Location and ultrastructure of spotted fever rickettsiae in nymphal *Haemaphysalis hystricis* ticks

Yasuhiro YANO^{1*}, Nobuhiro TAKADA¹, Hiromi FUJITA², Mutsuyo GOKUDEN³ and Shuji ANDO⁴

¹Faculty of Medical Sciences, University of Fukui, Eiheji Fukui 910-1193 Japan

²Mahara Institute of Medical Acarology, Anan Tokushima 799-1510 Japan

³Kagoshima Prefectural Institute for Environmental Research and Public Health, Kagoshima 892-0836 Japan

⁴National Institute of Infectious Diseases, Tokyo 162-8640 Japan

ABSTRACT

The *Haemaphysalis hystricis* ticks we collected contained rod-shaped rickettsiae (length, 1.3 μ m; diameter, 0.3 μ m) in midgut epithelial cells, rectal sac cells, muscle cells surrounding trachea, cells of the central nervous system, and granular cells of the salivary glands. These microorganisms, which were defined as *Rickettsia japonica* by PCR analysis, existed freely in the cytoplasm of the cells and were surrounded by an electron-lucent (halo) zone. Nuclear localization and proliferation of the rickettsia were present in salivary gland cells. The rickettsial species we evaluated also demonstrate trans-stadial transmission because we used nymphal ticks, which molted from engorged larvae. This report is the first to describe the location and ultrastructure of spotted fever rickettsiae in *H. hystricis* ticks.

Key words: ultrastructure, spotted fever, rickettsiae, *Haemaphysalis hystricis*

INTRODUCTION

During a epidemiologic survey of Japanese spotted fever in July 2011 at Fukuoka City, Japan, 20 engorged larval *Haemaphysalis hystricis* ticks were collected from an *Apodemus speciosus* rodent. These ticks molted into nymphs under the laboratory conditions. Ten of the 20 ticks collected were emulsified and individually inoculated into L-929 cultured cells (for 3 wk at 33°C). Rickettsiae were isolated from the ticks according to the methods described in our previous report (Takada, et al., 1994). PCR analysis (Gokuden, et al., 2011) identified these rickettsiae as *Rickettsia japonica*, a causative agent of Japanese spotted fever. The remaining 10 ticks were used to evaluate the location and ultrastructure of the rickettsiae in various tick organs.

Tetsuo GOTOH and DeMar TAYLOR (eds.), Acarology XIV: Proceedings of the International Congress. *Journal of Acarological Society of Japan* 25 (Suppl. 1): 1-192.

* Corresponding author: tel: +81 776 61 8331, e-mail: yhyano@u-fukui.ac.jp

DOI: 10.2300/acari.25.Suppl_185

MATERIALS AND METHODS

Tick internal organs including midgut, rectal sac, trachea, central nervous system, and salivary gland were dissected in cold 3 % glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.4), fixed for 6 h, and washed in fresh buffer. The tissues were postfixed for 3.5 h in 1 % OsO_4 in 0.1 M sodium cacodylate buffer, dehydrated in an acetone series, and embedded in epoxy resin. Thin sections (about 70 nm) were cut on a microtome, by using a diamond knife, and doubly stained with uranyl acetate and lead acetate before examination in an electron microscope (75 kV, model H-7650, Hitachi High-Technologies Corp., Tokyo).

RESULTS AND DISCUSSION

The *H. hystricis* ticks we collected contained rod-shaped rickettsiae (length, $1.3\mu\text{m}$; diameter, $0.3\mu\text{m}$) in midgut epithelial cells (Fig. 1), rectal sac cells (Fig. 2), muscle cells surrounding trachea (Fig. 3), cells of the central nervous system (Fig. 4), and granular cells of the salivary glands (Figs. 5 and 6). These microorganisms, which were defined as *R. japonica* by PCR analysis (Gokuden, et al., 2011), existed freely in the cytoplasm of the cells and were surrounded by an electron-lucent (halo) zone (Fig. 5, inset). The cell walls of the rickettsiae exhibited a well-defined structure with the inner electron-dense layer thicker than its outer layer (Fig. 5, inset). Nuclear localization and proliferation of the rickettsiae were present in salivary gland cells (Fig. 6). The ultrastructural features of the isolated rickettsiae resembled those of rickettsiae in the

Fig. 1 Cross section and longitudinal section of rickettsiae (R) in a midgut epithelial cell.

Fig. 2 Rickettsiae (R) in a rectal sac cell.

Fig. 3 Rickettsiae (R) in a muscle cell along the trachea.

Fig. 4 Rickettsiae (R) in a central nervous system cell.

spotted fever group (Silverman et al., 1978; Yano et al., 1993; Yano et al., 2004). Specifically, the current rickettsiae were found in the cells of every organ evaluated. A similar distribution was noted for the spotted fever group rickettsia (subsequently identified as *Rickettsia tamurae*) in *Amblyomma testudinarium* tick larvae (Yano et al., 2000). The presence of rickettsiae in the salivary gland suggests that they are transmitted with salivary secretions to hosts during feeding by *H. hystricis* ticks. Nymphal *H. hystricis* ticks are known to bite humans, and *H. hystricis* is thought to be the most important vector of *R. japonica* to humans (Yamauchi et al., 2009).

Furthermore, the rickettsial species we evaluated also demonstrate trans-stadial transmission because we used nymphal ticks, which molted from engorged larvae. This report is the first to describe the location and ultrastructure of spotted fever rickettsiae in *H. hystricis* ticks.

Fig. 5 Rickettsiae (R) in a salivary gland granular cell. The inset shows a cross section of a rickettsia. CW, cell wall; *, halo zone

Fig. 6 Proliferating rickettsiae (R) in the nucleus (N) of a salivary gland granular cell.

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