AAVLD - Serology Committee Serology Interpretations

The following interpretive summaries have been provided by members of the AAVLD, in cooperation with ongoing efforts of the Interpretive Serology Committee to provide standardization and interpretation of serologic assays. The summaries are organized as follows;

Glossary of terms final version from Serology chair Peter Wright

I. Agent

II. Serology Test(s)

1. Tests available
2. Advantages/disadvantages of each
3. Comparative Results

III. Serology Results

1. Sensitivity and Specificity if available
2. Vaccination, exposure, duration of response
3. Serologic response in relation to clinical signs

IV. Uses of Serology Test Results

1. Single serum/paired serum/herd serology
2. Magnitude of response if applicable
3. Appropriate/Inappropriate use of serology results
4. Overall benefit and use of the test results

The summaries focus on interpretation of selected serologic tests used in AAVLD accredited laboratories, noting however that standard serum banks and protocols are not available for many, if not all of the assays discussed. The interpretive summaries are offered as guidelines only, and reflect the experience and opinion of the authors. The serology committee cautions that interpretation of any serologic test requires a working knowledge of the test, the animal(s) history, and an understanding of the agent in question.

CANINE BRUCELLOSIS (B. CANIS)
BOVINE VIRAL DIARRHEA VIRUS (BVDV)
CAPRINE ARTHRITIS-ENCEPHALITIS VIRUS CAPRINE LENTIVIRUS
AVIAN CHLAMYDIA
EQUINE RHINOPNEUMONITIS
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SEROLOGY INTERPRETATION

CANINE BRUCELLOSIS (*B. CANIS*)

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1. CANINE BRUCELLOSIS (*BRUCELLA CANIS*)

Canine brucellosis was first recognized in the U.S. in 1966 during outbreaks of abortions and reproductive failures in large, commercial breeding kennels and in packs of field dog~, mainly beagles. Since that time, the disease been recognized in several countries. The infection is common in Mexico and Central and South America where seroprevalence rates of nearly 30% have been reported. Seroprevalence rates of ~6% have been reported in the Southern States of the U.S., especially in areas where stray dogs are allowed to roam freely. In the U.S., the impact of canine brucellosis is greatest in breeding kennels and field-trial dogs. Although originally recognized in beagles, several additional breeds have been affected, especially in breeding kennels where large numbers of dogs are bred for research use, or the pet shop trade.

The natural disease has been reported only in domestic and wild canids. Domestic livestock and chimpanzees have only limited infections. Several human cases were reported during the initial decade after canine brucellosis was recognized, but few reports have issued in the past 20 years. The prevalence of canine brucellosis in dogs is not known, but it is probably as high now as in 1966 when the disease was first described. Most cases are unreported since only a few states require the disease to be reported. After oral-nasal infection, a leukocyte associated bacteremia is commonly detected within 2- 3 weeks, with more than 103 bacteria present per milliliter of blood. The bacteremia may be as short as 6 months, but it commonly persists longer than 1 to 2 years and has been observed as long as 5 years after infection. Brucellae localize in the lymph nodes, spleen, bone marrow and reproductive tracts of males or gravid bitches. Organisms have been recovered from the prostate glands of infected males for as long as 5 years post-infection (PI), months after agglutination tests become suspicious or negative. Infected, non-pregnant females are usually asymptomatic, except for enlarged lymph nodes; however, pregnant dams commonly suffer abortions

at -45-55 days of gestation. Early embryonic deaths and abortion 2 to 3 weeks after a mating have been reported and are often regarded as 'conception failures'. Infected males may become sterile. They usually have epididymitis, scrotal edema, and reduced sperm viability . More than 90% of sperm may be abnormal by 5 months PI. Chronically infected males often have atrophy of one or both testes. Spontaneous recovery of either sex may occur as early as 1 year PI, but it is more common after 2 to 3 years of infection; however, active infections may last more than 5 years, the longest period studied. Transmission of *B. canis* may occur at the time of an abortion, during a breeding, at estrus or, less commonly, through prolonged contact with contaminated urine of male dogs. Infected males harbor organisms in the prostate gland and epididymides for many months after the bacteremia has ceased and may disseminate the disease in semen at the time of a breeding or, as noted, via urine. The most common source of infection in kennels is contact with organisms shed for 4 to 6 weeks in the vaginal discharges following an abortion and during estrus. *B. canis* is relatively short-lived outside the dog and is readily inactivated by common disinfectants. Clinical signs are not adequate to diagnose canine brucellosis, although the disease must be considered whenever there is a history of abortions or poor reproductive performance in either sex. Most infected dogs will have enlarged and firm lymph nodes which are most readily demonstrated by palpation of the retropharyngeal or external inguinal nodes. The possibility of canine brucellosis also should be considered in cases of diskospondylitis.

2. SEROLOGIC TESTS

Brucella canis, like *B. ovis,* is naturally rough (R). Unlike *B. ovis,* growth of wild-type *B. canis* on liquid or solid media is mucoid (M+), a property that adversely affects antigen preparation and the development of specific diagnostic tests.

Tests Available	Antigen	Seropositivity	Comments
1. 2-ME Rapid Slide Agglutination using *B.*	Cell wall	~5-8 weeks post-infection until dogs	Sensitive, rapid Negative reactions accurate. Additional

ovis or *B. canis*(**M**-) (2-ME RSAT)		Become abacteremic. Results variable.	tests required. False-positives less with *B.canis* (**M**-).
2. 2-ME Tube Agglutination (2-ME TAT)	Cell wall	Similar to RSAT.	Semiquantitative. False-positives common. Titers>1:200 generally indicate infection.
3. Agar-gel immuno-diffusion (AGID1)	Cell wall (LPS)	Similar to RSAT; antibodies may be detected~1-2wks earlier.	Highly sensitive. Difficult to interpret. Not recommended. False positive reactions common.
4. Agar gel immuno-diffusion (AGID2)	Cytoplasmic proteins infection.	~8-12weeks post-Antibodies persist several months bacteremia subsides.	Highly specific. Onset of precipitins variable. Detects chronic cases. Detects infections by other *Brucella sp.*
5. Enzyme-linked immunosorbent assay (ELISA)	Cell wall (LPS) or CPAg (*B. abortus)*	Unknown	Experimental. Good results reported with mutant (M-) *B. canis* cell wall extracts or *B. abortus* CPAg
6. Indirect fluorescent antibody (IFA).	Cell wall (LPS)	Unknown	No published data. Sensitivity unknown.

3. SEROLOGIC RESULTS

Tests are further confounded by the cross-reactivity of *B. canis* with other, mostly unknown, organisms. The canine brucella is similar to *B. suis* in its biochemical reactions, but it is antigenically similar to B. ovis, lacking the somatic lipopolysaccharide (LPS) side chains of S-brucella.

Of the tests noted above, four types are most commonly used by diagnostic laboratories: the 2-ME RSAT, a presumptive (screening) test (D-Tec*CB) that uses *B. ovis* as antigen and is produced commercially by *Synbiotics Corp.* (Kansas City, MO); the modified 2-ME RSAT that employs the M-(less mucoid) mutant *B. canis;* the agar-gel immunodiffusion test (AGm2) that utilizes cytoplasmic proteins from *B. canis;* and an

indirect fluorescent antibody (IFA) test. Unfortunately, there is no published information on the IF A test in regard to sensitivity or specificity . Formerly, the 2-ME TAT was used extensively, employing an antigen formerly produced by the USDA, but antigen is no longer available since results were similar to those obtained with the 2-MB RSAT.

Advantages of the 2MB- RSAT is its commercial availability as a screening test, for the test is highly sensitive and false negative results are rare. Specificity has been substantially increased with the use of the 2MB-RSAT that employs the *B. canis(M-)* antigen. It is highly sensitive and more specific than the 2-MB RSAT *(B. ovis* antigen) or the 2-MB TAT *(B. canis* antigen). The modified 2-MB (M-)RSAT has resulted in the reduction of false-positive reactions to less than 10%. It is now used by the Diagnostic Laboratory at Cornell as a screening test.

The AGm2 test is used by a few laboratories that utilizes soluble protein antigens extracted from the cytoplasm of *B. canis,* or other *Brucella sp.* . The cytoplasmic protein antigens (CP Ag) are highly conserved in the genus *Brucella* and are completely cross-reactive with antibodies to other members of the Genus *Brucella*.

Other serological methods have been used occasionally, or in experimental studies. They include counter-immunoelectrophoresis, indirect immunofluorescence, complement-fixation, and enzyme-linked immunosorption tests (ELISA), using wild type *B. canis* or the more specific variant as antigen. With the exception of the 2-MB RSAT, 2-MB (M-)RSAT, and the AGm2 test that utilizes CP Ag, the other tests have not been critically evaluated. As a consequence, difficulties in the interpretation of test results are common. The most important test criteria are the nature, purity and specificity of the antigen used, and its sensitivity - not the type of test used. No animal should be judged as "positive" (infected) on the basis of tests that utilize cell wall antigens, i.e. agglutination tests. Because of the prolonged bacteremia, blood cultures should be done on all seropositive dogs that have not received antibiotic treatment.

Several experimental ELISA test procedures have been reported, but two methods have been published that appear to be sensitive and specific. One employs LPS extracted from *B. canis* (Mstrain); the other utilizes cytoplasmic proteins of *B. abortus.* Published results indicate significant advantages of both tests over the commonly used

agglutination methods and they appear to warrant serious consideration for further development and use in diagnostic laboratories, or as rapid ELISA kits for veterinary office tests. Such tests would require the critical study of sera from known infected, false-positive and normal dogs before they could be recommended. As noted, an indirect fluorescent antibody (IFA) test is used by some laboratories, but data on its accuracy have not been published. The limited data available suggests that false-positive reactions are common and that the sensitivity of the FA test is relatively low

4.USE OF SEROLOGIC RESULTS

The current status of the serodiagnosis of *B. canis* infection poses several problems for veterinarians and diagnosticians because little published data is available on several of the tests used by some diagnostic laboratories. Also, there is dissimilarity in the nature of the antigens used, experience in performing the tests and the failure of many diagnosticians to insist on use of more definitive follow-up procedures such as the AGm2 test and hemoculture. On the other hand, certain laboratories employ appropriate antigens, have experience and are aware of the pitfalls of serological methods in common use.

Contributed by: Sang Shin and Leland Carmichael

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SEROLOGY INTERPRETATION

BOVINE VIRAL DIARRHEA VIRUS (BVDV)

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1. BOVINE VIRAL DIARRHEA VIRUS

Disease in cattle induced by BVDV was first reported in 1946. Initial reports described a clinically severe enteric disease accompanied by fever and leukopenia. Most infections with BVDV result in subclinical to mild disease. In addition to the enteric disease that was described originally, BVDV also induce respiratory disease, reproductive failure, thrombocytopenic purpura, persistent infection, and mucosal disease. The virus is widespread and infects cattle of all ages. Two biotypes of BVDV, termed noncytopathic and cytopathic, are identified based on cytopathic effect in cell culture. Cytopathic BVDV induce cytoplasmic vacuolation and cell death. This occurs within 24 to 48 hours after infection with cytopathic BVDV that have been adapted to cell culture; however, field isolates of BVDV may require a longer incubation period before cytopathic effect is observed. Noncytopathic BVDV infect cell cultures without inducing an adverse effect. The majority of BVDV are noncytopathic in cell culture. Viral biotype does not correlate with virulence in cattle, and cytopathic BVDV are not antigenically distinct from noncytopathic BVDV. Two viral genotypes of BVDV, termed type 1 and type 2, have been identified based on differences in nucleic acid sequence of viral genomic RNA. Type 1 BVDV are antigenically related to type 2 BVDV, but the viral genotypes can be differentiated serologically. Noncytopathic BVDV of either genotype can induce persistent infection following fetal exposure with virus during the first four months of fetal development. Persistent infection is rare, lifelong induration, and an important factor in spread of BVDV. Outbreak of disease may occur after introduction of a persistently infected animal into a herd of susceptible cattle. Current taxonomic classification places BVDV in the Flaviviridae family. Hog cholera virus of swine, border disease virus of sheep, and BVDV form the Pestivirus genus. All pestiviruses are antigenically related but can be differentiated serologically and genetically.

2. SEROLOGIC TESTS

Viral Neutralization Test -- Most veterinary diagnostic laboratories rely on the viral neutralization test to detect antibody against BVDV. This test usually is done in microtitration plate wells that contain serial two fold dilutions of serum and approximately 100 to 1,000 cell culture infective doses of laboratory adapted cytopathic BVDV. The endpoint of viral neutralizing antibody titer is determined as the last dilution of serum that protects against cytopathic effect in cells. Duration of the test varies among laboratories from 2 to 7 days. Laboratories also vary in choice of cytopathic reference virus and cell type used in the test. Variation in choice and dosage of virus, cell type, and duration of test might explain the range of titers reported when the same samples of sera are tested in several different laboratories. Viral neutralizing antibody titer may be used to verify recent viral exposure by testing paired samples of sera, and to determine the viral genotype responsible for a recent disease outbreak.

ELISA Test -- Several ELISA tests for BVDV have been described, but few veterinary diagnostic laboratories in the United States use ELISA tests for detection of antibody against BVDV. The ELISA test is more rapid than the viral neutralization test, it is less expensive, and it can be automated. The sensitivity and specificity of most reported ELISA tests are approximately 95% when compared with viral neutralization tests. The disadvantages of ELISA tests are that they are more susceptible to technical problems than the viral neutralization test, and results from an ELISA test are not as clinically useful as viral neutralizing antibody titer. This is because protective immunity against BVDV appears to correlate with viral neutralizing antibody titer. Most ELISA tests for BVDV do not distinguish between neutralizing and non-neutralizing antibody; therefore, results from an ELISA test may not correlate with viral neutralizing antibody titer.

Other Serologic Tests -- Complement fixation tests, immunodiffusion tests, hemagglutination tests, and immune electron microscopy are seldom used for detection of antibody against BVDV. Indirect fluorescent antibody staining or indirect immunoperoxidase staining also are used infrequently for detection of antibody against BVDV.

Commercial Serologic Tests -- None are federally licensed for detection of antibody against BVDV.

3. SEROLOGIC RESULTS

Viral Neutralizing Antibody -- In general, BVDV stimulate high titer of viral neutralizing antibody after natural exposure of cattle ($\geq$1,024). The viral neutralizing antibody titer stimulated by modified-live virus vaccines usually approaches that detected after natural exposure with field virus. Viral neutralizing antibody titer stimulated by inactivated-virus vaccines often is $\leq$128. However, there is tremendous individual animal variation in titer of viral neutralizing antibody stimulated by BVDV or by vaccines for BVDV. This makes diagnostic interpretation difficult if based on serologic results from a single viral neutralization test. Paired samples of serum obtained from multiple animals at intervals of 2 to 3 weeks are preferred for purposes of diagnostic interpretation of events related to outbreaks of clinical disease. Also, antigenic differences between BVDV of different viral genotype can complicate diagnostic interpretation of viral neutralizing antibody titer.

A differential viral neutralization test that uses a cytopathic BVDV from each viral genotype is useful with convalescent serum to identify the viral genotype responsible for a disease outbreak in nonvaccinated herds. The titer of viral neutralizing antibody usually is 10 to 100 fold higher against the viral genotype of exposure (2,048 vs 64). In vaccinated herds, the differential viral neutralization test yields results similar to those obtained in nonvaccinated cattle when the viral genotype of exposure is identical to the viral genotype contained in the vaccine administered to the herd. When the viral genotype of exposure is different from the viral genotype contained in the vaccine, there may not be an appreciable difference in antibody titer between viral genotypes. Alternatively, the "original antigenic sin" phenomenon may occur and the antibody titer against the vaccine virus may be extremely high ($\geq$16,383) and the antibody titer against the viral genotype of exposure may be $\geq$1,024. In the absence of exposure with field virus, the viral neutralizing antibody titer stimulated by recent vaccination with inactivated viral vaccines usually varies from 16 to 256 against the vaccine virus, and 0 to 32 against BVDV of the genotype not included in the vaccine. The viral neutralizing

antibody titer stimulated by live virus vaccines usually varies from 64 to 2,048 against the vaccine virus, and 8 to 256 against BVDV of the viral genotype not included in the vaccine.

Duration of viral neutralizing antibody in serum after natural exposure with BVDV is unknown; however, current speculation is that antibody induced by natural exposure persists for the life of the animal. Similarly, duration of viral neutralizing antibody in serum after vaccination with a live virus vaccine is unknown, but it is thought that antibody induced by live virus vaccines remains in serum for at least one year. The duration of viral neutralizing antibody stimulated by inactivated vaccines is less than one year when measured against BVDV antigenically different from the vaccine virus.

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SEROLOGY INTERPRETATION

CAPRINE ARTHRITIS-ENCEPHALITIS VIRUS

CAPRINE LENTIVIRUS

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1. Caprine Arthritis - Encephalitis Virus (CAEV)

The CAEV was first described by Crawford, et al, in the late 1970's from goats with chronic arthritis, and on rare occasions, encephalitis. The seroprevalence of infection in the domestic goat population in the United States is approximately 85%. Goats with CAEV antibodies are usually subclinical, but may progress onto disease (arthritis, mastitis, pneumonia, and encephalitis). There may be a genetic predisposition for goats to progress on to disease (progressor).

There is currently no vaccine for CAEV. Once goats seroconvert to CAEV, they are regarded as seropositive/infected for life. False positives may occur in goat kids which have ingested colostrum from CAEV positive does. For this reason, kids should be retested after 6 months of age.

Due to the high rate of subclinical infections (70-80%), seropositive status is a marker for infection and when observed in conjunction with clinical signs of arthritis, a tentative diagnosis can be made. The use of histopathology on affected regions of joints serves to confirm hyperplastic lymphoid response rather than a septic (bacterial, mycoplasma, chlamydial) response.

2. SEROLOGIC TESTS/ 3. SEROLOGIC RESULTS

a. There are several serologic assays available for the detection of exposure to CAEV. These are the agar gel immunodiffusion (AGID); ELISA, K-ELISA, and immunoprecipitation (IP). The IP is primarily used as a research tool.

b. The assays measure a range of antibodies to the envelope gp135, and to the core protein, p28. The test results are generally reported as positive for negative. The AGID assays is widely used due to its simplicity and rapid test time (48 hours). The majority of commercial assays use a cross-reacting antigen, ovine progressive pneumonia (OPP). There is a lack of sensitivity resulting in 15 to 25% false negatives. More current assays used CAEV antigens and the sensitivity approaches 98% 2-4 weeks after infection by AGID. The ELISA/K-ELISA can detect infected goats as early as 2-3 weeks after infection.

c. The K-ELISA using CAEV antigens appears to be the most sensitive and specific compared to the ELISA or AGID.

d. The sensitivity/specificity of the K-ELISA is 95% and 100% respectively compared to IP. A suspect zone includes about 3% of the samples tested. Suspect goats are retested within 90 days to determine their serologic status.

4. USE OF SEROLOGIC TEST RESULTS

a. Single serum results indicate infection or not. A second serum within 90d covers the range of possible incubation period for CAEV. The serology test has clinical utility when used as part of herd surveys and assessment of management intervention (pasteurization of colostrum).

b. The assays are reported out as positive or negative. Suspect or indeterminate results may be reported by some labs if ELISA results are in the gray zone.

c. Positive serology results for CAEV indicate infection has occurred. A disease prognosis cannot be made at this time. The results can be used to manage herds, screen replacements, etc.

d. Overall benefit and use of serology results is primarily in herd management and eradication of CAEV infection.

Key References

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Contributed by: Jim Evermann, Ph.D.

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SEROLOGY INTERPRETATION

AVIAN CHLAMYDIA

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1. CHLAMYDIA

Chlamydia psittaci of avian hosts has worldwide distribution. It is known to infect over 150 species of birds and it is of public health importance, especially to pigeon fanciers, caged psittacine bird owners, poultry slaughter plant workers, wildlife biologists, and aviary caretakers. Inapparent infections are a hallmark of chlamydial infections in birds.

The prevalence is variable depending upon the extent of shedding of the agent and presence of a susceptible population. Incidence varies from 2 to 5% in wild psittacine bird populations and may be 30 to 50% in feral pigeons. In birds crowded together in aviaries, during shipment, or other situations the incidence may reach 75 to 100%.

Transmission from bird to bird or from bird to human normally occurs by inhalation of infectious aerosols from dried nasal secretions or feces, but may occur directly during close contact. Clinical signs in birds vary from none in the so-called silent or inapparent carriers to one or more of the following: severe depression, ruffled appearance, dyspnea, biliverdinuria, conjunctivitis, purulent nasal discharge, and inappetence.

Pathogenicity is variable depending upon the particular "strain" and upon susceptibility of different types of birds. Pathogenicity differences in birds apparently is no indication of pathogenicity for humans. In humans, the disease may be acute or chronic with an insidious onset. The most effective treatment is the tetracyclines which are chlamydiastatic and must be administered over an extended period of time.

2. SEROLOGIC TESTS

Elementary body agglutination (EBA) is recommended as the primary testing method because it detects IgM activity in birds. A titer $\geq$10 in budgerigars, cockatiels, or lovebirds is considered to be indicative of a current infection, but this titer is considered

to be suspicious of a current infection in other types of birds. An EBA titer of ≥20 in any type of bird is considered as positive evidence of a current infection.

Direct complement fixation (DCF) detects only IgG activity in birds. It is not of much value in making a diagnosis unless a first serum sample is negative (<10) after which antibody activity usually is detectable in 5 to 7 days in acutely infected birds.

One laboratory does some testing by indirect ELISA for their own research. Another laboratory offers indirect immunofluorescent testing for diagnostic purposes. Those two methods need additional comparative testing to evaluate their usefulness. There are no USDA- approved commercial serologic testing kits in the U.S.A.

3. SEROLOGIC RESULTS

The sensitivity of serologic methods for avian antibody activity probably is high (95 to 100%). Specificity probably is fair to good (85 to 90%) although it has not been accurately determined due, in part, to the culturing for chlamydiae being a poor "gold standard." That is because of intermittent shedding, especially in low-grade chronic infections in which antibody activity is detectable.

Serologic results should not be affected because of vaccine administration because, currently (1996), no efficacious vaccine exists. Exposure of naive birds to infected birds apparently requires rather close contact for transmission to occur. The duration of contact prior to transmission evidently depends upon numbers of chlamydiae being shed in secretions and excretions.

The IgM response usually decreases fairly rapidly after infected birds are treated with chlamydiastatic antibiotics. The time required for decreasing to an undetectable level apparently depends upon the magnitude of the titer attained prior to antibiotic administration. The IgG titer usually declines slowly over several months to a few years, depending upon its magnitude attained prior to treatment, and is indicative of a past infection whenever an IgM titer is no longer positive. In cockatiels, the IgG production most often decreases rapidly following treatment.

Serologic results are usually positive in infected birds that are not clinically ill. The magnitude of the titers may vary depending upon the type of bird. Clinically ill birds are likely to have higher titers then those without signs of infection and they are more likely to be shedding chlamydiae. Increased antibody production apparently results because of greater antigenic stimulation due to increased multiplication of chlamydiae.

Extraordinarily long maintenance of IgM and IgG titers oftentimes occurs in birds after apparent spontaneously resolved infections and in those cured by antibiotic administration. This occurs in various species and the phenomenon is not understood at present (1996). The prolonged titer maintenance, especially in clinically normal birds, may necessitate clinical laboratory testing such as WBC counts, measurement of liver function enzyme or bile acid levels in serum, or even chlamydial isolation attempts.

4. USE OF SEROLOGIC TEST RESULTS

Single serum samples which yield positive EBA results can identify infected birds.

Testing a second sample may be necessary if a chlamydial infection is strongly indicated by signs of clinical illness and results on the first are negative. Negative results on a second sample are usually indicative of a diseases in which signs are similar to chlamydiosis, e.g., salmonellosis.

Testing of a second sample in 2 to 3 months after chlamydiastatic antibiotic treatment of an infected bird may be used to assess the efficacy of the treatment as indicated by significant, i.e., ≥4-fold titer decreases.

The DCF method may be used to determine that a past infection has occurred whenever EBA results are negative.

Magnitude of the serologic response is variable, apparently depending upon the length of time the infection has been present, the immunocompetence of the individual bird, and possibly the pathogenicity of the chlamydial "strain" involved.

Inappropriate uses of serologic testing occur whenever paired of multiple samples are not collected at appropriate intervals, whenever an additional serum sample is not

tested even though that is indicated, and testing of a serum sample that is collected as an afterthought following several days of administering chlamydiastatic antibiotics which may delay or even preclude antibody production to a detectable level.

The overall benefit of serologic testing by EBA is that serologic testing yields results quicker than and is less expensive than culturing. In virtually all cases, serologic testing is more sensitive than and more specific than culturing. Results of DCF, however, may not be practical to use for making a diagnosis without undue delay whenever a first sample has a moderate to high titer because DCF titers usually change slowly.

Contributed by: James E. Grimes, Ph.D.,

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SEROLOGY INTERPRETATION

EQUINE RHINOPNEUMONITIS

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1. Equine Rhinopneumonitis

Equine Rhinopneumonitis (ER) is a term used to describe a highly contagious infection of horses caused by two closely related herpesviruses, equine herpesvirus-1 (EHV-1) and equine herpesvirus-4 (EHV-4). The viruses are worldwide in distribution, infect members of Equidae family, and occasionally other species, i.e. llamas.

Infection is characterized primarily by respiratory tract disease with severity affected by age and immune status of the animal. Respiratory tract disease is usually seen in young animals, foals, yearlings, young animals in training or racing. Subclinical infection is common in all ages of horses. Following recovery from infection, many animals become latently infected with the virus. EHV-1 may spread beyond the respiratory tract, producing viremia and causing central nervous system infections or abortion in pregnant mares. EHV-4 is primarily associated with respiratory disease and occasionally has been isolated from aborted fetuses.

2. SEROLOGIC TESTS

Virus neutralization test is probably the most frequently used test and is relatively easy to perform in a laboratory equipped to do cell culture. The disadvantage of the test is that it may be several days before results are available.

ELISA testing can be done with less time and expense involved, however, there is no commercially available test in the U.S. and development of the test in an individual laboratory could be expensive.

Complement fixation test is a labor intensive test requiring considerable technical skill but may be the test of choice when only a single serum sample is available to detect recent infection. However, this test is not widely available.

3. SEROLOGIC RESULTS

Virus neutralization can be a sensitive and specific test for detecting antibodies to EHV-1/EHV-4. Acute and convalescent serums are necessary. Infection and vaccination are common, making interpretation of a single serum sample difficult. Significant change in antibody titer may not occur with reinfections. Susceptibility to or protection from infection cannot be associated with any definite level of neutralizing antibody.

In cases of abortion, a single serum sample taken at the time of abortion may not be of diagnostic value. Any change in antibody titer may have e already occurred at the time of the abortion. Abortion due to ER can occur in the presence of neutralizing antibody. A wide range of antibody titers can be found in mares that have aborted. ER antibodies detected in the cerebral spinal fluid of horses with CNS signs ARE of diagnostic significance.

Because complement fixing antibodies are present after recent infection and are of relatively short duration, this test can be used when only a single serum sample is available.

Serologic differentiation between EHV-1 and EHV-4 infections may only be possible on primary infection because of the close relationship between the two viruses. All of the serologic tests detect cross reacting antibodies between EHV-1 and EHV-4.

Contributed by: Mary Lynne Vickers, Ph.D.

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SEROLOGY INTERPRETATION

FELINE ENTERIC CORONAVIRUS /

FELINE INFECTIOUS PERITONITIS VIRUS

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1. Feline Enteric Coronavirus (FECV)/Feline Infectious Peritonitis (FIP) Virus

Feline coronavirus was initially described in the mid 1960's as being associated with FIP based upon electron microscopy. In 1979, coronaviruses were isolated in culture from both FIP and from enteric infections of cats. Coincident with viral culture was the observation that cats with FIP were seropositive to other coronaviruses of swine (TGE) and dogs (CCV). In the early 1980's it was determined that there was a second coronavirus of cats referred to as feline enteric coronavirus (FECV). Based upon the information presented, there is a group of antigenically related coronaviruses of swine, dogs, and cats, which cross-react on serologic testing. This observation has direct bearing on the interpretation value of feline coronavirus based serologic assays.

The ecology of FECV in the domestic cat population indicates that as high as 85% of the domestic cats are seropositive to feline coronavirus. Exotic species of felids, such as the cheetah, have been reported to be highly susceptible to FIP and the seroprevalence rate may be as high as 65% in selected captive populations.

Pederson speculated that FIPV was a mutation of the more common FECV due to the occurrence (1-2% of infected cases) of FIP in closed cat colonies. Recent studies support this idea.

Cats infected with FECV are usually subclinical, but may progress onto mild diarrhea. Virus is shed primarily fecal-oral and is relatively labile outside the cat. There is evidence that FECV can be transmitted to dogs. Cats that develop FIP succumb within 3 weeks to 3 months in 100% of the cases. In addition to viral mutation (FECV ÷FIPV), there is mounting data to support a genetic predisposition to FIP.

2. SEROLOGIC TESTS/ 3. SEROLOGIC RESULTS

There are several types of serologic assays for detection of feline coronavirus antibody. None of the assays are specific for FIPV. The assays include indirect fluorescent antibody (IFA), ELISA, SN, and western blot (WB).

The IFA measures group specific antibodies. The height of the titer may be used to predict cats at risk to develop FIP. The K-ELISA is very sensitive, but the specificity is subject to question and may pick-up cross-reacting antibodies to FBS. The C-ELISA is commercially available (IDEXX, Inc.) and sets a threshold at or about 1:3200. A positive reaction is therefore considered to be at $\geq$1:3200. The SN test has been run on a limited research basis. Based on the profiles of limited numbers of cats, they may be IFA seropositive, but SN seronegative. WB profiles have been done on cats and cheetahs. The WB results correlate well with IFA.

4. USE OF SEROLOGIC TEST RESULTS

The sensitivity of the assays for detecting serum coronavirus antibody approaches 95%. The problem with the assays is when the attempt is made to use the results to diagnose FIP. At this time there is no serologic assay that will diagnose FIP. The predictive value of a high antibody titer offers 65-75% specificity when cats are clinical.

The clinical value of feline coronavirus serology results are debatable, since none of the assays currently being used can make a diagnosis of FIP. Serology can be used in conjunction with electron microscopy and/or polymerase chain reaction to detect carriers/shedders of feline coronavirus. These assays may be of some value if a cattery is striving to be feline coronavirus- free. The serologic assays in use can be used to monitor cats pre-sale or pre-breeding. At this time, the benefit of coronavirus serology is primarily on a population basis to assist in management.

The use of commercially available ts mutant vaccine given ocular-nasal (Primucell7) will result in antibody titers within the normal range (1:25 IFA to 1:3200 IFA).

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SEROLOGY INTERPRETATION

JOHNE'S DISEASE IN CATTLE

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1. JOHNE'S DISEASE IN CATTLE

Johne's disease caused by *Mycobacterium paratuberculosis*, initially infects the ileum and gradually spreads to regional lymph nodes and other body organs. Initial clinical signs, following prolonged incubation period of two to ten years, include gradual weight loss despite normal appetite. Other than the loose consistency the feces appear normal. As the disease progresses, the affected animals become increasingly lethargic and emaciated. Cachexia and ?waterhose? diarrhea characterize the terminal stages of the disease.

2. SEROLOGIC TESTS/ 3. SEROLOGIC RESULTS

Three serologic tests for the detection of antibodies in serum of cattle infected with *M. paratuberculosis* are available. These include the agar gel immunodiffusion test (AGID), the complement fixation test (CFT) and enzyme-linked immunosorbent assays (ELISA). The AGID test has the highest specificity and a reasonable sensitivity (90% plus) in cattle with clinical signs compatible with Johne's disease. Infected cattle without clinical signs rarely are positive on AGID.

The CFT, which is required by many countries for export or for import. Is considered intermediate in sensitivity and specificity to AGID and ELISA. With many false positives and false negatives, the CFT is not recommended for routine use. It is important to emphasize that the antigens used in the different assays vary considerably in composition depending on the method of preparation.

ELISAs detecting antibodies in the sera of cattle with Johne's disease have been described. Several of the ELISA tests have not been widely proven nor accepted by many investigators since published data and critical review has not been conducted in

several laboratories. There are ELISA tests for Johne?s commercially available, with USDA approval.

A commercially available ELISA has been widely used for screening herds. Positive ELISA tests occur most frequently in cattle with high numbers of *M. paratuberculosis* in the feces. One paper describes the ELISA sensitivity for the clinical cases as 87%, while the sensitivity was about 15% for subclinical light shedding cattle. In several published reports, the overall sensitivity ranged from 40% to 50%. However, most of these reports did not include cattle in the earliest stages of infection, cattle known to be infected but not yet positive by fecal culture. Thus, the ELISA sensitivity to detect infected cattle seems correlated with the stage of disease. The more advanced the disease, the greater the test sensitivity. Conversely, animals in the early stages of infection, especially those less than two years of age are often ELISA negative. Specificity was excellent for the cattle not infected with *M. paratuberculosis*. ELISA positive animals can be confirmed infected with an organism based test, such as fecal culture or a DNA probe. With a specificity of 99%, one animal in a hundred uninfected animals will be ELISA false positive. Alternately, animals ELISA negative should not be regarded as not infected with *M. paratuberculosis*, but as infection not detected. The ELISA should not be used to indicate animals are not infected such as may occur with pre-purchase examinations, prior to introduction into a herd. The primary use for the ELISA is to screen herds of cattle. If all cattle are negative on ELISA, the herd history is clean for the past five years and it is likely the herd is Johne's negative.

4. USE OF SEROLOGIC TEST RESULTS

Cattle vaccinated for Johne?s as neonates often give false positive ELISA results. However, it is not easy to differentiate the positive ELISA due to vaccination or true infection with *M. paratuberculosis*. Most states allow the vaccine to be used in herds that are known to be infected with *M. paratuberculosis*.

The serologic tests are most useful for identifying cattle with clinical disease and are of limited value in detecting animals with subclinical disease with the exception of animals

shedding large numbers of *M. paratuberculosis* in feces. It has been suggested that the ELISA test may be used to identify herds free of disease; however, three or more complete herd negative tests (animals over two years of age) conducted at 12 month intervals are necessary. Serologic tests are not typically acceptable to confirm a diagnosis of Johne's disease. Confirmation of Johne's disease should be made with an organism based test (i.e. fecal culture).

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SEROLOGY INTERPRETATION

LEPTOSPIRA

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1. *LEPTOSPIRA*

Leptospirosis is caused by the genus *Leptospira*, of which there are more than 200 serovars. The disease was first diagnosed in the US in humans and rats in the early 1900's. From the 1930's through the 1950's, it was reported from nearly all wild and domesticated animal species except birds. The disease is found worldwide, generally transmitted via contaminated water, and usually has two distinct phases: 1) leptospiremia and fever for about a week, 2) then a leptospiruria, which may persist for 2 to 3 months. Various clinical signs are noted, but fever, icterus, kidney failure and abortion (reproductive failure) are the primary ones observed.

2. SEROLOGIC TESTS

The microscopic agglutination test (MAT), using live leptospires, is the primary serologic test in use. Other available techniques include ELISA, indirect hemagglutination, complement fixation, and the macroscopic plate agglutination tests. There are currently no commercially available tests.

The MAT is sensitive and serovar/serogroup specific; the disadvantage is the maintenance of live leptospiral cultures and the associated health risks. The other test mentioned are not universally accepted and are not routinely used in diagnostic facilities.

3. SEROLOGIC RESULTS

A vaccination history is extremely important. Vaccination of animals will usually result in serologic titers, which may persist for 2 to 3 months. If the vaccine status is unknown, it

is presumed that titers are vaccine related when 3 or more serovars react in a single sample. In dogs, if *icterohaemorrhagiae* and *canicola* both react, that is probably vaccination. Protection due to vaccination should last at least a year, with the exception of *hardjo*, where cows may have to be given a booster dose every 6 months.

A clinically significant titer for most serovars is generally greater than or equal to 1:800. One exception is with *hardjo* in cattle, where titers of cows with reproductive failure may be as low as 1:100 to 1:200. Secondly, dogs with *grippotyphosa* may be 1:200 to 1:800. Titers are usually maximal at the time of abortion, and may persist indefinitely if the animals are continually exposed to leptospires, e.g., contaminated pond water, etc.

4. USE OF SEROLOGIC TEST RESULTS

A single sample is helpful if there is a titer greater than or equal to 1:800 to a single serovar. Since the titer is usually at a maximum at the time of abortion, paired samples are generally unrewarding and usually difficult or impractical to obtain from livestock. When paired samples are tested, both sera must be tested at the same time with the same antigen lot. The most practical method for serologic diagnosis is to get at least 10 samples from a herd that include both known affected and nonaffected animals. This approach is also preferred by the livestock producer from an animal handling standpoint.

One example of successful interpretation is manifested with reproductive problems in swine. A swine practitioner has stated that he has been very successful in correcting these problems by recommending vaccinations with a leptospiral bacterin containing *bratislava* in clinically affected, unvaccinated herds that have *bratislava* titers in multiple serum samples.

With the "relatively recent" report of leptospirosis in Kentucky horses, interest in the equine disease has increased but care should be exercised in evaluating leptospiral titers in horses. Many horse serum samples will react, with fairly high titers, to multiple serovars and interpretation becomes very difficult. Therefore, diagnosis of equine leptospirosis should be based on a very high serologic titer ($\geq$ 1:6400), or microscopic

examination (FA, histopathology, or dark field microscopy) on the fetus. Any positive MAT titer on fetal fluid is significant.

In summary, serology can be of value if vaccine and clinical history are known, and proper sampling of animals in the herd is done.

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SEROLOGY INTERPRETATION

NEOSPORA CANINUM IN CATTLE

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1. *NEOSPORA CANINUM*

Neospora caninum, identified in 1984 in association with paresis in puppies, was first described in a bovine abortion epidemic in 1989. Prior reports of encephalomyelitis in calves due to *Toxoplasma*-like protozoal infections have since been retrospectively diagnosed as due to *Neospora caninum*. Though relatively recently described, serologic evidence suggests that neosporosis in cattle is not a new disease. Clinically, *Neospora caninum* is associated with abortions in the 3rd to 7th month of gestation. Other than abortion, infected cows do not exhibit clinical signs of disease, however lower milk production and earlier culling risks have been associated with infected cows. Vertical transmission, from seropositive dam to fetus, is the only demonstrated route of transmission, and can account for ~95% of the seropositive animals in a herd. Congenital infection appears to have no detrimental affect on calf health, and clinically inapparent infection persists for the life of the animal. *Neospora caninum* is genetically related to *Toxoplasma gondii*, and a similar life-cycle that would include a definitive host such as carnivorous cats, dogs, rodents, or birds. The dog has been suggested as the definitive host, but experimental or natural transmission between dogs, or any definitive host, and cattle has not been documented. Direct contact with infected fetal tissues has also been postulated, but never proven, as a source of post-natal infection.

Neospora caninum occurs in cattle worldwide. Seroprevalence within herds varies widely, from serologically negative herds to herds with >90% of cows affected. The average dairy herd seroprevalence appears to be ~35%. Beef herds have a lower overall seroprevalence, ranging from unaffected to 15%, with an average of <5% of cows in the herd seropositive.

2. SEROLOGIC TESTS

Indirect fluorescent antibody test -- *Neospora caninum* tachyzoites (most commonly the NC-1 isolate) propagated in cell culture, are used as antigen in *Neospora* IFA tests. Approximately $3X10^4$ intact tachyzoites per well or spot have been recommended as a standardized test concentration. Sera is tested in serial dilutions ranging from 1:40 to 1:20480, or at a screening dilution of 1:200, dependent on specific protocol. A positive reaction is visualized as complete peripheral fluorescence of the tachyzoites. Fluorescence of the apex of the tachyzoite is considered non-specific, and is not interpreted as a positive reaction in the absence of peripheral fluorescence. Apical fluorescence is presumed to occur due to cross-reaction with other members of the Apicomplexa parasites, including *Eimeria* sp., *Cryptosporidia* sp., *Sarcocystis* sp., *Hammondia* sp., and *Toxoplasma* sp. IFA Slides are commercially available from VMRD, Inc.

ELISA Tests -- Various ELISA assays, including competitive ELISA and recombinant antigen ELISAs, have been reported in the literature, however are not currently available for routine diagnostic use. The kinetic ELISA is currently available in a limited number of veterinary diagnostic laboratories. All ELISA tests currently available in the U.S. utilize tachyzoite antigens from the *Neospora caninum* (NC-1 isolate).

3. SEROLOGIC RESULTS

Various endpoints for classification of a positive *Neospora* IFA reaction have been reported in the literature:

	Sensitivity	Specificity	Overall correct Classification
IFA at a dilution of 1:200	NR	NR	
IFA at a dilution of 1:320	90%	82%	86%

IFA at a dilution of 1:640	87%	92%	90%

*NR = not reported

Kinetic-ELISA: A Vmax S/P ratio of ≥0.45 is interpreted as positive for the kinetic ELISA.

	Sensitivity	Specificity	Overall correct Classification
kinetic ELISA	89%	97%	93%

IDEXX, Inc ELISA: utilizes a similar antigen as the kinetic ELISA, with S/P ratios >0.50 reported as positive. The commercial ELISA has reported similar or greater sensitivity and specificity.

4. USE OF SEROLOGIC TEST RESULTS

Serology is a useful addition to existing bovine abortion serology screens. A negative ELISA test for an aborted cow can be used to exclude *Neospora caninum* as the cause of abortion in individual cows. Because not all seropositive cows abort, a positive antibody test confirms infection, but not abortion. Positive fetal serology can be used to indicate infection, but should be interpreted as evidence of infection and not necessarily definitive for the cause of the abortion. To evaluate the role of *Neospora caninum* in abortion storms; an equal number of aborted and non-aborted cows at the same stage of gestation can be tested and seroprevalence compared between the 2 groups using a 2x2 table (chi-square, odds ratio analysis). Herds with an endemic form of *Neospora* abortion show a 2 to 4-fold higher risk of abortion among seropositive cows when compared to their seronegative herdmates. In *Neospora*-induced abortion epidemics, the abortion risk among seropositives may be as much as 40-fold higher when compared to seronegative herdmates. Seroconversion from seronegative to seropositive status, indicative of post-natal transmission has been observed rarely, and

been reported at <3% of cows per year in endemically infected herds. Seroconversion, from seronegative to seropositive or seropositive to seronegative in paired samples following abortion is not typically observed with natural infection, though have been reported in experimental infections and occasionally in association with an abortion epidemic. Serologic status persists for the life of the cow. Passively-derived antibody (colostral antibody) persists for ~4-5 months.

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SEROLOGY INTERPRETATION

PORCINE REPRODUCTIVE

AND

RESPIRATORY SYNDROME VIRUS (PRRSV)

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1. PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRUS

The porcine reproductive and respiratory syndrome virus (PRRSV) is now endemic in the United States, Canada, Europe, and Asia. As diagnosticians in other areas of the world develop the techniques to diagnose this disease, the virus will most likely be found, confirming our suspicion that this virus has a world-wide distribution. For the swine practitioner, the key to the cascade of events that leads to a confirmed diagnosis of porcine reproductive and respiratory syndrome (PRRS) is the ability to recognize this disease. Because of the complex nature of the clinical signs and the fact that this disease can affect both the respiratory and the reproductive systems, and it can infect and affect a wide age range of swine, one must include PRRS in the tentative list of diagnoses of many clinical syndromes.

In the mid to late 1980's when PRRSV was infecting our naive swine populations, the clinical syndrome was more consistent. Many of these herds that were affected by PRRS had both reproductive and respiratory disease with the reproductive disease being the more severe. Typically, one would observe sows with moderate fevers (104 to 106), transient anorexia, early farrowing (108-110 days) resulting in a marked increase in stillborn pigs, followed by abortions of sows in earlier stages of gestation and an increase in mummified fetuses. Many of the pigs that were born alive were weak and died within hours. Those that survived were unthrifty, succumbing to bacterial diseases that were normally under control in that particular herd. These would include diseases such as streptococcosis, rhinitis and Glasser's disease (*H. parasuis*) and others. Often, preweaned and post-weaned pigs developed a severe respiratory disease, manifested by a rapid abdominal type of respiration, often referred to as "thumping". The PRRSV

replicates in the lung causing an interstitial pneumonia. Although this lesion suggests a PRRS infection, other viruses can cause similar lesions, so one must not immediately conclude that interstitial pneumonia equals PRRS.

As PRRS "matured" in our swine population, the clinical picture also changed. Classical cases of PRRS with reproductive and respiratory signs, as previously described, still occur. However, it is common to see either reproductive or respiratory disease independent of each other. Today, the respiratory syndrome of PRRS is more apparent than the reproductive syndrome.

One must include PRRS in the list of tentative diagnoses when dealing with difficult respiratory problems in nursery and grow/finishing pigs, especially those that are unresponsive to antibiotic therapy. Even respiratory diseases that have a known etiology like *Actinobacillus pleuropneumoniae* or *Salmonella choleraesuis* may be triggered or exacerbated by a PRRS infection.

2. SEROLOGIC TESTS

The indirect fluorescent antibody (IFA) test, serum virus neutralization (SVN) test, immunoperoxidase monolayer assay (IPMA) and enzyme-linked immunosorbent assay (ELISA) have been developed for the detection of antibodies specific for PRRSV. Among those tests, the IFA, SVN and ELISA tests are currently being used in most North American veterinary diagnostic laboratories.

The IFA is thought to have a high specificity (99.5%) but unknown sensitivity for individual animals. An advantage of the IFA test compared to ELISA is that the magnitude of the titer can be determined. A titer of 16 or 20, depending upon an initial serum dilution for the test, is considered positive. However, the endpoint of IFA titer would vary among technicians and laboratories performing the test since it is subjectively determined. Such subjective determination of the titer also limits the use of IFA test to testing small scale samples. Furthermore, test results (negative vs. positive, or titers) could be confounded by the PRRSV strain used in the test because of the antigenic diversity among PRRSV.

An IFA test for the detection of PRRSV-specific IgM antibodies has recently been developed. This test was reported to be useful to detect acute/recent PRRSV infection. A titer of 16 or 20 is considered positive. It is of interest that research data demonstrated a high percentage (81%) of isolation of PRRS from IgM-positive samples. However, false positive results due to the possible nonspecificity of the IgM IFA test are of concern and overall performance of the test, including the specificity and sensitivity, must be evaluated before the test will be widely used for routine diagnosis.

Several formats of ELISA have been described: indirect ELISA using sample to positive ratio (S/P) system, indirect ELISA using direct OD value, and a blocking ELISA. With respect to the ELISA kit from IDEXX laboratories Inc., a S/P ratio of 0.4 or greater is considered positive. The test is reported to be sensitive (100%) and specific (99.5%). Although the magnitude of the S/P ratio might be correlated with the magnitude of IFA titer, the manufacturer does not recommend interpreting the S/P ratio in this way at this time. Many veterinary diagnosticians/practitioners consider a S/P ratio of 2.5 or greater to be indicative of a recent or active infection. Automation which results in less variation and high quality control are considered to be a strong merits of the test. In addition, several other advantages of ELISA are: 1) detecting antibody against both American and European PRRSV strains; b) fast turnaround time; and c) licensure by USDA and AgCanada.

The SVN test is also considered to be a specific test, but previous studies have suggested that the SVN test is less sensitive than the IFA and ELISA tests. Currently, a titer of 4 is considered positive. A recent report indicated that the sensitivity of the SVN test could be increased by adding fresh normal swine serum to serum being assayed. Even so, the SVN test is best used as a research tool rather than a routine diagnostic test.

3. SEROLOGIC RESULTS

In pigs exposed to PRRSV under experimental conditions, virus-specific antibodies are reported to be first detected by the IgG-IFA, ELISA, and the SVN test at 7-11, 9-13, and

9-28 days PI respectively, and reach their peak value by 30-50, 30-50, and 60-90 days PI, respectively. PRRSV specific IgM antibodies are reported to be detected within 5 days PI and persist 21-28 days PI. It is generally estimated from experimental and field observations that the IFA, ELISA and the SVN antibody titers would approach undetectable levels by 4-5, 4-10, and 12 months, respectively. The same time frame should be expected in animals vaccinated without a history of previous exposure.

With respect to PRRS, the following information have been obtained based upon evaluation of 30,000 sera tested at Rollings Laboratory, NC.

1. Nonvaccinated gilts, replacement boars and adult breeding swine with S/P ratios of <2.25 may be viremic at the time of blood sampling. Sera with ratios of 3.5 to >5.0 are likely to be from recently infected animals. The same is observed for immature swine.

2. In evaluating infected herd that have been vaccinated, the following patterns emerged:

- For stabilizing breeding herds that have essentially negative nurseries (pigs are not being vaccinated), sows and boars will have S/P ratios from negative up to the low 2's but rarely greater than 2.5. Experimental vaccination of naive feeder age pigs results in S/P ratios in excess of 3.5. That magnitude of antibody response to vaccination, has not seen in vaccinated sows and boars in a stable, previously infected herd that has the desired farrowing rate and number of pigs weaned per sow per year.
- An ideal target to strive for vaccinated breeding swine is an average S/P ratio of 1.00. Typically, up to 20% of the vaccinates will be negative within 30 to 60 days after vaccination. A typical range of S/P ratios for a well vaccinated herd will be from 0.7 to 1.8, with some animals having a response above 2.0. When over 20% of the vaccinated breeding swine are negative within 60 days after vaccination, vaccine administration and handling should be evaluated if the breeding herd is receiving 2 doses of vaccine per year.
- In herds that receive 4 or more doses per year, it is not uncommon to see seronegative vaccinated animals exceed 20%. The basis of the lack of detectable humoral antibody is not known at this time. If those seronegative, probable immune vaccinates are subsequently infected with a field strain of PRRSV, most of them will become positive. When vaccinated herds have a reoccurrence of reproductive failure or an increase in poor-doing pigs associated with amplification of introduction of a field strain, the antibody levels shift. It is not uncommon to have sows with antibody levels of >3.0 due to superimposed infection, and as stated above, it is common to have all sows and boars positive by ELISA.

3. Regarding decay of maternal antibody in pigs from vaccinated sows, in pigs from sow herds with litter virus circulation in the breeding animals, 3 to 4 week-old piglets S/P ratios are between 0.7 and negative. Antibody levels of >1.8 are probably from viremic pigs that were weaned into the nursery. In a nursery with very little virus circulation, most piglets will be negative by 6 weeks of age. If many viremic animals are in the nursery, by 7 weeks of age S/P ratios will be increasing and many pigs will be positive with ratios of >1. By 9 to 10 weeks of age, pigs in a nursery with very active viral circulation will all be positive. Again, most of the positive pigs in the nursery from 7 to 10 weeks of age are likely to be shedding PRRSV.

4. Nursery pigs 30 to 80% positive when transferred to finishing will usually continue to become infected and seroconvert in the finishing floor facility. Mean S/P ratios will not necessarily increase during that time. They frequently do, but we see many floors with a high incidence of positive animals that rarely have S/P ratios of >1.8. That may be due to building design and pig flow.

4. USE OF SEROLOGIC TEST RESULTS

Several problems or limitations should be taken into account when interpreting PRRS serology. Serological information from a single sample is not sufficient for diagnosing clinical PRRS in an individual animal because PRRS virus infection is highly prevalent (80%) in US swine herds. For example, a serologically negative animal sampled one time has several possible interpretations:

- the pig was not infected with PRRSV;
- the pig was recently infected with PRRSV and not yet seroconverted;
- the pig was infected with PRRSV but has become seronegative; or
- the test employed was negative because of low sensitivity or laboratory error.

Therefore, it has been suggested that PRRS serology should be used primarily to determine if a herd has been exposed to PRRS virus.

Antibodies specific for PRRS virus may not persist for the lifetime of an animal. The relatively short duration of IFA and/or ELISA antibodies has led to the recommendation

to test young pigs in order to establish herd status of PRRS virus infection within herd. In single-site, farrow-to-finish swine herds, the seroprevalence of PRRSV infection is usually highest in the grow-finish unit. Serum from 10 finishing pigs is usually considered to be an adequate sample size to determine whether the herd has been exposed to PRRSV. For multi-site production systems, each stage of production represents a single population, so each site should be sampled.

Diagnosis of PRRSV infection as the cause of reproductive failure or respiratory disease can be achieved by showing seroconversion using paired samples. For a more definitive diagnostic evaluation of PRRS with respect to current infection, it is recommended that serological information should be interpreted in combination with results from virus isolation (e.g., viremia).

Using IgM IFA, IgG IFA and SVN tests, 3 categories of serologic profile have ben described to exist among pigs in herds exposed to PRRSV; noninfected, acutely infected, and pigs with antibody decay. Noninfected pigs can be identified as negative by all 3 tests. Acutely infected pigs were defined as pigs with IgM and/or IgG IFA titers of 64 but no detectable SVN antibody.

Some research data suggest that SVN antibody specific for PRRSV plays a role in clearing the virus from the blood circulation. However, prolonged viremia and persistent infection of PRRSV in the presence of circulating antibody, as well as antibody dependent enhancement of PRRSV infection by the presence of low concentration of virus-specific antibody, bring the protective role of antibody into question. Consequently, it is important to determine if the presence of neutralizing antibody correlates with protective immunity.

Broad antigenic variation among PRRS virus isolates is a concern in interpreting the serological information because false negative results may be due to the strain of virus in use at a diagnostic laboratory. Such potential problem may be overcome using the commercial ELISA kit because the IDEXX kit contains antigens from several different isolates of PRRS virus. In fact, this kit was shown to detect antibodies against both North American and European PRRS virus isolates.

Although many limitations and pitfalls of PRRS serological testings exist, the PRRS serology is still recommended to be used in serologic profiling of herds for: a) monitoring clinical disease process; b) evaluating donor and recipient herds PRRS status; and c) evaluating vaccine efficacy.

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APPENDIX

Most North American veterinary diagnostic laboratories have been using the IFA test, while in European laboratories have relied on the IPMA using PRRS virus-infected porcine alveolar macrophages. The recent licenser of a commercial ELISA is changing this picture. Advantages and disadvantages of each test are summarized in Table 1.

Table 1. Comparison of PRRS serologic assays

Tests	Advantages	Disadvantages
IFA	high specificity early detection	variable sensitivity subjective endpoint confound by antigenic variation
IPMA	high specificity and sensitivity early detection	subjective endpoint high background confound by antigenic variation
SVN	longer detection	low sensitivity time-consuming confound by antigenic variation

ELISA	automation high quality control	high background*

* This is a potential problem for ELISA in general but not with the commercial PRRS ELISA kit.

In a recent experimental study, it was estimated by regression analysis that the ELISA, IFA, and SVN antibody titers would approach the lower limits of detection by approximately days 137, 158, and 356 PI, respectively, assuming a constant rate of antibody decay.

IFA antibodies (IgG) is detected by 7-11 days post infection (PI), peak at 30-50 days PI, and may decline to undetectable levels by 4-6 months.

IgM antibodies can be detected by the IFA test within 5 days PI and persist 21-28 days PI.

Experimental evidence using the commercial ELISA kit indicates that PRRSV-specific antibodies appear by 9-13 days PI, rise to peak values by 30-50 days PI, and decline to undetectable approximately by 4-10 months.

Neutralizing antibodies against PRRSV develop slowly (9-28 days PI) and rise slowly for approximately 2-3 months. The SVN antibodies are estimated to exist at detectable levels for at least 1 year.