure 4).

Among coyotes fed 2 500-g meals of treated goat meat one week apart (days 9 and 16), we noted an initial increase in serum iodine concentrations similar to that in the first trial, followed by a second, but much smaller, increase following the second feeding (Figure 5).
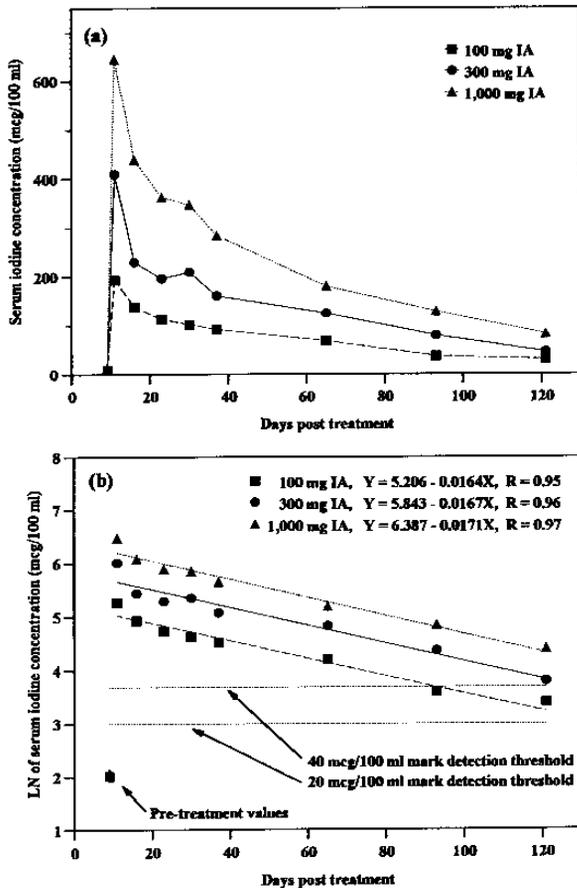
Figure 3. Mean serum iodine concentrations in coyotes over a 112-day period following ingestion of 500 g of meat from goats that had been injected with iophenoxic acid (IA) at 3 dose levels, expressed in terms of (a) the serum concentration and (b) the natural logarithm of the serum concentration. The regression equations associated with dissipation of the elevated serum iodine concentrations are based on individual measurements.
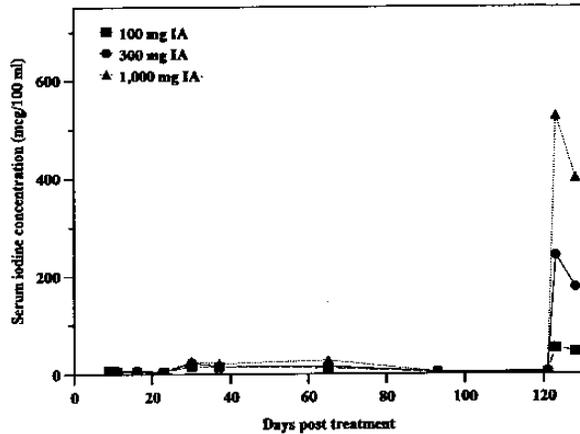
Figure 4. Mean serum iodine concentrations in coyotes used as a control treatment, followed on day 121 with a single 500-g feeding of meat from goats injected with one of 3 doses of iophenoxic acid 120 days earlier.

to goats could be detected for 134, 170, and 199 days, respectively. The unexplained elevation in serum iodine levels among control animals between days 30 and 65 caution against this. To avoid unambiguous "marks," we recommend using doses that will create a secondary mark at least 5 times greater than base levels at the time the assay samples are obtained. In the case of coyotes, a more conservative threshold to detect the mark would be a serum iodine level of 40 mcg/100 ml. Using this threshold of detection would reduce the effective duration of marks to 93, 129, and 158 days, respectively (Figure 3b). Although decreasing the acceptable threshold for recognizing the mark increases the working longevity of the mark, it also

Discussion

We demonstrated that the elevated serum iodine level associated with an injection of IA can be readily transferred, by ingestion, from a mammalian herbivore to a mammalian carnivore and provide an effective, long-term physiologic "mark" to study carnivore feeding patterns. The slow but systematic dissipation of the primary and secondary marks also provides a basis to estimate potential longevity of such marks. We initially anticipated that a threshold of 20 mcg of iodine/100 ml serum (2.5 times baseline) would be adequate to detect the mark. Assuming this value and extrapolating via the regression equations (Figure 3b) suggest that biomarks in coyotes resulting from eating a single 500-g meal from the 100-, 300-, and 1,000-mg treatments
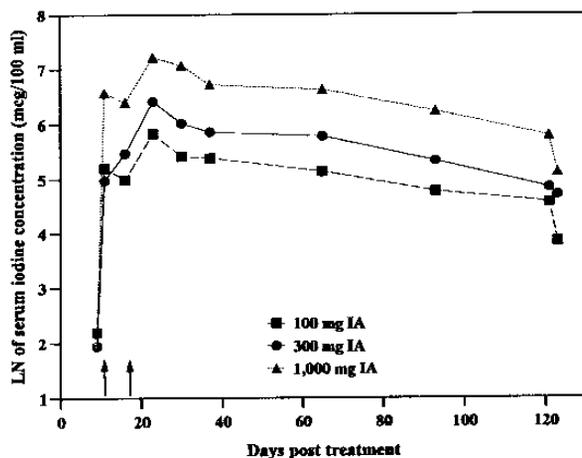

Figure 5. Mean serum iodine concentrations among coyotes fed 500 g of meat from iophenoxic acid-treated Angora goats (3 dose levels) on days 9 and 16 (arrows).

increases risks associated with properly identifying the presence or absence of the mark.

Subsequent to our study, Stoddart and Olmstead (1992) demonstrated that the elevated serum iodine levels we noted between days 30 and 65 among non-IA-treated animals were likely related to a seasonal ingredient or additive in the "fur industry diet" upon which animals were maintained, and not with some seasonal physiologic function among coyotes. The year following our study, they acquired a stock of the "fur industry diet" in mid-summer and froze it. They then fed one group of coyotes from the frozen stock and a second group on fresh supplies of the diet obtained 3 times weekly from the distributor. Serum iodine levels of coyotes fed the frozen stock did not become elevated, whereas those fed from fresh supplies of the diet displayed a rise in serum iodine concentrations comparable, in date and degree, to what we observed. This suggests a seasonal component of the diet caused the elevated serum iodine levels. This "dietary mark" disappeared quickly without the systematic decline we noted for an IA mark. We obtained ingredient records from the food distributor but were unable to ascertain the specific cause of the elevated serum iodine levels. Resolution of this issue might permit reducing the threshold for identifying the mark and thus potentially extend the useful working duration of the mark.

Several aspects of dosing are relevant. Differential dose responses and mark deterioration rates are apparent among species (Larson et al. 1981, Baer et al. 1985, White et al. 1995), suggesting a need to test intensity and persistence of marks when contemplating applications for other species. This would be particularly important if transfer of the mark from one species to another is planned because IA uptake may differ. While IA elevates serum iodine levels in many carnivores and some ungulates, it apparently does not do so among some avian species (Larson et al. 1981). Our dose-response data suggested IA saturation in Angora goats occurs at about 26 mg/kg. Dosing above this level may not appreciably increase intensity or duration of the mark. On the other hand, the slow but predictable deterioration of the mark should facilitate calculations to estimate doses required to meet objectives associated with specific studies. Incorporating IA into a slow-release matrix might provide another means of maintaining high levels among primary subjects.

Because IA elevates protein-bound iodine in serum (Baer et al. 1985), transfer of the mark to mammalian carnivores presumably could result from consuming of any blood-bearing tissue. Our use of blood to sample seemingly corroborates this interpretation. Whether the intensity of a secondary mark (degree to which serum iodine concentrations are elevated) might be differentially associated with ingestion of various body tissues remains to be tested.

Subsequent to our study, Windberg et al. (1997) used IA injections to estimate the proportion of a coyote population that fed on a flock of domestic goats and to determine whether specific segments of the coyote population were involved. While they were able to discern whether specific coyotes fed on the goats, they could not identify which coyotes killed the goats. Similarly, because parameters of dose and time were confounded, these authors were unable to calculate how much goat meat individual coyotes consumed. A more sophisticated study design would be required to determine the latter.

Pentachlorobenze, another long-term physiological marking agent (Kimball et al. 1996), may be an alternative biomarker meeting the requirements for similar predation-related studies. However, additional aspects associated with creating and transferring the biomark from one species to another need to be assessed as well as the physical distribution of the mark within the body of the primary species.

Conclusions

Iophenoxic acid, which causes significant elevations in serum iodine levels, can be used as a long-term biomarker that transfers effectively from one mammal to another through ingestion. This technique may not work among avian species. Among mammals, intensity of the mark is directly dependent on dose rate, but a saturation affect may become relevant at greater dose rates. Although systematic dissipation of the elevated serum iodine levels provides a mechanism to assess the amount of IA acquired or the time period in which it was acquired, these 2 parameters are confounded.

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Literature cited

BAER, G. M., J. H. SHADDOCK, D. J. HAYES, AND P. J SAVARIE. 1985. Iophenoxic acid as a serum marker in carnivores. Journal of Wildlife Management 49:49-51.

CHAMBERLAIN, C. P., J. D. BLUM, R. T. HOLMES, Z. FENG, T. W. SHERRY, AND G. R. GRAVES. 1997. The use of isotope tracers for identifying populations of migratory birds. Oecologia 109:132-141.

EASON, C. T., AND D. BATCHELOR. 1991. Iophenoxic and iopanoic acid as bait markers for feral goats. Wildlife Research 18: 85-90.

FISHER, P. 1999. Review of using Rhodamine B as a marker for wildlife studies. Wildlife Society Bulletin 27:318-329.

HYAMS, D. 1997. CurveExpert 1.34:A comprehensive curve fitting system for windows. Shareware, Starkville, Mississippi, USA.

KIMBALL, B. A., L. A. WINDBERG, C. A. FURCOLOW, M. ROETTO, AND J. J. JOHNSTON. 1996. Two new oral chemical biomarkers for coyotes. Journal of Wildlife Diseases 32:505-511.

KNOWLTON, F. F., P. J. SAVARIE, C. E. WALGREN, AND D. J. HAYES. 1988. Retention of physiologic marks by coyotes ingesting baits containing iophenoxic acid, mirex, and rhodamine B. Pages 141-147 in R. W. Bullard and S. M. Schumache, editors. Test methods for vertebrate pest control and management materials. American Society for Testing and Materials, Special Technical Publication 974, Philadelphia, Pennsylvania, USA.

KNOWLTON, F. F., L. C. STODDART, R. L. CRABTREE, AND J. W. BLATT. 1989. Evaluation of some radioisotopes as marking agents for monitoring bait consumption. Pages 52-62 in K. A. Fagerstone and R. D. Curnow, editors. Test methods for vertebrate pest control and management materials, American Society for Testing and Materials, Special Technical Publication 1055, Philadelphia, Pennsylvania, USA.

LARSON, G. E., P. J. SAVARIE, AND I. OKUNO. 1981. Iophenoxic acid and mirex for marking wild, bait-consuming animals. Journal of Wildlife Management 45:1073-1077.

LINDSEY, G. D. 1983. Rhodamine B: a systemic fluorescent marker for studying mountain beavers (Aplodontia rufa) and other animals. Northwest Science 57:16-21.

LINHART, S. B., AND J. J. KENNELLY. 1967. Fluorescent bone labeling of coyotes with demethylchlortetracycline. Journal of Wildlife Management 31:317-321.

PELTON, M. R., AND L. C. MARCUM. 1975. The potential use of radioisotopes for determining densities of black bears and other carnivores. Pages 221-236 in R. L. Phillips and C. Jonkel, editors. Proceedings of the 1975 Predator Symposium. Montana Forest and Conservation Experiment Station, University of Montana, 16-19 June 1975, Missoula, USA.

SAUNDERS, G., S. HARRIS, AND C. T. EASON. 1993. Iophenoxic acid as a quantitative bait marker for foxes. Wildlife Research 20: 297-302.

SHAPIRO, R., AND E. B. MAN. 1960. Iophenoxic acid and serum-bound iodine values. Journal of the American Medical Association 173:132.

STODDART, L. C., AND S. R. OLMSTEAD. 1992. Evaluation of an observed short term increase in coyote serum iodine level. Final Report of Pilot Study PS-018. Denver Wildlife Research Center, Denver, Colorado, USA.

TAYLOR, M., AND J. LEE. 1994. Tetracycline as a biomarker for polar bears. Wildlife Society Bulletin 22:83-89.

TILMAN, D. 1982. Resource competition and community structure. Princeton University, Princeton, New Jersey, USA.

VAN BRACKLE, M. D., S. B. LINHART, T. E. CREEKMORE, V. F. NETTLES, AND R. L. MARCHINTON. 1994. Oral biomarking of white-tailed deer with tetracycline. Wildlife Society Bulletin 22:483-488.

WHITE, L. M., D. P. HUETTER, S. B. LINHART, P. J. SAVARIE, AND M. D. VAN BRACKLE. 1995. Iophenoxic acid as an oral biomarker in white-tailed deer. Wildlife Society Bulletin 23:194-197.

WINDBERG, L. A., F. F. KNOWLTON, S. M. EBBERT, AND B. T. KELLY. 1997. Aspects of coyote predation on small experimental flocks of Angora goats. Journal of Range Management 50:226-230.

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