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Systemic herpes-like virus in catfish *Ictalurus melas* (Italy) differs from Ictalurid herpesvirus 1 (North America)

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ABSTRACT: A herpesvirus was isolated during 2 occurrences of mass mortality among adult catfish *Ictalurus melas* raised in different farms in northern Italy. The agent replicated in the channel catfish ovary (CCO) cell line from channel catfish *I. punctatus*, inducing a cytopathic effect similar to that caused by Ictalurid herpesvirus 1 (also referred to as channel catfish herpesvirus, CCV). The new herpesvirus, designated *I. melas* herpesvirus (IcmHV) did not react with polyclonal rabbit or monoclonal antibodies directed to CCV in either neutralization or indirect immunofluorescence assays. The virions of IcmHV possessed a hexagonal nucleocapsid of 107 nm in diameter surrounded by an envelope with a diameter of 227 nm (n = 20) typical for members of the family *Herpesviridae*. Virions of IcmHV purified from infected CCO cells contained 17 polypeptides ranging in size from 17.5 to 175 kDa and most differed in molecular weight from those found for CCV. The IcmHV was also distinct from CCV when compared by restriction fragment length polymorphisms (RFLP) of genomic DNA following digestions with the endonucleases *Kpn* I and *Sac* I. Lastly, the virulence of IcmHV for channel catfish fry and juveniles, respectively, was demonstrated by experimental infections induced by bath exposure or intraperitoneal injection that resulted in 78 to 96% cumulative mortality in groups of exposed fish. Preventing the introduction of this agent into geographic regions where significant channel catfish production occurs should be a high priority.

KEY WORDS: Catfish · Herpesvirus · *Ictalurus melas*

INTRODUCTION

Alborali et al. (1996) described the initial isolation of a herpes-like virus (IcmHV) associated with mass mortality of black bullhead *Ictalurus melas* in 2 farms in northern Italy in 1994. Black bullheads are relatives of the channel catfish *I. punctatus*, and the latter species forms the basis of the largest freshwater aquaculture industry in the USA. Black bullheads are viewed more as pests or invasive species in North America, but a small industry associated with their culture is present in parts of Europe.

Several features of the new disease described among black bullheads in Italy were similar to channel catfish virus disease (CCVD), a systemic hemorrhagic condition that occurs among juvenile channel catfish infected with Ictalurid herpesvirus 1 (IcHV-1) in North America (Fijan et al. 1970, Plumb & Gaines 1975). Gross signs of fish infected with the IcmHV include spiral swimming movements and hanging in a vertical position in the water column prior to death (Alborali et al. 1996). Hemorrhages are evident in the epidermis of the abdomen and at the base of the fins and internally in the kidney and other major organs. Microscopic lesions in IcmHV-infected black bullheads are most severe in the kidney and include hemorrhage and necrosis of the hematopoietic cells. Focal necrosis, congestion and hemorrhages also occur in the spleen and

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liver (Alborali et al. 1996). The apparent similarities between the diseases caused by IcmHV and CCV prompted us to compare the 2 agents. In this report we compare IcmHV from Italy with a reference strain of CCV from North America, with respect to antigenic responses to anti-CCV polyclonal and a monoclonal antibody, virion polypeptide composition, restriction fragment patterns of genomic DNA, and virulence for channel catfish fry and juveniles.

MATERIALS AND METHODS

Viruses and cell lines. The herpes-like virus IcmHV from Italy used in this study was isolated from adult catfish in 1994 as described by Alborali et al. (1996). The CA80-5 strain of Ictalurid herpesvirus 1 or channel catfish virus (CCV) was isolated from an epizootic among juvenile channel catfish in California in 1980 (Arkush et al. 1992). Both herpes-like viruses were propagated in the channel catfish ovary (CCO) cell line in minimum essential media (MEM) supplemented with 7.5% fetal bovine serum (FBS), 50 IU penicillin ml^{-1}, 50 µg streptomycin ml^{-1}, and 2 mM L-glutamine (MEM-7.5). The FBS concentrations of the growth medium were reduced to 2% (MEM-2) when CCO cells were infected with IcmHV or CCV. Cells were incubated at 25°C following virus inoculation. Concentrations of either IcmHV or CCV as present in cell culture media or tissues of virus-exposed fish were estimated by the method of Reed & Muench (1938) by 50% end-point tissue-culture infective dose ($TCID_{50}$) analyses in 96 well plates containing CCO cells incubated at 25°C for 10 d.

Potential antigenic relationships between viruses. The reactions of IcmHV and CCV to anti-CCV polyclonal rabbit and a mouse monoclonal antibody (Mab 38) were compared in neutralization and immunofluorescence assays using methods identical to those described by Arkush et al. (1992).

Virus purification and DNA extraction. Both IcmHV and CCV were partially purified for virion polypeptide and restriction fragment analyses of genomic DNA. Batch propagations of each virus in 150 cm^2 flasks began when cytopathic effects were extensive. The virus was purified as described by Gilad et al. (2002). The purified virus was placed directly into either TNE (50 mM Tris-HCl, 150 mM NaCl, 1 mM disodium ethylenediaminetetraacetic acid, pH 7.5) at a final protein concentration of 1 mg ml^{-1} for sodium-dodecyl sulfate polyacrylamide gel electrophoresis, SDS PAGE (virion polypeptide analyses) or suspended in molecular biology-grade water and treated with DNaseI and RNaseA at a final concentration of 15 µg ml^{-1} for later genomic DNA comparisons. Genomic DNA from both viruses was extracted as described by Gilad et al. (2002). The concentration of DNA was determined by a spectrophotometer (Pharmacia Biotech, GeneQuant II), and samples were then stored at –20°C until use.

Electron microscopy. Preparations of IcmHV, both following viral purification as described above or as observed directly following collection by ultracentrifugation (100 000 × *g* for 1 h) from the culture media of infected CCO cells, were examined by transmission electron microscopy. Virus suspensions were stained with 2% phosphotungstate and then examined with a Zeiss 10C electron microscope at 80 kV. A total of 20 virions with intact envelopes were measured to assess virion size.

Analysis of virion polypeptides. Purified virus (1 mg protein ml^{-1}) in TNE, was mixed 1:1 with 2× sample application buffer, heated to 100°C for 2 min, and then centrifuged for 2 min at 16 000 × *g*. Virion polypeptides were separated by SDS-PAGE under reducing conditions in 15% gels or 4 to 20% Bio-Rad Ready Gels (Bio-Rad) according to the system of Laemmli (1975). Novex Mark-12™ molecular weight standards (Novex) were included in each gel. After electrophoresis, the gels were stained with Coomassie Blue G-250 and the approximate molecular weight of the virion polypeptides was estimated by their relative mobility compared to the molecular weight standards.

Restriction fragment length polymorphism (RFLP) comparisons. The genomic DNA for both IcmHV and CCV were compared by restriction fragment length polymorphism (RFLP) analyses with 2 endonucleases. Aliquots of each viral DNA (1 µg) were digested with 10 U of either *Kpn* I or *Sac* I for 1 h at 37°C. The different DNA fragments were separated by electrophoresis on 0.8% agarose gels and observed after staining with 1% ethidium bromide. Also included in comparisons were 2 additional herpesviruses from fish, the cyprinid herpesvirus Type 1 (CyHV-1 or CHV) and the koi herpesvirus (KHV). The preparation of the viral DNA from all 4 herpesviruses compared was identical to that described by Gilad et al. (2002).

Fish for experimental virus exposures. Channel catfish were obtained from a farm in northern California that has been inspected annually for the past 20 yr. There have been no outbreaks of CCVD, nor has the virus been isolated or anti-CCV neutralizing antibodies detected from broodstock examinations at this farm. Examinations of fish from this farm by PCR assays specific for CCV have also been consistently negative (authors' unpubl. data). Fish were obtained as fry. Groups of fish were used immediately for bath exposures or, after further growth, for injection challenges with the virus. Growth from fry to juveniles occurred in 130 l aquaria receiving 20°C well water at

1.8 l min^{-1}. Fish were fed a commercial diet dispensed by automatic feeders.

Virus exposures. The virulence of IcmHV for channel catfish exposed by bath (fry experiments) or intraperitoneal injection (juvenile experiment) was evaluated in 3 experimental trials. In the first trial, channel catfish fry had a mean weight of 0.02 g and were exposed to IcmHV or CCV propagated in the CCO line as described above (subsection 'Viruses and cell lines'). In the second trial, the channel catfish fry were 0.03 g and were exposed only to IcmHV. In the first fry exposure trial, 6 replicate groups of 50 channel catfish were placed in 10 l aquaria containing 3 l of 24 to 25°C well water; 2 aquaria received only culture media containing no virus (control). Two additional aquaria received IcmHV added to the water at a dose of $10^{4.39}$ ml^{-1}, and the other 2 aquaria received CCV in the water at a dose of $10^{4.31}$ ml^{-1}. To maintain water quality, 2 l of water were changed each day. Fish were fed a commercial diet sparingly, once a day. Dead fish were removed for analyses during twice-daily examinations of the aquaria. Water temperature was maintained at a constant 24 to 25°C. After 6 d, the experiment was terminated and all remaining fish were collected for analyses. The second fry trial was conducted under the same conditions except that only 4 replicate groups of catfish were used, with 2 receiving IcmHV exposure and the other 2 serving as unexposed controls; the duration of the trial was 10 d. The concentration of IcmHV was $10^{3.38}$ l^{-1} during the 1 h exposure period. In the third trial, juvenile channel catfish (15.8 g) received intraperitoneal injections of IcmHV at 2 doses, $10^{4.45}$ and $10^{3.45}$ $TCID_{50}$ $fish^{-1}$. A total of 10 fish were injected at each virus dose and 10 control fish received injections with MEM only. The fish were held in static aquaria under the same conditions as described above. Duration of the juvenile catfish trial was 6 d.

Whole individual dead fish (catfish fry) or excised tissues including the kidney, spleen, liver, and intestine (juvenile catfish) were examined for the presence of virus by standard virus isolation methods on CCO cells (Ganzhorn & LaPatra 1994). The concentrations of virus in fish tissues during peak mortality (3 d post-exposure) were determined from 6 to 10 dead fish from each of the IcmHV- and CCV-exposed groups in the fry trials and from 3 IcmHV-exposed fish in the juvenile trial. At the termination of the fry experiments all remaining fish in the IcmHV group and 10 fish from the CCV exposed and unexposed control groups were examined for the presence of virus. The remaining control fish from the juvenile trial were examined for the presence of virus as individual fish with a pool of organs that included kidney, spleen, gill, and intestine inoculated onto CCO cells.

Tissues, including the kidney, spleen, liver, and intestine for histopathological examinations were collected only from newly dead fish in the juvenile trial during the peak mortality period. Tissues were processed for hematoxylin- and eosin-staining (Humason 1979). In addition, at the termination of the juvenile trial, surviving IcmHV-exposed fish and 5 control fish were evaluated for the presence of microscopic lesions.

RESULTS

Electron microscopy

Virions of IcmHV observed by electron microscopy were typical in size and shape of members of the family *Herpesviridae* (Fig. 1). The capsids were 107 nm in diameter and were surrounded by a loose envelope 227 nm in diameter.

Antigenic comparisons

The IcmHV was not neutralized by polyclonal rabbit anti-CCV serum at any dilution tested. In contrast, neutralization of CCV was observed with anti-CCV serum at a dilution of 1:160. Monoclonal antibodies to CCV (Mab 38) in mouse ascities neutralized CCV at a dilution of 1:12800. No neutralization of IcmHV with any dilution of Mab 38 was observed (lowest dilution tested was 1:100). Indirect fluorescent antibody tests with both rabbit polyclonal antibodies (1:20 dilution) or Mab 38 (1:1000 dilution) provided specific staining of CCV-infected but not IcmHV-infected CCO cells.

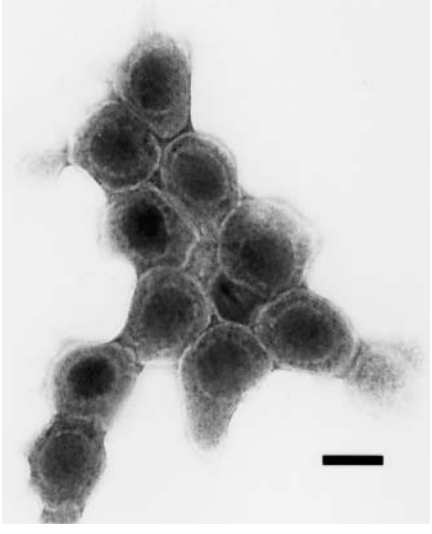

Fig. 1. *Ictalurus melas* herpesvirus (IcmHV). Electron micro graph of negatively stained virions. (Scale bar = 100 nm)

Virion polypeptides

A total of 17 polypeptides ranging in size from 17.5 to 175 kDa were identified from purified preparations of IcmHV by SDS PAGE (Fig. 2). The major polypeptides of IcmHV were found at 144, 113, 70, 44, 32, 31, 30, 28 and 21 kDa. The most prominent polypeptides from CCV ranged in size from 115 to 15 kDa. Approximately 7 of the polypeptides observed in IcmHV had similarly sized homologs in CCV. The most prominent polypeptides in both viruses were at 113 and 115 kDa for IcmHV and CCV, respectively.

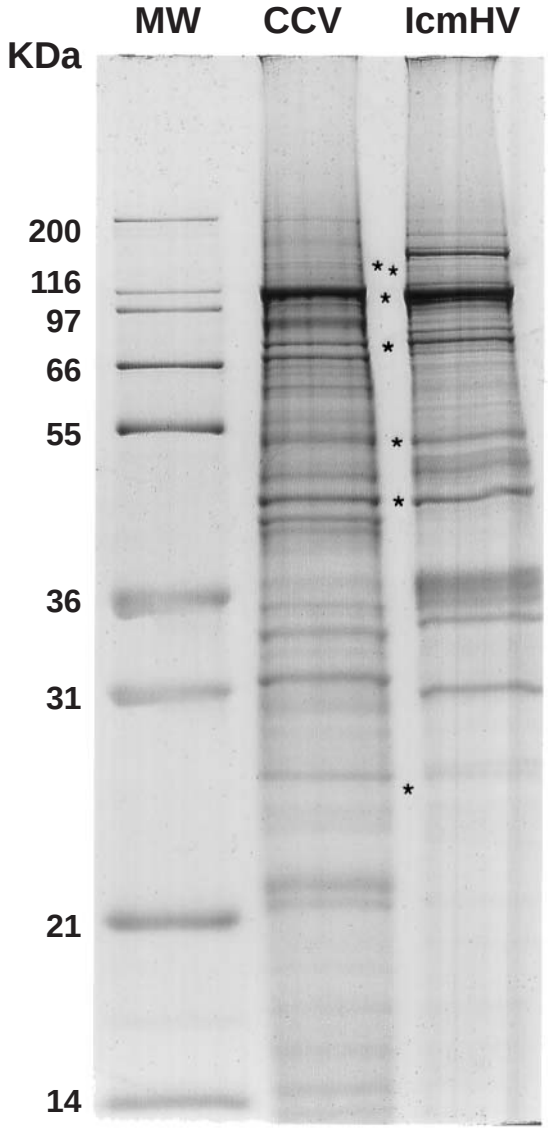

Fig. 2. *Ictalurus melas* herpesvirus (IcmHV). Comparison of virion polypeptides of IcmHV with those of channel catfish virus (CCV). Asterisks: polypeptides of similar size in both viruses; molecular weight (MW) standards are given on left

RFLP analyses

The RFLP comparisons of IcmHV with CCV and 2 additional herpesviruses from fish demonstrated that each virus was clearly distinguishable with both enzymes tested. The results for the comparison with the endonuclease *Kpn* I are shown in Fig. 3.

Susceptibility of channel catfish fry and juveniles

Bath exposures of channel catfish fry in 2 separate experiments with IcmHV at concentrations of $10^{4.39}$ and $10^{3.38}$ $TCID_{50}$ ml^{-1} resulted in 96 and 83% cumulative mortality, respectively (Table 1). Fish began dying 2 d following virus exposure, and by 4 to 5 d nearly all fish had died in virus-exposed groups compared to 5 and 16% of the control fish in Expts 1 and 2, respectively. In the first experiment, replicate groups of channel catfish fry bath-exposed to CCV at $10^{4.31}$ $TCID_{50}$ ml^{-1} had a cumulative mortality of 52%, and most mortality occurred between Days 3 and 5 post-virus exposure. Either IcmHV or CCV was recovered from all

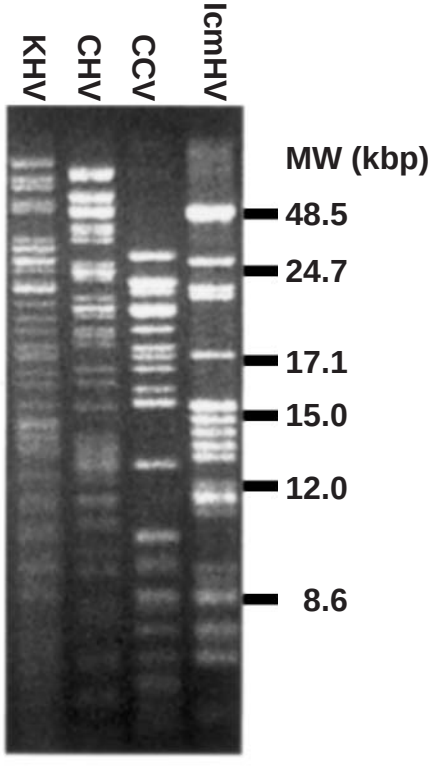

Fig. 3. *Ictalurus melas* herpesvirus (IcmHV). Restriction fragment length polymorphisms resulting from digestion of viral DNA from IcmHV, channel catfish virus (CCV), cyprinid herpesvirus 1 (CHV) and koi herpesvirus (KHV) with *Kpn* I. DNA molecular weight (MW) standards are given on right

Table 1. *Ictalurus punctatus.* Cumulative mortality of channel catfish following exposures to herpes-like virus (IcmHV) isolated from adult *I. melas*. Virulence of IcmHV for channel catfish fry at mean weights of 0.02 and 0.03 g was evaluated following bath exposure to virus in Expts 1 and 2, respectively. A replicate group of channel catfish fry was exposed to the channel catfish virus (CCV) under the same conditions as that used for IcmHV in Expt 1. Concentrations of virus found in dead fish were also determined by $TCID_{50}$ analyses. In a third experiment, mortality was evaluated among juvenile channel catfish (15.8 g) that received intraperitioneal injections of IcmHV at doses of $10^{4.45}$ or $10^{3.45}$ $TCID_{50}$ $fish^{-1}$

Virus Dose	No. dead fish/ no. fish exposed		Range of virus conc. ($TCID_{50}$ g^{-1})
	Replicate 1	Replicate 2	
Expt 1			
IcmHV ($10^{4.39}$)	48/50	48/50	$10^{4.66}$ to $10^{5.45}$
CCV ($10^{4.31}$)	23/50	18/50	$10^{7.93}$ to $10^{8.68}$
Control	4/50	1/50	0
Expt 2			
IcmHV ($10^{3.38}$)	44/50	39/50	$10^{4.48}$ to $10^{6.15}$
Control	8/50	8/50	0
Expt 3			
IcmHV ($10^{4.45}$)	8/10	–	
IcmHV ($10^{3.45}$)	9/10	–	
Control	0/10	–	

dead virus-exposed fish and no virus was recovered from control fish including 5 dead fish in Expt 1 and 10 dead fish in Expt 2. Several fry in the control group in both experiments that died were emaciated and we suspect this contributed to the mortality observed. The concentrations of virus found among IcmHV-exposed fry in both experiments ranged from $10^{4.66}$ to $10^{6.15}$ $TCID_{50}$ g^{-1}. Concentrations of virus from $10^{7.93}$ to $10^{8.68}$ $TCID_{50}$ g^{-1} were found among channel catfish fry exposed to CCV that died during the experiment. Virus was not isolated from any of the CCV-exposed survivors examined in Expt 1. No IcmHV was detected among virus-exposed channel catfish fry surviving at 10 d in Expt 2.

Groups of juvenile channel catfish injected with IcmHV at 2 doses in the third experiment also experienced mortality of 80 to 90% (Table 1). There was no mortality among the control fish. Virus was recovered from all dead virus-exposed fish but not from virus-exposed survivors nor any control fish examined at the termination of the study. Pathological examinations of 4 freshly dead IcmHV-injected juvenile channel catfish (2 from the higher dose and 2 from the lower exposure dose) revealed hemorrhages present in the kidney, spleen, and to a lesser extent in the liver. Microscopic lesions were most severe in the spleen and liver (Fig. 4). Multi-focal lesions in regions of major blood vessels in the liver were prominent in 2 fish (Fig. 4A). These lesions were characterized by mild to moderate necrosis of hepatocytes. Severe necrosis of the parenchyma of the spleen was the most prominent lesion found in all 4 fish (Fig. 4B). Proliferation of the interstitial hematopoietic cells of the kidney and necrosis of the mesangial cells of the glomeruli were observed in 2 fish (Fig. 4C). The muscularis of the small intestine and the serosal surfaces of most organs in contact with the peritoneal cavity demonstrated moderate to severe necrosis. Vascular spaces in these areas contained macrophages with ingested debris. Similar macrophage accumulations were evident in blood vessels in the spleen (Fig. 4D). These histopathologic changes found in IcmHV-injected catfish were not observed among control fish.

DISCUSSION

Characteristics of a herpes-like virus associated with high mortality (80 to 90%) of farmed black bullhead in Italy demonstrate that it clearly differs from CCV, the agent causing CCVD among farmed channel catfish in North America. The virus from black bullhead, termed *Ictalurus melas* virus (IcmHV), however, does replicate in a cell line from channel catfish ovary (CCO) and is virulent for fry and juvenile channel catfish. In contrast to CCV, IcmHV fails to react with polyclonal and monoclonal antibodies to CCV and has distinctly different virion polypeptides and genomic DNA restriction fragment length polymorphisms. IcmHV should be considered as a newly characterized herpes-like virus that represents a potentially significant pathogen for not only black bullheads but also channel catfish.

Herpesviruses are complex viruses with linear double-stranded DNA genomes of 125 to 245 kbp (Davison 2002). The genome is contained within an icosahedral capsid that ranges in size from 100 to 125 nm and lies within a tegument or matrix surrounded by a lipidcontaining membrane or envelope. Fully enveloped virus particles range in diameter from 200 to 250 nm. The Ictalurid herpesvirus 1 (IcHV-1) or CCV is currently the type species of an unassigned genus 'ictalurid herpes-like viruses' in the family *Herpesviridae* (Van Regenmortel et al. 2000). All the remaining herpesviruses known from fish are currently unassigned members of the family *Herpesviridae*. The entire genome of 1 CCV isolate has been sequenced, and in-depth studies have investigated both the structure and function of selected genes and gene products of the virus (Davison 1992, 1998, 2002, Hanson et al. 1994, Davison & Davison 1995, Booy et al. 1996, Kucuktas & Brady 1999, Vanderheijden et al. 1999).

The size and shape of the virions of IcmHV are similar to those of other known herpesviruses from other

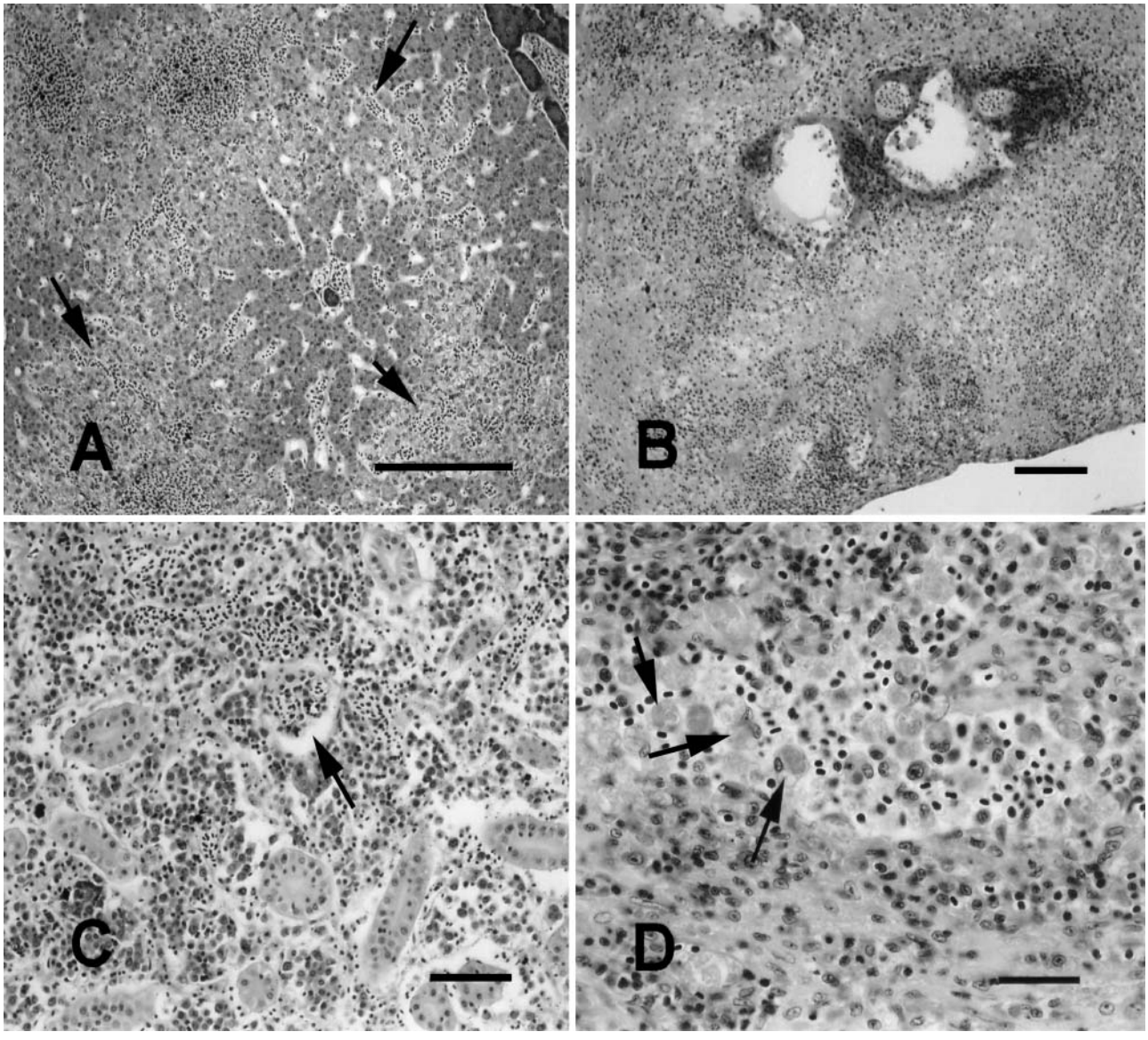

Fig. 4. *Ictalurus melas* herpesvirus (IcmHV). Microscopic lesions observed in tissues of juvenile channel catfish receiving intraperitoneal injections of IcmHV. (A) Multi-focal necrosis (arrows) of hepatocytes in region of central vein in the liver (scale bar = 100 µm); (B) severe depletion of parenchyma surrounding 2 large blood vessels in the spleen (scale bar = 100 µm); (C) proliferation of cells and necrosis of a glomerulus (arrow) in the kidney (scale bar = 60 µm); (D) macrophages (arrows) with ingested debris in vein of the spleen (scale bar = 10 µm)

animal species, including CCV (Fig. 1). These morphologic characteristics are consistent with traditional criteria used to group newly discovered viruses in the family *Herpesviridae* (Van Regenmortel et al. 2000). The restricted host-species adaptation found among most herpesviruses, particularly the fish herpes-like viruses (Hedrick & Sano 1989), initially suggested that IcmHV might be related to CCV, as both are found in ictalurid hosts. However, certain of the early characteristics of IcmHV indicated it might differ from CCV (Alborali et al. 1996). Differences included causing disease in adult catfish rather than just in young catfish and the ability of the new virus to replicate in cell lines derived from centrarchid and cyprinid fishes rather than solely on cell lines of ictalurid or clarid catfish species.

The absence of anti-CCV polyclonal and monoclonal antibody binding to IcmHV was the first evidence that the 2 viruses were distinct. These antigenic differences most probably reflected significantly different epitopes present on the major structural proteins of IcmHV compared to CCV. The major protein composing the

pentons and heptons of the capsid of CCV has an apparent molecular weight of 115 kDa and is comparable to VP 5 of the human herpes virus simplex HHV-1 (Booy et al. 1996). A major polypeptide observed at 113 kDa in IcmHV is presumed to be the counterpart to VP 5 and the 115 kDa capsid protein of CCV (Fig. 2). If we assume that several faint bands revealed by SDS-PAGE analyses of virion polypeptides of IcmHV are potential cellular contaminants, the virus would possess between 18 and 20 structural proteins. This compares to 18 known structural proteins observed with CCV (Dixon & Farber 1980, Davison & Davison 1995).

Differences between the genomic DNA of IcmHV and CCV were also evident by RFLP analyses with both *Sac* I and *Kpn* I (Fig. 3). The banding patterns of restriction fragments were considerably different between IcmHV and CCV than those observed between 12 isolates of CCV compared by the same method by Colyer et al. (1986).

A characteristic shared by both IcmHV and CCV was their virulence for fry and juvenile channel catfish (Table 1). Under the experimental conditions in our infection trials with channel catfish fry, IcmHV was more virulent than the isolate of CCV. For these trials we used a water temperature of 24 to 25°C, which was similar to that at the time of the initial outbreaks of IcmHV infections in Italy (Alborali et al. 1996). The water temperatures used in our experimental trials are at the lower end of the range most probably optimal for CCV infections, since most seasonal outbreaks in channel catfish farms and more severe experimental infections with CCV occur at water temperatures greater than 25°C (Plumb 1973, 1978, Zhang & Hanson 1995). The concentrations of IcmHV recovered from infected channel catfish fry were similar to those reported from fingerling channel catfish dying from experimental or natural infection with CCV (Plumb & Gaines 1975). The channel catfish fry with CCV in our trial generally had 1000-fold more virus than IcmHV-infected fry. Microscopic lesions observed in IcmHV-infected juveniles resembled but were less severe than those reported for CCV infections in fingerling channel catfish (Wolf et al. 1972, Major et al. 1975, Plumb & Gaines 1975). Considering the high mortality, recovery of significant concentrations of virus, and microscopic lesions observed in channel catfish following infection with IcmHV, the virus should be considered as a potentially serious threat to the channel catfish industry. Precautions, including inspections and controlled movements of fish (Hedrick 1996), to prevent the introduction of the virus to growing areas in North America, are therefore warranted.

The origin of the virus in black bullhead in Italy is currently unknown. Compared to other known herpes-like viruses from fishes (Hedrick & Sano 1989, Hetrick & Hedrick 1993), IcmHV has unique phenotypic properties, including a more broad host-range both for cell lines and catfish by a natural infection route (bath). IcmHV replicates in cell lines of ictalurid, cyprinid, and centrarchid origin and causes mortality following natural routes of infection in at least 2 species of catfish. In contrast, CCV is known only to infect clarid or ictalurid cell lines *in vitro* and only channel catfish by natural routes of infection (Wolf & Darlington 1971, Noga & Hartman 1981, Plumb et al. 1985, Boon et al. 1988, Wolf 1988). These unusual characteristics of IcmHV may indicate that it has yet to fully adapt to the black bullhead host and/or that it originates from another fish or other species of aquatic vertebrate. Perhaps exchanges between fish and amphibian hosts in the aquatic environment also occur with herpesviruses, a phenomenon shown for members of the family *Iridoviridae* (Mao et al. 1999) which are second only to herpesviruses as the most frequently encountered group of DNA viruses in fishes (Hetrick & Hedrick 1993). That the ranid (frog) and fish herpesviruses are considerably more closely related than either is to the mammalian herpesviruses has recently been shown by complete genome comparisons (Davison 2002).

Apparently IcmHV remains restricted in its geographic distribution, perhaps due to the relatively minor trade in live black bullheads in Europe. The spread of the virus from the current known geographic range could have potentially serious economic and ecologic consequences, particularly if it reaches regions with large-scale channel catfish production. The virulence of IcmHV for fry, juvenile and adult catfish is also alarming and contrasts to that of CCV, which is viewed mainly as a disease of fry and, to a lesser extent, fingerling channel catfish. Thus increased vigilance to control movements of fishes and perhaps amphibians infected with IcmHV to regions where channel catfish may be present are warranted.

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