Isolation of Serotype 2 Marek’s Disease Virus from a Cell Line of Avian Lymphoid Leukosis

Hiroshi HIHARA, Kunitoshi IMAI, Kenji TSUKAMOTO and Kikuyasu NAKAMURA

National Institute of Animal Health, 3-1-1 Kannondai, Tsukuba, Ibaraki 305 and "Hokkaido Research Station, National Institute of Animal Health, 4 Hitsujiyucho, Toyohira, Sapporo 062, Japan

(Received 25 June 1997/Accepted 12 September 1997)

ABSTRACT. To determine a serotype of Marek’s disease virus (MDV) persistently infected in a lymphoid leukosis (LL)-cell line, LSCC-BK3, clone A (BK3A), and to examine the pathogenicity of the virus, an attempt was made to isolate MDV from culture fluid of the LL-cell line, using chick embryo fibroblast cultures resistant to infection with subgroup A avian leukosis virus (ALV) to eliminate subgroup A ALV. The MDV isolate, serologically identified as serotype 2 MDV and designated as the 2H strain, was free from ALV and reticuloendotheliosis virus. A serotype 2-specific antigen of MDV was detected in 44% of BK3A, but antigens of serotypes 1 and 3 were not detected in this cell line, by the indirect immunofluorescent antibody test using serotype-specific monoclonal antibodies. MDV antigens were undetectable in LSCC-BK3, clone 2C; both cell lines were established from the same chicken. Neither clinical signs nor macroscopic lesions were observed in any of 19 chicks inoculated with the 2H strain. Three out of 19 chicks histopathologically examined had no lesions. These results suggest that serotype 2 MDV can persist in B cells transformed by ALV without cytopathic effect at a high rate, and the isolate may become a candidate for MD vaccine strains. — Key words: lymphoid leukosis cell line, Marek’s disease virus, serotype 2 Marek’s disease virus.

Marek’s disease virus (MDV) isolates are classified into three classes based on serological characteristics, the pathogenic MDVs (serotype 1), nononcogenic MDVs (serotype 2), and non-oncogenic herpesvirus of turkey (HVT) (serotype 3) [3, 6].

When the chicks were inoculated with a concentrated avian leukosis virus (ALV) material prepared from culture fluid of a lymphoid leukosis (LL)-cell line, LSCC-BK3, clone A (BK3A), most chicks developed LL, ascites complicated with LL, ascites, erythroblastosis and others less than 100 days after inoculation [11]. However, some of the inoculated chicks had lesions possibly caused by MDV infection, such as mild lymphocytic infiltration in peripheral nerves and the central nervous system, although MD tumor lesions were not noticed. These results prompted us to isolate MDV from concentrated ALV material prepared from culture fluid of the LL-cell line, and to characterize the MDV isolate.

In this report we describe the isolation of serotype 2 MDV from concentrated ALV material, the detection of serotype 2 MDV antigen in the cell line by indirect immunofluorescent antibody (IFA) test using serotype-specific monoclonal antibodies (MoAbs), and the measurement of the pathogenicity of the MDV isolate in chicks. We also discuss the interaction in pathogenicity between serotype 2 MDV and ALV.

MATERIALS AND METHODS

Chicks: All the chicks used were derived from a specific-pathogen-free (SPF) flock of White Leghorn chickens: line 151, obtained in 1960 from the Regional Poultry Research Laboratory, East Lansing, Mich. [4, 14].

Cell cultures: Cultures of chick embryo fibroblast (CEF) from 10-day-old chick embryos were prepared in petri-dishes as described previously [14, 20]. The embryos were derived from 2 flocks of chickens, line 151 and BK [14]. Both flocks were free of ALV [14]. C/AE (resistant to infection with ALV of subgroups A and E) phenotype CEF cells, originally derived from a flock of line 151, were recovered from a frozen state in liquid nitrogen [12]. The CEF cells were cultured in F10 medium containing 10% tryptose phosphate broth (TPB), 5% bovine serum, 0.5% chicken serum and antibiotics.

Two LL-cell lines (LSCC-BK3, clone A: BK3A; clone 2C: BK3C2) which had been established from an enlarged bursa of Fabricius (bursa) of an LL-chicken (No. 4484) [13] were cultivated in the F10 medium containing 10% TPB, 5% fetal calf serum, 0.5% heat-inactivated chicken serum and antibiotics.

All cultures were incubated stationary in a 2% CO2 incubator at 38°C.

The material for MDV isolation: The culture fluid, when the LL-cell line (BK3A) grew actively, was collected by low speed centrifugation, and passed through a 450 nm membrane filter. The filtrate was concentrated 1:100 using polyethylene glycol (PEG) 6,000 by a modification of the methods described previously [2, 11].

The concentrated virus material contained 106 tissue culture infective dose (TCID) per ml of subgroup A ALV and 10 plaque-forming units (PFU) per ml of MDV when titrated later [11].

Viruses: Representative reference MDV strains, serotype 1 (GA strain) [8, 16], serotype 2 (SB-1 strain) [17, 21],
serotype 3 (FC-126 strain of hVT) [24], and the MDV isolate (2H strain) were propagated in CEF cultures and used as antigens for IFA tests.

BH-RSV pseudotype A [BH-RSV(RAV-1)] was used for the assay of ALV in the resistance-inducing factor (RIF) test [12, 20].

Reticulon endotheliosis virus, strain T (REV-T) [19], was used for the examination of REV in the isolate of MDV by IFA as a reference strain [16, 26].

Antibodies: Three serotype-specific MoAbs (FC-62C2 of serotype 1, FH-4-6610 of serotype 2, Hv.8 of serotype 3) and one group-specific MoAb to MDV-related viruses (FC3-IB3, common to serotypes 1, 2 and 3) [17] were supplied by Dr. T. Kondo, Department of Epizootiology, Faculty of Veterinary Medicine, Hokkaido University, Sapporo, Japan.

Chicken sera immune to MDV (SB-1 strain) or REV-T were prepared by the methods already described [16].

IFA test: CEF cells infected with one each of MDV, HVT or REV-T were grown in wells of a Heavy Teflon Coating Slide (Bokusu Brown Co., Tokyo). After washing in phosphate buffered saline (PBS), the cells were air-dried, fixed in cold acetone and dried again. The LL-cell suspension was adjusted at $1 \times 10^7$ cells per ml in PBS was deposited in wells of the slide to make a smear, air-dried, fixed in cold acetone, and dried in air. These fixed antigens were stored at $-40^\circ$C in a refrigerator before use.

To examine the reactivity of the antigens with MoAbs, the antigens were stained with two-fold serial dilutions of mouse ascites containing MoAb against MDV and a 1:160 dilution of fluorescein-isothiocyanate (FITC)-conjugated rabbit anti-mouse immunoglobulin (Ig) preparation (Seikagaku Kogyo Co., Tokyo).

To examine the infection with REV, the antigens of CEF cells infected with the MDV isolate (2H strain) or with REV-T (positive antigen as a reference) were stained with 1:40 dilution of anti-REV chicken serum and 1:80 dilution of FITC-conjugated rabbit anti-chicken Ig (Cappel Lab., PA., U.S.A.).

The stained cells were mounted with glycerin for ultraviolet microscopy.

In the observation of LL-cells, the percentage of cells exhibiting the characteristic patterns of antigen immunofluorescence was determined after at least 100 total cells were counted by epi-fluorescence systems. A Nikon fluorescence microscope equipped with HB-201AN high-mercury source was used throughout.

Agar-gel precipitation (AGP) test: An AGP antigen of MDV was prepared from CEF cultures infected with the 2H strain by the method already described [26]. The AGP test was carried out to detect antibodies of chicks inoculated with the 2H strain of MDV as described previously [26].

Enzyme-linked immunosorbent assay (ELISA): The antigens for detecting antibodies of chicks inoculated with the 2H strain of MDV were prepared by the method of Cheng et al. [7]. Briefly, monolayers of primary CEF were infected with the 2H strain. When the cytopathic effect (CPE) confluently appeared, the infected cells were harvested and washed three times with PBS. After counting the cell number, the cell density was adjusted to $5 \times 10^5$ per ml in PBS, and one-tenth ml of the cell suspension was delivered into each well of Nunc module plates (No. 469957; A/S, Nunk Roskilde, Denmark). The plates were centrifuged at 1,500 rpm for 10 min, the supernatant flied off, and the cells were then allowed to dry at room temperature before storage at $-80^\circ$C. ELISA procedure was carried out by the method already described [23]. Sera collected from an SPF chicken flock of 100-day-old were used for determining the cut-off level of A values.

Pathology: The pathogenicity of the 2H strain was examined in chicks of line 15i. Two groups of chicks inoculated with the 2H strain were kept in negative-pressure isolators, and examined for clinical signs for 43 days and 67 days, respectively. After that, all birds were necropsied, and liver, spleen, thymuses, bursa, proventriculus, sciatic nerve and brain were collected from 3 out of 10 chicks in group 1. They were microscopically examined, using paraffin sections and hematoxylin-eosin (HE) staining techniques.

RESULTS

Isolation of MDV from an LL cell line: Concentrated culture fluid from the cell line (BK3A) was inoculated into C/CE CEF cultures, which were serially passaged 5 times. When herpesvirus-like CPE was noticeably evident in the culture, the culture fluid was collected and filtered through a 450 nm membrane filter. The filtrate was subjected to the RIF test and to examination for cell free MDV. The filtrate contained no MDV, but did have a small amount of subgroup A ALV (10 TCID/ml). Therefore, after three cycles of freezing and thawing the C/CE cells (about 2 $\times 10^6$ cells/ml) with CPE of MDV, one-tenth ml of the lysed cell suspension was inoculated again into the C/CE CEF culture, which was serially passaged 4 times until the CPE was noticeably evident in the culture. The culture fluid was passed through a 450 nm membrane filter, and 1 ml of the filtrate was inoculated into C/E CEF cells, which were serially passaged 3 times, for the examination of ALV, REV or cell-free MDV. The result was that the filtrate contained none of these viruses. The CEF cells with CPE were collected by trypsinization, stocked in liquid nitrogen as a seed virus and used for further experiments.

Identification of the isolate: The type of CPE on CEF induced by the isolate (Fig. 1a) was similar to that induced by SB-1 (serotype 2) (Fig. 1b), but differed from that induced by pathogenic MDV such as GA (serotype 1) which formed only small plaques (Fig. 1c) or HVT (Fig. 1d). The isolate, which showed CPE in CEF cells, was identified as MDV by IFA test using chicken immune sera to SB-1 strain (Fig. 2a) and serotype 2 specific MoAb (Fig. 2b). Table 1 shows the results of cross-IFA tests using serotype-specific MoAbs. The antigens of the 2H and SB-1 strain of MDV reacted strongly with serotype 2 MoAb, but not with those of types 1 and 3. These results indicate that the MDV
isolate from the LL-cell line was serotype 2. The isolate was designated as the 2H strain of MDV.

Presence of serotype 2 antigens in an LL-cell line: Two LL-cell lines (BK3A and BK3C2) established from the same chicken were examined for MDV antigens using chicken sera immune to the SB-1 strain of MDV (Fig. 2c) and serotype-specific MoAbs (Fig. 2d). Serotype 2 antigens were detectable in 44% of BK3A, but types 1 and 3 antigens were undetectable in the cell line. In contrast, BK3C2 did not react with any of the MoAbs or polyclonal antibodies against MDVs (Table 2).

Pathogenicity of the 2H strain in line 15I chicks: A 0.1 ml amount of inoculum containing 10^5 or 10^4 PFU of the 2H strain was inoculated subcutaneously into two groups of day-old chicks of line 15I, which were observed for 67 days (group 1) and 43 days (group 2), respectively (Table 3). Sera collected at necropsy were examined for AGP and ELISA antibodies to the 2H strain of MDV. The results were that neither clinical signs nor macroscopic lesions were observed in any of the chicks inoculated. Three (Nos. 751, 752 and 753) out of 10 chicks inoculated with 10^4 PFU of the virus were microscopically examined, but no lesions were observed in any of the organs of the chicks examined (data not shown). In both groups AGP antibodies were detected in all the chicks inoculated and their titers ranged from 1 to 16 or more, except 2 chicks (Nos. 749 and 752). ELISA antibodies were detected in all the chicks inoculated.

DISCUSSION

When we established the LL-cell line in 1980 [13], the LL chicken was kept in a conventional chicken house. AGP antibody against MDV was detected from about 60% of ALV-inoculated chicks, as well as uninoculated ones. However, the positive chicks showed no clinical signs and had no gross lesions of MD. Therefore, we presumed that the infection had been induced by avirulent MDV, or that the chicks of lines BK and 15I were resistant to clinical infection with MDV [14]. From the present work, it is concluded that the LL-chicken used for the establishment of the LL-cell lines was infected with serotype 2 MDV. Hiros et al. [15] reported that one of 7 field isolates of MDV was identified as a serotype 2 MDV, which was isolated for the first time in Japan in 1986, and Lin et al. [17] reported that serotype 2 MDV was isolated from apparently healthy birds belonging to genus Gallus in 1990 that had no history of vaccination with MDV or HVT. Our present work suggests that serotype 2 MDV infection had been common in chicken flocks and present in Japan before 1980. The 2H strain of MDV was isolated from the culture fluid of an LL-cell line. However, it is obscure if the viral material contained a small amount of infectious MDV or not. As the viral material

Fig. 1. Plaques induced by the representative strains and a new isolate (2H strain) of MDV in CEF. a) Large plaques of rounded cells of variable size caused by the 2H strain infection were like those induced by the SB-1 strain. ×400. b) Large plaques induced by the SB-1 strain of serotype 2 infection. ×400. c) Small plaques caused by the GA strain of serotype 1 infection. ×400. d) Plaques caused by the HVT FC-126 strain of serotype 3 infection. ×400.
Table 1. Serotype identification of the 2H strain of MDV by IFA test using serotype-specific MoAbs<sup>a</sup>

<table>
<thead>
<tr>
<th>Specific to</th>
<th>Virus strain (serotype)</th>
<th>Normal</th>
</tr>
</thead>
<tbody>
<tr>
<td>MoAb</td>
<td>GA (1)</td>
<td>SB-1 (2)</td>
</tr>
<tr>
<td>FC6-2C2</td>
<td>&lt;10</td>
<td>&lt;10</td>
</tr>
<tr>
<td>FH4-6E10</td>
<td>&lt;10</td>
<td>25,600</td>
</tr>
<tr>
<td>Hw.8</td>
<td>&lt;10&lt;sup&gt;b&lt;/sup&gt;</td>
<td>&lt;10</td>
</tr>
</tbody>
</table>

<sup>a</sup>Antigens were prepared from CEF infected with each MDV or HVT. <sup>b</sup>Titer of MoAb: Expressed as the reciprocal of the highest dilution of MoAb which showed positive reaction by IFA.

Table 2. Examination of MDV antigen in two LL-cell lines by IFA test using MoAbs

<table>
<thead>
<tr>
<th>Specific to serotype</th>
<th>LSCC-BK3A</th>
<th>LSCC-BK3C2</th>
<th>GA (CEF)</th>
<th>2H (CEF)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MoAb</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FC6-2C2</td>
<td>&lt;10 (&lt;1%)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>&lt;10 (&lt;1%)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1,600</td>
<td>&lt;10</td>
</tr>
<tr>
<td>FH4-6E10</td>
<td>12,800 (44%)</td>
<td>&lt;800 (&lt;1%)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>ND&lt;sup&gt;d&lt;/sup&gt;</td>
<td>12,800</td>
</tr>
<tr>
<td>FC3-1B3</td>
<td>12,800 (26%)</td>
<td>&lt;10 (&lt;1%)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>12,800</td>
<td>12,800</td>
</tr>
</tbody>
</table>

<sup>a</sup>Titer of MoAb: Expressed as the reciprocal of the highest dilution of the MoAb which showed positive reaction by IFA. <sup>c</sup>The number in parenthesis shows the ratio of MDV- or HVT-antigen positive cells. <sup>d</sup>Not done.

Fig. 2. IFA test. a) CEF cells infected with the 2H strain were stained with polyclonal antibodies against the SB-1 strain, ×800. b) CEF cells infected with the 2H strain were stained with serotype 2-specific monoclonal antibody, ×2,000. c) LSCC-BK3, clone A cells stained with polyclonal antibodies against the SB-1 strain, ×800. d) LSCC-BK3, clone A cells stained with serotype 2-specific monoclonal antibody, ×2,000.

The present results indicate that serotype 2 MDV can persist in B cells transformed by ALV without CPE at a high rate. This has already been reported by Fynan et al. [9, 10], although in their report [10] less than 2% of the tumor cells expressed MDV antigens.

In the present work, another cell line (BK3C2), contained a small amount of PEG used for the concentration of ALV, noninfectious MDV might be incorporated into CEF cells by the fusion effect of PEG [22]. The presence of subgroup A ALV in the culture fluid of 5-time passaged C/AE CEF cells after inoculation with the viral material suggests this possibility.
Table 3. Pathogenicity of the 2H strain of MDV isolated from an LL-cell line

<table>
<thead>
<tr>
<th>Inoculum dose (PFU)</th>
<th>Chicken No.</th>
<th>Clinical signs&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Day-old at sacrifice</th>
<th>Antibody titers AGP&lt;sup&gt;&lt;b&gt;6&lt;/sup&gt; ELISA&lt;sup&gt;&lt;b&gt;3&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>746</td>
<td>67</td>
<td>2</td>
<td>0.966</td>
<td></td>
</tr>
<tr>
<td>747</td>
<td>67</td>
<td>2</td>
<td>1.035</td>
<td></td>
</tr>
<tr>
<td>748</td>
<td>67</td>
<td>4</td>
<td>0.866</td>
<td></td>
</tr>
<tr>
<td>749</td>
<td>67</td>
<td>&lt;1</td>
<td>0.550</td>
<td></td>
</tr>
<tr>
<td>750</td>
<td>≥16</td>
<td>1.100</td>
<td></td>
<td></td>
</tr>
<tr>
<td>751</td>
<td>67</td>
<td>1</td>
<td>0.811</td>
<td></td>
</tr>
<tr>
<td>752</td>
<td>67</td>
<td>&lt;1</td>
<td>0.574</td>
<td></td>
</tr>
<tr>
<td>753</td>
<td>67</td>
<td>4</td>
<td>1.248</td>
<td></td>
</tr>
<tr>
<td>754</td>
<td>67</td>
<td>8</td>
<td>1.270</td>
<td></td>
</tr>
<tr>
<td>755</td>
<td>≥16</td>
<td>1.247</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 × 10^3</td>
<td>756</td>
<td>43</td>
<td>1.790</td>
<td></td>
</tr>
<tr>
<td>757</td>
<td>43</td>
<td>4</td>
<td>0.915</td>
<td></td>
</tr>
<tr>
<td>758</td>
<td>43</td>
<td>2</td>
<td>0.772</td>
<td></td>
</tr>
<tr>
<td>759</td>
<td>43</td>
<td>2</td>
<td>0.789</td>
<td></td>
</tr>
<tr>
<td>760</td>
<td>43</td>
<td>8</td>
<td>0.924</td>
<td></td>
</tr>
<tr>
<td>762</td>
<td>≥16</td>
<td>0.856</td>
<td></td>
<td></td>
</tr>
<tr>
<td>763</td>
<td>43</td>
<td>8</td>
<td>0.915</td>
<td></td>
</tr>
<tr>
<td>764</td>
<td>43</td>
<td>2</td>
<td>0.732</td>
<td></td>
</tr>
<tr>
<td>765</td>
<td>43</td>
<td>4</td>
<td>0.792</td>
<td></td>
</tr>
<tr>
<td>1 × 10^4</td>
<td>756</td>
<td>43</td>
<td>1.790</td>
<td></td>
</tr>
</tbody>
</table>

a) = No clinical signs. b) AGP titer is expressed as the reciprocal of the highest serum dilution which showed positive reaction. c) All sera were tested at 1:100 dilution in ELISA. The cut off value, the upper limit of an ELISA value (A=x + 3SD) for the SPF chicks (n=10) was determined as 0.450.

established from the same chicken, did not have MDV antigens. The reason of this heterogeneity in these cells lines is not clear. Taking into consideration the clonality of LL-tumor cells [18], the heterogeneity of these cell lines may be due to infection with serotype 2 MDV at a later stage of LL development, i.e., in the stage when some of the tumor cells were infected with serotype 2MDV and the remaining ones were not.

It has been reported that serotype 1 MDV antigens were usually detected in various lymphoid organs and feather follicles and that degenerative lesions were common in the bursa of the infected chickens. It has also been reported that replication of SB-1 and HVT in lymphoid organs such as bursa, spleen and thymus was rare, and necrosis in the organs was essentially absent [5, 21, 25]. In the present work, the 2H strain of MDV was pathogenic in line 151 chicks. However, in previous studies, dual infection with a large amount of subgroup A ALV and serotype 2 MDV microscopically induced mild lymphocytic foci in some visceral organs [11]. Schat and Calnek [21] reported that serotype 2 MDV, SB strain, could induce a lytic infection resulting in cell death and therefore degenerative lesions, but it reached pathogenic levels only when the host was immunologically incompetent. Therefore, we presume that lymphocytic foci observed in the previous experiment were caused by immunosuppression due to inoculation of a large amount of ALV, since our recent studies indicate that ALV from the LL-cell line induces immunosuppression in inoculated chicks. Bacon et al. [1] reported that serotype 2 MDV infection augments the incidence of LL in LL-susceptible strains of chickens. If so, there is a possibility that the development of LL in line 151 chickens in less than 100 days in our previous experiment was induced by augmentation of serotype 2 MDV infection. However, such augmentation does not seem to occur in rapidly induced LL, because ALV alone can rapidly induce LL in our recent experiment.

From the viewpoint mentioned above, the two LL-cell lines in which serotype 2 MDV does persist and does not persist will provide a good system for analyzing the interaction between ALV (retrovirus) and MDV (herpesvirus).

Neither clinical signs nor macroscopic lesions were observed in any of 19 chicks inoculated with the 2H strain of MDV. So, the new isolate of serotype 2 MDV (2H strain) may become a candidate for MD vaccine strains.

ACKNOWLEDGEMENT. This work was supported in part by Grants-in-Aid for Cancer Research from the Ministry of Education, Science and Culture of Japan.

REFERENCES

and reactivation of Marek’s disease virus in B lymphocytes transformed by avian leukemia virus. J. Gen. Virol. 74: 2163–
2170.
lymphoid leukemia and ascites by avian leukemia virus from a
Two strains of avian sarcoma virus newly isolated from chick
fibrosarcomas induced by lymphatic leukemia virus subgroup
from chickens with avian lymphoid leukemia. Avian Dis. 24:
971–979.
T. 1980. Oncogenicity of three strains of avian leukemia virus
Q. (Jpn.) 20: 87–95.
Preparation of monoclonal antibodies against Marek’s dis-
ease virus and herpesvirus of turkeys. Jpn. J. Vet. Sci. 48:
1263–1266.
16. Imai, K., Yuasa, N., Kobayashi, K., Nakamura, K., Tsukamoto,
K. and Hihara, H. 1990. Isolation of Marek’s disease virus
from Japanese quail with lymphoproliferative disease. Avian
Pathol. 19: 119–129.
17. Lin, J. A., Kitigawa, H., Ono, M., Iwanaga, R., Kodama, H.
and Mikami, T. 1990. Isolation of serotype 2 Marek’s disease
virus from birds belonging to genus Gallus in Japan. Avian
Dis. 34: 336–344.
leukemia virus DNA and RNA in bursal tumors: viral gene
expression is not required for maintenance of the tumor state.
avian reticuloendotheliosis virus (strain T). Avian Dis. 18:
278–288.
Cancer Inst. 60: 1075–1082.
22. Steimer, K. S. and Boettiger, D. 1977. Complementation res-
cue of Rous sarcoma virus from transformed mammalian cells
by polyethylene glycol mediated cell fusion. J. Virol. 23:
133–141.
A highly sensitive, broad-spectrum infectivity assay for
24. Witter, R. L., Nazerian, K., Purchase, H.G. and Burgoyne, G.
H. 1970. Isolation from turkeys of a cell-associated herpes-
virus antigenically related to Marek’s disease virus. Am. J.
key herpesvirus infection in chickens: Induction of lymphoproliferative lesions and characterization of vaccinal
reticuloendotheliosis virus from chickens inoculated with
16: 141–151.