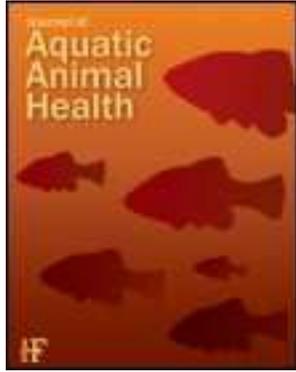


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An Enzyme-Linked Immunosorbent Assay (ELISA) for Detection of Antibodies to Channel Catfish Virus (CCV) in Channel Catfish

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Abstract.—An enzyme-linked immunosorbent assay (ELISA) was developed for the detection of antibodies against channel catfish virus (CCV) in the serum of channel catfish *Ictalurus punctatus*. The ELISA was used to test sera from 20 waterborne CCV-exposed juvenile channel catfish and 10 unexposed channel catfish for antibodies to CCV. Each serum was tested at a single dilution (1:10, volume:volume), and positive–negative cutoff values were established as the mean of negative sera plus either 2, 3, or 4 standard deviations. The resulting ELISA was highly reproducible and accurate with sensitivities and specificities of 100% and 90%, 95% and 90%, and 95% and 100%, respectively. Most fish seroconverted between 22 and 28 d after first exposure to CCV. This immunoassay is capable of handling large numbers of samples reliably and rapidly for detection and assessing prevalence of CCV exposure in channel catfish.

Channel catfish virus disease (CCVD) is a major cause of morbidity and mortality in young channel catfish *Ictalurus punctatus* (Plumb 1977). The etiologic agent is channel catfish virus (CCV), a herpesvirus that can be transmitted horizontally and vertically to healthy fish (Plumb 1978; Wise et al. 1988). It is suspected that most fish surviving an outbreak become carriers. However, the carrier state does not necessarily correlate with a history of clinical CCVD or the presence of neutralizing serum antibodies (Wise et al. 1985). Because carriers can potentially transmit the disease to other catfish as well as to offspring, the capability to determine and select for CCV-free broodstock is highly desirable.

In California, the diagnosis of CCVD depends primarily on the observation of clinical signs, isolation, and identification of CCV. Demonstration of serum antibodies to CCV in fish with no clinical signs of disease, and from which the virus cannot be isolated with standard procedures, is also used to assess prior exposures to the virus. Serum antibodies are detected with a serum neutralization assay (SNA) for initial screening followed by viral neutralization index (NI) determinations to confirm positive results. These procedures require 7 d for the SNA and an additional 7 d for NI confirmation. In addition to the 14 d required to evaluate the CCV status of a given fish, the number of samples that can be processed at a given time is limited. Furthermore, whereas a positive SNA

is considered a good indication of prior exposure to CCV (Plumb 1973; Heartwell 1975; McConnell and Austin 1978), many sera that empirically should be positive are considered negative after indexing (a 1:50 volume: volume, v:v, dilution of serum must neutralize $1.7 \log_{10}$ of virus to be considered positive). Here we describe an enzyme-linked immunosorbent assay (ELISA) for evaluating the CCV status of fish that has the capacity to screen hundreds of samples in 1 d. The parameters of this ELISA were optimized to obtain highly sensitive and specific results that can be compared with those of the SNA–NI. This ELISA was then used to determine time to seroconversion for juvenile channel catfish after experimental exposure to CCV.

Methods

Fish.—Juvenile channel catfish (aged 7 months) used in this study were from a private farm with no history of CCVD or anti-CCV neutralizing activity in the sera of broodstock. All fish were weighed and given an identification number (1–30) by tattooing on the abdominal skin. Weight of fish ranged from 107 to 325 g (mean = 172 g). Ten fish served as controls and 20 fish were used for treatment–exposure to virus. To minimize potential effects of handling and bleeding, control and treatment groups were each divided into two groups: control groups 1 and 2; treatment groups 1 and 2. Fish from alternating control and treatment groups were bled once per week, allowing 2 weeks between individual bleedings.

To ensure comparable weight distribution of

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control and treatment groups, fish were given random numbers from a table. The fish were ranked in order of weight, then divided serially into 10 groups of three fish. Within the groups of three, the fish with the highest random number was assigned alternatively to control group 1 or 2; the second highest was assigned to treatment group 1; and the fish with the lowest random number went into treatment group 2.

The fish were allocated according to respective treatment or control groups into six 130-L tanks supplied with well water at 2 L/min. Water temperature was increased during a 5-d period from 21°C to 25°C. The fish were acclimated for 2 weeks before exposure to virus. Blood was taken from all fish after 1 week of acclimation as a baseline for measurement of serum antibodies.

Serum collection and handling.—Approximately 0.3 mL of blood was drawn from the caudal vein at each handling and collected into individual non-heparinized containers. Blood was allowed to clot at room temperature for 30–60 min, then the clot retracted overnight at 4°C. Serum was removed by centrifugation at 3,000 × gravity (g) for 10 min and stored at –20°C until needed for both ELISA and NI.

Monoclonal antibodies (MAbs).—Anti-catfish immunoglobulin (Ig) monoclonal 1H12-7 (approximately 1 mg/mL) was obtained via cross-species ascites production in Balb/C mice for ELISA reagent (kindly provided by C. J. Lobb, University of Mississippi–Jackson). The MAbs were concentrated by ammonium sulfate precipitation (Harlow and Lane 1988) and then purified by passage through MAC Protein G Discs (Amicon, Bedford, Massachusetts).

Virus and virus titrations.—The CA80-5 strain of CCV was used for challenging the fish, serum neutralization indexing, and coating the microtiter plate wells for the ELISA. The virus was grown in channel catfish ovary (CCO) cells (Bowser and Plumb 1980) and purified as described by Dixon and Farber (1980). Virus was stored at –70°C. Estimations of virus concentrations were made by 50% tissue culture infectious dose (TCID₅₀) end-point titrations (Reed and Muench 1938) after incubation on CCO cells for 10 d at 25°C.

Exposure to CCV.—The 10 fish allocated to control groups were placed in a 100-L tank filled with 25°C well water. One milliliter of tissue culture media from uninfected CCO cells was added to the water. After 30 min, catfish were removed and placed into 130-L tanks. The 20 fish assigned to treatment groups were treated in the same manner

but received 1 mL of media containing CCV (final concentration, 5.5×10^2 TCID₅₀/mL). After 30 min, catfish were transferred to 130-L tanks.

No morbidity or mortality was observed within the first 10 d of exposure. To ensure adequate exposure to CCV, the fish in the treatment group were reexposed 17 d after initial exposure, in the manner previously described, to CCV at a final concentration of 3.8×10^3 TCID₅₀/mL. Although virus concentrations in the water have never been measured in an active outbreak, challenging with this level of virus under laboratory conditions has produced disease similar to that seen in field outbreaks (Hedrick and McDowell 1987; Hedrick et al. 1987).

ELISA procedure.—The ELISA procedure was modified from that described by Case et al. (1983), Arkoosh and Kaattari (1990), and Lorenzen and Dixon (1991). Purified CCV, quantified by ultraviolet absorption (Harlow and Lane 1988), was assayed at 40, 20, 10, 5, and 2.5 µg/mL diluted in carbonate buffer (0.1 M Na₂CO₃, 0.02% NaN₃, pH 9.6) and mixed by vortex. Each well of a 96-well flat-bottomed polystyrene microtitration plate (Falcon 3915, Becton Dickinson, Franklin Lakes, New Jersey) received 100 µL of the viral antigen in coating solution. Plates were covered and incubated overnight at room temperature in a humid chamber. Wells were washed five times with a phosphate-buffered saline solution supplemented with 0.1% Tween-20 (Polyoxyethylene sorbitan monolaurate, Sigma Chemical Co., St. Louis, Missouri) and 1% Thimerosal (PBST, pH 7.4) diluted 1:10 (v:v) in double-distilled water. All plates were washed five times after each step, except the substrate incubation, with an automated plate washer (Bio-Tek Instruments, Inc., Winooski, Vermont).

Each well of the ELISA plate was blocked for 1 h at 37°C with 200 µL of 10% (weight/volume, w/v) nonfat powdered milk (10% NFM) in PBST to prevent nonspecific protein binding. The plate was washed and reincubated for 1 h at room temperature with serum samples.

Serum was assayed by serial twofold dilutions from 1:10 to 1:5,120 (v:v) in 10% NFM–PBST. Each sample (50 µL) was added to four replicate wells. Each plate had four replicates of positive and negative control sera as well as four replicates with no sera (10% NFM–PBST blanks). Positive control serum came from a previously identified CCV-infected fish (high-NI fish from a CCV-identified outbreak). Negative control serum was obtained from fish on a farm consistently testing seronegative for CCV and having no reported clinical

outbreaks. Sodium azide and glycerin were added to the control sera to prevent contamination and for improved storage at -20°C .

After incubation and a washing, 50 μL of purified ascites containing catfish Ig-specific mouse MAbs at threefold serial dilutions from 1:50 to 1:109,350 in 10% NFM-PBST were added to each well. Plates were incubated for 30 min at room temperature and washed five times.

Antibody conjugate (biotin-labeled affinity purified goat antibody to mouse IgG), in serial twofold dilutions from 1:250 to 1:2,000 in 10% NFM-PBST was added (100 μL /well) and incubated for 30 min at room temperature. This was followed by a checkerboard titration of the enzyme conjugate, peroxidase-labeled streptavidin (100 μL /well; Kirkegaard & Perry, Gaithersburg, Maryland), also in serial twofold dilutions from 1:250 to 1:2,000 in 10% NFM-PBST. After incubation for 30 min at room temperature, the plate was washed five times. Substrate, 3,3',5,5'-tetramethyl-benzidine (Sigma), was prepared in 0.05 M citric acid (pH 4.8), and immediately before use, hydrogen peroxide was added for a final concentration of 0.01% (v/v). Substrate (100 μL) was added to each well and incubated for 5–15 min. The reaction was stopped with 50 μL of 1 M H_2SO_4 per well, and absorbance (A_{450}) was read at 450 nm with a MR700 Microplate Reader (Dynatech Laboratories, Inc., Chantilly, Virginia).

Assay analysis.—The optimal assay conditions were determined by using the positive and negative control sera. Optimal conditions were those that yielded the highest positive–negative absorbance ratio (PNR): the A_{450} value of the positive control divided by the A_{450} value of the negative control. The average A_{450} value of four replicates was used for each control value in the PNR. Once assay conditions were established, the 30 experimental sera and controls were assayed by using four replicates to determine a positive–negative cutoff. To account for plate-to-plate differences, the average ELISA optical density (OD) for each sample was divided by the average OD of the positive control on the respective plate. This gave each sample a percent of positive (PP) value and made among-plate comparisons valid. Reproducibility within plates was assessed by calculating coefficients of variation (CV) for each replicate. Sensitivity and specificity and exact 95% confidence limits were calculated at selected PP cutoff values with the EpiTable module of Epi Info (version 6.04, Centers for Disease Control and Prevention, Atlanta, Georgia).

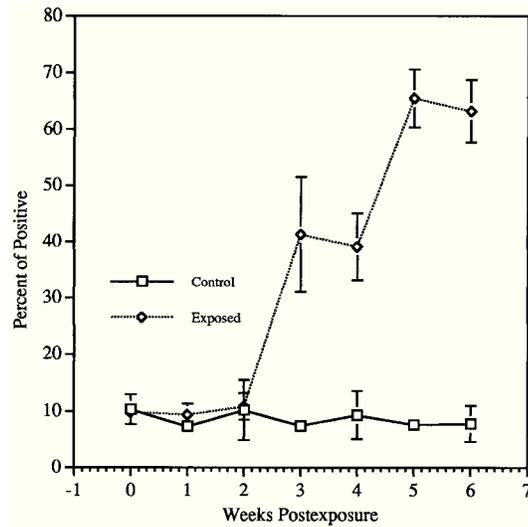


FIGURE 1.—Mean percent of positive ELISA values (\pm SE) for anti-channel catfish virus (CCV) neutralizing activity in the serum of juvenile channel catfish after waterborne exposure to CCV.

Neutralization index test.—The experimental sera were tested for their activity against the CA80-5 strain of CCV. The virus was prepared as 10-fold dilutions in minimal essential medium supplemented with 2% fetal calf serum (MEM-2) and 0.015 mM HEPES and distributed to replicate wells of 96-well microtiter plates. All sera were diluted 1:50 (v:v) in MEM-2-HEPES. Diluted serum (25 μL) was added to each of five replicate wells of a microtiter plate containing 25 μL of the diluted virus. As a control, virus was added to replicate wells of media without serum. After the microtiter plates were left for 1 h at room temperature, 50 μL of a suspension of CCO cells (approximately 3.7×10^4 cells) in MEM-2-HEPES were added to all wells. The plates were held at 25°C for 1 week, and the virus titers (TCID₅₀/mL) were calculated by the method of Reed and Muench (1938). The NI of each serum was expressed as the difference in $\log_{10}(\text{TCID}_{50}/\text{mL})$ between the virus dilutions incubated with MEM-2-HEPES alone and those incubated with serum.

Results

Juvenile channel catfish exposed to waterborne CCV produced anti-CCV antibodies in their sera that were detected by our ELISA (Figure 1). Infection from CCV remained subclinical (fish were asymptomatic) for the duration of the experiment. One control fish was seropositive (PP = 32.7) at baseline (day 0), and its PP value decreased at each

TABLE 1.—Mean and SD of optical density (OD) and percent of positive (PP) ELISA values for juvenile channel catfish exposed to channel catfish virus (CCV) and for control fish.

Time (week)	Exposed to CCV				Control			
	OD		PP		OD		PP	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
0	0.061	0.038	9.88	6.09	0.063	0.051	10.38	8.39
1	0.059	0.039	9.43	6.16	0.045	0.009	7.37	1.99
2	0.067	0.048	10.87	7.42	0.062	0.072	10.22	11.80
3	0.258	0.205	41.30	32.27	0.045	0.013	7.46	2.50
4	0.234	0.113	39.12	18.95	0.056	0.058	9.37	9.46
5	0.400	0.102	65.43	16.15	0.047	0.022	7.68	3.54
6	0.038	0.113	63.16	17.38	0.047	0.043	7.84	7.04

sampling to 20.4 by the end of the experiment (day 42). All other controls were and remained seronegative. Some fish began to show signs of *Flavobacterium columnare* infection 14 d after challenge. Subsequently, every tank was treated daily for 7 d with nitrofurazone at 10 mg/L. Treatment was suspended for 3 d, but *F. columnare* continued to be a problem. Therefore, treatment with nitrofurazone resumed for 11 d. Water temperature was also gradually increased to 27°C (reached at day 22) for the duration of the experiment to help abate further *F. columnare* growth.

Standardization of the ELISA

The optimal CCV antigen concentration for the ELISA was 10 µL/mL. Of the several blocking agents tested, 10% NFM yielded the least background reactivity. Assay conditions were optimal with a catfish serum dilution of 1:10, a MAb dilution of 1:4,000, and a conjugate dilution of 1:1,000 with a reading time of 5 min after addition of substrate. These conditions gave linear responses while yielding the highest PNR values for

the positive serum. Positive control sera OD values ranged from 0.544 to 0.666. Negative control sera OD values ranged between 0.005 and 0.035. Four independently purified preparations of CCV retained optimal ELISA values for at least 12 months at -70°C. No differences were found between antigen preparations by ELISA with the positive reference serum (CV < 2.3%).

Pooled control sera and samples from all exposed and control fish were evaluated in four replicates by using the optimized ELISA conditions. The OD and PP values for the ELISA are in Table 1. A high degree of reproducibility was indicated by CVs less than 10% for four replicate positive samples within plates, except three sera with CVs of 10.7, 11.1, and 23.9%. The three PP values for these sera were far above all cutoff points (54, 64, and 68, respectively). The positive-negative cutoff values (CO) of 23.0, 29.6, and 36.2 were calculated by adding the mean for all negative sera plus 2, 3, and 4 SDs, respectively. The number of false-positive and false-negative test results for these cutoff values were 1 and 0 (CO = 23.0), 1 and 1 (CO = 29.6), and 0 and 1 (CO = 36.2). These cutoffs were rounded to 20, 30, and 40 PP to calculate time to seroconversion (Table 2). Estimates of the sensitivity and specificity of the ELISA are shown in Table 3.

Most fish seroconverted between 22 and 28 d after first exposure to virus and this was independent of PP cutoff. Times to seroconversion ranged between 14 and 42 d when all cutoff values were considered.

Of the 20 fish exposed to CCV, only four fish (20%) were identified as positive by NI: two from those sampled on day 35 and two on day 42. The neutralization indices of the four positive fish ranged from 1.87 to 2.07. All nonexposed fish were negative by NI.

TABLE 2.—Time to seroconversion of 20 juvenile channel catfish exposed to channel catfish virus by using three positive-negative cutoff values equal to the mean percent of positive (PP) of known negatives plus either 2, 3, or 4 SDs.

Days	Number of fish at PP cutoffs of:		
	20	30	40
1-7	0	0	0
8-14	2	0	0
15-21	5	5	5
22-28	7	6	6
29-35	4	5	4
36-42	1	4	4
All	19 ^a	20	19 ^b

^a One fish had a baseline ELISA value greater than 20 PP.

^b One fish did not seroconvert with the 40-PP cutoff by termination of experiment on day 42.

TABLE 3.—Accuracy of ELISA for correctly classifying serum samples from CCV-exposed and control channel catfish by using positive–negative cutoff values equal to the mean percent of positive of all known negatives plus either 2, 3, or 4 SDs; CI = confidence interval.

ELISA cutoff	% sensitivity (95% CI)	% specificity (95% CI)
Mean + 2 SD = 23.0	100 (83.2–100)	90 (55.5–99.7)
Mean + 3 SD = 29.6	95 (75.1–99.9)	90 (55.5–99.7)
Mean + 4 SD = 36.2	95 (75.1–99.9)	100 (69.2–100)

Discussion

A wide range of CCV neutralizing activity has been found in channel catfish exposed experimentally and naturally to CCV (Plumb 1973; Heartwell 1975; Hedrick et al. 1987). Plumb (1973) and Heartwell (1975) observed highly variable serum anti-CCV neutralizing activity in adult and juvenile fish receiving intraperitoneal injections of CCV. Some fish produced little to no virus-neutralizing activity whereas others produced a significant amount. Neutralizing activity was detected as early as 1 week after injection and peaked between 3 and 9 weeks. The variability in antibody response after these experimental infections with CCV is similar to the wide range of neutralizing activity found in hatchery broodstock from which progeny have suffered CCV outbreaks (Plumb 1973). Fish in both studies did not show signs of CCVD. The variability in the time to detection of antibodies after exposure to CCV was also observed in our experiments: antibody was detected with the 20-PP cutoff for ELISA values as early as 14 d but as late as 42 d after exposure. Our results suggest that a quarantine and testing period of at least 42 d (with fish held at 27°C) should be observed to ensure seroconversion can be detected.

Reexposures to antigen (Plumb 1973; Heartwell 1975) and water temperature (reviewed by Bly and Clem 1992) can affect the antibody response in channel catfish. With respect to the response to CCV, conflicting data have been published. Collins et al. (1976) and Bowser and Munson (1986) found that increased water temperature raised the titers of anti-CCV neutralizing activity of naturally infected catfish. In contrast, Hedrick et al. (1987) found that both the titers and number of seropositive fish decreased with increased temperature among experimentally exposed catfish. Amend and McDowell (1983) found no seasonal differences in titers of anti-CCV neutralizing activity in farm-reared channel catfish broodstock. Klesius (1990)

found that levels of serum immunoglobulin in channel catfish were maintained whether fish were assayed during the winter or summer at water temperatures of 10°C and 30°C, respectively. The water temperatures in our study ranged from 24.5°C to 28°C, which are within the immunologically permissive temperature range for channel catfish B and T cell functions (Bly and Clem 1992).

Hedrick et al. (1987) reported anti-CCV neutralizing activity in several adult fish and clinical infections in two adult fish after waterborne exposure to low levels of CCV (final concentration, $10^{1.5}$ TCID₅₀/mL). In the same experiment, adults reared from CCV-exposed juveniles had anti-CCV neutralizing titers that did not increase after reexposure to CCV as adults (Hedrick et al. 1987). In our experimentally exposed fish, anti-CCV Ig, detected by the ELISA, increased during the time of the experiment in 17 of the 20 fish. In three fish, antibody values had peaked by 21 d (2 fish) and 28 d (1 fish). It is possible that the second exposure to virus may have shortened seroconversion times if the immune system had already been primed by the first exposure. One control fish was seropositive with the 20- and 30-PP cutoff values. This may have meant the fish was either previously exposed to CCV or had cross-reacting antibodies and was a false positive. Our experimental study employed waterborne exposures to induce infections, which may contrast with the more common mode of vertical transmission from infected broodstock. Hence, our estimates of the sensitivity and specificity of the CCV ELISA should be considered preliminary until they can be validated with data from natural field infections. However, we presume that our ELISA will be most useful to assess the CCV status of healthy asymptomatic carrier populations not unlike our asymptomatic experimentally exposed population. Currently, the CCV status of catfish populations in California is assessed by NI, which had a sensitivity of only 20% in the present study.

The choice of positive–negative cutoff values is subjective and relies on the needs of the user, given the potential economic consequences of misclassifying a fish or population of fish either positively or negatively. Also, depending on the prevalence of CCV in a given population, the users of this assay may want to emphasize either specificity or sensitivity. We calculated test accuracy at three cutoffs to give the flexibility needed when prevalence is unknown. Using the lowest ELISA cutoff—mean negative plus 2 SDs (100% sensitivity)—yielded a high negative predictive value

(Courtney and Cornell 1990), which is important when attempting to eradicate potentially CCV-infectious broodstock or to demonstrate that valuable genetic stocks are free of CCV. Using the highest ELISA cutoff—mean negative plus 4 SDs (100% specificity)—yielded a high positive predictive value, which is important to catfish producers when classification of fish or fish populations as false positives might adversely affect trade or result in unnecessary culling of broodstock. Until further assay validation is done, we recommend using the two cutoffs when analyzing ELISA data from catfish populations to obtain the clearest assessment of their exposure status and to determine whether classification of population status is dependent on choice of the ELISA cutoff value.

References

- Amend, D. F., and T. McDowell. 1983. Current problems in the control of channel catfish virus. *Journal of the World Mariculture Society* 14:261–267.
- Arkoosh, M. R., and S. L. Kaattari. 1990. Quantization of fish antibody to a specific antigen by an enzyme-linked immunosorbent assay (ELISA). Pages 15–23 in J. S. Stolen, T. C. Fletcher, D. P. Anderson, B. S. Roberson, and W. B. van Muiswinkel, editors. *Techniques in fish immunology*. SOS Publications, Fair Haven, New Jersey.
- Bly, J. E., and L. W. Clem. 1992. Temperature and teleost immune functions. *Fish and Shellfish Immunology* 2:159–171.
- Bowser, P. R., and A. D. Munson. 1986. Seasonal variation in channel catfish virus antibody titers in adult channel catfish. *Progressive Fish-Culturist* 48:198–199.
- Bowser, P. R., and J. A. Plumb. 1980. Fish cell lines: establishment of a line from ovaries of channel catfish. *In Vitro* 16:365–368.
- Case, J. T., A. A. Ardans, D. C. Bolton, and B. J. Reynolds. 1983. Optimization of parameters for detecting antibodies against infectious bronchitis virus using an enzyme-linked immunosorbent assay: temporal response to vaccination and challenge with live virus. *Avian Diseases* 27:196–210.
- Collins, M. T., D. L. Dawe, and J. B. Gratzek. 1976. Immune response of channel catfish under different environmental conditions. *Journal of the American Veterinary Medical Association* 169:991–994.
- Courtney, C. H., and J. A. Cornell. 1990. Evaluation of heartworm immunodiagnostic tests. *Journal of the American Veterinary Medical Association* 197:724–729.
- Dixon, R. A., and F. E. Farber. 1980. Channel catfish virus: physico-chemical properties of the viral genome and identification of viral polypeptides. *Virology* 103:267–278.
- Harlow, E., and D. Lane, editors. 1988. *Antibodies, a laboratory manual*. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.
- Heartwell, C. M. 1975. Immune response and antibody characterization of the channel catfish (*Ictalurus punctatus*) to a naturally pathogenic bacterium and virus. U.S. Fish and Wildlife Service Technical Papers 85.
- Hedrick, R. P., J. M. Groff, and T. McDowell. 1987. Response of adult channel catfish to waterborne exposures of channel catfish virus. *Progressive Fish-Culturist* 49:181–187.
- Hedrick, R. P., and T. McDowell. 1987. Passive transfer of sera with antiviral neutralizing activity from adult channel catfish protects juveniles from channel catfish virus disease. *Transactions of the American Fisheries Society* 116:277–281.
- Klesius, P. H. 1990. Effect of size and temperature on the quantity of immunoglobulin in channel catfish, *Ictalurus punctatus*. *Veterinary Immunology and Immunopathology* 24:187–195.
- Lorenzen, K., and P. F. Dixon. 1991. Prevalence of antibodies to lymphocystis virus in estuarine flounder *Platichthys flesus*. *Diseases of Aquatic Organisms* 11:99–103.
- McConnell, S., and J. D. Austin. 1978. Serological screening of channel catfish virus. U.S. National Marine Fisheries Service Marine Fisheries Review 40(3):30–32.
- Plumb, J. A. 1973. Neutralization of channel catfish virus by serum of channel catfish. *Journal of Wildlife Diseases* 9:324–330.
- Plumb, J. A. 1977. Channel catfish virus disease. U.S. Fish and Wildlife Service Fish Disease Leaflet 52.
- Plumb, J. A. 1978. Epizootiology of channel catfish virus disease. U.S. National Marine Fisheries Service Marine Fisheries Review 40(3):26–29.
- Reed, L. J., and H. Muench. 1938. A simple method of estimating fifty percent endpoints. *American Journal of Hygiene* 27:493–497.
- Wise, J. A., P. R. Bowser, and J. A. Boyle. 1985. Detection of channel catfish virus in asymptomatic adult channel catfish, *Ictalurus punctatus* (Rafinesque). *Journal of Fish Diseases* 8:485–493.
- Wise, J. A., S. F. Harrel, R. L. Busch, and J. A. Boyle. 1988. Vertical transmission of channel catfish virus. *American Journal of Veterinary Research* 49:1506–1509.