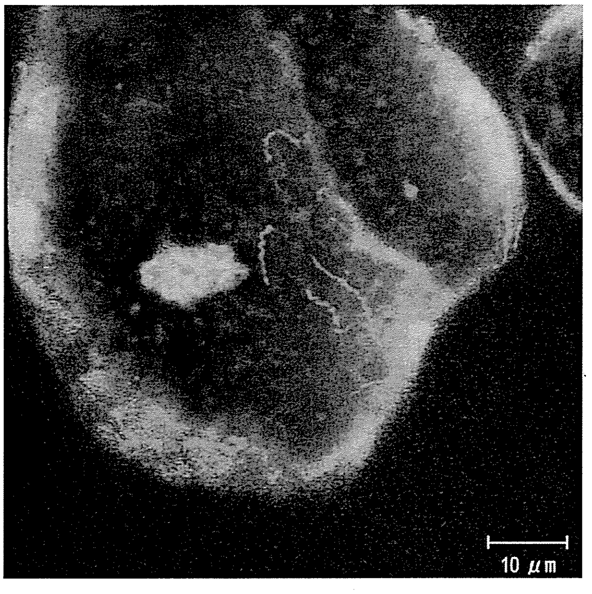

Fig. 1. Borrelial cells (red) in salivary glands of molted *A. geoemydae*. Immunofluorescence assay (IFA) was performed as previously described (Takano et al., 2010). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

ticks (Barbour, 2005). This phenotype of RF borreliae was thought to be required for borreliae to be transmitted by fast feeders (soft ticks). This phenotype was not observed, however, in LD borreliae (e.g. *B. burgdorferi*) and REP borreliae (*Borrelia* sp. tAG, our previous study), which are transmitted by hard ticks (Piesman et al., 2001; Takano et al., 2011). In this study, the borrelial phenotype (able to infect the salivary gland of molted ticks) was observed in hard tick-borne RF borreliae (*Borrelia* sp. AGRF in this study), although this phenotype might not be required for *Borrelia* sp. AGRF in *A. geoemydae* (slow feeders). From this result, we speculate that RF borreliae (including hard tick-borne RF borreliae) generally show this phenotype, and the transstadial transmission of *Borrelia* sp. AGRF in the salivary glands may be due to characteristics of the borreliae itself, rather than characteristics (e.g. blood-feeding time) of the vector tick.

In this study, it was suggested that the prevalence of *Borrelia* sp. AGRF was reduced through molting when compared to that of *Borrelia* sp. tAG (Table S1, chi-square test, $P < 0.01$). It was reported that the rate of transstadial transmission from larvae to adult ticks was only 61% in the case of the hard tick-borne RF *Borrelia* sp. in *I. scapularis* ticks (Scoles et al., 2001). In the case of soft tick-borne RF borreliae, a high rate of transstadial transmission has been suggested for *B. hermsii* in *Ornithodoros hermsi* (Lopez et al., 2011). Moreover, the transstadial transmission rate of LD borreliae in vector ticks was estimated at over 90% (Dolan et al., 1998; Piesman, 1995). Although the reason is unclear, the inefficiency in transstadial transmission of hard tick-borne RF borreliae compared to that of soft tick-borne RF borreliae and LD borreliae might be due to difference(s) in characteristics of the borreliae. Furthermore, in the case of hard tick-borne RF *Borrelia* sp., transovarial transmission occurred in *I. scapularis* (Scoles et al., 2001). Thus, we speculate that *Borrelia* sp. AGRF is transovarially transmitted in *A. geoemydae* because *Borrelia* sp. AGRF is phylogenetically related to hard tick-borne RF *Borrelia* spp. However, since we could not collect *Borrelia* sp. AGRF-positive engorged females, transovarial transmission of *Borrelia* sp. AGRF could not be evaluated. To elucidate the mechanism of transstadial transmission of RF borreliae in ticks, further analysis will be required. Our findings may contribute to the elucidation of transstadial transmission mechanisms of borreliae in ticks.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ttbdis.2012.06.003.

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