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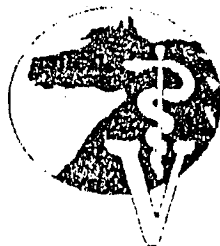

Refereed

PREDICTIVE METHODS TO DEFINE INFECTION WITH EQUINE INFECTIOUS ANEMIA VIRUS IN FOALS OUT OF REACTOR MARES

RS McConnico, DVM, PhD[1]; CJ Issel, DVM, PhD[2]; SJ Cook, MSc[2]; RF Cook, PhD[2]; C Floyd, DVM[3]; and H Bisson, MS[4]

SUMMARY

Equine infectious anemia (EIA) was diagnosed in a high percentage of horses gathered from public lands administered by the Bureau of Land Management in the area of the confluence of the White River and the Green River in Northeastern Utah in 1998. Twenty-three mares with foals by their sides were gathered in this area. The twelve mares that were positive on serologic tests for EIA were euthanatized. Their foals were moved to an isolation facility where they became subjects of a prospective study. The antibodies in the foals' serum against EIAV antigens were determined to be passive in origin, as levels declined and eventually became undetectable in official tests for EIA in all twelve foals. No evidence for the genetic material of EIA virus (EIAV) was found in sensitive reverse transcriptase-polymerase chain reaction (RT-PCR) assays of plasma samples from the foals collected at the time of separation from all test-positive adult horses, and during their 8 months in isolation. The passively acquired antibodies against EIAV antigens were detected with highest sensitivity in the immunoblot test. Of the official ELISA-based test kits, the competitive ELISA (CELISA) was highest in sensitivity, followed by the other ELISA assay (Vira-CHEK) for detection of antibodies against the major core protein (p26) of EIAV. These results correlated well with those obtained in the agar gel immunodiffusion (AGID or Coggins) test, the internationally recognized gold-standard serologic test for EIA, which also detects antibody against the p26 antigen. The least sensitive indicator for passive antibodies in these foals against EIAV was the SA-ELISA test, which detects activity against the transmembrane protein, gp45. From another perspective, the SA-ELISA test was the initial serologic indicator of lack of infection in these foals, with negative reactions a mean of ≥101 days earlier than in AGID tests. The results confirm that foals of test-positive mares can be raised free of infection with EIAV. The data indicate that the lack of detectable EIAV-RNA in plasma of foals with declining serum antibody titers against EIAV are excellent predictors for lack of infection in foals of test-positive dams.

Authors' addresses: [1]Oklahoma State University, College of Veterinary Medicine, Stillwater, OK 74078, [2]University of Kentucky, Gluck Equine Research Center, Lexington, KY 40546-0099, [3]Oklahoma Department of Agriculture, Oklahoma City, OK, [4]Bureau of Land Management, Washington, DC.
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INTRODUCTION AND MATERIALS AND METHODS

Equine infectious anemia (EIA) is caused by a lentivirus specific to members of the family Equidae which induces persistent infection in its hosts. Despite the existence of accurate tests with which to define the equid reservoirs of EIAV since the early 1970's, foci of infection continue to be uncovered.[1,2] EIA is viewed as a manageable disease in well-managed populations of equids. Recently, EIAV infection of wild free-roaming horses has been discovered in populations on the Shackleford Banks in North Carolina and on public lands administered by the Bureau of Land

Management in the Uinta Basin in Utah.[3-4]

Regardless of where test-positive equids are found, little definitive data have been reported to document the rate of spread of EIAV infection in populations of horses and the impact of the infection on the health, survival and breeding efficiency of equid populations. Despite the relative dearth of definitive data on these subjects, the persistent nature of infection with this mutable lentivirus and the lack of an available vaccine, have led to an overwhelming high acceptance of a "test and slaughter" mentality.[5,6]

When infection with EIAV was found in wild free-roaming horses in Utah in 1998, the adult test-positive horses were euthanatized. Disposition of the test-positive foals of 12 test-positive mares, however, was more problematic. Antibodies against EIAV in the foals could have been produced by the foals in response to active infection. However, research on bands of test-positive mares with no evidence of overt disease (inapparent carriers of EIAV) has shown that foals can be raised free of the infection, even when kept in areas with relatively high populations of known insect vectors of EIAV.[7,8] Therefore, it was considered more likely that antibodies to EIAV in foals had been passively acquired from their dams in colostrum. After considering the data, recommendations, and public sentiment, the BLM and several interest groups developed a cooperative plan to sponsor a prospective study of these foals relative to EIAV. In order to carry out this study, the foals were moved from Utah to an USDA-approved isolation facility for EIA leased by the BLM from Oklahoma State University where they were periodically tested for EIA. This paper documents the results of that study.

The 12 foals were gathered during the week of May 25, 1998, and confirmed as positive in ELISA assays for antibodies against the p26 antigen of EIAV and in the AGID test.[9-12] Subsequently, samples were collected from the foals for serologic testing and for testing in reverse transcriptase-polymerase chain reaction (RT-PCR) assays for the presence of the *gag* gene of EIAV.[13] Test kits for diagnosis of EIA were kindly provided by IDEXX Laboratories, Westbrook, ME (AGID and CELISA), Synbiotics Corporation, San Diego, CA (Vira-CHEK EIA) and Centaur Inc, Overland Park, KS (SA-ELISA). Reagents for the immunoblot procedure were as described earlier[12]; the prototype cell-adapted strain of EIAV was used as antigen. The RT-PCR assay was conducted as described below using appropriate primers to amplify a portion of the highly conserved *gag* gene of EIAV.[13]

The age of the foals was estimated on the basis of their stage of development relative to their respective dams. Regardless of their age, the foals were separated from the mares on June 29 and maintained in a quarantined area, separated by at least 200 yards from other equids and cared for by dedicated personnel. Foals were fed milk replacer (Foal-Lac™, Pet-Ag, Elgin, IL) in an amount equivalent to 20% body weight divided into 4 feedings over a 24-hour period. Hay and a creep feed ration was offered ad libitum in addition to the liquid diet. On July 22, 1999, the foals were divided into 2 groups of 6, and transported in 2 stock trailers to a United States Department of Agriculture (USDA) approved biocontainment building on the Oklahoma State University (OSU) campus. The facility used in this study was modified to meet and exceed the standards for approval by the USDA for the safe conduct of these studies, and was approved by the OSU Committee for Institutional Animal Care and Use. Prior to shipment, foals were administered 20 grams of KCl/NaCl (Lite Salt™) solution orally. Trailer doors were officially sealed by an Animal, Plant, Health Inspection Service (APHIS) veterinarian. The duration of the trip was 22 hours with stops every 4 hours to allow veterinarians to examine the foals. Grass hay was available free choice and ad libitum water was available at all times. An APHIS veterinarian was part of the team which escorted the foals from Vernal, Utah to Stillwater, Oklahoma and was available to remove and re-seal the trailers when necessary. State veterinarians from Utah, Colorado, and Kansas had authorized transport of the foals through their respective states for the days of July 22nd-23rd. One foal was administered 2 liters of a polyionic electrolyte solution by the intravenous route 10 hours into the trip as he had become clinically dehydrated and had not been observed to drink. After arrival on the OSU campus, the 12 foals were moved into and then housed in individual (2 foals/stall), 4 x 4-m shavings-bedded box stalls, were fed milk replacer (until 3-4 months of age), prairie grass hay and mixed concentrated grain ration, and had ad libitum access to a salt/mineral block and water. Foals were dewormed by use of an ivermectin product at 8-week intervals beginning in September. All foals were vaccinated against Eastern and Western Equine Encephalitis and tetanus at 5-6 months of age and were boosted at 8-9 months of age. At least twice each day, the foals were allowed access (2 at a time) to a 20 X 94 foot hallway with rubber matted flooring in the enclosed biocontainment building for exercise.

Blood samples were collected from the foals at the time of the initial gather on May 25-30. Subsequently, plasma and serum samples were collected on June 30, July 21 and at approximately 14-day intervals thereafter. At each collection, whole blood samples were taken in evacuated glass tubes and allowed to clot; serum was harvested and antibody-tested with official test kits by AGID and in the three ELISA-based formats as a routine. The samples were then aliquoted and stored at -20°C until additional testing was complete. As plasma samples were to be tested for EIAV RNA, which is very labile, blood was collected in evacuated glass tubes with EDTA, held on ice and processed within 30 minutes of collection. Aliquots of plasma were transferred to cryovials, frozen on dry ice in the field and kept at -70°C in the laboratory until tested.

The internationally accepted gold-standard serologic test for EIA is the AGID (or Coggins) test.[9] The AGID test is designed to be interpreted either as positive or negative, but reactions of positive samples vary in intensity with the level

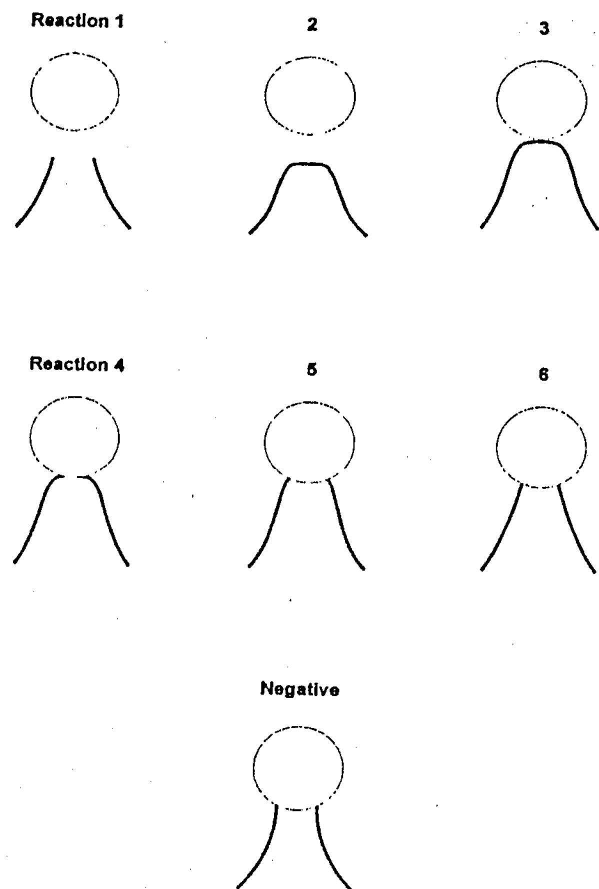

Figure 1. Reactions of positive samples with the highest amount of antibody against p26 fail to reach a zone of optimal proportions: a line of reaction is missing and the reference line terminates prematurely (reaction #1). Positive samples with progressively less antibody (or dilutions of a positive sample) have reactions like #246. In a negative reaction, the reference line makes a slight outward deflection (compare with the slightest inward deflection of positive sample reaction #6).

of antibody against the p26 antigen. In most cases, an intense line of precipitation forms in common with the reference positive sample, i.e., a line of identity. Interpretation of results with samples with low reactivity, however, present frequent problems. In samples with lower levels of anti-p26 antibodies, a line of identity is not formed; a deflection in the reference line of precipitation is observed with the amount of deflection relative to the level of antibody (see Figure 1). It is these so-called "weak positive," "very weak positive," or "very weak, weak positive" samples that are frequently reported falsely as negative.[11] In more qualified hands, these reactions are generally reported as indeterminate or equivocal, and another sample requested. For this study we developed a semi-quantitative numerical AGID test-scoring system to assess the change in antibody level in the foals (see legend for Figure 1). The sample was judged positive if 2 or more EIAV proteins were recognized. To ensure maximal accuracy and comparability between samples, the samples from each mare-foal pair or from each foal over time were tested with the same test kit, on the same agar plate, and at the same time.

Similarly, results in the ELISA tests are read visually and/or with the aid of a spectrophotometer, and reported as positive or negative. Samples with equivocal results in the ELISA tests are less numerous than in the AGID test because of the quantitative nature of the spectrophotometric result. The ELISA results are reported as positive or negative in this study. The SA-ELISA protocol was only performed on fresh samples, as the package insert specifies the need to run the test on fresh samples.

The presence of EIAV genetic material in the bloodstream of these foals was investigated using the nested RT-PCR and oligonucleotide primers designed to amplify a portion of the highly conserved viral *gag* gene. In this procedure, 2ml of plasma was centrifuged at 37,100xg for 2h at 4°C to pellet any virus particles, which may have been present. The resultant pellet was resuspended in 200ml of diethyl-pyrocarbonate treated distilled water and the RNA extracted using the High Pure™ Viral RNA kit (Roche, Indianapolis, IN). This potential extract-containing RNA was subjected to a nested RT-PCR as described previously.[13] Sensitivity of this procedure is between 10 to 100 molecules (RF Cook, unpublished data), this was monitored in each test by including known quantities of an RNA standard as a control.

RESULTS AND DISCUSSION

When first sampled, all the foals had reactions in the AGID test which were equivalent to or of lesser intensity than their dams (Table 1). Reactions of serum samples from foal #6 over the duration of the study are presented in Table 2. Selected samples from foal #6 tested in the immunoblot test gave positive reactions for 12-- 158 days longer than either AGID or ELISA tests (against the p26 and gp45 determinants, respectively). Data on the persistence of detectable antibody in

Table 1. Comparison of relative intensity of reactions of initial samples from mares and their foals in the AGID test (refer to Figure 1 for explanation of numbers)

Foal Number	AGID Reaction of Mare	AGID Reaction of Foal
1	2	2
2	2	4
3	2	2
4	2	2
5	2	2
6	2	2
7	4	5
8	2	2
9	3	3
10	2	4
11	2	4
12	2	3

Table 2. Comparative serologic test results from a foal of a test-positive mare

Foal #6	Reactions				
Age in Days(est)	AGID[1] (Dam)	CELISA	ViraCHEK	SA ELISA	Immunoblot 90 45 26
27	2 (2)	POS	POS	POS	ND
49	2	POS	POS	POS	ND
61	3	POS	POS	ND	ND
82	4	POS	POS	POS	5+ 4+ 2+
96	+	POS	POS	NEG	ND
111	+	POS	POS	NEG	4+ 4+ 2+
123	5	POS	POS	NEG	ND
139	+	POS	POS	NEG	ND
151	5	POS	POS	NEG	ND
166	+	POS	POS	NEG	ND
180	6	POS	POS	NEG	ND
193	?	POS	POS	NEG	ND
214	NEG	POS	NEG	NEG	2+ 2+ 1+
228	NEG	POS	NEG	NEG	ND
240	NEG	NEG	NEG	NEG	ND
253	NEG	NEG	NEG	NEG	1+ 1+ 1+

[1]Intensity of selected positive AGID test reactions is indicated numerically. Refer to Figure 1 for an explanation.

the 12 foals using the different serologic tests are presented in Table 3. Briefly, the last sample that was positive on any of the officially approved tests for EIA was collected at 81-336 days of age (mean of 203 days). Selected samples from each foal were tested by immunoblot, and samples were interpreted as positive for a mean of ≥231 days. Plasma samples collected at the beginning and end of isolation (7/21/98 and 3/23/99) were tested and found to contain no evidence of the *gag* gene of EIAV.

Sera from individual foals gave divergent results in the different serologic tests (see Table 3). Reactions were positive in SA-ELISA for a mean of ≤82 days, in the Vira-CHEK for 169 days, in the AGID for 183 days, and in the CELISA for 202 days. Sera tested by the immunoblot test were positive for ≥28 days longer than the last positive result using any of the officially approved tests.

The role of passive transfer of antibody in colostrum of ungulate species is to ensure an immediate presence of high levels of antibodies against antigens encountered by the dam. In most cases, these antibodies can neutralize an invading organism or toxin, and so provide a level of clinical protection against the agent. The persistence of effective levels of these antibodies has been assumed to be about 3-6 months in equids and many of our immunization regimens for young equids are based on this assumption.[14] However, we are beginning to realize that in horses, the persistence of even low levels of these colostral antibodies may prevent the recognition of immunogens and interfere with maximal primary responses following immunization.

In adults, the presence of antibodies against EIAV correlates with active infection and is used in surveillance

Table 3. Persistence of passive antibody against EIAV in twelve foals of test-positive mares

Last positive reaction in official test formats[1]					Immunoblot reactions			
Foal	AGID	CELISA	ViraCHEK	SA-ELISA	90	45	26	days[1]
1	202	228	192	≤27	3	2	1	111
					1	-	1	228
					1	-	1	253
2	172	184	145	≤61	2	1	2	145
					1	-	1	200
					-	-	1	226
3	229	229	167	126	4	4	3	99
					4	2	2	241
					2	1	1	274
4	221	235	199	235	5	4	3	117
					4	3	2	235
					3	3	1	260
5	260	≥336	260	129	5	4	3	118
					2	2	2	292
					1	1	1	336
6	192	229	192	82	4	4	2	111
					2	2	1	214
					1	1	1	253
7	81	81	ND	≤48	2	2	-	132
					1	1	-	143
					-	-	-	176
8	192	192	165	≤27	2	1	2	111
					1	-	1	214
					-	-	1	285
9	213	249	159	81	5	4	3	132
					3	2	2	235
					3	1	1	274
10	156	172	144	≤61	2	1	1	145
					1	1	1	172
					1	-	1	200
11	155	138	110	≤27	4	4	3	96
					3	2	1	179
					-	-	-	214
12	122	150	122	81	4	4	3	111
					3	2	1	155
					2	1	1	192
Mean	183	202	169	≤82				

[1]Approximate age in days.

and control programs to identify equid carriers of EIAV. Although the passive transfer of immunoglobulins against EIAV (serum from long-term carriers of EIAV) can provide a level of protection against clinical EIA, no firm evidence for protection against infection has been presented (Issel CJ, unpublished findings). Thus, the 12 foals could have been carrying passive antibodies and be actively but subclinically infected at the time of their capture.

The lack of amplifiable EIAV *gag* gene signal in the plasma from all 12 foals at the initiation of the prospective study (23 days after separation from their dams) was an

indication that no active infections were present. As the level of colostrally-acquired antibodies appeared to be declining over this period, the chance of active infection was thought to be low. The data generated over the additional period of study proved the foals were not infected. The passively-acquired antibodies declined to undetectable levels (see below), and no evidence for the *gag* gene of EIAV was found using the sensitive RT-PCR assay. Thus RT-PCR and prospective serologic testing was used to predict accurately the infection status of these 12 foals of EIA test-positive dams. These foals adjusted well to their early weaning, extended transport to Oklahoma and life in an isolation facility. They were further gentled by repeated exposure to caring veterinary students who were contracted by the BLM for their daily care and nurturing.

This prospective study raises a number of interesting questions related to sensitivity/specificity of serologic tests for EIA as they relate to the diagnosis of EIAV infection. For example, using the immunoblot test, antibodies against EIAV antigens were found for 20-160 days beyond their detection time using both of the officially approved tests for EIA. The highest positive correlation with the immunoblot results was seen with the CELISA test kit; the most divergent results with the SA-ELISA test kit. The immunoblot assay, interpreted as positive if 2 or more EIAV proteins were detectable, was positive from ≥130 to ≥336 days (mean of ≥231 days) in this group of 12 foals, longer than has previously been reported using the AGID test. The persistence and functional activity of low levels of passive antibody are active areas of research in several laboratories, especially as they impact on effective immunization to common pathogens.

As these foals in time lost their antibodies to EIAV and were evidently not infected with EIAV, the positive serologic test results could arguably be discussed as "false positive" because they did not correlate with infection, in contrast to samples from adult equids. If we restrict our discussion to detection of low levels of antibodies against EIAV antigens, the anti-p26 diagnostic test kits, CELISA, AGID and Vira-CHEK, were maximally sensitive for detection of antibodies. The mean persistence of antibodies against the p26 antigen in the AGID test in our report (183 days) must be interpreted carefully; we would suggest that the mean persistence would be ≤150 days in most diagnostic laboratories. Often, samples with the intensity of AGID reactions in the category 5 and 6 range would be reported by approved laboratories as negative on routine testing.[11] In fact the first sample from foal #7 (an AGID reaction of 5) was reported as negative when first officially tested. The interpretation of AGID reactions can give rise to errors in reporting. For this reason, samples with these types of AGID test reactions are included as a minority in the annual proficiency check-test panels from the USDA-VS-APHIS-NVSL.[10] As adult equids with category 5 and 6 AGID test-reactions are relatively rare in the population (we estimate <1% of infected equids have these reactions consistently), this is appropriate. Reporting of these samples falsely as negative, however, could have important consequences, especially if the animals in question move freely and put other equids at risk.

The anomalous and early negative SA-ELISA reactions were unexpected and did not correlate with the loss of detectable antibody against determinants of gp45 in the immunoblot test. As the SA-ELISA test uses only one synthetic peptide of the gp45 antigen, the divergent results could be explained as differential recognition of this determinant by the mares, or, perhaps, a lower than expected degree of conservation of this determinant among different field strains of EIAV. Alternatively, the immunoblot reactivity against gp45 can be accounted for by recognition of alternative epitopes that are known to occur on gp45. This result raises a potentially important point, as the SA-ELISA test has been reported to have a higher "false-negative" rate than the p26-based diagnostic tests, which thus far remains unexplained. It is also possible that the anti-gp45 activity monitored by the SA-ELISA assay requires a background of viral replication to remain positive. Thus, in the absence of virus infection, as in these foals, or in the absence of continued high levels of virus replication, as in some field infections, the SA-ELISA signal could become negative before the anti-p26 assays. These intriguing possibilities deserve further investigation.

When this study was initiated, the risks of these foals having contracted infection with EIAV could not be estimated with accuracy. The risks were assumed to be high because there was evidence of clinical EIA and active transmission of EIAV in the area. The clinical status of the mares of the 12 foals, however, could not be determined retrospectively to help establish risk estimates. Fortunately, the foals had limited exposure to infected equids (49-98 days; mean of 68) from March 26 (the earliest estimated birth date) to June 29. During this time, the most likely insect vectors of EIAV, the tabanids, were not observed in the area at the time of capture or at the xeric quarantine site. During the gather, foals were separated from the mares immediately upon capture and reunited after their transport and sampling. The chance of mechanical transmission mediated by man and by contact with blood was thus minimized.

CONCLUSION

The 12 wild foals, dubbed "The Fuzzy Dozen," have been adopted through the BLM adoption program and have found safe havens for their continued growth. The costs associated with management and research of these foals from their collection to their adoption were substantial. It is hoped that the knowledge gained and the lessons learned from this project will stimulate discussions leading to more logical and effective, and less costly, management of future EIAV "outbreaks." We hope that this project can

serve as a model for rational control programs for EIA (see recommendations – Table 4).

Table 4. Recommendations for handling and testing foals born to EIA reactor mares

1) Confirm EIAV status of mare by a second test (AGID). Mare should be identified (branded) – indicating EIA reactor status.
2) Obtain pre- (if possible) and post-suckle blood samples from peripheral vein of foal for serology (CELISA, AGID, & immunoblot testing) and PCR analysis. [University of Kentucky]
3) Maintain foal with mare under appropriate quarantine conditions (for maintaining EIA positive animals) and offer high-quality hay/feeds and water. Minimize stressful conditions for mare and foal.
4) Obtain follow-up blood samples for EIA testing on foal at 4-6 week intervals (AGID, CELISA, Immunoblot & PCR).
5) If foal shows a declining antibody titer and no evidence of EIAV genetic material via PCR, then foal can be weaned at 4-5 months of age.
6) Foal should be quarantined for at least 45 days after being separated from mare at a site at least 200 yards from any EIA positive animal (ie. secondary quarantine site) and re-tested (AGID, CELISA, immunoblot, & PCR).
7) Foal should be kept in quarantine until it is determined to be negative for EIAV antibody on all official EIA tests (ELISA, AGID). This may be up to 10(?) months of age.

REFERENCES

1. Campbell CL et al.: Communication on infectious diseases of horses: a prospectus on equine infectious anemia with guidelines. *US An Health Assn Proc* 1971;75:249–261.
2. Cordes TR, USDA-APHIS-VS-NAHP, personal communication.
3. Issel CJ, Cook SJ, Howell D, Nitschke-Sinclear J, Gardner D, Mathis JG, Marshall MR, Rogers LE: Equine Infectious Anemia in Wild Free-Roaming Horses in Utah, *US An Health Assn Proc* 1999;102:376–384.
4. Issel CJ, Cook SJ, Cordes TR, McConnico RS, Messer NT IV, Bisson H, Shea P: Equine infectious anemia: problem turned to opportunity. How the destruction of free roaming horses with EIA may lead to healthier populations and better management plans. *The Horse* 1999;16(8):27–40.
5. Issel CJ, McManus JM, Hagius SD, Foil LD, Adams WV Jr, Montelaro RC: Equine Infectious Anemia: prospects for control. *Dev Biol Stand* 1990;72:49–57.
6. Issel CJ, Cook SJ, Cook RF, McCoy HK, Lew AM, Montelaro RC: A search for the elusive protective determinants of equine infectious anemia virus. *Proceedings of the 8th International Conference on Equine Infectious Diseases*, R&W Publications (Newmarket) Limited, Great Britain, 1999;p. 408.
7. Issel CJ, Adams WV Jr, Foil LD: Prospective study of progeny of inapparent equine carriers of equine infectious anemia virus. *Am J Vet Res* 1985;46:1114–1116.
8. McConnico RS, Floyd C, Issel C, Lehenbauer TW, Rodgers S, Saliki J: Evaluation of techniques for the serologic diagnosis of equine infectious anemia in a naturally-infected, clinically inapparent herd of reproductively active horses. 1999 *Proc 8th International Conference on Equine Infectious Diseases*, R&W Publications (Newmarket) Limited, Great Britian, p. 400.
9. Coggins L, Norcross NL, Nusbaum SR: Diagnosis equine infectious anemia by immunodiffusion test. *Amer J V Res* 1972;33(1):11–18.
10. Pearson JE, Gipson CA: Standardization of equine infectio anemia immunodiffusion and CELISA tests and their applicati to control of the disease in the United States. *J Eq Vet S* 1988;8(1):60–61.
11. Issel CJ, Cook SJ, Cook RF, Cordes TR: Optimal paradigm for the serologic diagnosis of equine infectious anemia. *J Eq V Sci* 1999;19:720–724.
12. Issel CJ, Cook RF: A review of techniques for the serolog diagnosis of equine infectious anemia. *J Vet Diagn Inve* 1993;5(1):137–41.
13. Langemeier JL, Cook SJ, Cook RF, Rushlow KE, Montela RC, Issel CJ: Detection of equine infectious anemia viral RNA plasma samples from recently infected and long-term inappare carrier animals by PCR. *J Clin Microbiol* 1996;34(6):1481–7.
14. Jeffcott LB: The transfer of passive immunity to the fo and its relation to immune status after birth. *J Reprod Fertil Su* 1975;Oct(23):727–33.