Infection, Genetics and Evolution xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

Infection, Genetics and Evolution

journal homepage: www.elsevier.com/locate/meegid

Research paper

Effects of mammarenavirus infection (Wēnzhōu virus) on the morphology of *Rattus exulans*

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ARTICLE INFO

Article history:
Received 10 March 2017
Received in revised form 15 May 2017
Accepted 25 May 2017
Available online xxxx

Keywords:
Mammarenavirus
Wēnzhōu virus
Rattus exulans
Host morphologies
Teratogenic effects

ABSTRACT

The circulation of mammarenaviruses in rodent populations of the Mekong region has recently been established, with a genetic variant of Wēnzhōu virus, Cardamones virus, detected in two *Rattus* species. This study tests the potential teratogenic effects of Wēnzhōu infection on the development of a Murid rodent, *Rattus exulans*. Using direct virus detection, morphological records and comparative analyses, a link was demonstrated between host infection status and host morphologies (the spleen irrespective of weight, the skull shape and the cranial cavity volume) at the level of the individual (females only). This study demonstrates that mammarenavirus infections can impact natural host physiology and/or affect developmental processes. The presence of an infecting micro-parasite during the development of the rat may lead to a physiological trade-off between immunity and brain size. Alternatively, replication of virus in specialized organs can result in selective morphologic abnormalities and lesions.

1. Introduction

This paper compares the phenotype of infected and uninfected individual hosts to highlight the potential teratogenic effect of a pathogen on its hosts. The landmark-based geometric morphometric method implemented in this paper is relatively new to the study of host/parasite complexes but is very promising for use in this field because of its low technological requirements and quality of the information it provides.

Although well-known agents of human disease in certain regions of the world, rodent-borne arenaviruses have only recently been demonstrated to circulate in the rodent populations of south-east Asia (Blasdell et al., 2016). One of these viruses, Wēnzhōu virus, has been detected in rodents of several *Rattus* species in both China (Li et al., 2015) and Cambodia, where the Cardamones variant of this virus was detected during the CERoPath project (www.ceropath.org; (Blasdell et al., 2016)). Classified as an Old World mammarenavirus (mammalian arenaviruses; (Radoshitzky et al., 2015)), Wēnzhōu virus is phylogenetically most closely related to the African mammarenaviruses, Lassa and Ippy. Human infection with Wēnzhōu virus has been detected, with potential association with respiratory illness. In rodents, pathology, in the form of severe diffuse pneumonia, was observed only in the lungs, although high viral loads were detected in several organs, including the liver, spleen and lungs (Blasdell et al., 2016). However, only animals infected experimentally as adults were tested, so it is unknown what impacts Wēnzhōu virus infection may have on rodents infected naturally or at an earlier life stage (i.e. in utero or neonatally). Infections by other arenavirus species have been found to have negative effects on their rodent natural hosts, including decreased survival, reduced reproductive success, stunted growth, weight loss, reduced body mass index and developmental abnormalities (Bonthius and Perlman, 2007; Borremans et al., 2011; Lalis et al., 2015; Salvato, 1993; Webb et al., 1975). For example, lymphocytic choriomeningitis virus (LCMV), which has been used as a biological model for the study of virus infection on host brain development, has been found to cause severe malformations, microcephaly and cerebellar hypoplasia in neonatally infected rats (Bonthius and Perlman, 2007). Within natural conditions, Lassa virus (LASV) infection was associated with a lateral constriction of the skull and a decrease in size of the infected host *Mastomys natalensis* trapped in West Africa (Lalis et al., 2015). Persistent infection by a New World mammarenavirus, Machupo virus, of *Calomys* rodents was associated with haemolytic anaemia and splenomegaly and decreased survival time (Webb et al., 1975). This

Abbreviation: CERoPath, Community Ecology of Rodents and their Pathogens in South-East Asia; CS, centroid size; $F_{x,y}$, F statistic from an analysis of variance table with x, the degrees of freedom of factor and y, the residual degrees of freedom; IFA, immune fluorescence assay; LASV, Lassa virus; LCMV, lymphocytic choriomeningitis virus; $Pillai_{x,y}$, Pillai statistic from a multivariate analysis of variance; PCs, Principal Components; Pv, *P*-value; RCCV, Relative Cranial Cavity Volume; RSW, Relative Spleen Weight.

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http://dx.doi.org/10.1016/j.meegid.2017.05.023
1567-1348/© 2017 Published by Elsevier B.V.

non-exhaustive list suggests that mammarenavirus infections in their natural rodent hosts may induce host detrimental responses and that natural infection in rodents does not always result in silent and persistent infections.

While arenaviruses are often transmitted horizontally, vertical transmission can also occur for several arenavirus species (Mims, 1981). Vertical transmission has not yet been demonstrated experimentally for Wēnzhōu virus, but horizontal transmission was found to occur between pups and dams (Blasdell et al., 2016). Virus infection, through vertical transmission or horizontal transmission early in life, can interfere with several stages of host development, particularly as persistent infection can also result (Childs and Peters, 1993). Infection of the embryo or foetus through vertical transmission could allow the virus to act as a potential teratogen; defined as any environmental factor that can produce a permanent abnormality in structure or function, restriction of growth, or death of the embryo or foetus (Gilbert-Barness, 2010). Alternatively, infection during the growth stage of hosts could occur, promoted by the close and regular contact between pups and their dam, if maternal antibodies are not present or protective. This neonatal period is another critical period for host development, in which the host responses are highly plastic and dependent on the conditions of antigen exposure (Adkins et al., 2004).

The mechanisms whereby Wēnzhōu virus may act on host development are currently unknown. At an individual level it may act directly by impacting on some function of the host cells (Oldstone et al., 1984). Alternatively infection may impact development indirectly through a trade-off between investment in the development of particular organs and other "expensive" (energy demanding) functions. For example infected individuals may "focus" energy investment in immunity (use costs, maintenance and deployment (Schmid-Hempel, 2003)) at the expense of other tissues such as the brain (Bordes et al., 2011). The main goal of this study was to characterize the potential effects of Wēnzhōu virus infection on the morphology of individual naturally infected hosts (*Rattus exulans*). For this, we compared individual morphology, immune strength and infection status to Wēnzhōu virus between infected and uninfected individuals in a single population of hosts from southern Cambodia.

2. Material & methods

2.1. Sampling the population at risk

The study focused on a single population of *Rattus exulans* (Peale 1848), known to contain individuals actively infected with the Cardamones variant of Wēnzhōu virus (Blasdell et al., 2016). This population was collected live in the district of Veal Renh (Cambodia) and sampled as part of a larger study looking at the impact of rodent communities and the environment on rodent-borne pathogens, not specifically to sample for Wenzhou virus (CERoPath project). Forty-three adult or sub-adult individuals (27 females and 16 males) were sampled. Captured rodents were collected daily during two sampling sessions and serum and organs (lungs, spleen, liver, kidney) were harvested, stored temporarily in liquid nitrogen and then transferred to long-term storage at −80C° (Blasdell et al., 2016). To assess whether Wēnzhōu virus was present in the *R. exulans* individuals sampled, a previously described reverse transcription PCR developed for the detection of Old World mammarenaviruses was used (Vieth et al., 2007; Blasdell et al., 2016) on rodent lungs (brains were not collected for logistical reasons). In addition, an immune fluorescence assay (IFA) using LCMV-infected cells was also performed to test for anti-mammarenavirus antibodies in the serum of animals positive by reverse transcription PCR. During the CERoPath project sampling sessions, information was collected on the rats (Herbreteau et al., 2011) and used to build the morphological indicators used in this study: the individual weight (g), the body size (mm) and the spleen weight (mg). Moreover, the skulls of rats were collected to acquire information on the brain volume of rats, skull shape and size.

2.2. Host variables

The spleen development was used as a measure of the immune strength of individual rats (Corbin et al., 2008; Ponlet et al., 2011). To make the spleen weight independent of the individual's size, a ratio of the spleen weight and the individual rat weight was used as an estimator of immunity (RSW: Relative Spleen Weight).

The approximated volume of the cranial cavity was calculated by filling the rat skulls with fine sand and deducing the cranial cavity volume (cm^3) by scaling the sand weight (g) by an appropriate factor (=1.55). To make the cranial cavity volume independent of the individual's size, a ratio of the cranial cavity volume and the individual rat size was used as an estimator of cognitive capacity (RCCV: Relative Cranial Cavity Volume). Since one may expect allometric scaling between the two quantities in this ratio, the numerator and denominator were log-transformed. Some skulls were removed from the RCCV analysis because the auditory bullae (which are very fragile) were broken.

The rat skulls were photographed using a Pentax K200 camera ensuring that the same focal and distance were maintained between the specimens and the camera. The morphologies of skulls were studied with 22 two-dimensional landmarks (10 on the dorsal view and 12 on the ventral view) presented in Fig. 1. Landmarks were defined and digitized twice by a unique user on numerical pictures of each specimen in order to assess measurement error. The average shape between the two digitizations of the same individual was used for the analysis. A partial generalized Procrustes superimposition (Bookstein, 1991; Dryden and Mardia, 1998) was applied to the landmarks and provided skull shapes and centroid sizes (CS) for each view. For shape, the superimposed landmarks coordinates were projected in a Euclidean space tangent to the shape space and then summarized by a principal component analysis (Claude, 2008; Dryden and Mardia, 1998). The size and shape measurement error were estimated using analysis of variance of size (anova) and shape (manova), considering individuals as the source of

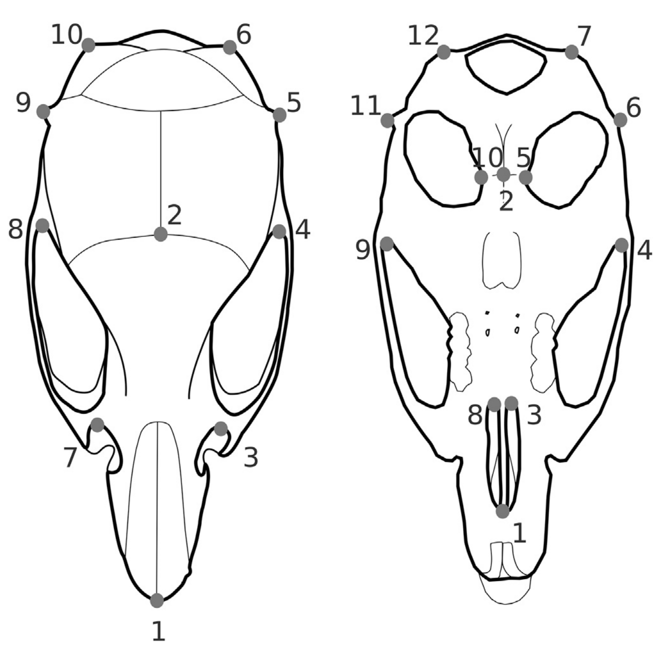

Fig. 1. Landmarks recorded on the dorsal (left) and ventral (right) surface of *Rattus exulans*. Dorsal view: anterior extremity of the suture between the nasals (1), parieto-frontal suture on the symmetry axis (2), posteriormost point of the curve of the infraorbital foramen (3, 7), posteriormost point of the zygomatic arch (4, 8), externalmost point of the lambdoid crest (5, 9), most posterior and lateral point on the braincase surface (6, 10). (B): Most anterior extension of the incisive foramen on the symmetry axis (1), basisphenoid–basioccipital suture on the symmetry axis (2), posteriormost point of incisive foramen (3, 8), posteriormost point of the zygomatic arch (4, 9), contact between tympanic bulla, basioccipital and basisphenoid (5, 10), externalmost point of the lambdoid crest (6, 11) and tip of the occipital condyle (7, 12).