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Temperature dependent replication of porcine parvovirus isolates

Brief Report

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Summary. The replication of four porcine parvovirus isolates, NADL-8, NADL-2, KBSH, and Kresse, in swine testes cells were compared at temperatures of 32, 37, and 39 °C. Replication of the Kresse isolate was restricted at 32 and 37 °C as evidenced by progeny virus, virus polypeptide and viral DNA synthesis, but not at 39 °C. In contrast, replication of KBSH was restricted at 39 °C, but not at 37 or 32 °C. Findings from this study support the contention that replication of KBSH and Kresse isolates are temperature dependent.

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Porcine parvovirus (PPV), a member of the autonomously replicating parvoviruses, causes reproductive failure in swine [14, 18]. Isolates of PPV have been made from a variety of sources including porcine tissues, both diseased and non-diseased [3, 13, 17, 18] and as cell culture contaminants [10]. The pathogenicity of four isolates, NADL-8, NADL-2, KBSH and Kresse, in swine have been extensively compared in our laboratory [4, 21–23] as well as in other laboratories [17, 25]. NADL-8 and NADL-2 were originally isolated from a mummified fetus [20] and normal pig leukocytes [19], respectively. Both NADL-8 and NADL-2 caused fetal infection and death when injected in utero, prior to immunocompetency, but higher concentrations of inoculum and longer incubation periods were required for NADL-2 to show these pathogenic effects [25]. KBSH was originally isolated from the human KB cell line where it had been passaged at least 300 times [10]. While KBSH is antigenically and molecularly related to prototype PPV strains such as NADL-8 [10, 23], KBSH fails to replicate in swine fetuses following in utero inoculation [23]. The Kresse isolate originated from skin lesions of young swine [17]. This virus isolate caused fetal death both before and after immunocompetency [4], in contrast to NADL-8 which was pathogenic only before immunocompetency [19].

Temperature dependent replication has been reported for various families of viruses including members of the orthomyxoviridae [24], herpesviridae [2, 7], picornaviridae [1, 9], paramyxoviridae [6], and coronavirus [16]. Temperature-sensitive (ts) mutants have also been described for parvovirus, H 1 [27], Kilham rat virus [28], and porcine parvovirus [8, 12]. Based on previous studies demonstrating temperature-dependent replication of parvoviruses as well as other families of viruses, we proposed to evaluate putative temperature-dependent replication of selected PPV isolates. Since the mean body temperature of a pig is 39.2 °C rather than 37 °C, a difference in temperature-dependent replication in vitro may help to explain the differences observed in the replication of these isolates in swine.

Established swine testes (ST) cells were used throughout this study for the propagation of PPV in vitro as previously described [22]. PPV isolates employed included NADL-8 and NADL-2, obtained from Dr. W. Mengeling (NADC, Ames, IA), KBSH from Dr. P. Tattersall (Yale University, New Haven, CT), and Kresse from Dr. J. Kresse (NVSL, Ames, IA). NADL-8 was isolated from a dead fetus and has been propagated solely in swine fetuses. NADL-2 had been passaged eight times in porcine embryo kidney cells before we obtained the isolate, and has been passaged an additional three times in ST cells at 37 °C in our laboratory before use in this study. KBSH, which had been previously propagated approximately 300 times in KB cells at 37 °C [10], had been passaged twice at 37 °C in ST cells in our laboratory before use in this study. Kresse was originally isolated from skin lesions of young pigs [17], was propagated twice in ST cells and once in swine fetuses. Using these PPV isolates, stock virus preparations was prepared by inoculating a multiplicity of infection (m.o.i.) of 0.01 fluorescent focus forming unit (FFU)/cell onto ST cells at 37 °C. Virus was extracted from cells by 3 cycles of freezing and thawing in TE buffer (50 mM Tris, 25 mM EDTA, pH 8.7), as previously described [4], and titrated by indirect fluorescent antibody (IFA) staining [21]. Virus titers were expressed in FFU. Stock virus preparations were stored at − 70 °C. The preparation of stock viruses of all four isolates was prior to the studies on temperature-dependent replication. Studies examining putative temperature-dependent replication were initiated by absorbing 1 m.o.i. of each of the 4 virus isolates onto 60 to 70% confluent monolayers of ST cells at either 32, 37, or 39 °C for 1 h. The virus inoculum was removed, medium was replenished and cell incubation was continued at the same temperatures. At 24 h intervals, for 6 days post-inoculation, aliquots of cell-free supernatants or cell extracts were tested directly for virus antigen by hemagglutination [15] or indirectly for infectious virus by IFA via the inoculation of 10-fold dilutions onto fresh ST cells [21]. To evaluate viral protein synthesis, [^{35}S]-methionine radiolabelled polypeptides were immunoprecipitated from infected cell lysates [21]. ST cells were infected at an m.o.i. of 1 for 20 h, pulsed with 150 μCi/ml of [^{35}S]-methionine for 6 h and immunoprecipitated with rabbit anti-PPV sera. The immunoprecipitated polypeptides were electrophoresed in SDS-containing 7.5% polyacrylamide gels, and were exposed

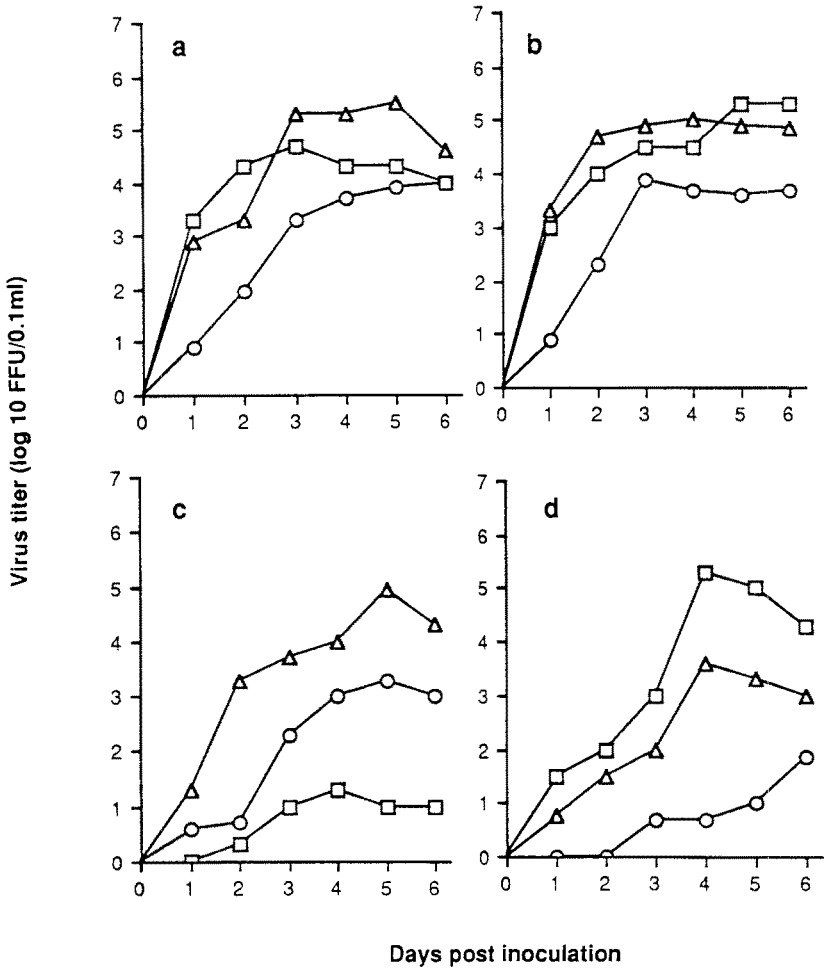

Fig. 1. Kinetics of cell-free infectious virus production. NADL-8 (**a**), NADL-2 (**b**), KBSH (**c**) and Kresse (**d**) isolates of PPV were inoculated onto ST cells at either 32 °C (○), 37 °C (△), or 39 °C (□) and at 1 day intervals, supernatants of infected cell cultures were tested for infectious virus production by IFA

to X-ray film (DuPont, Boston, MA). To evaluate PPV DNA synthesis, slot blot hybridization was performed on viral DNA extracted from cultured cells by a modified Hirt procedure [26]. Extracted DNA was blotted onto nylon membranes (Hybond-N, Amersham) in the Minifold II apparatus (Schleicher and Schuell, Keene, DH). Hybridization was performed by incorporating a ^{32}P radiolabelled RNA probe following procedures previously described [11]. The RNA probe was synthesized using Sp 6 RNA polymerase from a Sp 64 plasmid containing a 3,300 bp fragment of the PPV genome [11]. The specific activity of the probe was approximately 5.0×10^7 cpm/μg.

NADL-8 and NADL-2 isolates showed similar replication profiles for the production of infectious virus in cell-free supernatants from the 3 temperatures tested (Fig. 1 a, b). The concentration of infectious virus was lowest in cells

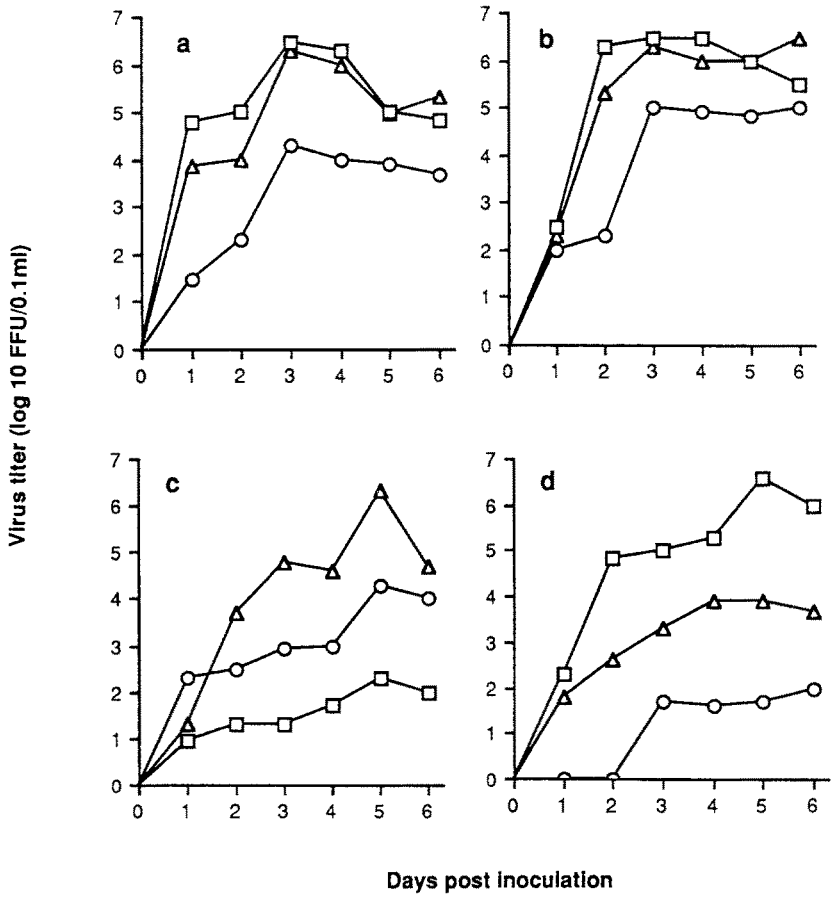

Fig. 2. Kinetics of cell-associated infectious virus production. NADL-8 (**a**), NADL-2 (**b**), KBSH (**c**) and Kresse (**d**) isolates of PPV, were inoculated onto ST cells at either 32 °C (○), 37 °C (△), or 39 °C (□) and at 1 day intervals cells were harvested, washed and extracts were tested for infectious virus production by IFA

cultivated at 32 °C. Markedly different growth profiles were observed in KBSH and Kresse infected cells (Fig. 1 c, d). Low titers of infectious virus (2×10^1 FFU/0.1 ml at 4 day PI) with no detectable HA titer were detected from KBSH-infected cells, propagated at 39 °C, but high titers (9×10^4 FFU/0.1 ml) of infectious virus were detected from KBSH-infected cells propagated at 37 °C. In contrast, Kresse isolate replicated with the highest titer (5×10^5 FFU/0.1 ml) at 39 °C and the lowest titer (7×10^1 FFU/0.1 ml) at 32 °C. Again, no HA antigen was detected from infected cells at 32 °C.

Intracellular or cell-associated infectious virus was evaluated by extracting virus from washed ST cells (Fig. 2). Similar patterns of cell associated virus were detected from both NADL-8 and NADL-2 infected cells at the 3 temperatures (Fig. 2 a, b), whereas marked differences were again observed for Kresse and KBSH-infected cells (Fig. 2 c, d). The pattern of cell associated virus from Kresse and KBSH-infected cells was similar to that described above for

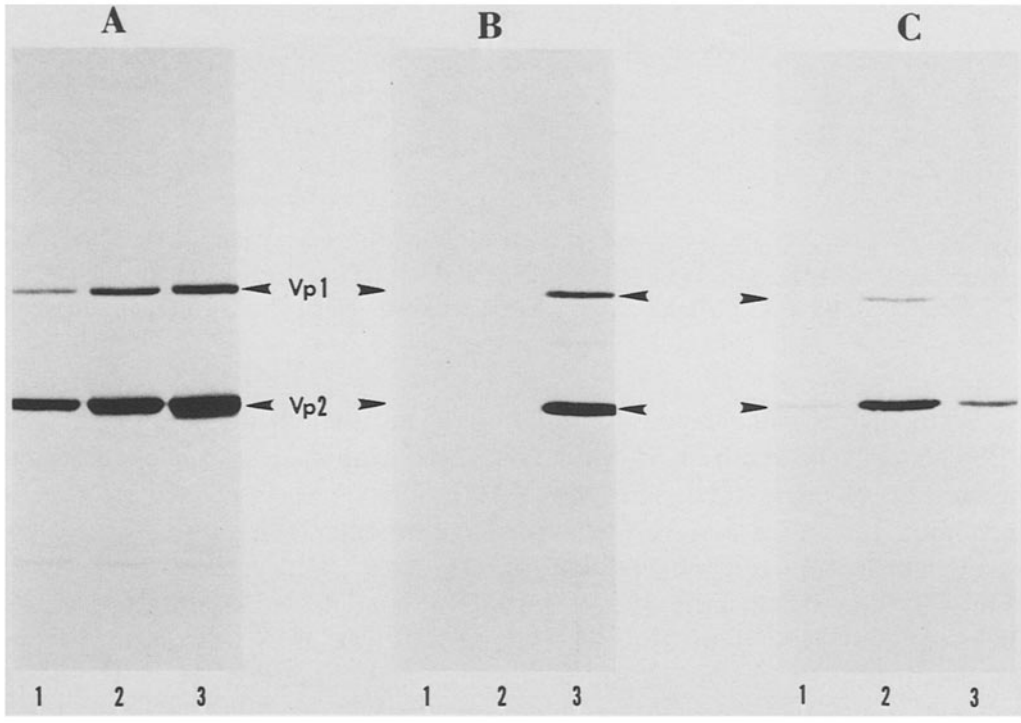

Fig. 3 A–C. Temperature dependent viral polypeptide synthesis of PPV isolates. ST cells were inoculated with either NADL-8 (**A**), Kresse (**B**), or KBSH (**C**) isolates for 20 h followed by pulsing the cells with [^{35}S] methionine for 6 h. Infected cells were incubated at either 32 °C (*1*), 37 °C (*2*), or 39 °C (*3*). After radio-labelling, cell lysates were immunoprecipitated with rabbit anti-PPV sera, and subjected to SDS-PAGE

extracellular virus with the exception that higher titers were observed than those of cell free virus. In addition, cell-associated HA antigen was detected from KBSH-infected cells after 5 day PI (data not shown), at which time no antigen was detected in cell free supernatant.

Since marked differences in replication of Kresse and KBSH isolates were observed at 37 and 39 °C evidenced by both intracellular and extracellular infectious virus and HA antigen, further characterization of viral polypeptide production was attempted. Synthesis of viral polypeptides at 32, 37, and 39 °C was examined by immunoprecipitation of radiolabelled polypeptides. Temperature dependent differences in the number and quantity of viral polypeptides were observed in cells infected with Kresse and KBSH and propagated at either 32, 37 or 39 °C (Fig. 3 A). Viral polypeptides (Vp 1 and Vp 2) were observed in ST cells infected with the Kresse isolate at 39 °C (Fig. 3 B, lane 3), but were present in lower concentrations from infected ST cells propagated at 37 °C and completely absent at 32 °C. Viral polypeptides (Vp 1 and Vp 2) were detected from KBSH-infected cells propagated at both 37 and at 39 °C, but at a higher concentration at 37 °C. No viral polypeptides were detected from uninfected control cells cultured at these temperatures.

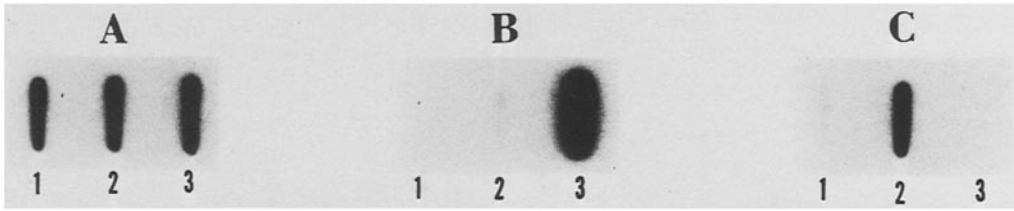

Fig. 4 A–C. Temperature dependent viral DNA synthesis of PPV isolates. ST cell were infected with either NADL-8 (**A**), Kresse (**B**), or KBSH (**C**) isolates for 13 h at either 32 °C (*1*), 37 °C (*2*), or 39 °C (*3*). Viral DNA was extracted from infected cells and then subjected to slot blot hybridization

To further examine the mechanism of temperature-dependent replication of PPV isolates, the synthesis of viral DNA was evaluated by nucleic acid hybridization of cell extracts (Fig. 4). NADL-8 DNA was detected from cells incubated at both 37 and 39 °C in equivalent amounts (Fig. 4 A). Viral DNA was detected from cells infected with Kresse isolate and propagated at 39 °C (Fig. 4 B, lane 3), but little or no viral DNA was detected from infected cells propagated at 37 °C (lane 2) or 32 °C (lane 1). Viral DNA was detected from ST cells infected with KBSH and propagated at 32 and 37 °C (Fig. 4 C, lanes 1 and 2), but little or no DNA was detected at 39 °C (Fig. 4 C, lane 3). Viral DNA was not detected from uninfected cells cultured at these temperatures.

In mouse coronavirus, wild type virus replicates rapidly in the brain and spreads to the liver causing lethal hepatitis. In contrast the ts 342 mutant replicates less extensively within the brain and could not be detected in the blood or liver [16]. Similarly, Gauntt et al. [9] have indicated that the difference in biological properties of 3 ts mutants of Coxsackie virus at temperatures above 4 °C may ultimately offer insight into differences in pathogenicity observed in neonate mice. These three mutants showed differences in viral RNA and polypeptide synthesis as well as virus assembly at 34 and 39 °C. Fujisaki et al. [8] and Izumida et al. [12] have established attenuated PPV strains by serial passages at low temperatures, 30 to 35 °C. No clinical signs, viremia or virus shedding were observed from sows inoculated with these viruses, but the pregnant sows immunized with these viruses and challenged with virulent virus gave birth to normal piglets. Present studies showed that NADL-8 and NADL-2 virus isolates, which are pathogenic to mid-term gestation swine fetuses, exhibited similar in vitro replication patterns at all 3 temperatures. In contrast, replication of KBSH, which is non-pathogenic to swine fetuses, was restricted at 39 °C but not at 37 °C. The replication of Kresse virus isolate, which shows a broad range of pathogenicities, causing fetal death and skin lesions in young pigs, was more favorable at 39 °C than at 37 or 32 °C. The mechanisms for restricted replication at non-permissive temperature appeared to be different between KBSH and Kresse isolates. Although both isolates showed comparable production of infectious virus at non-permissible temperatures, differences were noted at early stages of replication. At 24 h PI, infectious virus, albeit at very

low titers, were detected from KBSH-infected cells incubated at 39 °C. In contrast, no virus was detectable until 3 days PI for Kresse virus infected cells incubated at 32 °C. When viral polypeptides were evaluated at 20 h PI, lower concentration of polypeptides was detected from KBSH-infected cells at 39 °C, but were not detected at all from Kresse-infected cells incubated at 32 °C. In addition, lower concentrations of viral DNA were detected from KBSH-infected cells at 39 °C but there was no detectable viral DNA from Kresse-infected cells at 32 °C when tested at 13 h PI. These findings indicate that replicative restriction at non-permissible temperature may be due to a defect in virus assembly for KBSH, but replication of Kresse appeared to be compromised at the initial stage of replication. Differences between production of HA antigen and infectious virus were also noted. Intracellular HA antigens, but not extracellular antigen, from KBSH-infected cells was detectable only after 5 days PI at 39 °C (data not shown). Similar findings was found in Kresse-infected cells at 32 °C. While infectious virus was detectable after 3 days PI, no HA antigen was detecable up to 6 days PI. These findings indicate that greater than 10^2 FFU are required for 1 HA unit, coincident with a previous study [5]. Infectious PPV particles contain 3 structural capsid polypeptides (Vp 1, Vp 2, and Vp 3), while empty, non-infectious virus particles consist of only 2 capsid polypeptides (Vp 1 and Vp 2) [21]. Since viral polypeptides were evaluated at 24 h PI following a 6 h pulse, insufficient infectious virus was produced to demonstrate Vp 3 capsid polypeptides. In the early stages of PPV replication, empty particles are predominantly produced and afterwards, Vp 3 is post-translationally cleaved from Vp 2 [21].

Collectively, these results indicate that the ability of an isolate to replicate at temperatures near or above the mean physiological temperature of swine, 39.2 °C, may be essential for virulence. The mechanism for restriction of PPV isolates at non-permissive temperatures and whether adaptation of PPV isolates at non-permissive temperatures can be accomplished awaits further investigation.

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