Comparison of the protective effect of a commercially available western diamondback rattlesnake toxoid vaccine for dogs against envenomation of mice with western diamondback rattlesnake (Crotalus atrox), northern Pacific rattlesnake (Crotalus oreganus oreganus), and southern Pacific rattlesnake (Crotalus oreganus helleri) venom

Charles C. Cates DVM
Erika V. Valore MS
Marcelo A. Couto DVM, PhD
Gregory W. Lawson DVM, PhD
James G. McCabe BA

Received April 11, 2014. 
Accepted September 30, 2014. 

From the Division of Laboratory Animal Medicine, David Geffen School of Medicine, University of California-Los Angeles, Los Angeles, CA 90095. Dr. Cates’ present address is Cedars Sinai Medical Center, Comparative Medicine Department, 8700 Beverly Blvd, Davis Room 7004, Los Angeles, CA 90048. 
Address correspondence to Dr. Cates (charles.cates@cshs.org).

OBJECTIVE
To evaluate effectiveness of a commercially available toxoid manufactured from western diamondback (WD) rattlesnake (Crotalus atrox) venom against envenomation of mice with WD, northern Pacific (NP) rattlesnake (Crotalus oreganus oreganus), and southern Pacific (SP) rattlesnake (Crotalus oreganus helleri) venom.

ANIMALS
90 specific pathogen–free female mice.

PROCEDURES
Mice were allocated into 3 cohorts (30 mice/ cohort). Mice received SC injections of C atrox toxoid (CAT) vaccine (n = 15/group) or adjuvant (15/group) at day 0 and again at 4 weeks. At 8 weeks, mice were challenged–exposed with 1 of 3 venoms. Survival until 48 hours was evaluated by use of log-rank analysis of survival curves and the z test for proportions.

RESULTS
6 of 15 WD-challenged CAT-vaccinated mice, 3 of 15 NP-challenged CAT-vaccinated mice, and 0 of 15 SP-challenged CAT-vaccinated mice survived until 48 hours. All adjuvant-only vaccinates survived ≤21 hours. Mean survival time of CAT vaccinates was longer than that of adjuvant-only vaccinates for all venoms (1,311 vs 368 minutes for WD, 842 vs 284 minutes for NP, and 697 vs 585 minutes for SP). Results of the z test indicated a significantly increased survival rate for vaccinates exposed to WD rattlesnake venom but not for vaccinates exposed to NP or SP rattlesnake venom. Log-rank analysis revealed a significant difference between survival curves of vaccinated versus unvaccinated mice exposed to NP but not WD or SP venom.

CONCLUSIONS AND CLINICAL RELEVANCE
CAT vaccination improved survival rate and survival time after challenge exposure with WD rattlesnake venom and may offer limited protection against NP rattlesnake venom but did not provide significant cross-protection against SP rattlesnake venom. (Am J Vet Res 2015;76:272–279)

In 2011, 5,700 incidents of snake envenomation in humans were reported by the American Association of Poison Control Hotlines.1 The true number of envenomations likely is higher because reporting is not mandatory, many snakebites go unreported, some snake-bite victims do not seek treatment, and some treating physicians do not consult with a poison control center.2,3 Although the incidence of rattlesnake envenomation in the pet population has not been quantified, it is thought to exceed that for humans (> 150,000 bites/y by 1 estimate4) because of a high rate of outdoor exposure, unreported or unnoticed incidents, and a presumed limited-threat judgment for bitten animals.5–7 A conditionally licensed WD rattlesnake (Crotalus atrox) toxoid vaccine is available for administration to dogs and horses at risk for snakebite and is intended to aid in the reduction of morbidity and deaths attributable to rattlesnake envenomation.6,7 The authors are not aware of any data on evaluation of the effectiveness of the CAT vaccine in scientific journals.8 Manufacturer data and advertisements suggest this CAT vaccine is efficacious against bites from WD rattlesnakes and also provides cross-protection against envenomation from other rattlesnake species.8,9 However, analysis of snake venom reveals it to be a complex milieu of peptides and proteins, and venom from related species and subspecies of rattlesnakes can differ markedly in composition.10–13 A vaccine that

ABBREVIATIONS
ADE Antibody-dependent enhancement
CAT Crotalus atrox toxoid
NP Northern Pacific
OD Optical density
SP Southern Pacific
WD Western diamondback
comprises venom from a single species might provide only limited protection against envenomation by other species of rattlesnakes. In California, companion animals are not typically exposed to WD rattlesnakes because these rattlesnakes are found only in sparsely populated areas in the southeast region of the state. Rather, pets are much more likely to encounter NP rattlesnakes (*Crotalus oreganus oreganus*) and SP rattlesnakes (*Crotalus oreganus helleri*), which inhabit heavily populated and traversed regions of central and coastal California. Therefore, we hypothesized that the CAT vaccine might provide limited cross-protection against 2 important species of rattlesnakes found in California. The purpose of the study reported here was to use rattlesnake envenomation of mice to evaluate the comparative effectiveness of the CAT vaccine against the venom of WD, NP, and SP rattlesnakes.

**Materials and Methods**

**ANIMALS**

Ninety specific pathogen–free outbred female Swiss Webster mice (4 to 6 weeks old) were obtained from a commercial source. Mice were allowed to acclimate for 72 hours. Mice were housed in groups (5 mice/cage) on corncob bedding with cotton nesting material in individually ventilated cages in an Association for Assessment and Accreditation of Laboratory Animal Care International-accredited biocontainment facility. All mice were fed standard laboratory rodent chow and provided with ad libitum access to reverse-osmosis-purified acidified water. The room was maintained at 20° to 21°C with relative humidity of 30% to 70%, 10 to 15 air changes/h, and a photoperiod of 12 hours of light to 12 hours of darkness. Use of the mice in this study was approved by the Institutional Animal Care and Use Committee of the University of California-Los Angeles.

**EXPERIMENTAL PROCEDURES**

A randomized, blinded, placebo-controlled study was conducted. On the basis of an a priori power analysis (power = 0.8, 0% censoring, and 50-to-50 ratio of control mice to experimental mice), the 90 mice were randomly selected by an individual unaffiliated with the study and assigned to treatment and control groups (45 mice/group). Treatment mice received an injection (0.2 mL, SC) of CAT vaccine at day 0 and again at 4 weeks. Control mice received an injection (0.2 mL, SC) of pharmaceutical-grade aluminum hydroxide adjuvant at day 0 and again at 4 weeks. Four weeks after administration of the second injection of CAT vaccine or adjuvant, mice were challenge-exposed with rattlesnake venom.

**VENOM**

The Society for the Study of Amphibians and Reptiles classification of the western rattlesnake (*Crotalus oreganus*) was used for the present study. The NP and SP rattlesnakes are 2 of 5 recognized subspecies of western rattlesnake, and the WD rattlesnake is a monotypic species with no recognized subspecies. Lyophilized WD rattlesnake venom was obtained. The venom was collected from WD rattlesnakes throughout the range of these rattlesnakes within the United States. Venom of NP and SP rattlesnakes was collected from various regions throughout northern and southern California (Figure 1). Samples of NP rattlesnake venom were collected at Sanger (Fresno County), Acton, Castaic, Leona Valley, Topanga Canyon, Malibu Canyon, and Griffith Park (Los Angeles County); Acton, Phelan, Devil’s Canyon, and Big Bear (San Bernardino County); Idyllwild-Pine Cove and Garner Valley (Riverside County); and De Luz (San Diego County). Venom samples were processed in accordance with a standardized protocol. The final lyophilized venom product contained equal parts (vol/vol) from each sample location. In preliminary experiments, the LD<sub>50</sub> was estimated for each venom on the basis of the animal-sparing up-and-down LD<sub>50</sub> testing paradigm. Those LD<sub>50</sub> values then were used in the study as follows: WD rattlesnake venom, 2.8 mg/kg; NP rattlesnake venom, 1.7 mg/kg; and SP rattlesnake venom, 1.5 mg/kg. These LD<sub>50</sub> values are similar to those published previously.

**Figure 1** —Map of the distribution for WD rattlesnakes (*Crotalus atrox*; black-shaded area), NP rattlesnakes (*Crotalus oreganus oreganus*; light gray–shaded area), and SP rattlesnakes (*Crotalus oreganus helleri*; dark gray–shaded area) in California and locations for collection of venom samples (circles). The range of each of the rattlesnakes was obtained from previously published information. Notice that major metropolitan population centers are located exclusively in the ranges of NP and SP rattlesnakes.
edited response to stimuli and core body temperature had a prolonged period of moribundity (severely lim-

distress (agonal breathing or intermittent gasping), or

multinucleated mice were compared with serial serum an-

trum antibody titers of 3 randomly selected vac-

inated versus control mice. Second, to compare specificity of an-

tibodies generated, dilutions (1:8,000) of serum ob-

ained from 8 randomly selected vaccinated mice

test of & was used to compare Kaplan-Meier survival

curves were generated for the 3 venoms and

in a postmortem blood sample was

obtained via cardiocentesis.

**VENOM CHALLENGE EXPOSURE**

Three cohorts (30 mice/cohort [15 treated mice and 15 control mice]) were challenge-exposed with 1

of the 3 venoms at 4 weeks after the second injection of CAT vaccine or adjuvant. Venom was administered
to each mouse via IP injection at twice the calculated LD<sub>50</sub>. For injection, lyophilized venom was reconstitut-
ed in sterile water to create a stock solution of 5 mg/mL, which was then diluted as needed to provide the
dose for administration. Mice were closely monitored

for 48 hours after venom administration.

Before venom administration, body weight and

baseline core body temperature were recorded. Tempera-
ture was obtained with a 1.5-cm-long thermometer

probe inserted via the rectum into the colon; tempera-
ture was recorded once per hour for up to 10 hours

and thereafter as needed. An observer who was un-

aware of the venom administered or vaccination status

of the mice assessed their condition and determined

when a mouse would be euthanized. Mice were eutha-
nized by gradual-fill CO<sub>2</sub> inhalation when they became

nonresponsive to stimuli, were in marked respiratory
distress (agonal breathing or intermittent gasping), or

had a prolonged period of moribundity (severely limited

response to stimuli and core body temperature < 70% of the baseline core temperature for ≥ 2 hours).

Surviving mice were euthanized 48 hours after venom

administration, and a postmortem blood sample was

obtained via cardiocentesis.

**ANTIBODY TITERS**

Blood samples were collected from the retro-

orbital venous sinus of isoflurane-anesthetized mice

1 week before venom challenge exposure (ie, 3

weeks after the second injection of CAT vaccine

or adjuvant) for use in determination of 2 sets of

serum antibody titers. First, to verify that mice gen-

erated antibodies against the CAT vaccine, serial

serum antibody titers of 3 randomly selected vac-

inated mice were compared with serial serum an-


tibody titers of 3 randomly selected adjuvant-only

control mice. Second, to compare specificity of an-

tibodies generated, dilutions (1:8,000) of serum ob-

tained from 8 randomly selected vaccinated mice

were tested against each of the 3 venoms.

**STATISTICAL ANALYSIS**

Mean survival time in minutes and Kaplan-Meier survival curves were generated for the 3 venoms and

saline (0.9% NaCl) solution control samples. A z test of

proportions was used to compare survival rates of vac-

inated versus control mice for all venoms. Log-rank

analysis was used to compare Kaplan-Meier survival

curves of vaccinated versus control mice for all ven-

oms. Multilevel, mixed-effects linear regression mod-

eling<sup>†</sup> was used to compare specificity of an antibody

Table 1—Summary of survival data for mice inoculated with CAT vaccine or adjuvant only at 0 and 4 weeks and challenge-exposed 4 weeks later with venom of WD rattlesnakes (*Crotalus atrox*), NP rattlesnakes (*Crotalus oreganus oreganus*), and SP rattlesnakes (*Crotalus oreganus helleri*).  

<table>
<thead>
<tr>
<th>Variable</th>
<th>WD rattlesnake venom</th>
<th>NP rattlesnake venom</th>
<th>SP rattlesnake venom</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of mice injected with venom</td>
<td>15</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>No. of mice that survived to 48 h after venom injection</td>
<td>6</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Survival time (min)</td>
<td>Mean</td>
<td>Minimum</td>
<td>Maximum*</td>
</tr>
<tr>
<td></td>
<td>1,311</td>
<td>121</td>
<td>2,880</td>
</tr>
<tr>
<td></td>
<td>368</td>
<td>238</td>
<td>422</td>
</tr>
<tr>
<td></td>
<td>842</td>
<td>82</td>
<td>2,880</td>
</tr>
<tr>
<td></td>
<td></td>
<td>284</td>
<td>401</td>
</tr>
<tr>
<td></td>
<td></td>
<td>697</td>
<td>1,440</td>
</tr>
<tr>
<td></td>
<td></td>
<td>585</td>
<td>1,269</td>
</tr>
<tr>
<td>P value†</td>
<td>z test for proportions</td>
<td>0.006</td>
<td>0.068</td>
</tr>
<tr>
<td></td>
<td>Log-rank analysis</td>
<td>0.146</td>
<td>0.010</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.166</td>
</tr>
</tbody>
</table>

*An endpoint of 2,880 min (ie, 48 hours) for survival was determined prior to the study (ie, surviving mice were euthanized at 48 hours after venom injection). Despite the fact some mice were expected to live > 48 hours after venom injection, survival time was limited in this manner to avoid effects on reported mean survival times in surviving mice and is in accordance with commonly accepted practices for survival studies.†Values were significant at P < 0.05.

--- = Not applicable because there were no surviving mice in either of these groups.
titer of 1:8,000 for all venoms. Significance for all tests was set at $P \leq 0.05$.

**Results**

**SURVIVAL RATE AND SURVIVAL TIME**

Both survival rate and survival time were analyzed (Table 1). For mice vaccinated with CAT vaccine, 6 of 15 mice challenge-exposed with WD rattlesnake venom, 3 of 15 mice challenge-exposed with NP rattlesnake venom, and 0 of 15 mice challenge-exposed with SP rattlesnake venom were alive at 48 hours after venom injection, whereas adjuvant-only control mice survived $\leq 21$ hours after injection of any of the 3 rattlesnake venoms. Mean survival time of vaccinated mice was longer than that of adjuvant-only control mice for all venoms (1,311 vs 368 minutes for WD rattlesnake venom, 842 vs 284 minutes for NP rattlesnake venom, and 697 vs 585 minutes for SP rattlesnake venom). Survival analysis for individual venom revealed that results of the z test for proportions were significant ($P = 0.01$) only for WD rattlesnake venom. Log-rank analysis of survival curves revealed significant ($P = 0.01$) differences only for NP rattlesnake venom (Figure 2). Maximum survival time was greatest for vaccinated mice, compared with survival time for adjuvant-only control mice, for all venoms. Notably, minimum survival time was greater for adjuvant-only control mice than for vaccinated mice for both WD and NP rattlesnake venoms. This was evident on the Kaplan-Meier survival curve for WD rattlesnake venom as an initial increase in death of vaccinated mice, compared with that of control mice, at early time points ($< 300$ minutes after venom injection). Because of this finding, a log-rank analysis for WD rattlesnake venom that excluded early time points was conducted ($n = 7$ mice) and revealed a significant ($P = 0.004$) effect.

**ANTIBODY TITERS**

Antibody titers against all 3 rattlesnake venoms for the 3 vaccinated and 3 control mice were plotted (Figure 3). Dilutions tested were 1:4,000, 1:8,000, 1:16,000, 1:32,000, 1:64,000, and 1:128,000. Mice vaccinated with CAT developed measurable antibody titers against all 3 venoms, whereas mice receiving only adjuvant had no evidence of reactive serum antibodies against any venom. The OD for a 1:8,000 dilution of serum obtained from 8 additional randomly selected vaccinated mice tested against all 3 venoms was plotted (Figure 4). Comparison of OD for the various venoms suggested a decreasing reactivity as follows: the reactivity of WD rattlesnake venom was greater than that of NP rattlesnake venom, and the reactivity of NP rattlesnake venom was greater than that of SP rattlesnake venom. Analysis of a multilevel mixed-effects linear regression model with venom as the sole categorical predictor revealed significant ($P \leq 0.001$) differences in OD for each venom. Interindividual variation was also evident because the majority (6/8) of the mice had titers with OD values approaching or exceeding 1.0, whereas the remainder (2/8) had OD values $< 0.5$. 

![Figure 2](image_url)—Kaplan-Meier survival curves for vaccinated mice (dashed lines) and adjuvant-only control mice (solid lines) after challenge exposure with WD rattlesnake venom (A), NP rattlesnake venom (B), and SP rattlesnake venom (C). There were 15 mice in each group. Time of challenge exposure (injection of venom) was designated as time 0. There was a significant ($P = 0.01$; log-rank analysis) difference in survival curves of vaccinated versus adjuvant-only mice after injection of only NP rattlesnake venom. In panel A, notice the possible early death phenomenon attributable to ADE of WD rattlesnake venom.
In the present study, survival analysis after rattlesnake envenomation of mice was conducted in a randomized, blinded, placebo-controlled study to evaluate the comparative effectiveness of CAT vaccine against 3 rattlesnake venoms. The data reported included evaluation of survival rate (whether a mouse died ≤ 48 hours after venom injection) as well as evaluation of survival time (number of minutes a mouse survived after venom injection, up to 48 hours). Survival time is an important consideration in light of the fact a venom vaccine may be useful if it extends the course of the envenomation, thereby allowing additional time to seek primary medical treatments such as antivenin and intensive care. In addition, antibody titers of vaccinated and adjuvant-only control mice were compared as well as specificity of the antibodies generated against each of the 3 venoms. Overall, results of the challenge-exposure experiment indicated that CAT vaccination resulted in a significant increase in survival rate and survival time against injection with WD rattlesnake venom; equivocal results after injection of NP rattlesnake venom, which would likely require a greater number of mice to verify a difference; and no significant improvement in survival measures after injection of SP rattlesnake venom. Analysis of antibody titers revealed a clearly measurable antibody response in vaccinated mice, compared with that in adjuvant-only control mice, against all 3 venoms. The antibodies were most reactive against WD rattlesnake venom, with significantly less reactivity against venoms of the 2 other rattlesnake species.

Analysis of the data for the present study indicated that administration of CAT vaccine conferred an increase in survival rate and survival time in vaccinated versus control mice challenge-exposed with WD rattlesnake venom. Mean survival time was greater in vaccinated than in control mice, and survival rate improved significantly (P = 0.01; z test for proportions). Unexpectedly, results for log-rank analysis of
survival curves did not reveal significant differences. This result was particularly surprising because challenge exposure with NP rattlesnake venom had a significant effect, as determined by use of log-rank analysis, despite the fact there were only half as many survivors as for challenge exposure with WD rattlesnake venom. Notably, minimum survival time was greater for control versus vaccinated mice for both WD and NP rattlesnake venom (Table 1). This was also evident on the Kaplan-Meier survival curve for WD rattlesnake venom as an initial increase in death of vaccinated versus adjuvant-only control mice at early time points (< 300 minutes after venom injection; Figure 2). The early deaths may have sufficiently altered early time points of the curve of vaccinated mice after injection of WD rattlesnake venom such that statistical modeling resulted in a curve for vaccinated mice that was indiscernible from the curve for the control mice, despite the clear difference at later time points (P = 0.004 for log-rank analysis after 300 minutes). We propose that the early deaths could have been attributable to 1 factor or a combination of factors, such as genetic predisposition to venom sensitivity, injection near or into a vascular bed that hastened systemic exposure to venom, or an antibody-mediated early death phenomenon that has been observed in a laboratory setting when testing vaccines against viruses and bacterial toxins. 

Use of the vaccine may afford limited cross-protection against NP rattlesnake venom; however, the data are not entirely conclusive. Mean survival rate of vaccinated mice significantly (P = 0.01; log-rank analysis of survival curves) exceeded that of adjuvant-only control mice, which suggested a protective effect. However, results of the z test for proportions of survival time did not reveal significant (P = 0.07) differences. However, it is plausible that testing a larger population of mice may have allowed us to detect a more subtle effect by use of the z test of proportions.

The vaccine did not provide significant protection against SP rattlesnake venom, although the mice with the greatest survival time were in the vaccinated group. The CAT vaccine may have been less effective against SP rattlesnake venom because of the divergent molecular composition of that venom. For example, 1 population of SP rattlesnakes can produce Mojave toxin, a unique and powerful neurotoxin, which to date has not been found in WD or NP rattlesnake venoms. 

In addition to survival analysis, antibody titers were measured in a number of mice to verify an antibody response against the CAT vaccine (Figure 3). Compared with control mice, vaccinated mice had a variably robust antibody response, and initial titers suggested that the antibodies were more specific for WD rattlesnake venom than for the NP or SP rattlesnake venoms. On the basis of this observation, sera from 8 randomly selected vaccinated mice were evaluated for antibody specificity against each of the 3 venoms evaluated in the study (Figure 4). Linear regression analysis revealed significantly increased OD against WD rattlesnake venom, as compared with results against SP or NP rattlesnake venoms. The analysis indicated that antibodies generated by mice were most specific against the venom of manufacture (ie, WD rattlesnake venom), compared with specificity against the other 2 genetically distinct venoms. It should be emphasized that antibody titers were measured only to verify that mice generated an antibody response against the vaccine and to evaluate the specificity of that antibody response. The magnitude of the murine antibody response and how it may relate to survival of vaccinated dogs and horses (or the ability of clinicians to provide a prognosis for survival of vaccinated animals) in real-life situations were beyond the scope of the present study.

The present study had several potential confounders. First, on the basis of a previous manufacturer-designed study, mice in the present study were injected with a vaccine dose of 0.2 mL, which could be from 50- to 1,500-fold as high (by volume) as manufacturer-recommended doses for dogs and horses. Potentially, this could have resulted in a more robust antibody response and more enhanced protective benefit than typically would be afforded to companion animals. On the other hand, it should be mentioned that mice were challenge-exposed with an extremely high (twice the LD50) dose of venom administered via the IP route commonly used in venom studies on mice. In most naturally occurring scenarios, companion animals receive SC or IM injection of venom, which results in slower and less immediately severe systemic effects than were seen in the mice of the study reported here. In light of this, findings for the present study should be considered with the caveat that, in theory, the vaccine may improve survival rate and survival time, but these improvements remain to be definitively verified in practice settings for the specific species and situations of interest. Finally, it should be mentioned that we evaluated survival rate and survival time but did not directly assess morbidity. In actual envenomations, local effects such as severe necrosis, hemorrhage, and inflammation can cause substantial morbidity, which potentially can lead to severe incapacitation and death. It remains to be determined whether vaccination has substantial effects to prevent or reduce important local sequelae after snake envenomation. Despite these drawbacks, there are a number of reasons investigators should use the described method of envenomation of mice, including that it is a well-accepted technique for venom analysis and antivenin evaluation, adheres to the concept of replacement in research (ie, use of mice instead of dogs or horses), and has been used in experiments conducted by the manufacturer to obtain USDA licensing for the CAT vaccine.

Data from the rattlesnake envenomation of mice reported here indicated that administration of the CAT vaccine resulted in a significant increase in survival
rate and survival time after injection of WD rattlesnake venom, equivocal results after injection of NP rattlesnake venom (possibly requiring a greater number of animals to confirm a difference), and no significant improvement in survival variables after injection of SP rattlesnake venom. Analysis of antibody titers confirmed a measurable antibody response in vaccinated versus adjuvant-only control mice and confirmed that specificity of the antibody response was significantly greater against the venom of manufacture. Overall, results of the present study suggested that vaccination with the CAT vaccine may provide limited cross-protection against NP rattlesnake venom but no significant cross-protection against SP rattlesnake venom. Future studies should include more in-depth analysis of antibody titers, testing of alternative vaccination strategies involving other venoms, and investigation into early deaths seen in some of the vaccinated mice. Such studies will be useful in validating results of the present study and providing increased insight into the real-world effectiveness of a rattlesnake venom vaccine.

Acknowledgments

The authors thank Drs. Thomas Ganz and Elizabeta Nemeth for assistance with laboratory procedures and statistical analysis; Brian Rasnow, Tina Rasnow, Bill Dundas, Thomas Masi, Jim Brockett; Wayne J. Fowlie, Erick Briggs, Gary Kessler, Marisa Schierjohn, Thomas M. McCabe, Nicholas S. McCabe, Tim Turmezei, Andrew Sioslac, Jim Harrison, and Kristen Wiley for assistance with venom acquisition; and Dr. Matthew Moyle for assistance with development of the protocol for determining antibody titer.

Footnotes


c. Alhydrogel 2%, lot No. AHG35-01, InvivoGen Inc, San Diego, Calif.

d. Lot No. CAT 1412, Kentucky Reptile Zoo, Slade, Ky.

e. STATA, version 13, StataCorP, college station, Tex.

References


