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KBSH Parvovirus: Comparison with Porcine Parvovirus

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We compared the molecular, antigenic, and pathogenic properties of KBSH parvovirus to those of porcine parvovirus (PPV) isolate NADL-8. KBSH, propagated in swine testes cells in culture, possessed two major capsid polypeptides of 83 and 64 kilodaltons that were similar in size to those of PPV. KBSH-infected cells also contained an 86-kilodalton nonstructural polypeptide that was identical in size to the PPV nonstructural polypeptide (NS-1). The KBSH polypeptides were structurally similar but not identical to the corresponding PPV polypeptides, as revealed by partial proteolysis mapping. Viral replicative-form DNA from KBSH-infected cells was similar in size to PPV replicative-form DNA and exhibited similar but not identical restriction endonuclease cleavage patterns to that of PPV replicative-form DNA. Antigenically, the two viruses were also very closely related. By using heterologous and homologous antisera, the two viruses were indistinguishable in hemagglutination inhibition and immunoprecipitation assays. However, pathogenically these viruses were dramatically different. NADL-8 caused fetal death when injected into swine fetuses in utero and viremia and high persisting antibody titers when administered orally to weaning-age swine. KBSH-inoculated fetuses were normal in appearance, and pigs orally exposed to KBSH failed to establish viremia and demonstrated only transient antibody titers. Thus, KBSH appears to be a PPV that is very closely related to a highly pathogenic PPV isolate, yet is itself nonpathogenic in swine. This reduced pathogenic potential of KBSH may be attributable to its poor ability to replicate in swine.

Isolates of porcine parvovirus (PPV) were first made in the late 1960s from various tissues and as contaminants in cell cultures (2, 9). The first experimental evidence that PPV caused reproductive failure in swine was provided 7 years later (10, 16, 17). NADL-8, an abattoir isolate of PPV from a diseased fetus, has been studied in some detail. NADL-8 was able to reproducibly cause fetal death both by in utero inoculation of 40- to 50-day-old (gestational age) fetuses and by oral administration to pregnant PPV seronegative swine (16, 17). We have recently reported the various molecular characteristics of this highly pathogenic PPV isolate (19, 20).

In the early 1970s, a number of parvoviruses were isolated from normal human cell cultures (6). One of these viruses, designated KBSH, was isolated from human cells and was found to be antigenically related to PPV isolate 59e/63 by hemagglutination (HA) inhibition (6). KBSH was found to replicate in swine cell cultures as well as human cell cultures (7). Although KBSH was shown to possess a nucleic acid characteristic of parvoviruses (23) and appeared to be closely related to PPV by serological criteria, comparisons of KBSH and PPV at the molecular and pathogenic levels had not been undertaken. In this paper, we compare the molecular, antigenic, and pathogenic features of KBSH and NADL-8 in swine.

MATERIALS AND METHODS

Virus and cells. KBSH was obtained from P. Tattersall (Yale University, New Haven, Conn.) and was originally from the laboratory of G. Siegl (Bern, Switzerland). KBSH was twice passaged in swine testes (ST) cells in our laboratory before the following studies, and supernatant fluids from this second passage were used as seed stock for all in vitro and in vivo inoculations. The NADL-8 isolate of PPV (17) (fourth passage in ST cells) was used for comparative analyses. ST cells were used for NADL-8 as well as for KBSH propagation. Conditions for ST cell culture and virus growth were as described previously (13, 20).

Antisera. Antisera to NADL-8 and KBSH were prepared by injecting 0.5 ml (1,024 HA units [HAU]/50 μl [12]) of either virus, emulsified in complete Freund adjuvant, into New Zealand White rabbits (1.0 to 2.0 kg). A booster injection containing 0.5 ml of virus in incomplete Freund adjuvant was given at 3 weeks after the initial injection. Sera were collected from all rabbits 2 weeks after booster injection. The sera were tested for antibodies to KBSH and NADL-8 viruses by HA inhibition (11). Fetal pig antisera were obtained from fetuses injected in utero at day 85 of gestation with PPV and collected 21 days later (T. W. Molitor, H. S. Soo, and M. S. Collett, J. Virol., in press). All antisera used in this study are summarized in Table 1.

Radiolabeling of cell cultures, immunoprecipitation, and protein analyses. Infected ST cell cultures were radiolabeled with either [^{35}S]methionine or $^{32}P_i$ as described elsewhere (Molitor et al., in press) and were prepared for immunoprecipitation by procedures previously reported (1). Samples of radiolabeled, immunoprecipitated polypeptides were electrophoresed in sodium dodecyl sulfate-containing 7.5 or 10% polyacrylamide gels (14) and were either fluorographed (3) and exposed to X-ray film (for [^{35}S]methionine label) or autoradiographed (for ^{32}P label) with Cronex 4 X-ray film and Lightning-Plus intensifying screens (Du Pont Co., Wilmington, Del.). The one-dimensional partial proteolysis mapping procedure of Cleveland et al. (4) was employed as previously described for the NADL-8 polypeptides (19).

Viral RF DNA isolation and analysis. Viral replicative-form (RF) DNA was isolated from NADL-8- and KBSH-virus-infected ST cells by a modified Hirt extraction procedure (8)

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TABLE 1. Various antisera used in the present study

Antisera	Method of production
Normal rabbit sera	Preimmunized sera of rabbits used below
Rabbit anti-PPV (NADL-8)	Immunized with NADL-8 virus, emulsified in Freund adjuvant
Rabbit anti-KBSH	Immunized with KBSH virus, emulsified in Freund adjuvant
Fetal 4 pig anti-PPV	Experimental virus infection by amniotic injection of pregnant sow; reactive with both structural and nonstructural polypeptides (Molitor et al., in press)
Fetal 3 pig anti-PPV	Experimental virus infection by amniotic injection of pregnant sow; reactive with only PPV structural polypeptides (Molitor et al., in press)

as detailed previously for PPV (20). Gel purification of parvovirus RF DNA, restriction endonuclease digestion and restriction fragment radiolabeling, and agarose and polyacrylamide gel analyses have all been previously described (20).

Inoculation of swine fetuses with parvovirus. Fetuses at 40 to 50 days of gestation (total gestation period for swine is 114 days) were inoculated in utero with either NADL-8 or KBSH. Procedures for laparotomy and fetal inoculation were followed as previously described (19, 22). In one experiment, fetuses from one uterine horn of one sow were inoculated via the amniotic sac with the NADL-8 virus (0.2 ml, 1,024 HAU/50 μl), and fetuses from one uterine horn of a second sow were inoculated with KBSH (0.2 ml, 1,024 HAU/50 μl). Other fetuses from the uninoculated horn in each animal served as controls. In a second experiment, fetuses of one horn were inoculated with NADL-8, and fetuses from the other horn were inoculated with KBSH. Tissues (lung, liver, and kidney) from all fetuses were collected 10 days after in utero inoculation. Half of each set of tissues was pooled, minced, sonicated (2 to 30-s bursts, 20 W), and tested for HA titer (11). By sterile techniques, the remaining half of each tissue was minced, frozen and thawed twice, and inoculated onto ST cells in culture for virus amplification and isolation. Supernatant fluids from inoculated cell cultures were tested at 5 days postinfection for the presence of virus by HA. Cells were blind passaged and tested again 5 days later.

Oral administration of parvoviruses to weaning-age swine. Fourteen-week-old pigs, seronegative to PPV, were inoculated orally with either KBSH or NADL-8. In one pen, three pigs were inoculated with 2 ml of 1,024 HAU of KBSH per 50 μl; one pig remained as an uninoculated control. In a separate pen, three pigs were inoculated with 2 ml of 1,024 HAU of NADL-8 virus per 50 μl, and one pig in this pen remained uninoculated. Heparinized blood samples were collected every other day for the first 10 days. A portion of the plasma was removed and tested for antibodies to PPV by HA inhibition (11), and the remaining portion was inoculated onto ST cells to attempt virus isolation. Serum samples were collected weekly from all pigs for an additional 5 weeks to measure viral antibody levels on HA inhibition.

RESULTS

Protein composition of KBSH. KBSH was purified from culture fluids of infected ST cells as previously described for the purification of NADL-8 (19). A peak of HA activity appeared at a density in CsCl of 1.39 g/ml, a position identical to that for NADL-8 (19). The polypeptide composition of these virus particles was evaluated by electrophoresis of the material in a sodium dodecyl sulfate-containing polyacrylamide gel (Fig. 1A). Only two major proteins were apparent, having molecular sizes of 83 and 64 kilodaltons (kDal). These polypeptides are identical in size to the PPV A and B structural proteins (19) and therefore most likely represent the capsid proteins of KBSH. We have previously described a third structural protein for PPV (polypeptide C, 60 kDal) present in virus preparations purified from infected fetuses (19). However, when grown in cultured cells, this protein is generally not observed or is found in very low amounts.

The antigenic relatedness of KBSH to PPV had previously been reported for PPV isolate 59e/69 (6). To establish this relationship for the NADL-8 isolate, we generated antisera against NADL-8 and KBSH and used these specific antisera in two ways to compare the antigenic relatedness of these two viruses. First, we tested the ability of the specific antisera to inhibit the HA of erythrocytes by the homologous and heterologous virus. We were unable to demonstrate any differences between the two sera. Each exhibited the same serological titer for either virus. To further demonstrate the antigenic relatedness of the two viruses, KBSH- and NADL-8-infected cells were radiolabeled with [^{35}S]methionine and used to generate lysates for immunoprecipitation analyses. Portions of the radiolabeled extracts from each of the

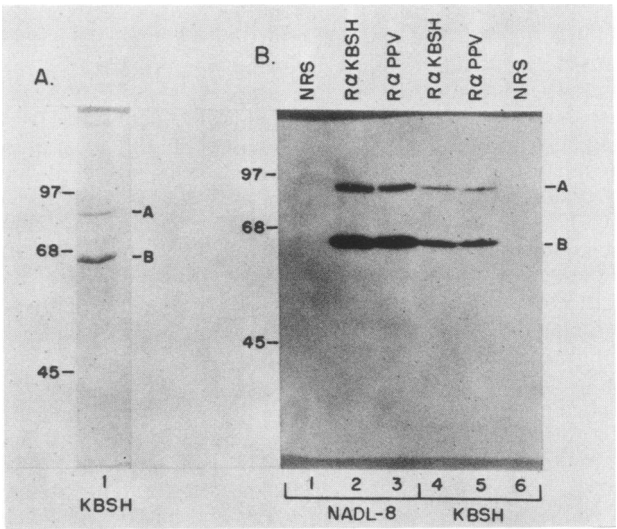

FIG. 1. Protein composition of KBSH. (A) Polypeptide composition of CsCl-purified KBSH virion particles. KBSH virus was propagated in ST cells in vitro and purified as described in the text. Fractions corresponding to a buoyant density of 1.39 g/ml were pooled and dialyzed against 50 mM Tris (pH 8.7)–25 mM EDTA. Approximately 10 μg of purified virus was electrophoresed in a sodium dodecyl sulfate-containing 10% polyacrylamide gel, stained with Coomassie blue, and destained. (B) Immunoprecipitation of radiolabeled cell lysates. KBSH- and NADL-8-infected cell lysates were radiolabeled with [^{35}S]methionine and prepared for immunoprecipitation as described in the text. Sera used to immunoprecipitate portions of the NADL-8 cell extracts and the KBSH cell extracts are listed in Table 1. After electrophoresis in a sodium dodecyl sulfate-containing 10% polyacrylamide gel, the gel was prepared for fluorography and exposed to X-ray film. The numbers in the left margin represent molecular size standards (in kilodaltons).

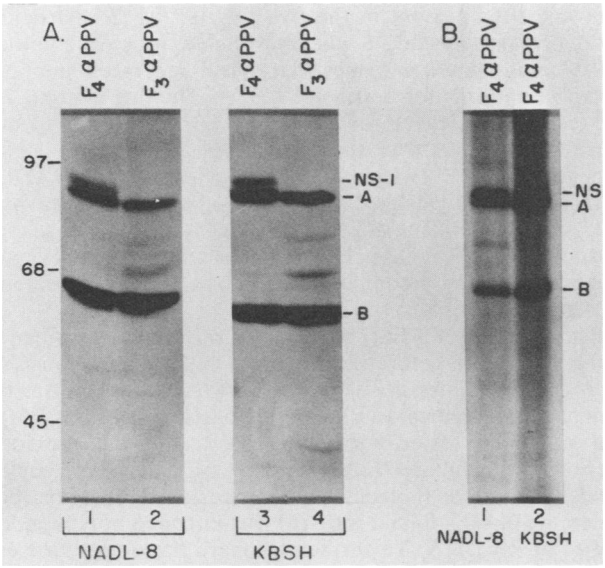

FIG. 2. Immunoprecipitation of radiolabeled virus-infected cell extracts with fetal pig antisera. ST cells were infected with either NADL-8 or KBSH, radiolabeled with either [^{35}S]methionine (A) or $^{32}P_i$ (B), and prepared for immunoprecipitation as described in the text. Two swine fetal antisera were used, $F_4\alpha$ PPV and $F_3\alpha$ PPV (Table 1). The numbers in the left margin indicate approximate molecular sizes (in kilodaltons).

virus-infected cells were immunoprecipitated with either the anti-KBSH or the anti-PPV antiserum (Table 1), and the immunoprecipitated polypeptides were resolved on polyacrylamide gels. Both antivirus antisera were able to immunoprecipitate the A and B capsid proteins of either virus (Fig. 1B).

Recently, several autonomous parvoviruses have been shown to code for nonstructural polypeptides in addition to their structural proteins (5, 18, 21). We have recently identified and characterized a nonstructural polypeptide of 86 kDal (NS-1) from NADL-8-infected cells (Molitor et al., in press). This identification was facilitated by the use of certain antisera generated in swine fetuses (Molitor et al., in press). To further compare KBSH with NADL-8, we used an antiserum (F_4 PPV, Table 1) that recognizes the NADL-8 NS-1 protein along with an antiserum that recognizes only the capsid proteins of this virus (F_3 PPV, Table 1) to immunoprecipitate proteins from radiolabeled KBSH-infected cell lysates. Both antisera readily recognized the KBSH capsid proteins (Fig. 2A). Furthermore, the F_4 antiserum was able to immunoprecipitate an 86-kDal polypeptide from KBSH-infected cell lysates in an identical fashion as from NADL-8-infected cell lysates. The three NADL-8 polypeptides, A, B, and NS-1, have recently been shown to exist in phosphorylated forms in infected cells (Molitor et al., in press). With these same antisera, the three corresponding proteins from KBSH-infected cells were also phosphorylated (Fig. 2B).

From the preceding experiment, it was clear that KBSH and NADL-8 possessed both structural and nonstructural polypeptides that were antigenically related. To further compare the polypeptides of these two viruses, we subjected

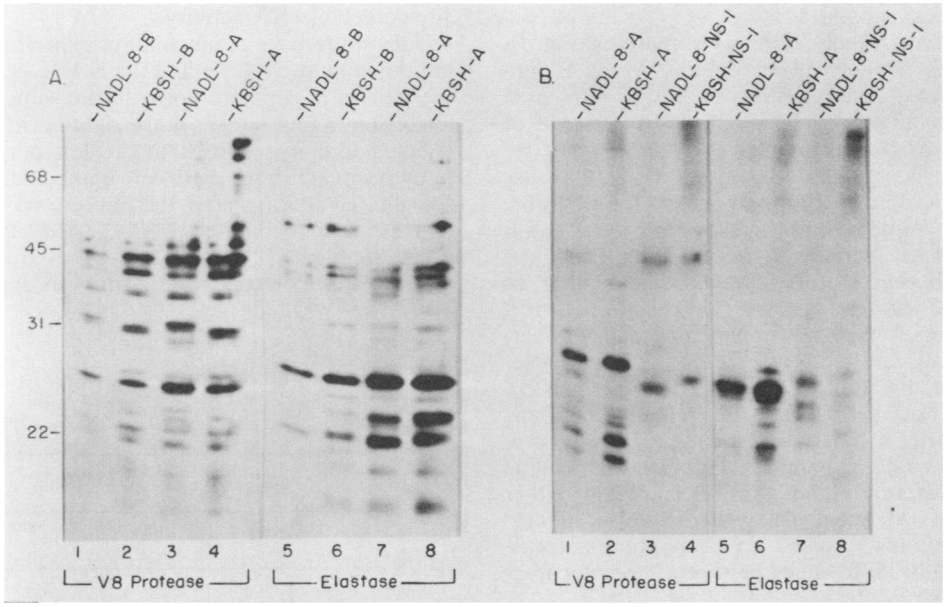

FIG. 3. Partial proteolysis mapping of the KBSH and NADL-8 polypeptides. Virus-infected cell cultures were radiolabeled with either [^{35}S]methionine (A) or $^{32}P_i$ (B) and prepared for immunoprecipitation. [^{35}S]methionine-radiolabeled lysates were immunoprecipitated with Rα PPV antiserum, and $^{32}P_i$-labeled lysates were immunoprecipitated with fetal antiserum F_4 (Table 1). The immunoprecipitated polypeptides were electrophoresed in sodium dodecyl sulfate-containing 7.5% polyacrylamide gels, localized by autoradiography, excised, individually subjected to a second cycle of gel electrophoresis, and then employed for partial proteolysis analyses as previously described (4, 19). Two proteases were used, *Staphylococcus aureus* V8 protease (0.1 μg/track) and elastase (1.0 μg/track). Electrophoresis in the presence of the proteases was in a sodium dodecyl sulfate-containing 15% polyacrylamide gel. The numbers in the left margin represent approximate molecular sizes (in kilodaltons).

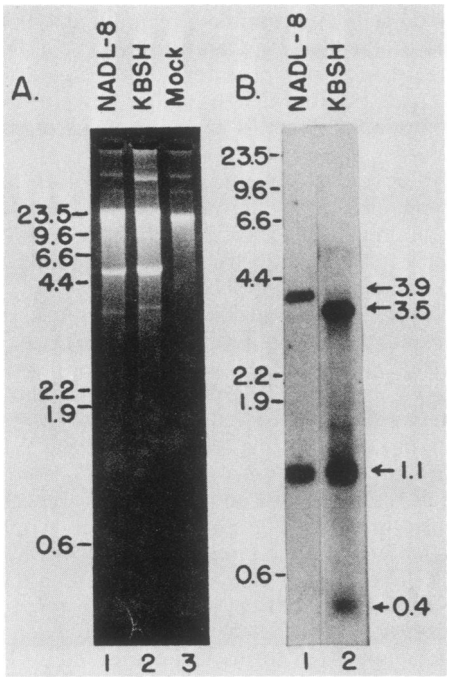

FIG. 4. KBSH RF DNA. (A) ST cells were either mock infected or infected with NADL-8 or KBSH and at 24 h postinfection harvested and used for the purification of viral RF DNA (20). A portion of each DNA preparation was electrophoresed in a 1% agarose gel and visualized by ethidium bromide fluorescence. (B) The RF DNAs of KBSH and NADL-8 were gel purified, digested with the restriction endonuclease *Msp*I, radiolabeled, electrophoresed in a 1% agarose gel, and autoradiographed as previously described (20). Numbers in the margins indicate DNA size (in kilobase pairs) with the *Hin*dIII endonuclease digestion fragments of bacteriophage lambda DNA as standards.

each of them to partial proteolysis mapping analyses (Fig. 3). The [^{35}S]methionine-labeled capsid proteins A and B, immunoprecipitated from either KBSH- or NADL-8-infected cells, were partially hydrolyzed by either V8 protease or elastase, and the resultant proteolysis products were resolved by gel electrophoresis (Fig. 3A). What was immediately clear was that the proteolytic peptide patterns, generated by either V8 protease or elastase, for the A and B proteins of both viruses were quite similar. Thus, the A and B proteins of KBSH were closely related to one another, as they were to the A and B proteins of NADL-8. However, although the patterns were very similar, differences in certain peptides were discernible (e.g., see Fig. 3A, V8 protease, 31-kDal region), indicating that the KBSH proteins were not identical to the NADL-8 proteins. A similar analysis was carried out comparing the partial proteolytic fragments of the phosphorylated viral polypeptides (Fig. 3B). The peptide patterns of the A protein from both viruses employing either protease were nearly identical. Analysis of the phosphorylated viral NS-1 proteins revealed similar peptide patterns, but with clear mobility differences between the major fragments (Fig. 3B).

RF DNA of KBSH. To compare the genetic makeup of the KBSH genome with that of the NADL-8 genome, we isolated the viral RF DNA of each virus from infected cells as described previously for NADL-8 (20). In a neutral agarose gel, the RF DNA from both virus-infected cells migrated very similarly at a size of ca. 5,000 base pairs (Fig. 4A). We performed a limited comparison of restriction endonuclease cleavage sites present in the two DNAs. Of 15 restriction endonucleases tested, 6 enzymes failed to cleave either DNA, and of the 9 enzymes that did, 8 generated identical cleavage patterns for both viral DNAs. The one endonuclease tested that cleaved the KBSH RF DNA differently from the PPV RF DNA was *Msp*I (Fig. 4B). NADL-8 RF DNA appeared to have a single *Msp*I site, whereas KBSH DNA possessed this same *Msp*I as well as one additional cleavage site. The positions along the PPV genome of the tested restriction endonuclease cleavage sites have been previously mapped (20), and a comparison with the KBSH genome is presented in Fig. 5.

Pathogenicity of KBSH in swine. KBSH was described a number of years before the reporting of PPV as a cause of reproductive failure in swine (6). KBSH was thought to be similar if not identical to PPV on the basis of serology, but it had not been tested for pathogenicity. In the previous sections, we compared KBSH and NADL-8 at the molecular level. Only minor molecular differences were observed between the NADL-8- and KBSH-virus-encoded polypeptides and viral RF DNA. To further compare these two viruses, the replicative capabilities and pathogenic effects of each were tested both by in utero injection of virus into fetuses and by oral inoculation of virus to PPV-seronegative pigs.

In one experiment, the fetuses of one uterine horn of a pregnant sow were inoculated with KBSH, and the fetuses of one uterine horn of a second pregnant sow were similarly inoculated with NADL-8. At day 10 after virus inoculation, the sows were slaughtered, and the fetuses were recovered. All uninoculated fetuses (from the uninoculated horn of each sow) were normal in appearance. Each of the fetuses from the NADL-8-inoculated horn were either mummified or hemorrhagic. The tissues from these diseased fetuses displayed high HA titers (4,000 to 8,000 HAU/50 μl). This was in contrast to the KBSH-inoculated fetuses which all appeared normal; tissues from these fetuses failed to exhibit any detectable HA activity.

This in utero virus inoculation experiment was repeated, but this time the KBSH and the NADL-8 were injected into the fetuses of separate horns in the same sow. KBSH was injected into four fetuses in the right horn, and NADL-8 was injected into five fetuses in the left horn. The remaining fetuses in each horn were left uninoculated. The sow was slaughtered 10 days after the in utero inoculations, and the fetuses were recovered. NADL-8-inoculated fetuses were again hemorrhagic, whereas fetuses from the KBSH or uninoculated controls were normal in appearance (Fig. 6).

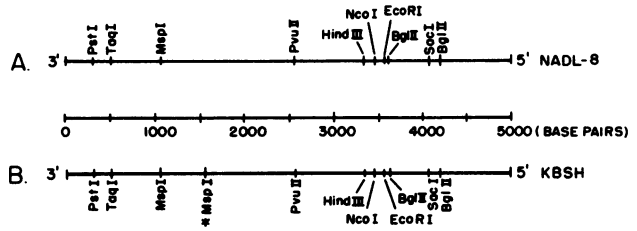

FIG. 5. Composite restriction endonuclease map of NADL-8 and KBSH RF DNA. The maps are oriented with respect to the single-stranded virion DNA, with the 3' terminus at the left. Restriction endonucleases tested that failed to cleave either RF DNA were *Ava*I, *Bam*HI, *Bgl*I, *Sal*I, *Xba*I, and *Xho*I. The single restriction site difference noted (an additional *Msp*I site) is highlighted (*).

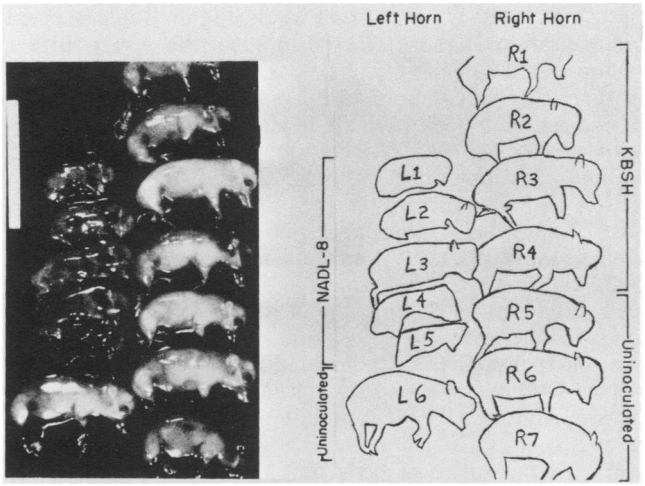

FIG. 6. Swine fetuses inoculated in utero with either KBSH or NADL-8. Fetuses from a single sow were inoculated with either NADL-8 (left horn) or KBSH (right horn) as described in the text. One fetus in the left horn (L6) and three fetuses in the right horn (R5 to R7) remained as uninoculated controls. The sow was sacrificed at day 10 after virus inoculation, and the fetuses were recovered.

The tissues from all NADL-8-injected fetuses had HA titers of greater than 2,000 HAU/50 μl; uninoculated and KBSH-injected fetuses were negative by HA. Virus isolation was then attempted on ST cell cultures from tissues of the KBSH-injected and the uninoculated control fetuses from the right horn. Virus was isolated from all tissues tested from three of the four KBSH-injected fetuses (Table 2). Virus was not isolated from any of the tissues from control fetuses.

We repeated the above experiment in which three fetuses in one horn were inoculated with KBSH and three fetuses from the other horn were inoculated with NADL-8. However, instead of allowing the infection to proceed for 10 days, it was allowed to proceed 21 days before termination. The results were the same. NADL-8-inoculated fetuses were mummified or hemorrhagic, and virus was easily isolated.

TABLE 2. Isolation of KBSH virus from fetal tissues

Fetus no.[a]	Tissue	Virus isolation[b]	Fetus no.[a]	Tissue	Virus isolation[b]
R_1	Lung	Pos	R_5	Lung	Neg
	Liver	Pos (2)		Liver	Neg
	Kidney	Pos (2)		Kidney	Neg
R_2	Lung	Neg	R_6	Lung	Neg
	Liver	Neg		Liver	Neg
	Kidney	Neg		kidney	Neg
R_3	Lung	Pos (2)	R_7	Lung	Neg
	Liver	Pos (2)		Liver	Neg
	Kidney	Pos (2)		Kidney	Neg
R_4	Lung	Pos			
	Liver	Pos (2)			
	Kidney	Pos (2)			

[a] Fetuses R_1 to R_4 were injected with KBSH; fetuses R_5 to R_7 were uninoculated.

[b] Virus isolation was attempted by inoculation of 0.2 ml of fluid from respective tissues into ST cell cultures. Pos, Cell cultures virus positive by HA of culture fluids; number in parenthesis indicates that detection of virus was not until the second cell culture passage; Neg, culture fluids were negative for HA activity after the second cell culture passage.

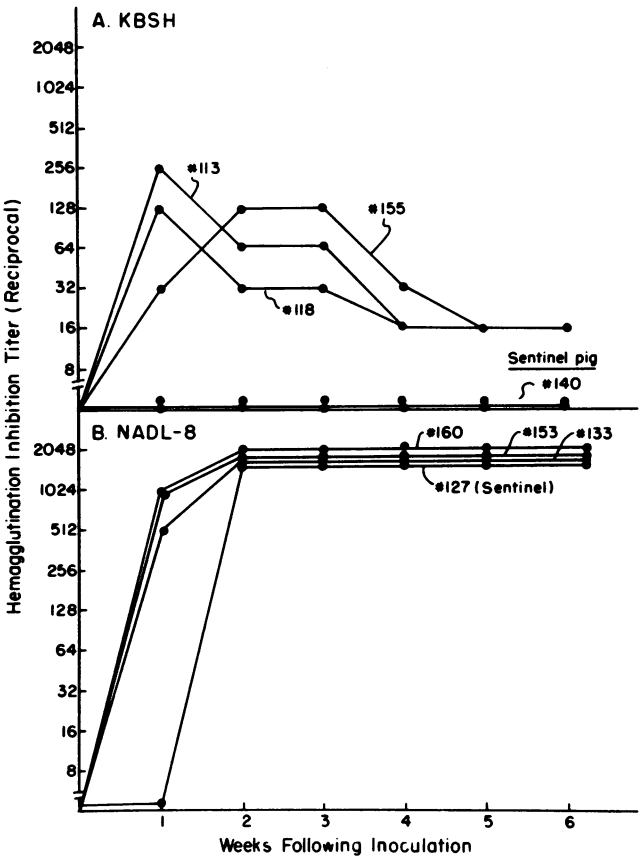

FIG. 7. Antibody response of pigs orally inoculated with viruses. Three pigs in each of two groups were orally inoculated with either KBSH or NADL-8. A fourth pig in each group served as an uninoculated control. The two groups were housed separately. Sera were collected weekly, and viral antibody levels were measured by HA inhibition (11).

KBSH-inoculated fetuses were normal in appearance, but virus could be isolated from the fetal tissues on the second cell culture passage.

The preceding studies suggested that the clear inability of KBSH to cause fetal disease upon in utero inoculation was related to the very poor ability of the virus to replicate in fetal tissues. In the pregnant animal, PPV-induced disease requires transplacental crossing of the virus from the infected sow (15). A viremic state in the sow is a prerequisite for this transplacental crossing (15). To evaluate this aspect of pathogenicity, we set out to determine whether, when orally administered to susceptible pigs, the KBSH could replicate in the animal and establish a viremic state. Fourteen-week-old seronegative pigs were inoculated orally with either KBSH or NADL-8. Blood samples were collected every other day for the first 2 weeks to test for virus replication (viremia) and weekly thereafter for 5 weeks to follow viral antibody levels. Virus was readily isolated from blood samples of all three NADL-8-inoculated animals at 3 to 7 days. Furthermore, the one control animal in this group became infected, indicating that NADL-8 was being shed from the infected animals. In contrast, we were unable to detect or isolate virus from blood samples from the KBSH-infected pigs.

Viral antibody titers were also followed in all animals (Fig. 7). All NADL-8-infected animals rapidly developed high-

level antibody titers that were maintained throughout the course of the experiment. The control animal in this group, after a slight delay, also developed high levels of PPV-specific antibody that persisted. Viral antibody titers in the three KBSH-infected pigs also rose 1 week postinoculation. However, the levels in these animals were much lower than in the NADL-8-infected animals (Fig. 7). Furthermore, they gradually declined over the course of the experiment. At no time did the control animal in this group exhibit a virus antibody response.

DISCUSSION

PPV is a major cause of reproductive failure in swine (10, 15, 16). KBSH, isolated from human KB cells grown in culture, was described as a parvovirus related to PPV, based on serological criteria (6, 24). However, little information addressing the molecular features or pathogenic potential of this isolate was available. Thus, we have undertaken these studies. We have found the physical characteristics and molecular composition of KBSH to be very similar, but not identical, to those of NADL-8. Purified, infectious KBSH virion particles banded at a density in CsCl of 1.39 g/ml and were composed of two major capsid proteins, A and B (Fig. 1A), identical in size to those of NADL-8. We have found that KBSH was antigenically indistinguishable from NADL-8 (Fig. 1 and 2), supporting its previously described close serological relationship to PPV isolate 95e/63 (6). Furthermore, KBSH-infected cells contained a nonstructural polypeptide identical in size to the NS-1 protein recently described in NADL-8-infected cells (Fig. 2B; Molitor et al., in press). All three viral proteins (A, B, and NS-1) from both viruses existed in phosphorylated forms in virus-infected cells (Fig. 2B). Structural comparisons of the three proteins by partial proteolysis mapping procedures revealed that the A and B capsid proteins of KBSH were very closely related to one another as they were to the A and B capsid proteins of NADL-8 (Fig. 3). However, minor differences between the peptide patterns of the KBSH and NADL-8 proteins could be discerned, indicating that although the capsid proteins of these two viruses were closely related structurally, they were not identical. Similar results were obtained from comparisons of the nonstructural protein from each of the virus-infected cells (Fig. 3B).

The genome of KBSH was a largely single-stranded DNA of ca. 5,000 bases (data not shown). We have compared the KBSH RF DNA to that of NADL-8, which has been previously described (20). Using a limited number of restriction endonucleases, we found that the enzyme cleavage patterns of the two RF DNAs were identical, with one exception: the KBSH RF DNA had an additional *Msp*I site (Fig. 4B and 5). Although these analyses have involved only a small number of sites along the DNA of these viral genomes, and additional studies are clearly needed, it is interesting that the one endonuclease site difference detected lies in the region that presumably encodes the NS-1 polypeptide (5). We were also able to readily detect differences between the NS-1 polypeptides of the two viruses by limited proteolysis mapping (Fig. 4B).

In summary, the molecular and antigenic features of KBSH and NADL-8 appeared to be largely very similar but not identical. However, upon examination of the replicative and pathogenic capabilities of KBSH in swine, this virus was quite different from the highly pathogenic isolate NADL-8. NADL-8 readily caused disease on in utero inoculation of swine fetuses. Virus replication was easily demonstrable. On the other hand, KBSH-inoculated fetuses showed no signs of disease or extensive virus replication (Fig. 6, Table 2). Oral administration of these viruses to susceptible pigs further demonstrated the inability of KBSH to replicate in swine. At no time after inoculation were we able to detect or isolate virus from the blood of KBSH-inoculated animals, although as soon as 3 days after NADL-8 inoculation, animals became viremic. The antibody response of pigs inoculated with KBSH further confirmed the poor replication of the virus in vivo. The type of serological response observed (Fig. 7) was similar to that observed in animals injected with adjuvanted, killed vaccines (12, 13, 25).

Explanations for the inability of KBSH to replicate extensively in porcine fetuses or weaning-age pigs remain speculative. One possibility may be related to the extensive passage history (300 passages) of this virus in human KB cells in culture. Although KBSH is readily propagated in ST cells in culture, such prolonged in vitro culture adaptation may have restricted the range of cells that this virus is capable of infecting. Screening of a variety of porcine cell types for their ability to support KBSH replication in cell culture should help to address this possibility. In conclusion, further investigation of KBSH, including more refined comparative molecular analyses (DNA sequences and protein sequence) with NADL-8, may help elucidate the molecular requirements associated with parvovirus replication and pathogenesis in infected host animals.

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LITERATURE CITED

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