

EXPERIMENTAL INFECTION OF BUCKS AND PREGNANT DOES WITH BOVINE HERPESVIRUS-4

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Abstract

Fifty-six does were inoculated with bovine herpesvirus-4 (BHV-4) or normal cell culture fluid by intravaginal (groups 1 & 2, early gestation) or intravenous routes (groups 3 & 4, late gestation). Forty bucks were inoculated with BHV-4 or normal cell culture fluid by intratesticular (groups 5 & 6) or intravenous (groups 7 & 8) routes. Clinical signs following inoculation of the virus in does included abortion in 2 pregnant does, decreased conception rate (25% conception rate in group 1 vs. 41.66% conception rate in control group 2), and mild vaginitis (2/24, group 1). No clinical signs were observed with viral infection in bucks. Microscopic lesions included marked placentitis, mild suppurative endometritis, moderate multifocal endometrial hemorrhages, endometrial oedema, oophoritis, mesenteric lymph node hyperplasia, and mild multifocal necrotizing hepatitis. Histopathological examination of intratesticularly inoculated rabbits revealed mild to moderate interstitial lymphocytic and acute necrotizing orchitis, with segmental to diffuse testicular degeneration, and mild interstitial oedema. The virus was isolated from one testicle of inoculated rabbit. Viral antigen was detected by immunohistochemistry only in the mesenteric lymph nodes and spleen in infected does, but virus was not isolated. Polymerase chain reaction (PCR) detected BHV-4 genome in two bucks. Transmission electron microscopy (TEM) revealed viral nucleocapsids in the uterus, vagina, spleen, and mesenteric lymph nodes of infected does and in the testis, spleen and mesenteric lymph node of infected bucks. Gross, microscopic, TEM, PCR, and immunohistochemical examination of tissues suggested that BHV-4 had a mild pathogenic role in bucks inoculated intratesticularly.

Key words: Abortion; bovine herpesvirus-4; conception rate; endometritis; immunohistochemical; rabbits; PCR, specific-pathogen-free; transmission electron microscopy; vaginitis.

INTRODUCTION

Bovine herpesvirus-4 (BHV-4) is a herpesvirus grouped under the family Herpesviridae, subfamily Gammaherpesvirinae and includes viruses that have a slow replication cycle in cell cultures and are highly cell-associated (Goltz *et al.*, 1994). Experimental studies of BHV-4 in both natural and experimental hosts indicated that, only certain isolates of BHV-4 can potentially cause clinical disease (Naeem *et al.*, 1991a). Clinical signs of BHV-4 infection ranged from mild febrile illness to respiratory disease or abortions. BHV-4 is suspected to cause abortion (Castrucci *et al.*, 1986), postparturient metritis (Theodoridis, 1985), infertility, stillbirth (Castrucci *et al.*, 1986), vaginitis, and repeat breeders in cows (Biolatti *et al.*, 1991). In bulls, further studies have implicated BHV-4 in orchitis (Dubuisson *et al.*, 1987) and epididymitis (Theodoridis, 1985). Concurrent infectious agents have been thought to be necessary to produce disease during BHV-4 infection (Castrucci *et al.*, 1992). Others suggested that BHV-4 causes immunosuppression, allowing secondary invaders to produce various clinical diseases (Wellems *et al.*, 1985). Because of the uncertain role that intercurrent infection may occur during the development of clinical disease in cattle exposed to BHV-4, it is important to develop a model of BHV-4 infection without concurrent infection by other pathogenic organisms. Rabbits are susceptible to BHV-4 infection, and are suitable laboratory species to examine this problem (Naeem *et al.*, 1991b). The objective of this study was to elucidate the pathogenic role of BHV-4 in the reproductive tract of experimentally inoculated specific-pathogen-free (SPF) bucks and does.

MATERIALS AND METHODS

Experimental animals

Fifty-six does and forty bucks, sexually mature, SPF (free of *Pasteurella multocida*, *Psoroptes cuniculi*, *Eimeria stiedae* and *Encephalitozoon cuniculi*), Dutch-belted rabbits were housed in individual cages within isolated animal rooms. All animals were kept under observation for at least 10-14 days before initiation of the experiment.

Virus inoculum

A field isolate of BHV-4 (strain 87-8363), recovered from an aborted bovine foetus, was obtained (Dr. Jim Evermann, Washington State Animal Disease Diagnostic Laboratory, Pullman, WA.). The virus was propagated in primary bovine embryonic lung (BEL) cells. When 90% of the cell monolayer showed CPE, the cells were frozen at -70°C. After thawing, the cell suspension was centrifuged to remove cell debris, and the

supernatant was further clarified by centrifugation at 10,000 x g for 20 minutes, aliquoted, and stored at -70°C until used. The virus inoculum was titrated, then diluted to contain 10^6 TCID₅₀/ml. The virus used for group 3 pregnant rabbits was strain 87-8363 BHV-4 reisolated from the testis of an infected buck (group 5), then, inoculated onto rabbit kidney (RK-13) cells for virus adaptation to rabbit tissue.

Experimental design

Does (Groups 1-4) and bucks (Groups 5-8) were inoculated with 1 ml of BHV-4/animal or 1 ml of uninfected tissue culture media by the schedule, virus dose, and routes indicated in Table 1. Intravaginally inoculated rabbits were purchased nonpregnant and artificially inseminated, while, intravenously inoculated rabbits were purchased pregnant at 16 days of gestation. Rabbits were sedated by intramuscular administration of acepromazine maleate (0.5 mg/kg b.w.), xylazine (10 mg/kg b.w.), and ketamine HCl (15 mg/kg b.w.). Intratesticular inoculation was divided equally into each testicle.

Monitoring and sample collection

Rabbits were observed daily for clinical signs of disease, uterine or nasal discharges, abortion, and gross testicular changes. Group 1 showed vaginal secretions, smears were done from the secretion, and then stained. Two does from group 1 were monitored for 5 days postkindling before samples collection. Rectal temperatures were recorded every other day in all rabbits. Semen samples from group 7 rabbits were collected on days 10, 17, 24, and 31 postinoculation (PI) for virus isolation and immunohistochemistry. Concentrated semen samples were smeared, air dried, fixed with 95% ethanol, and stored at room temperature until stained.

Postmortem sampling

Inoculated does were euthanatized by an overdose of barbiturate (Beuthanasia-D; Schering-Plough Animal Health Corp. Kenilworth, NJ.) on days 10, 17, 24, 27, 33 PI, 5 days postkindling, or immediately after abortion in groups 1 and 2; and just following abortion or normal parturition in groups 3 and 4. Bucks were euthanatized on days 10, 17, 24, and 31 PI in groups 5 and 6, and on day 31 PI in groups 7 and 8. Complete necropsies were performed immediately after death. Tissues for histopathological examination were fixed in 10% neutral-buffered formalin overnight, embedded in paraffin, sectioned at 5 µm, stained with hematoxylin and eosin (HE) and examined. Tissue specimens collected included ovary, uterus, cervix, vagina, vulva, placenta, testes, epididy-

mis, liver, spleen, and mesenteric lymph node. Tissues were aseptically collected for virus isolation using sterile whirlpac bags, and stored at -70°C until processed for virus isolation. Tissue specimens (listed in Table 2) for TEM examination were cut into thin strips with razor blades immediately following euthanasia and placed in cold (4°C) 3% glutaraldehyde fixative in 0.1 M phosphate buffered saline (PBS, pH 7.2) overnight, and then, fixed in 1% osmium tetroxide before dehydration and embedding in plastic.

Table 1. Experimental infection of specific-pathogen-free does and bucks with bovine herpesvirus-4 (strain 87-8363).

Group	No. of Rabbits	Route of inoculation	Virus Dose (TCID ₅₀)
Group 1	24	Intravaginal (4 days postinsemination)	10^6 TCID ₅₀ /rabbit
Group 2	24	Intravaginal (4 days postinsemination)	1 ml normal T.C. fluid/rabbit*
Group 3	5	Intravenous (16th day of gestation)	10^6 TCID ₅₀ / rabbit
Group 4	3	Intravenous (16th day of gestation)	1 ml normal T.C. fluid/rabbit*
Group 5	16	Intratesticular	10^6 TCID ₅₀ / rabbit
Group 6	16	Intratesticular	1 ml normal T.C. fluid/rabbit*
Group 7	4	Intravenous	10^6 TCID ₅₀ / rabbit
Group 8	4	Intravenous	1 ml normal T.C. fluid/rabbit*

* Each of the controls received a volume of virus-free tissue culture equivalent to the infectious inocula following the procedure of the inoculation route in each case.

PI = post-inoculation

Immunohistochemistry

Indirect immunoperoxidase (IP) staining using the biotin-antibiotin system was performed to detect antigens of BHV-4 in formalin-fixed, paraffin-embedded tissue or semen samples of group 7 infected and control rabbits as previously described (Katz *et*

al., 1977). Slides were rinsed in PBS, pH 7.8 followed by incubation in 0.05% enzyme solution of protease VIII (P-5380, Sigma, St. Louis, MO.) for three minutes at 37°C. Diluted primary antibody (polyclonal bovine anti-serum to BHV-4, NVSL, lot No. 352 BDV 8201) was added to the sections and incubated, washed, and monoclonal biotinylated secondary antiserum (B-9780, Sigma, St. Louis, MO.) was then added to the sections. Peroxidase labeled monoclonal antibiotin (A-0185, Sigma, St. Louis, MO.) was added, followed by immersion in a substrate solution containing 3,3' diaminobenzidine tetrahydrochloride dihydrate (Aldrich, 189-0.).

Tissue culture chamber slides of BHV-4 infected monolayers of RK-13 cells were fixed in 95% ethanol or 10% neutral-buffered formalin for 1 hour and processed in the same way as tissues from rabbits. These slides served as virus positive control slides included during staining. Virus specific primary antibody was replaced with fetal bovine serum (FBS) in tissue sections used as negative controls during staining.

The same procedures were followed during staining of semen slides prepared from group 7 bucks.

Transmission electron microscopy

Ultrathin sections were cut with glass knives (Knife maker 7801B, LKB, Stockholm, Sweden) on an ultramicrotome (MT2-B ultramicrotome, Sorvall Kukont Instruments, Wilmington, DE.). Thin sections were stained with uranyl acetate and lead citrate stain before examination using an electron microscope (Hitachi, HU-12A, Tokyo, Japan).

Virus isolation

Mesenteric lymph node, uterus, placenta, vagina, and spleen were taken separately from 17 does in group 1 and 5 does from group 3 for virus isolation. A pooled sample of lymph nodes and individual parts of spleen, liver, uterus, vagina, and placenta were selected for virus isolation from does that aborted. A pool of liver, kidney, heart, and lungs from aborted foetuses were also collected for virus isolation. A pooled sample of uterus and placenta from one control animal was processed as a negative control. Individual or pooled samples of lymph nodes, spleen, liver, and testicle from 11 bucks in group 5 and 2 bucks in group 7 bucks and semen from 4 bucks in group 7 were collected for virus isolation. Tissues were processed by preparing a 10% (wt/vol) homogenized suspension in MEM with antibiotics by mechanical disruption using a blender (Dynatech Laboratories, Alexandria, VA.). Inoculums for tissue culture were

cleared by centrifugation and filtration before inoculation onto monolayers of bovine turbinate (BT) and RK-13 cells in 4-well plates. All plates were checked for CPE after 10 days incubation. All samples were passaged twice before being called negative. Positive samples for viral isolation were confirmed by immunofluorescent antibody technique (IFAT).

Polymerase chain reaction

Polymerase chain reaction (PCR) was applied on formalin-fixed, paraffin embedded sections of tissue. Blocks of tissue were also examined histologically. The method of reaction was performed as previously described, with primers from thymidine kinase gene (Egyed *et al.*, 1996). Formalin-fixed, paraffin embedded tissue sections were rehydrated, removed from the glass slides into a microfuge tube into 20 ul of water. Twenty microliters of Gene-Releaser Bioventures Inc., Knoxville, TN.) was then added, and the mixture was microwaved for 7 minutes. To this, a PCR reaction mix containing buffer was added (66 mM Tris pH 8.8, 16.5 ammonium sulfate, 0.01% Tween 20), 2.5 mM MgCl₂, 200 uM nucleotide triphosphates, 1 mg/ml bovine serum albumin, 0.3 uM primers, and 0.5 units Taq polymerase in a final volume of 100 ul.

Data analysis and statistical tests

Statistical correlation between experimental groups (infected vs. controls) were evaluated for each of the variables using Chi-squared (X^2) or Fisher's exact tests whenever appropriate (Norman and Streiner, 1994).

RESULTS

Abortion occurred in one doe in group 1 (26 days of gestation), and in one doe in group 3 (24 days of gestation). Premature delivery occurred in one control doe (group 2), and in one infected doe (group 3). Kits of the two does (control & infected) were born alive but died after delivery and were smaller than full term kits. The conception rate of intravaginally inoculated rabbits (group 1) was 25% (6/24) versus 41.66% (10/24) in the inoculated controls (group 2). Depression and anorexia were noticed in two intravenously inoculated rabbits (group 3) for two days starting 9 days PI. One doe became febrile (40.5°C), had a slight clear nasal secretion, became recumbent, then, died on the next day (11 days PI). A mild, suppurative, vaginal secretion was observed for 5 days in two intravaginally inoculated does (group 1) on the 10th day PI.

- Inoculated bucks of groups 5-8 did not show signs of illness.

Gross pathology

Gross lesions were observed in one litter of aborted foetuses (group 1). Subcutaneous hemorrhages on the head and hind quarters were noted in 4/6 aborted foetuses. Aborted foetuses of group 3 doe were autolytic. Gross lesions were not detected in any buck.

Histopathological changes

The uterus of group 1 doe that aborted had moderate multifocal hemorrhages and oedema of the endometrium with mild focal hemorrhages and marked oedema in the lamina propria of the vaginal mucosa. There was multifocal hemorrhage in the placental tissue of one infected doe. Mild endometritis was evident in one BHV-4 inoculated doe. Placentomes of control and BHV-4 inoculated does were similar with necrotic trophoblastic epithelium.

The histological findings of genital tracts of control and infected does euthanatized on 10, 17, 24, 27, and 33 days PI were limited to congestion and dilation of blood vessels of the tunica muscularis and mild oedema in the lamina propria of the uterus. No gross or microscopic lesions were noted in tissues from two infected does euthanatized 5 days postkindling.

The histological changes noted in an intravenously inoculated does and the corresponding control does that had normal term kits consisted of mild hemorrhages and oedema in the mucosa of uterus, cervix, and vagina consistent with normal parturition.

Mesenteric lymph nodes in 16/24 (66.7%) intravaginally inoculated and in 5/5 (100%) of intravenously inoculated does had mild to moderate hyperplasia

One doe in group 3 had random multifocal necrotizing hepatitis that did not have associated viral inclusion bodies. This lesion was interpreted to reflect septicemia. No significant microscopical findings were observed in the spleen.

Microscopical examination of testes of rabbits of group 5 euthanatized on 10 days PI revealed focal areas of mild to marked degeneration of seminiferous tubules, loss of most germinal epithelium, with sertoli cells remaining (Fig. 1). Mild infiltration of the degenerate seminiferous tubules with heterophils and lesser numbers of lymphocytes, monocytes, and plasma cells was observed in addition to oedema and mild perivascular cuffing. One control rabbit euthanatized 10 days PI had one to two small foci of lymphocytes and heterophils in the interstitial tissue of testis. Orchitis associated

with tubular degeneration was prominent in 3/4 group 5 rabbits that were euthanatized on 17 days PI. The histopathological findings were marked multifocal lymphocytic infiltration (Fig. 1) and interstitial perivascular cuffs. Random foci of degenerated seminiferous tubules infiltrated with heterophils and lymphocytes were also observed in one rabbit inoculated with the virus. The prominent microscopic lesions observed in testicles of animals euthanatized on 24 days PI were lymphocytic orchitis associated with degeneration of the seminiferous tubules and interstitial oedema. Diffuse hypospermatogenesis was noted in one rabbit. The microscopic alterations noted in the testicular tissue of infected animals euthanatized on 31 days PI were similar to those animals euthanatized on 10, 17, 24 days PI with active germinal epithelial cellular necrosis.

No significant microscopical findings were detected in testicles from rabbits in groups 6, 7, or 8. Spermatid giant cells were occasionally observed in the testicles of infected and control rabbits. The epididymal head, body, and tail of all infected rabbits in group 5 and 7 did not have visible alterations.

Lymphoblastic proliferation in germinal centers of most lymphatic nodules of mesenteric lymph nodes was noted in 3/20 (15%) of the virus inoculated animals (group 5 and 7). No significant microscopic alterations were observed in spleens. Livers of both control and infected animals showed mild to severe vacuolar degeneration of centrilobular hepatocytes.

Immunohistochemistry

Tissue sections of spleen and lymph nodes were the only samples that showed distinct positive immunostaining reaction for BHV-4 antigen in groups 1, 3, 5, and 7 (Table 2). In all positive cases, multiple foci of brown pigment deposition were seen in the cytoplasm of mononuclear cells. Lymph nodes showed prominent immunostaining reaction in the mononuclear cells of the medullary sinuses, subcapsular sinuses, and peritrabecular sinuses, with occasional macrophage staining at the corticomedullary junction. The immunostaining reaction in the spleen was often seen in the mononuclear cells in the red pulp, splenic cords, and in the marginal zone. All tissue sections from the control animals were negative by IP staining. Semen slides prepared from group 7 rabbits were negative by IP staining for BHV-4.

Transmission electron microscopy

Transmission electron microscopy of inoculated does and buck tissues revealed the presence of 80-90 nm hexagonal virus nucleocapsids in 7/12 samples (Table 2).

Virus isolation

Except for one rabbit of group 5 (Table 2), tissue specimens subjected to conventional virus isolation in cell culture were negative for BHV-4. Virus was not isolated from the pooled semen samples collected at different stages PI from any buck of group 7.

Polymerase chain reaction

The PCR was positive in 2 bucks (Table 2, Fig. 2).

Table 2. Recovery of BHV-4 by immunoperoxidase, virus isolation, PCR, and transmission electron microscopy.

Tissue Specimen	Immunoperoxidase	Virus Isolation	Virus PCR	Electron Microscopy
Group 1 doe that aborted - Vagina - Lymph node	Negative Positive	Negative Negative	Not Done	Positive Negative
Group 1 doe - Uterus	Negative	Negative	Negative	Negative
Group 3 doe that aborted - Vagina - Lymph node	Negative Negative	Negative Negative	Negative Negative	Positive Negative
Group 3 doe - Uterus - Placenta	Negative Negative	Negative Negative	Negative Negative	Negative Negative
Group 3 doe - Uterus - Lymph node	Negative Positive	Negative Negative	Negative Negative	Positive Positive
Group 5 buck - Testis	Negative	Positive	Negative	Positive
Group 5 buck - Spleen	Positive	Negative	Positive	Positive
Group 7 buck - Lymph node	Positive	Negative	Positive	Positive

DISCUSSION

Late-stage abortions occurred in only two virus inoculated does without premonitory clinical signs. The macroscopic reproductive tract findings in both aborted animals

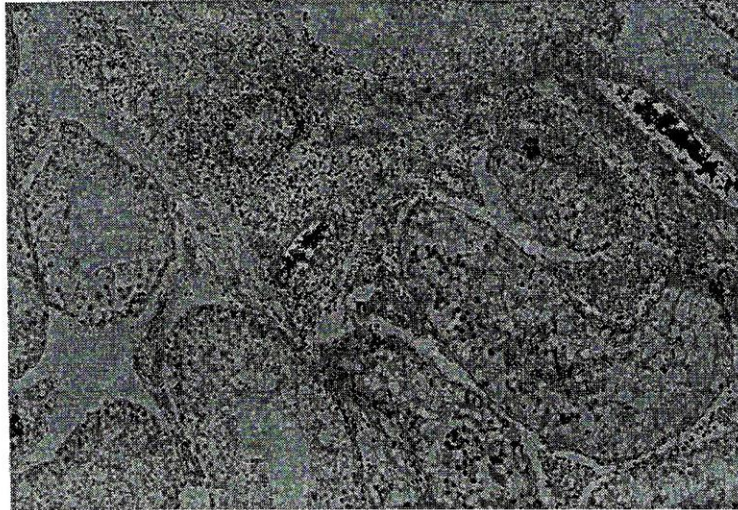


Fig. 1. Testicle from a buck of group 5 inoculated with BHV-4 and sampled on day 10 postinoculation. There is perivascular and interstitial accumulations of plasma cells, heterophils, and lymphocytes. Degeneration of the germinal epithelium of the seminiferous tubules is evident. H & E, X100.



Fig. 2. Agarose gel electrophoresis of PCR products to the TK gene region from rabbit tissues. Lanes 8 and 9 from the same rabbit exhibit a positive reaction product. Lane 12 from a different rabbit exhibits a positive reaction product as well. Lane 17 is a BHV-4 positive control. Lanes 1, 7, 10, 13, and 16 loaded with water. Markers on right from the top: 600, 500, 400, 300 bp ladder.

and their control does that did not abort or had premature deliveries were similar, and suggested that the abortions were not related to BHV-4. Immunoperoxidase staining did not localize virus to reproductive tissue, also, suggested that abortions were not due to virus effect. Results of both virus isolation and IP staining applied on tissue sections from reproductive tract of one aborted doe was negative. Failure of detection of BHV-4 from tissues could be related to the highly cell-associated nature of the virus and the large number of tissues combined in the inoculum homogenate for virus isolation (Naeem *et al.*, 1991b). The system of virus isolation and detection used here is routinely used for successful culture of BHV-4 from nasal swabs by our laboratory. Absence of both unique macroscopic and microscopic alterations in the reproductive tract and liver, spleen, and mesenteric lymph nodes of the infected does and the presence of the same clinical findings in a control doe suggested that BHV-4 was not the etiology of premature delivery in any BHV-4 inoculated doe.

Group 1 infected does had a lower conception rate (25%) than the control animals of group 2 (41.66%). This supported the possible role of BHV-4 as a cause of infertility (Biolatti *et al.*, 1991). Endometritis in these rabbits may prevent implantation of embryos and leading to early embryonic death and resorption as previously observed (Naeem *et al.*, 1991a and Naeem *et al.*, 1991b). The lower conception rate may be due to transient endometritis. Endometritis was not evident at necropsy, but 2 does had transient suppurative exudate from the vulva 10 days PI.

Transmission electron microscopic examination of the reproductive tract, liver, and mesenteric lymph nodes revealed viral nucleocapsids of BHV-4 in 4/9 tissue samples examined, although the IP staining and PCR of these tissues were negative.

Inoculated bucks of both groups 5 and 7 did not exhibit clinical disease in this study, although BHV-4 was found by TEM, IP, and PCR in tissues. Histopathological examination of tissue sections from testes of the inoculated bucks at various periods PI in group 5 consistently revealed the presence of orchitis and testicular degeneration. These findings were similar to experimental studies in bulls (Dubuisson *et al.*, 1987). Testicular oedema was observed on 24 and 31 days PI and in only one testicle in 1/4 animals on 10 days PI. These observations are similar to those observations made in the testicles of a bull which exhibited oedematous orchitis, azoospermia, and had BHV-4 isolated from tissue.

Light microscopic examination of the testicular tissue of bucks of group 7 was similar to group 8 control rabbits. The BHV-4 used in this study may not have a tro-

pism for testicular tissue, although the rabbits in this study seroconverted following inoculation (Essmail *et al.*, 1999).

Giant spermatid cells observed in testicles of both infected and control bucks have been demonstrated in previous studies of normal bucks (Morton *et al.*, 1986). The presence of these cells is not specific for any type of injury and they were frequently present in normal seminiferous tubules.

No histopathological changes were observed in the epididymis of the inoculated bucks in the present study, although epididymitis has been previously reported in bulls (Dubuisson *et al.*, 1987). Potential variation among BHV-4 isolates and species differences could account for this finding (Naeem *et al.*, 1991a).

While most strains of BHV-4 are able to induce infection in cattle, they are generally unable to cause clinical disease in experimental studies (Castrucci *et al.*, 1992, Dubuisson *et al.*, 1987, and Katz *et al.*, 1977). The present study corroborates these findings. The exaggerated clinicopathological manifestations noted in other experimentally infected rabbits could be referred to the use of non-SPF rabbits with significant intercurrent disease (Naeem *et al.*, 1991b). The effect of synergistic pathogens with BHV-4 has been shown in one study conducted in cats. Inoculation of SPF male cats with calicivirus in association with BHV-4 resulted in reduced time for the development of clinical signs and increased complications (Fabricant, 1977). Additionally, reactivation of bovine viral diarrhea virus has been observed when calves were superinfected with BHV-4 (Castrucci *et al.*, 1992).

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عدوى تجريبية بفيروس الهيريس البقري-٤ في ذكور وإناث الأرانب العشار

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أجريت هذه الدراسة على عدد ٩٦ من ذكور وإناث الأرانب العشار الخالية من الأمراض البكتيرية النوعية من نوع الألماني بيلتيد. تم تقسيم إناث الأرانب (عدد = ٥٦) إلى ٤ مجموعات، المجموعة ١ و ٢ شملت كل منها على عدد ٢٤ حيوان في مرحلة العُشر المبكر والمجموعة ٣ (عدد = ٥) والمجموعة ٤ (عدد = ٣) كانت في المرحلة المتأخرة من العُشر، تم إجراء العدوى التجريبية بحقن فيروس الهيريس البقري-٤ في حيوانات المجموعة ١ و ٢ عن طريق الحقن المهبلي والحقن الوريدي على التوالي، بينما أستخدمت حيوانات المجموعة ٢ و ٤ كضوابط وتم حقنها بمزارع خلوية لا تحتوي على الفيروس عن طريق الحقن المهبلي والحقن الوريدي على التوالي. أيضاً تم تقسيم ذكور الأرانب (عدد = ٤٠) إلى ٤ مجموعات، المجموعة ٥ و ٦ شملت كل منها على عدد ١٦ حيوان والمجموعة ٧ و ٨ شملت كل منها على عدد ٤ حيوانات، تم إجراء العدوى التجريبية بحقن فيروس الهيريس البقري-٤ في حيوانات المجموعة ٥ و ٧ عن طريق الحقن في الخصية والحقن الوريدي على التوالي، بينما أستخدمت حيوانات المجموعة ٦ و ٨ كضوابط وتم حقنها بمزارع خلوية لا تحتوي على الفيروس عن طريق الحقن في الخصية والحقن الوريدي على التوالي.

شملت الأمراض الإكلينيكية في إناث الأرانب المحقونة بالفيروس حدوث إجهاض في عدد ٢ من إناث أرانب المجموعة ١ و ٣، انخفاض معدل الإخصاب في إناث المجموعة ١ حيث بلغ معدل الإخصاب في إناث المجموعة ١ نسبة ٢٥٪ مقابل ٤١,٦٦٪ في إناث المجموعة ٢ (المجموعة الضابطة) وحدث التهاب مهبل طفيف في عدد ٢ من إناث المجموعة ١، لم تظهر أي أمراض إكلينيكية على أي من ذكور أرانب المجموعة ٥ و ٧.

أوضح الفحص بالميكروسكوب الضوئي لأنسجة إناث الأرانب المحقونة بالفيروس حدوث التهاب واضح في المشيمة، التهاب صديدي طفيف في بطانة الرحم، انزفة متعددة في بطانة الرحم، ارتشاحات في بطانة الرحم، التهاب في المبايض، تكاثر عددي في خلايا الليمفوسايت للغدة الليمفاوية الحشوية والتهاب تنكروزي متعدد في الكبد. وفي ذكور الأرانب أظهر الفحص الميكروسكوبي لأنسجة الخصية حدوث التهاب ليمفاوي بدرجة طفيفة في النسيج البيني للخصية وكذلك التهاب تنكروزي حاد مصحوباً بتحلل في الخلايا الجرثومية الذكرية المبطننة للأنيبيبات المنوية بصورة متفرقة أو منتشرة وارتشاح طفيف في النسيج البيني للخصية. بينما أوضح فحص العينات بالميكروسكوب الإلكتروني تواجد نيكلوكابسيدات الفيروس في أنسجة المهبل،

الرحم، الغدة الليمفاوية الحشوية، الطحال، والخصية. تم عزل الفيروس بطرق العزل التقليدية فقط من خصية أحد ذكور الأرناب المحقونة بالفيروس بينما تم استبيان الفيروس بواسطة اختبار الإمينوبيرواوكسيداز في أنسجة كل من الغدة الليمفاوية الحشوية والطحال في إناث (المجموعة ١ و٢) وذكور الأرناب (المجموعة ٥ و٧). كذلك تم الكشف عن وجود جينوم الفيروس (الحامض النووي) بواسطة اختبار إنزيم البلمرة المتسلسل (ال-PCR) في أنسجة الطحال والغدة الليمفاوية الحشوية في عدد ٢ من ذكور الأرناب المحقونة بالفيروس (المجموعة ٥ و٧).

ومن نتائج جميع الاختبارات التي أجريت في هذه الدراسة يُمكن استخلاص أن الدور الذي يلعبه فيروس الهيريس البقري-٤ في إحداث أعراض إكلينيكية أو تغيرات باثولوجية في الجهاز التناسلي للإناث المحقونة دوراً ضعيفاً وأن الفيروس قادر على إحداث تغيرات باثولوجية في خصية ذكور الأرناب المحقونة مما يؤثر على كفاءة إنتاج الحيوان للسلائل المنوي واحتمالية إفران الفيروس في السلائل المنوي للحيوانات المصابة.