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Identification of Midgut and Salivary Glands as Specific and Distinct Barriers to Efficient Tick-Borne Transmission of *Anaplasma marginale*▽

Massaro W. Ueti,[1]* James O. Reagan, Jr.,[1,2] Donald P. Knowles, Jr.,[1,2] Glen A. Scoles,[2] Varda Shkap,[3] and Guy H. Palmer[1]

Program in Vector-borne Diseases, Department of Veterinary Microbiology and Pathology, Washington State University, Pullman, Washington[1]; Animal Diseases Research Unit, U.S. Department of Agriculture, Agricultural Research Service, Pullman, Washington[2]; and Division of Parasitology, Kimron Veterinary Institute, Bet Dagan, Israel[3]

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Understanding the determinants of efficient tick-borne microbial transmission is needed to better predict the emergence of highly transmissible pathogen strains and disease outbreaks. Although the basic developmental cycle of *Anaplasma* and *Ehrlichia* spp. within the tick has been delineated, there are marked differences in the ability of specific strains to be efficiently tick transmitted. Using the highly transmissible St. Maries strain of *Anaplasma marginale* in *Dermacentor andersoni* as a positive control and two unrelated nontransmissible strains, we identified distinct barriers to efficient transmission within the tick. The Mississippi strain was unable to establish infection at the level of the midgut epithelium despite successful ingestion of infected blood following acquisition feeding on a bacteremic animal host. This inability to colonize the midgut epithelium prevented subsequent development within the salivary glands and transmission. In contrast, *A. marginale* subsp. *centrale* colonized the midgut and then the salivary glands, replicating to a titer indistinguishable from that of the highly transmissible St. Maries strain and at least 100 times greater than that previously associated with successful transmission. Nonetheless, *A. marginale* subsp. *centrale* was not transmitted, even when a large number of infected ticks was used for transmission feeding. These results establish that there are at least two specific barriers to efficient tick-borne transmission, the midgut and salivary glands, and highlight the complexity of the pathogen-tick interaction.

Arthropod vectors transmit a wide diversity of microbial pathogens, including viruses, bacteria, and protozoa, to humans and animals (2, 12, 20, 25, 28). Although simple mechanical transmission may occur, efficient transmission usually requires pathogen replication and development within the vector (1, 10, 16). In most pathogen-vector interactions, this involves the invasion of one or more organs of the vector, survival in face of the arthropod immune response, intra- or extracellular replication, and the development of infectivity prior to transmission effected by the vector biting or feeding on a susceptible human or animal host (2, 9, 27). Understanding how the pathogen mediates each of these steps and how this varies among pathogen strains is fundamentally important for better predicting vector-borne disease patterns and for developing new strategies to block transmission.

Pathogens in the genera *Anaplasma* and *Ehrlichia* are transmitted by ixodid ticks that initially feed on an infected animal host and then, following interhost transmission, feed on a susceptible human or animal (8, 9, 14, 17). This transmission reflects a complex development within the tick. Following the initial acquisition feeding and ingestion of the blood meal into the midgut lumen, the bacterium enters the midgut epithelial cells and undergoes a first round of replication within a membrane-bound vacuole (11, 22). This is followed by migration to and invasion of the salivary glands (9, 10). A second round of replication in the salivary gland acinar cells, apparently dependent on resumption of tick feeding on a mammalian host, is followed by transmission via the saliva (9, 13). Although this basic development cycle is broadly conserved among the *Anaplasma* and *Ehrlichia* spp., it is also clear that not all pathogen strains are equally or efficiently tick transmitted. Our interest is to identify the specific developmental steps within the tick vector that affect pathogen transmissibility, with the long-term goal of mapping specific pathogen determinants of high versus low transmissibility.

Within these two genera, only a single pathogen strain with a defective transmission phenotype has been examined in detail to date. The Florida strain of *Anaplasma marginale* is nontransmissible by *Dermacentor andersoni*, *Dermacentor variabilis*, *Rhipicephalus (Boophilus) microplus*, and *Rhipicephalus (Boophilus) annulatus*, all competent vectors for other *A. marginale* strains and the natural vectors within North America (6, 23). The defect in the Florida strain occurs at the level of the midgut following initial acquisition feeding. In vitro studies suggest a failure to bind to the midgut epithelial cells, while in vivo studies are more consistent with entry into the midgut epithelium followed by clearance without detectable first-round replication (6, 23). Whether this defect represents the sole determinant of transmissibility or transmission efficiency is unknown. In the present study, we use additional pathogen strains to demonstrate that transmission efficacy is determined at the levels of both the tick midgut and salivary glands.

* Corresponding author. Mailing address: Department of Veterinary Microbiology and Pathology, Washington State University, Pullman, WA 99164-7040. Phone: (509) 335-6325. Fax: (509) 335-8529. E-mail: massaro@vetmed.wsu.edu.

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MATERIALS AND METHODS

Pathogen and vector strains. Three strains of *A. marginale* were used: the St. Maries and Mississippi strains are *A. marginale* senso stricto strains, and the Israel vaccine strain is currently classified as *A. marginale* subsp. *centrale* (7). The St. Maries strain was used as a reference strain, as it has previously been shown to be efficiently acquired and transmitted by adult male *Dermacentor* and *Rhipicephalus* (*Boophilus*) ticks (9). The Mississippi strain was obtained from an acute clinical case, and it has not been shown to be transmitted by *D. andersoni* (G. H. Palmer, unpublished data). *A. marginale* subsp. *centrale* was derived from the Theiler vaccine strain and has been used continuously as a vaccine in Israel since 1953 (7, 19). The transmission phenotype of *A. marginale* subsp. *centrale* by *D. andersoni* is unknown; however, it lacks the major surface protein 1a (MSP1a) N-terminal repeat region (24) proposed to be required for entry into the tick midgut and thus was predicted to be nontransmissible (5, 6). The colony of *D. andersoni* was originally collected from Owyhee County, ID, and has been maintained at the USDA-ARS Animal Disease Research Unit (23). The colony efficiently transmits multiple strains of *A. marginale*, including the St. Maries strain, and is free of other pathogens (8, 9, 26).

Tick acquisition feeding on *A. marginale*-infected animals. Age-matched (5 to 6 months old) Holstein calves were utilized for infection with the three strains and subsequent tick acquisition feeding. The calves were confirmed to be free of *A. marginale*, as determined by an MSP5 serologic competitive enzyme-linked immunosorbent assay (cELISA) (VMRD, Pullman, WA) and nested PCR targeting *msp5* (22). These assays will detect all *A. marginale* strains, including *A. marginale* subsp. *centrale*. Calves were inoculated with one of the three *A. marginale* strains by intravenous inoculation of 10^9 organisms. The development of acute *A. marginale* bacteremia was assessed by microscopic examination of Giemsa-stained blood smears. During acute bacteremia, defined as levels of $>10^8$ organisms per ml of blood, adult male *D. andersoni* ticks were allowed to acquisition feed on each calf for 7 days. Immediately after removal, a cohort of ticks was dissected and the total midgut, including the luminal blood meal, was collected and DNA extracted, as previously described (13, 28), to confirm the ingestion of *A. marginale* by using quantitative PCR (see below).

Development and replication of *A. marginale* in the tick following transmission feeding. Following the acquisition feeding, the ticks were incubated at 26°C in 94% relative humidity for an additional 7 days prior to transmission feeding (9, 23). This interval ensures that the blood meal is completely digested, which occurs within the first 72 h, and thus, subsequent detection of bacteria within the midgut can be used to confirm entry into the epithelium and quantification can be used to test whether replication has occurred (9). After this incubation period, *D. andersoni* ticks were transmission fed on naïve age-matched (5 to 6 months old) Holstein calves, confirmed free of *A. marginale* as determined by MSP5 cELISA and nested *msp5* PCR (22). As a positive control using the known transmissible St. Maries strain, 10 ticks infected with the St. Maries strain were fed on one calf and 35 on a second calf. In contrast, 100 *D. andersoni* ticks acquisition fed on the Mississippi strain-infected calf were transmission fed on each of two naïve calves. As with the Mississippi strain, 100 *D. andersoni* ticks acquisition fed on the *A. marginale* subsp. *centrale*-infected calf were transmission fed on each of two naïve calves. Following 7 days of transmission feeding, the ticks were removed and individual salivary glands and the midgut were dissected separately. DNA was extracted and used both for quantitative PCR and for confirmation of strain identity from positive samples.

Detection and quantification of *A. marginale*. Bacteria were detected using a specific *msp5* PCR as previously described (9). The *msp5* sequences are identical in the St. Maries and Mississippi strains and 87% identical in *A. marginale* subsp. *centrale* (15). Importantly, the primer sequences (forward, 5′-TACACGTGCC CTACCGAGTTA-3′; reverse, 5′-TCCTCGCCTTGGCCCTCAGA-3′) are conserved in all examined *A. marginale* strains and *A. marginale* subsp. *centrale* and amplify a fragment of 343 bp (22). The identity of the *msp5* amplicons was confirmed by sequencing.

For quantification of the St. Maries and Mississippi strains, the previously described TaqMan real-time PCR assay was used (9, 23). The primer sequences (forward, 5′-CTTCCGAAGTTGTAAGTGAGGGCA-3′; reverse, 5′-CTTATC GGCATGGTCGCCTAGTTT-3′) were selected to amplify a fragment of 202 bp and a TaqMan probe (5′-GCCTCCGCGTCTTTCAACAATTTGGT-3′) designed to bind within the amplicon. Full-length *A. marginale msp5* cloned into pCR-4 TOPO vector (Invitrogen Corporation, Carlsbad, CA) was utilized to construct the standard curve, as previously described (23). Due to the polymorphism in the *msp5* gene of *A. marginale* subsp. *centrale* (15), the real-time assay was modified. The primer sequences (forward, 5′-CACCTCCGAGGTTGTGA GTGA-3′; reverse, 5′-GGTAGGGCACGTGTACTTGCA-3′) were selected for the amplification of a fragment from nucleotides 114 to 264, and a fluorogenic probe (5′-TTACCGTCAGCAGCAGCGATTTTGG-3′) was designed to anneal between nucleotides 182 and 207 of *A. marginale* subsp. *centrale msp5*. The real-time PCR was carried out under the following conditions: 95°C for 10 min, 55 cycles of 95°C for 20 s, 59°C for 10 s, and 72°C for 10 s, final extension at 72°C for 30 s, and holding at 10°C. The real-time PCRs were conducted using a PCR mixture containing 10 mM Tris (pH 8.3), 50 mM KCl, 2.0 mM $MgCl_2$, 200 μM of each dATP, dCTP, dGTP, and dTTP, 0.2 μM of each primer, 0.2 μM fluorogenic probe, and 1.25 U of AmpliTaq Gold (PE Applied Biosystems, Foster City, CA). All reactions were performed using the iCycler iQ real-time PCR detection system (Bio-Rad, Hercules, CA). Full-length *A. marginale* subsp. *centrale msp5* was cloned into the pCR-4 TOPO vector and used to construct a standard curve for the real-time assay. As an internal standard for the extraction of DNA, amplification, and probe binding, known numbers of organisms of each strain were used as a source for DNA extraction and amplification and the results from the real-time assay compared.

Confirmation of strain identity. The identity of *A. marginale* subsp. *centrale* in the blood and tick tissues could be confirmed by sequencing of the amplified *msp5* due to the polymorphism compared to that of the *A. marginale* St. Maries and Mississippi strains. To distinguish between the St. Maries and Mississippi strains, the repeat region of *msp1*α was amplified and sequenced. This region differs in the number and sequence of repeats among strains. Briefly, the primer sequences (forward, 5′-GTGCTTATGGCAGACATTTC C-3′; reverse, 5′-CTCAACACTCGCAACATTGG-3′) were designed to amplify the conserved regions flanking *msp1a* repeats of *A. marginale* strains (18). The strain identity was confirmed by amplicon sequencing.

Transmission of *A. marginale* to naïve animals. The development of infection in the calves following transmission feeding was monitored by microscopic examination of Giemsa-stained blood smears, *msp5* PCR, and MSP5 cELISA. Weekly examination continued until 100 days post-tick feeding, representing >3 standard deviations from the mean time to *A. marginale* detection in the calves transmission fed with ticks infected with the positive control St. Maries strain.

TABLE 1. *A. marginale* levels in the peripheral blood and within the *D. andersoni* total midgut during acquisition feeding

Strain	No. of bacteria/ml of blood	% of *A. marginale*-positive total midguts (no. positive/total no. examined)[a]	Mean no. of bacteria per total midgut (±SD)[a]
A. marginale Saint Maries	1.0×10^8	100 (5/5)	$10^{6.36(\pm 0.85)}$
A. marginale Mississippi	3.2×10^8	100 (20/20)	$10^{3.7(\pm 0.59)}$
A. marginale subsp. *centrale*	1.3×10^8	100 (20/20)	$10^{5.4(\pm 0.69)}$

[a] Includes both undigested blood meal in the midgut lumen and organisms that have entered the midgut epithelium.

RESULTS

***Anaplasma* level in the peripheral blood and tick midgut during acquisition feeding.** Ticks were acquisition fed on infected calves during acute bacteremia, with levels of $>10^8$ organisms per ml of blood (Table 1). All fed ticks ingested *A. marginale* as demonstrated by PCR amplification of *msp5* from the total midgut dissected from a cohort of ticks immediately after removal. The total midgut includes both the epithelium and lumen and thus cannot discriminate between *A. marginale* bacteria that have already entered and replicated in epithelial cells and those remaining in the luminal blood meal. Quantification of the total midgut *A. marginale* bacteria revealed the highest levels in ticks acquisition fed on the St. Maries strain-infected calf and the lowest levels in ticks fed on the *A. marginale* Mississippi-infected calf (Table 1). There was no positive association between the total midgut levels immediately after

TABLE 2. *A. marginale* infection rates and levels within *D. andersoni* following transmission feeding

Strain	% of *A. marginale*-positive midguts (no. positive/ total no. examined)[a]	Mean no. of bacteria per midgut (±SD)	% of *A. marginale*-positive salivary glands (no. positive/ total no. examined)	Mean no. of bacteria per salivary gland pair (±SD)
A. marginale Saint Maries	100 (35/35)	$10^{6.8(\pm 0.45)}$	100 (35/35)	$10^{7.7(\pm 0.45)}$
A. marginale Mississippi	2 (3/150)	NQ[b]	0 (0/150)	NQ
A. marginale subsp. *centrale*	54 (81/150)	$10^{5.4(\pm 0.67)}$	71 (107/150)	$10^{7.4(\pm 0.80)}$

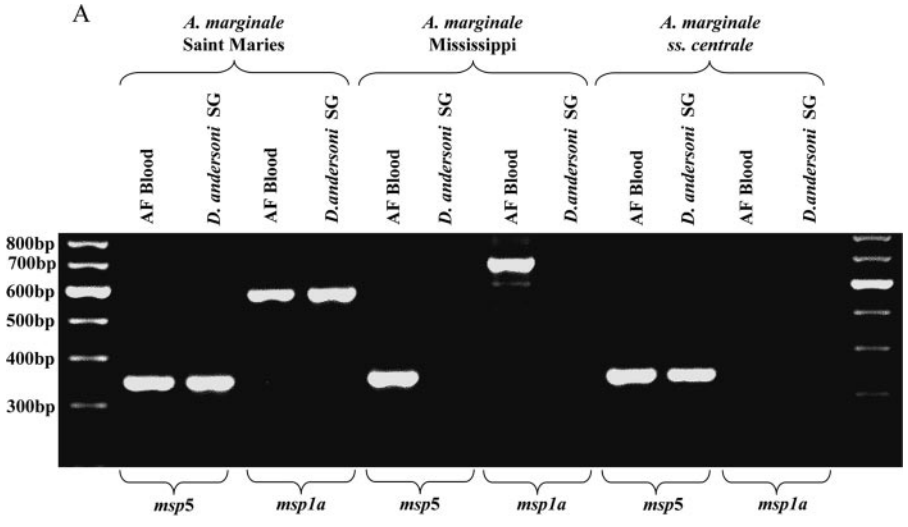
[a] Midguts positive by *msp5* PCR. This reflects only organisms that have entered the midgut epithelium, as this was determined at 14 days postingestion of the acquisition feed blood meal.
[b] NQ, nonquantifiable (below the minimum linear range detectable level of 5×10^1 bacteria).

removal and the *A. marginale* bacteremia levels in the calves during acquisition feeding (Table 1).

Development and replication of *A. marginale* in the tick following transmission feeding. The ability of each strain to colonize the midgut epithelium was measured by both determining the infection rate and quantifying the *A. marginale* load in positive midguts. Consistent with the high transmissibility of the St. Maries strain, 100% of the ticks exposed to this strain were *msp5* positive and contained a mean of $10^{6.8}$ organisms per midgut (Table 2). *A. marginale* subsp. *centrale* also colonized the midgut epithelium although with a lower efficiency (Table 2). In contrast, only 3 of 150 ticks that ingested the Mississippi strain had positive midguts and the levels in these three were below the level of detection ($<5 \times 10^1$ organisms) by real-time PCR.

Both the St. Maries strain and *A. marginale* subsp. *centrale* effectively colonized the salivary glands during transmission feeding (Table 2). The levels exceeding 10^7 organisms per salivary gland pair reflect replication following invasion, as previously shown for efficiently transmitted strains (9, 13). Consistent with the lack of colonization in the midgut epithelium, a prerequisite to invasion of the salivary glands, the Mississippi strain was not detected in the salivary glands of any of the 150 fed ticks examined.

Confirmation of strain identity. The identity of the *A. marginale* strains colonized in the salivary glands was confirmed using amplification and sequencing of *msp5* and *msp1*α. For comparison, *msp5* amplicons were generated from the blood of each of the calves used for original tick acquisition feeding (Fig. 1A). Similarly, the *msp1*α repeat region was am-



B

Saint Maries strain of *A. marginale*

MSAEYVSSQS**AD**SSLAGGQQQESSVSSQSDQASTSSQLG**AD**SSSAGGQQQESSVSSQSDQASTSS
QLG**AD**SSSAGGQQQESSVSSQSDQASTSSQLGTDWRQEMRS

Mississippi strain of *A. marginale*

MSECVSLQQ**AD**SSSASGQQQESSVSSQSEASTSSQLGG**AD**SSSASGQQQESSVSSQSEASTSSQLG
G**AD**SSSASGQQQESSVSSQSEASTSSQLGG**AD**SSSASGQQQESSVSSQSEASTSSQLGG**AD**SSSAS
GQQQESSVSSQSEASTSSQLGTDWRQEMRS

FIG. 1. (A) Confirmation of *A. marginale* strain identity in the acquisition feed blood and tick salivary glands by PCR amplification of *msp5* and *msp1*α. AF blood, blood collected at time of acquisition feeding; *D. andersoni* SG, salivary glands following transmission feeding; *ss.*, subsp. (B) MSP1a N-terminal repeat region sequences of the St. Maries and Mississippi strains. The amino acid repeat regions are underlined, and the start of each repeat is in boldface.

TABLE 3. Tick transmission of *A. marginale* to naïve calves

Strain	Result of test on indicated day[a]											
	Giemsa-stained blood smears[b]				MSP5 cELISA				*msp5* PCR			
	25	45	75	100	25	45	75	100	25	45	75	100
A. marginale subsp. *centrale*	−	−	−	−	−	−	−	−	−	−	−	−
A. marginale Saint Maries	+	+	±[c]	−	±[d]	+	+	+	+	+	+	+
A. marginale Mississippi	−	−	−	−	−	−	−	−	−	−	−	−

[a] Number of days after the removal of ticks.
[b] Microscopic examination of a minimum of 50 high-power fields.
[c] One animal remained Giemsa stain positive; the second animal had *A. marginale* levels below the limit of microscopic detection.
[d] One animal had seroconverted at day 25; the second animal had not yet seroconverted at this time point.

plified from the blood of the calves infected with the St. Maries and Mississippi strains but not with *A. marginale* subsp. *centrale*, which completely lacks this repeat region (24). The *msp1*α amplicons represented the expected size for the three repeats and five repeats of the St. Maries and Mississippi strains, respectively (Fig. 1A). *msp5* was amplified from the salivary glands colonized by both the St. Maries strain and *A. marginale* subsp. *centrale*, and *msp1*α was amplified from the salivary glands colonized only by the St. Maries strain. Neither *msp5* nor *msp1*α was amplified from the Mississippi strain, which failed to colonize the salivary glands (Fig. 1A). The sequences of the *msp5* and *msp1*α amplicons matched those previously reported for each strain, thus confirming strain identity. The *msp5* sequences of the St. Maries (blood and salivary glands) and Mississippi (blood) strains were identical to each other and to the genome sequence of the St. Maries *msp5* strain (GenBank accession no. NC_004842). These two strains were discriminated and the identity of the St. Maries strain within the salivary glands confirmed by sequencing of the *msp1*α amplicon; the amplicon sequence from the salivary glands was identical to that in the blood of the St. Maries-infected calf and to that previously reported for this strain (GenBank accession no. NC_004842). This *msp1*α sequence differs from that of the Mississippi strain in both the number and sequence of repeats (GenBank accession no. AY010243) (Fig. 1B). The identity of *A. marginale* subsp. *centrale* in the salivary glands was confirmed by the *msp5* sequence; the sequences from the blood and salivary glands were identical to each other and to that previously reported for the Israel vaccine strain (GenBank accession no. AY054384).

Transmission of *A. marginale* to naïve animals. *D. andersoni* colonized with the St. Maries strain successfully transmitted infection to naïve calves following transmission feeding of either 10 or 35 ticks (Table 3). Infection followed a typical course of acute bacteremia, detectable by microscopic examination of Giemsa-stained blood smears and accompanied by seroconversion. Both animals progressed to persistent infection within 60 days, characterized by bacteremia of $\leq 10^7$ *A. marginale* bacteria per ml of blood, detectable by PCR but not microscopic examination of blood smears (Table 3). In contrast, none of the calves, each transmission fed with 100 ticks exposed to either the Mississippi strain or *A. marginale* subsp. *centrale*, were infected as documented throughout the 100-day observation period following attempted transmission.

DISCUSSION

We have identified the tick salivary glands as a second and distinct barrier, in addition to the midgut, for efficient *Anaplasma marginale* transmission. The entry and replication of *A. marginale* subsp. *centrale* in the midgut and then salivary glands recapitulated the basic developmental cycle of the pathogen within the tick (8, 9); however, this was not sufficient for transmission. As a positive control, the St. Maries strain of *A. marginale* was transmitted using either 10 or 35 *D. andersoni* ticks, consistent with a previously published study in which three infected ticks were sufficient for transmission (21) and with data (G. A. Scoles, unpublished data) showing that a single infected tick transmitted the St. Maries strain. In contrast, feeding of 100 *D. andersoni* ticks, of which >70% had positive salivary glands, did not transmit *A. marginale* subsp. *centrale*. Notably, this did not reflect a failure to replicate within the salivary glands during transmission feeding. Replication in the salivary glands to levels of $\geq 10^4$ *A. marginale* bacteria has previously been associated with successful transmission (9, 13). In the present study, *A. marginale* subsp. *centrale* replicated to a mean level of $10^{7.4}$ bacteria per salivary gland pair and each individual positive tick had $>10^6$ *A. marginale* bacteria per salivary gland pair, a minimum of 100 times the number of organisms previously linked with successful transmission (9). While there was no significant difference in the numbers of organisms within the salivary glands at transmission feeding between the St. Maries strain and *A. marginale* subsp. *centrale*, the transmission of only the St. Maries strain reveals that a previously unsuspected mechanism within the salivary glands is required for efficient transmission. This may involve the site of replication (i.e., at the cellular level within the correct acinar cell type or intracellular compartmentalization), vector pathogen signaling for the development of infectivity, efficient secretion in the saliva, or early survival in the mammalian host following transmission tick feeding. The identification of *A. marginale* subsp. *centrale* as being replication competent but defective for efficient transmission by *D. andersoni* provides a needed tool to identify these required mechanisms.

The tick midgut epithelium also represents a barrier to *A. marginale* transmission but, unlike the salivary glands, functions at the time of tick acquisition feeding (6, 23). This was originally described using the Florida strain of *A. marginale* (6, 23), and in the present study, the transmission phenotype of the Mississippi strain displayed a similar early defect. Although

the Mississippi strain-infected calf had the highest level of bacteremia during acquisition feeding and all fed ticks ingested *A. marginale* (Table 1), the levels within the total midgut (lumen and epithelium) were significantly lower than in ticks that ingested either the St. Maries strain or *A. marginale* subsp. *centrale*. This suggested that while the last two strains entered the midgut epithelium early and initiated replication, the Mississippi strain total midgut levels reflected only the luminal *A. marginale* ingested with the blood meal. Consistent with this early defect, the Mississippi strain did not progress to invade the salivary glands and was not transmitted.

Whether the very few (3/150) Mississippi strain-positive midgut epithelium samples (Table 2), all of which had fewer than 50 bacteria per midgut, indicate that entry into the midgut epithelium did occur but was inefficient, or that entry occurred but there was no subsequent survival or replication, is unknown and highlights the current gap in knowledge regarding these early events. Similar to what has been observed with the Mississippi strain, the failure of the Florida strain to be transmitted has been proposed to reflect either an inability to bind the midgut epithelial cells for subsequent internalization or a failure to survive and replicate within the midgut epithelial cells (6, 23). These possibilities are not necessarily mutually exclusive, as blood digestion in ixodid ticks occurs intracellularly within the midgut epithelium, rather than in the lumen, and thus passive entrance of cell-associated *A. marginale* into the epithelium may occur in addition to any receptor-ligand interactions of cell-free bacteria.

The outer membrane protein MSP1a has been proposed to mediate the binding of cell-free *A. marginale* to the midgut epithelial cells and to allow for the prediction of the transmission phenotype of *A. marginale* strains based on the number and sequence of MSP1a repeats (5, 6). Our results are not entirely supportive of this proposed mechanism. First, *A. marginale* subsp. *centrale* completely lacks the MSP1a repeat region (24) yet invades and replicates within the midgut epithelium prior to colonization of the salivary glands. This demonstrates that MSP1a N-terminal repeats are not uniformly required for entry and colonization in *Dermacentor* sp. ticks. Whether this phenotype will be representative of other *A. marginale* strains is unknown. Multiple genome-level comparisons have revealed an unexpected high level of polymorphism among *A. marginale* strains (3), a wide spectrum that may well include *A. marginale* subsp. *centrale*. However, to date all *A. marginale* senso stricto strains encode the MSP1a N-terminal repeats, and thus, *A. marginale* subsp. *centrale* could represent an outlier that uses an alternative, non-MSP1a mechanism to invade the midgut epithelium as a cell-free bacterium. Second, the Mississippi strain used in the present study has an MSP1a repeat type identical to that identified as being transmission competent (5), yet this strain failed to colonize the *D. andersoni* midgut and was consequently not transmitted. This indicates that even within *A. marginale* senso stricto strains, the sequence of MSP1a N-terminal repeats is not consistently predictive of midgut invasion in *Dermacentor* spp. The previously reported predictive association between the MSP1a repeat sequence and transmission phenotype was based on transmission data for *D. andersoni* as well as a closely related tick, *D. variabilis* (4, 5, 6); thus, it is possible that an association remains valid for the latter species. While studies with the St. Maries, South Idaho, Puerto Rico, and Florida strains of *A. marginale* and both *Dermacentor* and *Rhipicephalus* species suggest that the transmission phenotypes, both positive and negative, are conserved, there may be differences among tick species in the efficiency or mechanism of the interaction at the midgut level (9, 20, 23, 26).

In summary, there are both early (midgut) and late (salivary glands) barriers to efficient tick-borne transmission of *A. marginale*. The identification of specific strains that fail to traverse these barriers provides needed tools to dissect the mechanisms involved in transmission not only to define the defect of these specific strains but to better understand the epidemiology of highly transmissible strains responsible for disease outbreaks.

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