

Research article

Survey of Adult *Dermacentor variabilis* (Say) (Acari: Ixodidae) Collected in Northern Mississippi for Spotted Fever Group Rickettsiae

Jerome Goddard^{1*}, Lauren Goltz¹, Kristine T. Edwards¹, Whitney Smith², and Andrea Varela-Stokes²

¹Department of Biochemistry, Molecular Biology, Entomology and Plant Pathology, 100 Old Highway 12, Clay Lyle Entomology Building, Mississippi State University, Mississippi State, MS 39762, U.S.A.

²Department of Basic Sciences, College of Veterinary Medicine, Mississippi State University, Mississippi State, MS 39762, U.S.A.

*Corresponding author e-mail: jgoddard@entomology.msstate.edu

Received: 04--III-2014 Accepted: 28-IV-2014

Abstract: One of the primary tick vectors of Rocky Mountain Spotted Fever (RMSF), *Dermacentor variabilis* (Say), is found throughout much of North America, including Mississippi, where numerous cases are reported annually. Although RMSF has long been documented in the US, it may be confused with other zoonoses, which sometimes delays treatment. Therefore, detailed documentation of the distribution and infection rates of this tick is important to public health officials. In this study, two sites in northern Mississippi, the Sam D. Hamilton Noxubee National Wildlife Refuge and Wall Doxey State Park, were surveyed weekly by drag cloth for a one year period for the presence of *D. variabilis*. The sites were chosen because northern Mississippi has more cases of RMSF than other parts of the state. Sixty-four adult *D. variabilis* ticks were collected in these sites and subsequently examined for presence of Spotted Fever Group Rickettsiae (SFGR), which includes the causative agent of RMSF, *Rickettsia rickettsii*. None of the ticks collected showed evidence of *R. rickettsii* or any other SFGR. Despite continued case reporting of RMSF in Mississippi, we found no evidence for natural infection of *D. variabilis* with *R. rickettsii* in these two sites in Mississippi during this sampling period.

Keywords: Ticks, Rocky Mountain spotted fever, vectors, zoonoses, *Dermacentor variabilis*

Introduction

In the eastern U.S., *Dermacentor variabilis* (Say), the American dog tick, is the primary vector of Rocky Mountain Spotted Fever (RMSF) which is caused by *Rickettsia rickettsii*. This tick species is capable of transmitting several pathogens of medical significance across a variety of vertebrate hosts, including humans (Burgdorfer 1975). As for RMSF in Mississippi, there are numerous historical and current reports of cases every year (Sexton and Burgdorfer 1975; Sexton et al. 1976; MSDH 2012), rising sharply after

the pesticide DDT was banned in 1972 (Figure 1). There were 25 cases of RMSF in Mississippi during 2012 (0.8/100,000) and 24 cases in 2011 (0.8/100,000)(MSDH 2012). Transmission through the bite of an infected *D. variabilis* tick requires at least a 4-6 hour attachment period with a subsequent incubation period of 3-14 days. There is no evidence of person to person transmission. In nature, reservoir hosts include small rodents such as chipmunks, squirrels, and white-footed mice (Burgdorfer 1975). Clinical presentation of RMSF is typical of many zoonoses with acute onset of fever, severe headache, malaise, myalgia, nausea, and vomiting, and also may include a macular or maculopapular rash on the extremities, including the palms and soles, which usually spreads over the entire body. Fatality occurs in approximately 13-25% of untreated cases or those with delayed recognition (Poole 1997; MSDH 2012).

Previous tick and rickettsia surveys in Mississippi have demonstrated spotted fever group rickettsiae (SFGR) infection rates in *D. variabilis* ranging from 1.2 – 3.0% (Nause and Norment 1984; Norment et al. 1985). However, publications from other states have recently cast doubt upon the role of *D. variabilis* in spotted fever-like infections because the causative agent seems to be more rare in the tick than once supposed (Moncayo et al. 2010; Fritzen et al. 2011; Stromdahl et al. 2011; Nadolny et al. 2014). Therefore, this study was undertaken to assay field-collected *D. variabilis* from two sites in northern Mississippi for the presence of SFGR, and especially *R. rickettsii*.

More RMSF cases are reported from northern Mississippi than the rest of the state (Figure 2), therefore two 0.5 hectare sites in that region were selected for tick sampling – one located at Wall Doxey State Park (WD) near Holly Springs in Marshall County (34.6567° N, 89.4664° W) and the other approximately 217 km southeast at the Sam D. Hamilton Noxubee National Wildlife Refuge (NR) (33.2944° N, 88.7788° W), which extends into three counties (Noxubee, Oktibbeha, and Winston). These sites were chosen because they are wooded with a medium-dense canopy, contain abundant leaf litter, and have numerous suitable host animals for a variety of tick species. Previous studies have demonstrated high numbers of ticks in these two areas (Norment et al. 1985; Goddard et al. 2003).

Materials and Methods

During a one-year period from August 2, 2010 through August 2, 2011, each site was sampled once per week, totaling 104 site visits. In order to collect from the plots in an efficient manner, each site was visually divided into 25 lanes and collecting was performed by transecting the sites in these predetermined lanes. A drag cloth was used for sampling and examined for ticks every 10 meters; all ticks found attached to the cloth were removed and placed in vials containing 95% ethanol. Once in the lab, ticks were identified to species and life stage, and adults were sexed. Only adult *D. variabilis* were analyzed since immatures do not bite people. One pair of ticks from this study was sent to Dr. Richard Robbins (Armed Forces Pest Management Board, Washington DC) for identification to species and these specimens have been deposited as vouchers in the Mississippi Entomological Museum, Mississippi State University.

In the lab, ticks were removed from ethanol, dried, placed in individual, labeled microcentrifuge tubes where they were macerated using a new, sterile #11 scalpel blade in 50µl lysis buffer and 10µl Proteinase K. Tick DNA was then extracted using the Illustra tissue and cells genomic prep mini spin kit (GE Healthcare, Piscataway, NJ) per manufacturer's protocol. All samples of extracted DNA from adult ticks were tested by PCR amplification of a portion of the tick mitochondrial 16S rRNA gene (Black and Piesman 1994) to confirm that tick DNA was present and extraction was successful. All samples were tested using a SFG-wide (SFGW) single PCR assay for amplification of RNA polymerase beta-subunit-encoding (*rpoB*) gene according to a published protocol (Paddock et al. 2010). All PCR assays were performed in Bio-Rad DNA Engine Thermal Cyclers and all products were visualized by electrophoresis in 2% agarose gels containing ethidium bromide. The positive control for tick mitochondrial 16S rRNA gene PCR assays was genomic DNA from a male Gulf Coast tick, *Amblyomma maculatum*. For all assays, distilled water was used as a negative (non-template) control, and DNA extracts of cultivated *R. parkeri* were used as positive a control for SFGR. Amplified products were purified using a DNA Clean and Concentrator Kit (Zymo Research, Irvine, CA), bidirectionally sequenced (Eurofins MWG Operon, Huntsville, AL, USA), and aligned using Clustal X2 (Larkin et al. 2007). Consensus sequences were compared to sequences in GenBank using the Basic Local Alignment Search Tool (National Center for Biotechnology Information www.ncbi.nlm.nih.gov).

Results and Discussion

A total of 64 adult *D. variabilis* ticks were collected in the sites over the one-year period; two were submitted for voucher specimens and the remaining 62 were examined for presence of SFGR (Table 1). *Dermacentor variabilis* collected during this study showed distinct seasonality with 70% (45/64) of all ticks collected between August 2 and September 16, 2010; 91% (31/34) from NR and 47% (14/30) from WD (Figure 3). At both sites, the peak of tick activity was mid-August. Interestingly, seasonality of the ticks roughly matches seasonal distribution of RMSF cases in northern Mississippi (April through October). Amplification of the tick mitochondrial 16S rRNA gene was detected in 100% of tick samples (62/62) examined, indicating that DNA extractions were successful. No tick samples were positive for DNA of *Rickettsia rickettsii* or any other SFGR (Table 1). Despite continued case reporting of Rocky Mountain spotted fever in Mississippi, we found no evidence for infection of *D. variabilis* with *R. rickettsii* in these two sites in Mississippi (during this sampling period). Perhaps RMSF case reports actually represent something else, such as infection with *R. parkeri*, which has been suggested (Openshaw et al. 2010; Ekenna et al. 2014), or some other vector such as the brown dog tick, *Rhipicephalus sanguineus*, is involved in Mississippi RMSF cases (Elchos and Goddard 2003; Demma et al. 2005). Despite historical reports of SFGR in *D. variabilis* in Mississippi (Nause and Norment 1984; Norment and Burgdorfer 1985), these negative data are consistent with recent surveys in other states revealing absolutely no *R. rickettsii* in *D. variabilis* (Moncayo et al. 2010; Fritzen et al. 2011; Nadolny et al. 2014). Further research is warranted to explore why so few samples of the reported “primary” vector of RMSF are actually positive for the agent.

Acknowledgements

This article has been approved for publication as Journal Article No. J-12485 of the Mississippi Agriculture and Forestry Experiment Station, Mississippi State University.

Table 1. Number of adult *Dermacentor variabilis* ticks collected and week collected at the Sam D. Hamilton Noxubee National Wildlife Refuge and Wall Doxey State Park, Mississippi (Aug. 2010 – Aug. 2011). All ticks were 16S rRNA positive for tick DNA but *RpoB* negative for spotted fever group rickettsiae.

Month	Number Collected (Noxubee Refuge)	Number Collected (Wall Doxey Park)
Jan	--	--
Feb	--	--
Mar	1	0
Apr	0	5
May	1	5
Jun	0	4
Jul	0	2
Aug	24	13*
Sep	7	1
Oct	0	0
Nov	1	0
Dec	0	0
Total	34	30

*Two ticks from the August 26 collection were kept as voucher specimens and not tested. All ticks from each collection date were sexed, extracted, and PCR tested separately.

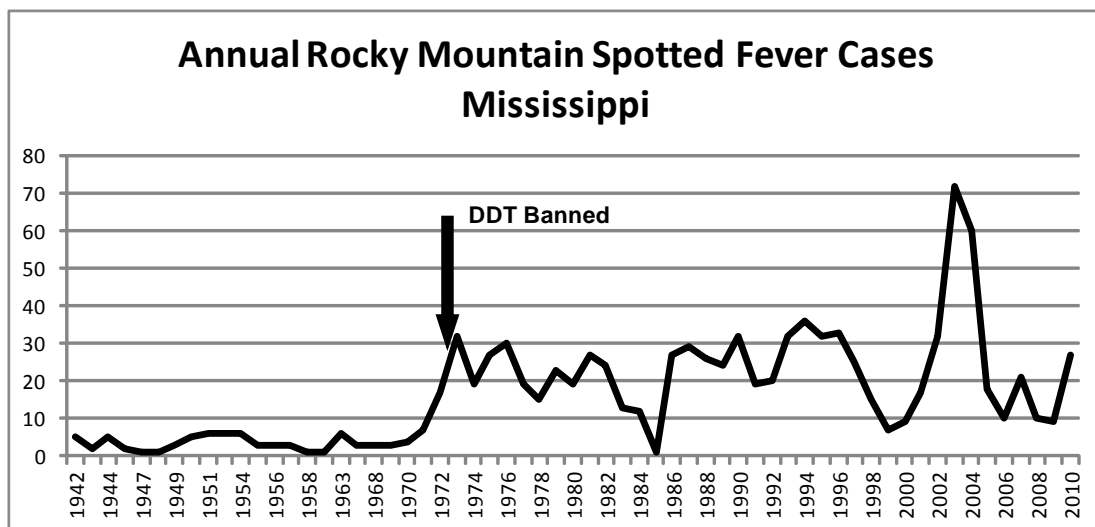


Figure 1. Number of human cases of RMSF reported to the Mississippi Department of Health, 1942-2010 (Data courtesy Mississippi State Department of Health).

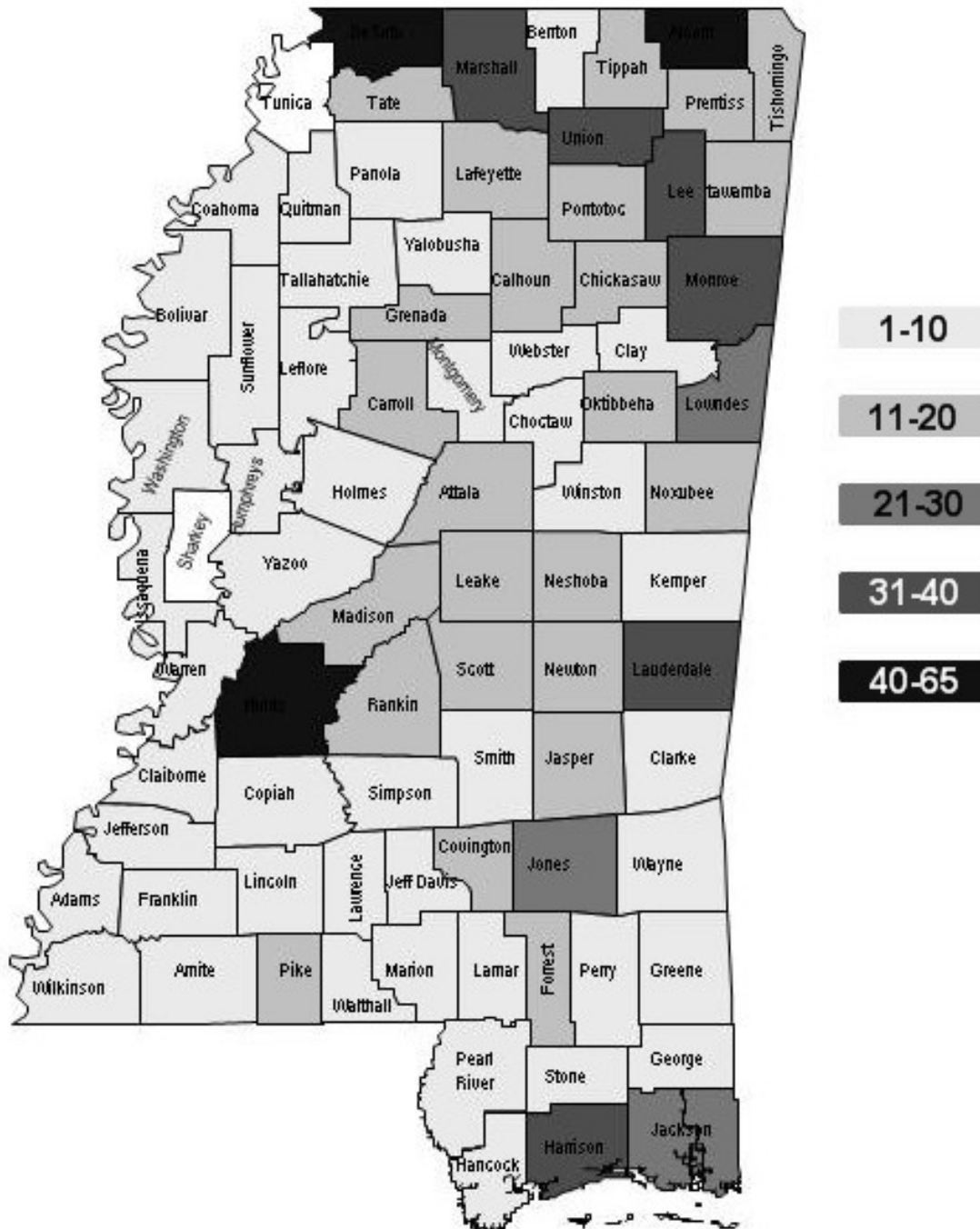


Figure 2. County distribution of human Rocky Mountain Spotted Fever cases reported to the Mississippi Department of Health, 1942-2010. Note: counties in northeast Mississippi are relatively unpopulated (Data courtesy Mississippi State Department of Health).

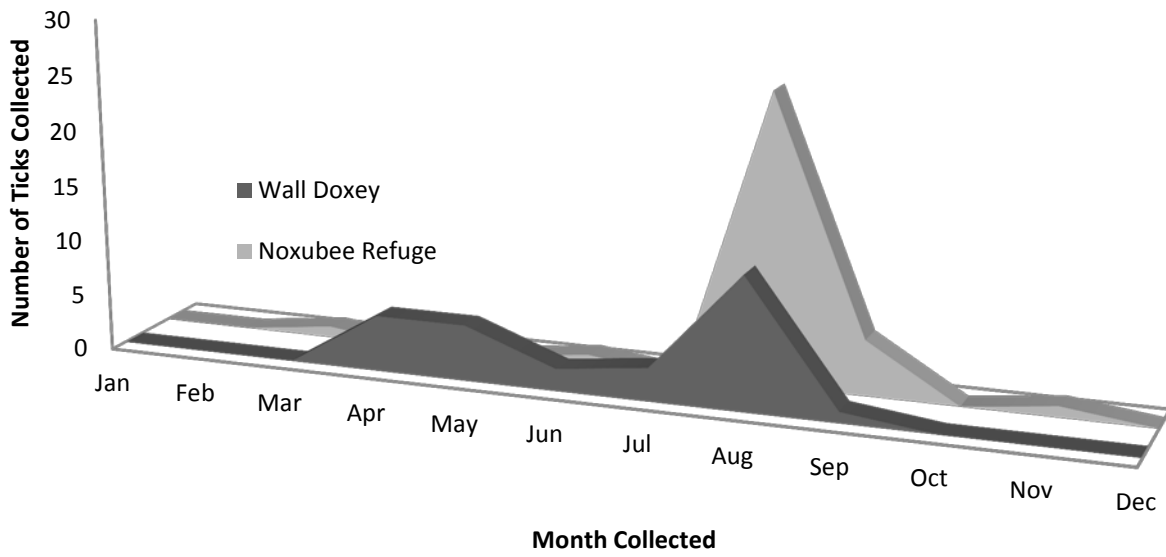


Figure 3. Number of adult *Dermacentor variabilis* ticks collected and week collected at Sam D. Hamilton Noxubee National Wildlife Refuge and Wall Doxey State Park, Mississippi (Aug. 2010 – Aug. 2011).

References

- Black, W. C. and Piesman, J. 1994.** Phylogeny of hard and soft-tick taxa (Acari: Ixodidae) based on mitochondrial 16S rDNA sequences. Proc. Natl. Acad. Sci. USA. 91.
- Burgdorfer, W. 1975.** A review of Rocky Mountain spotted fever: its agent, and its vectors in the U.S. J. Med. Entomol. 12: 269-278.
- Demma, L. J., Traeger, M. S., Nicholson, W. L., et al. 2005.** Rocky Mountain spotted fever from an unexpected tick vector in Arizona. New England Journal of Medicine. 353: 587-594.
- Ekenna, O., Paddock, C. D. and Goddard, J. 2014.** Gulf Coast tick rash illness caused by *Rickettsia parkeri*. J. Mississippi St. Med. Assoc. (in press).
- Elchos, B. N. and Goddard, J. 2003.** Implications of presumptive fatal Rocky Mountain spotted fever in two dogs and their owner. Journal of the American Veterinary Medical Association. 223: 1450-1452.
- Fritzen, C. M., Huang, J., Westby, K., et al. 2011.** Infection prevalences of common tick-borne pathogens in adult lone star ticks (*Amblyomma americanum*) and American dog ticks (*Dermacentor variabilis*) in Kentucky. Am. J. Trop. Med. Hyg. 85: 718-723.
- Goddard, J., Sumner, J. W., Nicholson, W. L., et al. 2003.** Survey of ticks collected in Mississippi for *Rickettsia*, *Ehrlichia*, and *Borrelia* species. J. Vector Ecol. 28: 184-189.
- Larkin, M. A., Blackshields, G., Brown, N. P., et al. 2007.** Clustal W and clustal X, version 2.0. Bioinformatics: 2947-2948.
- Moncayo, A. C., Cohen, S. B., Fritzen, C. M., et al. 2010.** Absence of *Rickettsia rickettsii* and occurrence of other spotted fever group rickettsiae in ticks from Tennessee. Am. J. Trop. Med. Hyg. 83: 653-657.
- MSDH. 2012.** Annual summary of selected reportable diseases - 2012. No. 30 (1):103.
- Nadolny, R. M., Wright, C. L., Sonenshine, D. E., et al. 2014.** Ticks and spotted fever group rickettsiae of southeastern Virginia. Ticks Tick Borne Dis. 5: 53-57.
- Nause, C. L. and Norment, B. R. 1984.** A tick/rickettsia survey of canines in Hinds, Lafayette, and Oktibbeha counties, Mississippi. J. Med. Entomol. 21: 405-408.
- Norment, B. R. and Burgdorfer, W. 1985.** Susceptibility of small mammals to nonvirulent spotted fever group rickettsiae. J. Med. Entomol. 22: 200-203.
- Norment, B. R., Stricklin, L. S. and Burgdorfer, W. 1985.** Rickettsia-like organisms in ticks and antibodies to spotted fever-group rickettsiae in mammals from Northern Mississippi. J. Wildl. Dis. 21: 125-131.
- Openshaw, J. J., Swerdlow, D. L., Krebs, J. W., et al. 2010.** Rocky Mountain spotted fever in the United States, 2000-2007: interpreting contemporary increases in incidence. Am. J. Trop. Med. Hyg. 83: 174-182.
- Paddock, C. D., Fournier, P. E., Sumner, J. W., et al. 2010.** Isolation of *Rickettsia parkeri* and identification of a novel spotted fever group *Rickettsia* sp. from Gulf Coast ticks (*Amblyomma maculatum*) in the United States. Appl. Environ. Microbiol. 76: 2689-2696.
- Poole, T. 1997.** Virus kills siblings. The Laurel Leader-Call Newspaper, Laurel, MS, October 15 edition, p. 1 No.
- Sexton, D. J. and Burgdorfer, W. 1975.** Clinical and epidemiologic features of Rocky Mountain spotted fever in Mississippi. South. Med. J. 68: 1529-1535.
- Sexton, D. J., Burgdorfer, W., Thomas, L., et al. 1976.** Rocky Mountain spotted fever in Mississippi: survey for spotted fever antibodies in dogs and for spotted fever group rickettsiae in dog ticks. Am. J. Epidemiol. 103: 192-197.
- Stromdahl, E. Y., Jiang, J., Vince, M., et al. 2011.** Infrequency of *Rickettsia rickettsii* in *Dermacentor variabilis* removed from humans, with comments on the role of other human-biting ticks associated with spotted fever group Rickettsiae in the United States. Vector Borne Zoonotic Dis. 11: 969-977.

