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Prevalence of antibodies against Kilham virus in experimental rat colonies of Argentina

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ABSTRACT

The Kilham rat virus (KRV) is a parvovirus originally isolated from a rat sarcoma in the late 1950s. The clinical signs associated with a natural KRV infection include foetal resorption in dams, runtin, ataxia, cerebellar hypoplasia and jaundice in suckling rats, and sudden death, scrotal cyanosis, abdominal swelling and dehydration in juvenile rats. The ability of this virus to produce persistent infections has resulted in a high frequency of contamination of cell cultures and transplantable-tumor system. In addition, the virus may interfere with research in other ways. The remarkable resistance to environmental conditions determines the importance of the detection and control of this agent, especially in the laboratory animal production. This study determines the seroprevalence of Kilham antibodies from sera of adult rats from conventional facilities, using the haemagglutination inhibition test. The seroprevalence varied between 27.8% and 75%. This result confirms that the virus is circulating in Argentinean conventional facilities and might be interfering with research. The recognized Kilham virus may be prevented from supply sources by implementing a health monitoring schedule including a regular serological surveillance, and by keeping the animals under barrier systems.

Key words: diagnosis, haemagglutination inhibition test, Kilham rat virus

RESUMEN

Prevalencia de anticuerpos contra el virus Kilham en colonias experimentales de ratas de Argentina. El virus Kilham es un parvovirus aislado originalmente a partir de un sarcoma de rata, a fines de la década del 50. Los signos clínicos que produce son reabsorción fetal, disminución del crecimiento, ataxia, hipoplasia cerebelar, ictericia en lactantes, muerte súbita, cianosis escrotal, hinchazón abdominal y deshidratación de ratas jóvenes. La capacidad del virus para producir infecciones persistentes hace que los cultivos celulares derivados de ratas puedan estar contaminados. Además, el virus puede interferir con los ensayos de investigación de diferentes formas. La resistencia a las condiciones ambientales determina la importancia de la detección y el control de este agente, particularmente en la producción de animales de laboratorio. En este estudio se analizó la prevalencia de anticuerpos contra el virus Kilham en ratas adultas provenientes de bioterios convencionales de Argentina utilizando la prueba de inhibición de la hemoaglutinación. La seroprevalencia varió entre 27,8% y 75%. Este resultado confirma que el virus está circulando en los bioterios convencionales de Argentina, por lo que podría estar interfiriendo con las investigaciones. La infección por este virus debe ser prevenida aplicando sistemas de vigilancia y control, y manteniendo a los animales con sistemas de barrera.

Palabras clave: diagnóstico, inhibición de la hemoaglutinación, virus Kilham de las ratas

The Kilham rat virus (KRV) is a parvovirus originally isolated from a rat sarcoma in the late 1950s (5). Later, in the 1960s, Toolan *et al*., (9) discovered a second parvovirus from human tumoral cell lines, designated as H-1. In 1998, a new small virus of laboratory rats identified as rat parvovirus type 1a (RPV-1a) was isolated and found to be antigenically distinct respect to KRV and H1 (2). Then, three viral variants were identified and these new parvoviruses named RMV-1a, 1b and 1c, closely related to each other, resulted antigenically and molecularly different from KRV, H1 and RPV-1a (3).

The pathogenesis of all these rat parvoviruses has been well studied. Natural and experimental KRV infections of rat foetuses and infant rats are highly pathogenic (7, 8). Experimental infections with H-1 can cause lesions similar to those caused by KRV. RPV-1a appears to be non-pathogenic when using experimentally infected infant rats (2). The potential effects of RMV-1 infection on research are still unknown (10).

The clinical signs associated with natural KRV infections include foetal resorption in dams, runtin, ataxia, cerebellar hypoplasia and jaundice in suckling rats, and sud-

den death, scrotal cyanosis, abdominal swelling and dehydration in juvenile rats. The ability of KRV to produce persistent infections has resulted in a high frequency of contamination of cell cultures and transplantable-tumor system. In addition, KRV may interfere with research in other ways. KRV has been reported, for example, to alter immune responses (3) as well as studies of foetal development and teratogenesis since it can cross the placenta and cause cerebellar hypoplasia, hepatitis, and death in rat fetuses (4). The remarkable resistance of KRV to environmental conditions determines the importance of the detection and control, especially in the laboratory animal production. World KRV seroprevalence is ~ 80% (6, 7, 11). There are several serological tests for the diagnosis of KRV antibodies (Ab). Gel immunodiffusion and complement fixation tests are the least sensitive. The immunofluorescence and enzyme-linked immunosorbent assays are generally used as screening assays. Both techniques are sensitive but lack specificity because there is cross-reaction with non-structural proteins that are conserved among rodent parvoviruses. The serum neutralization test is specific but takes several days to be completed and is therefore not suitable for routine use. Haemagglutination-inhibition (HAI) is the most commonly used serological test because it is specific, does not cross-react with the other groups and is useful to analyse a high number of samples in a short time. The purpose of this study was to determine the seroprevalence of KRV-Ab in rats from conventional facilities of Argentina, using the HAI test.

Sera from healthy adult rats submitted from twenty different conventional facilities (n=392) between 2006 and 2008 for microbiological monitoring were selected for this study. Twenty sera of one specific pathogen-free (SPF) animal facility were introduced as controls. Animal handling and all experimental procedures were carried out in compliance with the recommendations of the “Guide for the Care and Use of Laboratory Animals of the National Research Council” (Academy Press, 2002, Washington, USA). The antigen (Ag) used in the HAI test was prepared in accordance with previously described methodology (1). The serum samples were heat-inactivated and stored at -20 °C. The HAI test was performed in accordance with standard methods. The serum samples were diluted serially twofold with phosphate buffered saline containing 0.1% of bovine seroalbumine (PBS-BSA). U-bottomed 96-well microplates were given 25 µl per well of each diluted serum and mixed with an equal volume of Ag containing eight haemagglutinating units (HAU). After 1 h incubation at 37 °C, 50 µl of 1% suspension of red blood cells from guinea pigs in PBS-BSA was added to

Table 1. Kilham rat virus seroprevalence determined by the haemagglutination inhibition (HAI) test in Argentinean conventional facilities

Number	Negative samples	Positive samples	Sample size	Positive samples %	CI [(1)] (95%)
1	12	8	20	40.0	18.5 - 61.5
2	12	8	20	40.0	18.5 - 61.5
3	10	10	20	50.0	28.1 - 71.9
4	5	13	18	72.2	51.5 - 92.9
5	7	11	18	61.1	38.6 - 83.6
6	6	13	19	68.4	47.5 - 89.3
7	9	11	20	55.0	33.2 - 76.8
8	7	13	20	65.0	44.1 - 85.9
9	6	14	20	70.0	49.9 - 90.1
10	6	14	20	70.0	49.9 - 90.1
11	5	15	20	75.0	56.0 - 94.0
12	12	8	20	40.0	18.5 - 61.5
13	12	8	20	40.0	18.5 - 61.5
14	13	5	18	27.8	7.1- 48.5
15	12	7	19	36.8	15.2 - 58.5
16	11	9	20	45.0	23.2 - 66.8
17	10	10	20	50.0	28.1 - 71.9
18	12	8	20	40.0	18.5 - 61.5
19	11	9	20	45.0	23.2 - 66.8
20	11	9	20	45.0	23.2 - 66.8
TOTAL	189	203	392	51.8	46.8 - 56.7

[(1)] Confidence interval (determined by standard error of the percentage and the Z-score of 1.96)

each well and further incubated for 2 h at room temperature before reading. One positive and one negative reference sera were used as controls in all reactions. The HAI titre of the individual serum sample was determined to be the inverse of the last dilution where cells were not agglutinated.

The HAI test revealed 51.8% (203/392) of positive samples (HAI titre: 1/10 to 1/640). The seroprevalence of KRV-Abs varied between 27.8% and 75% for the different conventional facilities. The confidence interval for a 95% determined by standard error of the percentage and the Z-score of 1.96, gave a range between 46.8% and 56.7% (Table 1). The sera of the SPF facility were negative.

An important finding that emerged of the results from the present study was that all Argentinean rat colonies analyzed were found seropositive. This has three implications: 1) KRV is circulating in Argentinean conventional facilities, 2) the virus might be interfering with researches by, for example, contaminating transplantable rat tumor lines and tissue culture cell lines and inducing production of interferon or altering leukocyte activity, and 3) It is necessary to develop a rapid, specific and sensitive method as polymerase chain reaction for detection of viral DNA in rat colonies.

The recognized KRV may be prevented from supply sources by implementing a health monitoring schedule including a regular serological surveillance, and by keeping the animals under barrier systems.

It is also important to recommend scientists that they should use laboratory rats that are known to be free of KRV. The determination and study of the prevalence of the pathogens that affect laboratory rats should be considered as a significant aspect which will result in better and more reliable results of animal experiments with fewer animals.

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REFERENCES

1. Ayala MA, Laborde J, Milocco S, Carbone C, Cid de la Paz V, Galosi CM. Desarrollo de un antígeno para diagnóstico del parvovirus de las ratas (Virus Kilham) por la técnica de inhibición de la hemoaglutinación (IHA). Rev Argent Microbiol 2004; 36: 16-9.
2. Ball-Goodrich LJ, Hansen G, Dhawan R, Paturzo FX, Vivas Gonzalez, BE. Validation of an enzyme-linked immunosorbent assay for detection of mouse parvovirus infection in laboratory mice. Comp Med 2002; 52: 160-6.
3. Besselsen DG, Besch-Williford CL, Pintel DJ, Franklin CL, Hook JR Jr, Riley LK. Detection of H-1 Parvovirus and Kilham rat virus by PCR. J Clin Microbiol 1995; 33: 1699-703.
4. Jacoby RO, Bhatt PN, Jonas AM. Viral Disease. In: Baker HJ, Lindsey JR, Weisbroth SH, editors. The Laboratory Rat Vol 1: Biology and Diseases. New York, Academic Press, 1979, p. 272-83.
5. Kilham L, Olivier LJ. A latent virus of rats isolated in tissue culture. Virology 1959; 7: 428-37.
6. Redig AJ, Besselsen DG. Detection of rodent parvovirus by use of fluorogenic nuclease polymerase chain reaction assays. Comp Med 2001; 51: 326-31.
7. Robey RE, Woodman DR, Hetrick FM. Studies on the natural infection of rats with Kilham rat virus. Am J Epidemiol 1968; 88: 139-43.
8. Salzman LA, Jori LA. Characterization of the Kilham rat virus. J Virol 1970; 5: 114-22.
9. Toolan HW, Dalldorf G, Barclay M, Chandra S, Moore AE. An unidentified filterable agent isolated from transplanted human tumors. Proc Natl Acad Sci USA 1960; 46: 1256-8.
10. Wan C-H, Söderlund-Venermo M, Pintel DJ, Riley LK. Molecular characterization of three newly recognized rat parvoviruses. J Gen Virol 2002; 83: 2075-83.
11. Weisbroth SH. Rat parvovirus. In: Waggie K, Kagiyama N, Allen AM, Namura T, editors. Manual of Microbiologic Monitoring of Laboratory Animals, 2nd edition. Maryland, USA, NIH Publication 94-2498, 1994, p. 93-8.

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