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# Protection of pigs by vaccination of pregnant sows against eastern equine encephalomyelitis virus

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**Abstract**

Serum-virus neutralizing antibodies were detected in serum and colostrum of sows vaccinated during pregnancy with commercially available vaccines against eastern equine encephalomyelitis virus (EEEV), and antibodies were detected in serum from nearly all pigs from vaccinated sows following colostrum uptake. Serum-virus neutralizing antibody (SVN) test titers were measured in colostrum and pigs at the next farrowing, and additional vaccination of sows prior to the third farrowing led to elevated SVN titers in serum, colostrum and all pigs.

Six pigs from vaccinated sows challenged at 8 to 9 days of age with $1 \times 10^6$ $TCID_{50}$ EEEV did not develop the high temperatures or signs of central nervous system disease that 6 pigs from non-vaccinated sows developed. Virus was isolated from blood and oropharyngeal swabs from all pigs from non-vaccinated sows with blood virus titers as high as $9.3 \times 10^4$ $TCID_{50}$, while only low levels of virus were detected in blood and oropharyngeal swabs from pigs from vaccinated sows. Virus was also isolated from tonsils collected at necropsy from 3 pigs from non-vaccinated and 1 pig from vaccinated sows.

Vaccination of pregnant sows leads to development of maternal antibodies that are transmitted via colostrum to pigs and are protective against clinical EEEV related disease after experimental challenge with EEEV. In addition, vaccination prevents amplification of virus in infected pigs and could result in protection of animals and farm labor in the environment of infected pigs.

*Keywords:* Eastern equine encephalomyelitis virus; Pig; Vaccination; Antibody; Experimental infection

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## 1. Introduction

Eastern equine encephalomyelitis (EEE) is recognized as an arthropod borne viral disease primarily affecting the central nervous system of mammalian hosts. Several species of birds are considered to be the reservoir for EEE virus (EEEV). The disease is endemic in the eastern United States, and appearance in mammals is correlated to climatic conditions which influence population dynamics of vectors (Scott and Weaver, 1989; Freier, 1993).

Swine producers can suffer substantial losses from EEE outbreaks in their herds (Pursell et al., 1972; Elvinger et al., 1994a; Anon., 1995) and also farm labor is at risk of contracting the disease (Elvinger et al., 1994b). Exposure of swine to EEEV is widespread in endemic areas (Feemster et al., 1958; Karstad and Hanson, 1958, 1959; Elvinger et al., 1996), although death losses seem to occur only in nursing pigs. In many instances EEEV infection could remain undiagnosed as no histopathologic lesions in brain tissues are apparent in pigs with an acute course of the disease and diagnosticians in veterinary diagnostic laboratories generally do not test swine for EEEV infection. Furthermore, EEEV usually does not grow in cell lines commonly used for isolation of virus from pigs.

Most horses in endemic areas are vaccinated once or twice per year against EEE. Vaccination of sows could be an option for protecting swine producers from catastrophic losses due to EEEV infection in pigs. The objectives of this study were to induce anti-EEEV antibodies in pregnant sows through vaccination, to monitor passive transfer of maternal anti-EEEV antibodies via colostrum and titer deterioration in pigs from vaccinated sows, and to evaluate protection of pigs from vaccinated sows against experimental infection with virulent EEEV.

## 2. Materials and methods

### 2.1. Sows and pig litters

Twenty-five [Yorkshire × Hampshire] × Duroc sows and litters housed at the Coastal Plain Experiment Station Swine Research Unit were available for inclusion in experiment I. Four sows initially included were eliminated from consideration when they were found not to be pregnant. Litters from 11 sows from experiment I and three gilts were available for experiment II.

### 2.2. Treatments and sampling schedules

#### 2.2.1. Experiment I

Eighteen of the remaining 21 sows were given one of two commercially available formalin inactivated, bivalent (eastern and western strains of equine encephalomyelitis virus) equine vaccines of avian origin, HV (Encevac® with Havlogen® Encephalomyelitis Vaccine, Haver, Mobay Corp., Animal Health Division, Shawnee, KS 66201, U.S.A.) and FD (Encephaloïd® I.M., Encephalomyelitis Vaccine Eastern and

Table 1
Treatment allocation of 18 vaccinated sows [a]

| | Inoculation route | | |
|---|---|---|---|
| | i.m. | i.d. | i.m. + i.d. |
| HV | 3 | 2 | 4 |
| FD | 2 | 3 | 4 |

[a] 3 non-vaccinated controls.

Western Killed Virus, Al–P absorbed Bio 169, Fort Dodge Laboratories, Fort Dodge, IA 50501, U.S.A.). Vaccines were administered by the intra-muscular (i.m., 1 ml), intra-dermal/subcutaneous (i.d., 1 ml) or i.m. + i.d. (2 ml) routes at days (±5) −58, −39, and −16 prior to farrowing day (day 0). Three sows did not receive vaccines and served as non-vaccinated controls (C). Treatment allocation of 18 vaccinated sows is presented in Table 1. Vaccinated sows and controls farrowed a total of 238 live pigs in a 10 day range.

Blood samples from sows were collected by intra-thoracic venipuncture (Lawhorn, 1988) at days (±5) −58, −39, −16 and −6 prior to farrowing, and after farrowing at days (±5) 9, 61, 151 (15 sows, 2nd farrowing), 278 (11 sows) and 292 (14 sows; 3rd farrowing, Experiment II). Colostrum samples were obtained at farrowing on day 0 from 16 sows, at the next farrowing approximately 5 months after day 0 from 13 sows, and from 12 sows at the third farrowing (Experiment II).

Blood samples from pigs were collected by orbital sinus bleeding (Huhn et al., 1969): the first sample was collected immediately after delivery, prior to first colostrum uptake (227 pigs). One (11 pig litters) to two (10 pig litters) blood samples (323 samples) were collected between 11 and 219 h following delivery, and another sample (all litters, 190 pigs) was collected on day 15 ± 5. Additional blood samples were collected from a group of randomly chosen pigs in the nursery on days (±5) 32 (94 pigs), 50 (86 pigs) and a final sample was collected on day 80 ± 5 (83 pigs) just prior to sale. Blood samples also were collected following colostrum uptake from 93 pigs from 14 litters at the second farrowing.

### 2.2.2. *Experiment II*

Blood samples were collected from 8 vaccinated and 3 non-vaccinated sows from experiment I and three gilts, prior to administration of 1 ml i.m. + 1 ml i.d. of FD vaccine to all 8 vaccinated sows. At farrowing, colostrum samples were collected from all 14 sows and gilts, and blood samples were collected 2 days after farrowing from 8 sows. Blood samples were collected from 125 pigs following colostrum uptake from 13 litters within 48 h after farrowing.

Antibody titers to EEEV were determined immediately following blood collection, and 2 pigs with elevated titers from each of 3 litters from vaccinated sows (V) and 2 pigs with negative titers from each of 3 litters from non-vaccinated control sows (C) were selected for experimental challenge study. Pigs were simultaneously weaned at 7 to 8 days of age and were adapted for 24 h to medicated milk replacer, (Soweena Litter

Life™, Merrick's, Middleton, WI; contains 200 g Neomycin Sulfate and 140 g Oxytetracycline per ton) prior to intra-dermal inoculation of 1 ml EEEV suspension adjusted to $1 \times 10^6$ $TCID_{50}$ per ml (day 0). Rectal temperatures were measured and clinical evaluations were performed at 6 h intervals. Rectal and oropharyngeal swabs were collected at the same time. Blood samples were collected just prior to inoculation, at 6 h intervals for 24 h, at 12 h intervals until hour 72 post challenge, and at hour 100 and 116 (Fig. 2). Two pigs were euthanized in extremis and surviving pigs were euthanized 116 h after experimental challenge. All pigs were necropsied and tissues were obtained for histopathologic and virologic examination.

### 2.3. Tests

#### 2.3.1. Serum-virus neutralization test

Serum samples were inactivated at 56°C for 30 min and serial twofold dilutions (1:2 to 1:256; 50 μl per well) were made in duplicate in modified MEM in standard 96-well flat bottom microtiter plates. Approximately 300 $TCID_{50}$ (in 50 μl) of virus suspension were added, and plates were placed in an incubator for 1 h at 37°C to allow neutralization of the virus. Finally, 100 μl of Vero cell suspension, containing a sufficient concentration of cells (approximately $10^5$ cells/ml) to give a complete monolayer within 48 to 72 h were added to each well. Plates were incubated at 37°C for 72 h, after which each well was examined for cytopathic effects (CPE). The titer of the virus challenge dose was determined by back-titration of the virus suspension. Tests were considered valid if the challenge dose of the virus was between 100 and 500 $TCID_{50}$. The antibody titer of the test serum was reported as the reciprocal of the highest dilution of serum causing 100% neutralization of the challenge virus.

#### 2.3.2. Virus isolation from serum and tissues from experimentally infected swine

Fifty, 100 and 200 μl serum or 0, 100, 200 and 300 μl of supernatant from homogenated, macerated and centrifugated tissues in modified MEM were mixed with 1 ml Vero cell suspensions ($100 \times 10^3$ cells/ml) in 24 well flat bottom tissue culture plates and incubated for 7 days at 37°C. Cell cultures were checked daily for CPE or toxicity. In case of non-virus related toxicity to cell cultures, or when no viral CPE was observed after 7 days incubation, 200 μl of supernatant from wells were inoculated into a second well containing 1 ml of Vero cells for subculture. The plates were incubated and checked for viral CPE for a second 7 day period. If CPE was detected, virus identification was confirmed by fluorescent antibody techniques.

#### 2.3.3. Identification of EEEV in cell culture by fluorescent antibody staining

Supernatants (0, 25, 50, and 100 μl) from wells with CPE were transferred in duplicate to eight chamber tissue culture slides. After addition of a 250 μl Vero cell suspension, slides were incubated at 37°C and monitored for CPE. When CPE was detected, slides were rinsed with phosphate buffered saline solution (PBS), immersed and fixed in cold acetone for 10 min and allowed to dry at room temperature. Cells were covered with horse anti-EEEV antiserum, incubated in a humidified chamber for 30 min at 37°C, washed with PBS before addition of fluorescein labeled goat anti-horse IgG

(H + L). Slides were incubated at 37°C for 30 min, followed by washing with PBS, counter stained for 5 min in 0.5% Evans Blue and rinsed in distilled water. Alternatively, sections were flooded with mouse anti-EEEV monoclonal ascites fluid followed by fluorescein labeled anti-mouse conjugate. Finally, stained cells were covered with glycerol-PBS mixture, cover slipped and examined for fluorescence on a UV microscope. The wells without supernatant (0 μl) served as negative controls.

*2.3.4. Histopathology of experimentally infected swine*

After in situ examination of organs, multiple coronal sections of brain and spinal cord, sections of heart from the interventricular septum, papillary muscles, both ventricles and atria, and single sections of lung, liver, kidney, spleen, pancreas, lymph nodes, tonsils, adrenal glands and intestine were fixed in Carson's modified phosphate buffered 10% formalin. Tissues were processed through a gradient of alcohols followed by xylene, infiltrated with and embedded in paraffin, sectioned at 4 μm and stained with hematoxylin and eosin for histopathologic examination.

*2.3.5. Bacteriologic and virologic examination of fecal and tissue specimen*

Fecal specimens were collected from pigs which developed diarrhea and examined by electron-microscope for rotavirus and coronavirus. All fecal specimens and tissue specimens collected at necropsy were aerobically cultured for bacterial pathogens commonly diagnosed in the veterinary diagnostic laboratory.

*2.4. Data analysis*

Treatments were designed to enhance chances of seropositive sows and pigs following absence of a detectable response in a prior experiment with the i.m. administration of one of the commercially available vaccines. The experiment was not designed to determine differences between treatment groups, i.e. to compare efficacy of commercial products or routes of inoculation. Results are presented as numbers of EEEV antibody positive pigs. Titer deterioration of maternally derived antibodies was determined on 70 pigs for which a minimum of 5 simultaneously analyzed samples were available. These pigs originated from 15 vaccinated sows. Titers were $\log_2$ transformed. Intercept, linear and quadratic regression coefficients were estimated using the general linear model procedure in SAS (1990), adjusting for effects of sow, treatment group and pig. Time was a continuous variable in the model designed as a split plot.

## 3. Results

*3.1. Experiment I*

*3.1.1. Neutralizing antibody titers in serum and colostrum of sows*

Antibody titers measured in serum and colostrum of vaccinated sows are presented in Table 2. One of the 3 vaccinated open sows had a SVN antibody titer of 1:4 three weeks after the second injection (day −16). A second of the 3 vaccinated open sows had a titer

Table 2
Serum virus neutralizing antibodies in serum of sows vaccinated with a commercially available equine anti-EEE vaccine [a]

| Day to farrowing | SVN titers | | | | | |
|---|---|---|---|---|---|---|
| | <1:4 | 1:4 | 1:8 | 1:16 | 1:32 | 1:64 |
| | Number of sows | | | | | |
| −58 | 18 | | | | | |
| −39 | 18 | | | | | |
| −16 | 18 | | | | | |
| −5 | 8 | 9 | 1 | | | |
| 0 [b] | | 5 | 4 | 4 | 1 | 1 |
| 9 | 15 | 2 | | | | |
| 61 | 4 | 6 | 9 | 2 | | |
| 151 | 13 | | | | | |
| 151 [b] | 6 | 2 | 1 | | | |
| 278 | 1 | 6 | 1 | | | |
| 290 [b] | 1 | 1 | 4 [c] | | 2 [c] | 1 [c] |
| 292 | | 4 | 2 | 1 | | 1 |

[a] All blood and colostrum samples from non-vaccinated controls were negative.
[b] Colostrum.
[c] Sows had received additional vaccine (2 ml i.m. + i.d.) on day 278.

of 1:8 10 days following the third vaccination (day −5), and 2 of the vaccinated open sows had titers of 1:8 on day 61. All blood and colostrum samples collected from non-vaccinated control sows were negative.

### *3.1.2. Neutralizing antibody titers in serum of pigs*

Antibody titers measured in serum of pigs are presented in Table 3. Deterioration of maternal antibody titers in 70 pigs pooled for all 6 vaccine treatment groups between hour 11 and hour 1,880 followed the equation $\ln_2(\text{titer}) = 3.83207 - 0.00203972 \times \text{time} + 0.000000377 \times \text{time}^2$ (Fig. 1; $r^2 = 0.79$). Half-life of maternal antibodies present at

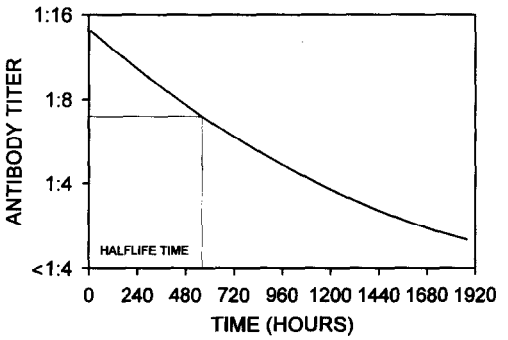


Fig. 1. Serum-virus neutralization test titers in pigs following colostrum uptake from sows vaccinated prior to farrowing against eastern equine encephalomyelitis virus.

Table 3
Serum virus neutralizing antibodies in serum of pigs from sows vaccinated with a commercially available equine anti-EEE vaccine [a]

| Day after farrowing | SVN titers | | | | | | |
|---|---|---|---|---|---|---|---|
| | < 1:4 | 1:4 | 1:8 | 1:16 | 1:32 | 1:64 | 1:128 |
| | Number of samples | | | | | | |
| 0 [b] | 187 | | | | | | |
| 5 [c] | 31 | 92 | 73 | 45 | 32 | 11 | 1 |
| 15 [d] | | 11 | 36 | 10 | 3 | | |
| 32 [d] | | 49 | 15 | 4 | 2 | | |
| 50 [d] | 17 | 51 | 2 | | | | |
| 80 [d] | 47 | 23 | | | | | |
| Experiment II [e] | | 4 | 19 | 29 | 22 | 6 | 4 |

[a] All 19, 27 and 26 samples taken prior to colostrum uptake, at days 5 and 15 from pigs from non-vaccinated sows were negative.
[b] Within 15 min of delivery, prior to colostrum uptake.
[c] Results pooled for pigs from 11 litters sampled once and 10 litters sampled twice between 11 and 219 h post delivery.
[d] ± 5 days.
[e] Experiment II: 84 pigs from revaccinated sows at third farrowing; all 41 pigs from non-vaccinated control sows and gilts were negative.

hour 24 was 23 days. Twenty-three of 70 pigs had an antibody titer of 1:4 on day 80 ± 5 (Table 3). All serum samples collected from pigs from non-vaccinated control sows were negative.

*3.1.3. Neutralizing antibody titers in serum of pigs at second farrowing*

Antibodies were not detected in serum of 13 vaccinated sows at the second farrowing after vaccination (day 151 ± 5), but were detected in 3 of 9 tested colostrum samples (Table 2). Eighty-four pigs from those 13 litters were screened for antibodies to EEEV by SVN test at a 1:4 dilution, and 34 (40.5%) pigs from 7 litters were positive. Antibody titers of pig sera were not determined. Nine pigs from one control sow were negative.

*3.2. Experiment II:*

*3.2.1. Neutralizing antibody titers in serum and colostrum of sows following additional vaccination*

Seven of the 8 previously vaccinated sows had detectable neutralizing antibodies when revaccinated 294 (278 + 16) days after the last immunization (Table 2). All 8 sows had increased titers following farrowing and had detectable antibodies in colostrum. One of 2 sows that were not revaccinated had a titer of 1:4 in colostrum at the third farrowing. No non-vaccinated control sows or gilts had detectable antibodies in blood or colostrum.

*3.2.2. Neutralizing antibody titers in serum of pigs*

Antibody titers measured in serum of pigs are presented in Table 3. Pigs chosen for experimental infection had titers of 1:32 to 1:128. Five days after experimental chal-

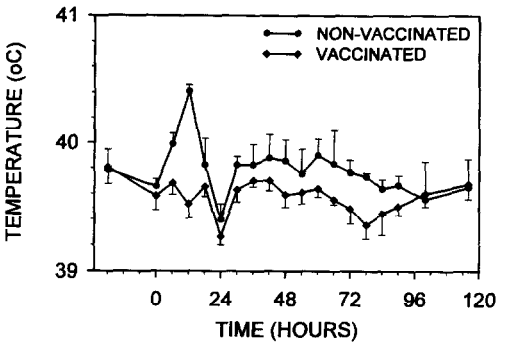


Fig. 2. Rectal temperatures of pigs from sows non-vaccinated and vaccinated against eastern equine encephalomyelitis virus following experimental challenge infection with virulent eastern equine encephalomyelitis virus.

lenge, titers were 1:4 to 1:16. Pigs from non-vaccinated sows had no detectable antibodies to EEEV.

### *3.2.3. Clinical response of pigs to experimental challenge with EEEV*

Rectal temperature increased in all 6 pigs from non-vaccinated sows to reach a peak at 12 h for 5 pigs and at 18 h for 1 pig (range of peak temperatures: 40.3 to 40.8°C; Fig. 2). None of the pigs from vaccinated sows had increased temperatures. During the study, 5 pigs in each treatment group developed diarrhea which lasted less than 12 to 116 h. No other clinical signs were noticed in the pigs from vaccinated sows. Five pigs from non-vaccinated sows also showed clinical signs of central nervous system disease which ranged from lethargy occurring in 3 pigs between 66 and 116 h after experimental inoculation to ataxia in 1 pig at 66 h and death in 2 pigs at < 36 (pig 66-4C) and 74 h

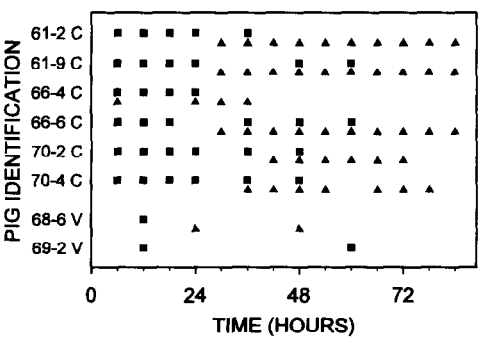


Fig. 3. Virus isolation from blood (■) and oropharyngeal swabs (▲) from pigs from sows non-vaccinated (C) and vaccinated (V) against eastern equine encephalomyelitis virus following experimental challenge infection with virulent eastern equine encephalomyelitis virus. Blood specimens were collected at hour 0, 6, 12, 18, 24, 36, 48, 60, 72, 100 and 116 after virus inoculation, and oropharyngeal swabs were collected at 6 h intervals from hour 6 to hour 116 after virus inoculation. Virus was not isolated from live pigs beyond hour 84 after virus inoculation.

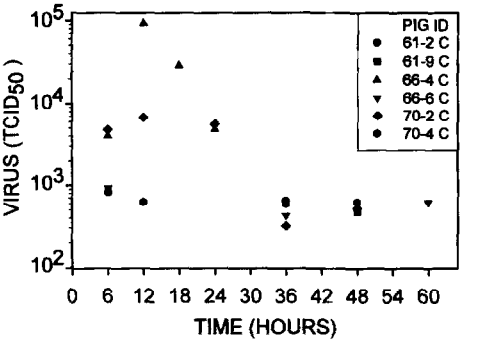


Fig. 4. Virus titers in blood from pigs from sows non-vaccinated against eastern equine encephalomyelitis virus following experimental challenge infection with virulent eastern equine encephalomyelitis virus. Blood specimens were collected at hours 0, 6, 12, 18, 24, 36, 48, 60, 72, 100 and 116 after virus inoculation. Virus was not titrated beyond hour 60 after virus inoculation.

(pig 70-2C; euthanized) after inoculation. Pig 66-4C also had bloody stools, emesis and was severely depressed. Pig 70-2C had elevated temperatures up to 40.9°C at hour 66.

*3.2.4. Virus isolation*

Eastern equine encephalomyelitis virus was isolated from blood and oropharyngeal swabs from 2 pigs from vaccinated sows and from all 6 pigs from non-vaccinated sows (Fig. 3). Virus was not isolated from rectal swabs. Recovered virus was titrated in 18 blood samples collected between hour 6 and 60, and titers ranged from $3.2 \times 10^2$ to $9.3 \times 10^4$ $TCID_{50}$ (Fig. 4).

Following necropsy, virus was isolated from the tonsils of pigs 66-4C, 70-2C, 70-4C and 63-1V, and from the cerebrospinal fluid from pig 70-2C.

*3.2.5. Pathologic examination*

Moderate to severe lesions of encephalitis were noted in all pigs from non-vaccinated sows. Mild to severe myocarditis was observed in 4 pigs from non-vaccinated sows. Multifocal necrotizing hepatitis was detected in pig 66-4C that died within 36 h after experimental challenge infection. Two pigs from vaccinated sows had mild coccidiosis.

*3.2.6. Bacteriologic examination*

α-streptococci and *Streptococcus suis* were isolated from brain, lung or spleen specimens from 3 and 2 of the pigs from non-vaccinated and vaccinated sows, respectively. *Klebsiella pneumoniae* was isolated from brain, kidney or liver specimens from 2 pigs from non-vaccinated sows. No histopathologic lesions compatible with *Sc. suis* or *K. pneumoniae* infection were noted.

## 4. Discussion

To prevent EEE in horses, live-attenuated and killed EEEV vaccines have been developed. Presently only killed vaccines of avian tissue-culture origin are commercially

available in the United States. Most horses develop neutralizing and hemagglutination inhibiting (HI) antibodies to EEEV within one week following vaccination, and antibodies can be detected in all horses one week after a second vaccination (Barber et al., 1978). In this study, neutralizing antibodies were detected in most sows only after a third vaccination. Colostrum from all vaccinated sows had elevated neutralizing antibody titers to EEEV. Colostrum from vaccinated mares had highest HI antibody titers, while serum from foals one week after birth and mares had similar but lower HI titers than colostrum (Ferguson et al., 1979). Piglets from all litters, regardless of product or route of inoculation, had elevated neutralizing antibody titers in serum. At the next farrowing, 167 days after the last vaccination, none of 13 vaccinated sows, but 3 of 9 tested colostrum samples had anti-EEEV antibody titers (Table 2). Thirty-four of 84 tested pigs had antibody titers to EEEV, regardless of serologic status of the dam. A booster vaccination of sows led to a rise in detectable antibody titers. Antibodies could be detected in all vaccinated horses up to 6 months after vaccination, while one year after vaccination, no HI or neutralizing antibodies could be detected in 94% and 53% of the horses, respectively (Barber et al., 1978). However, all vaccinated horses in that study were refractory to challenge infection 12 months after vaccination. It appears that measurable serologic status is a poor indicator of the immune status of the mammalian host, and that piglets from sows exposed to EEEV in the field or through vaccination could be protected from clinical EEE, even if no antibodies can be measured in the serum of the dams.

Half-life of maternally derived antibodies present at 24 h after birth was estimated at 23 days, and antibodies persisted in some pigs up to the last possible sampling date at 12 weeks of age when pigs were sold. Half-life of maternal antibodies in foals between 1 and 100 days of age was estimated at 20 and 33 days (Ferguson et al., 1979; Gibbs et al., 1988). It is possible that maternal antibodies derived from colostrum of vaccinated mares interfere with active immunity of foals after vaccination (Ferguson et al., 1979; Gibbs et al., 1988). Seventy percent of foals with moderate HI antibody titers did not show a rise in titer when vaccinated at three months of age, while all foals aged 4 months and older showed evidence of response to the vaccine (Gibbs et al., 1988). However, vaccination of nursing or growing pigs would not be practical and interference of maternal antibodies with vaccines should be of no concern in swine. Epidemiologic evaluation of outbreaks indicates that older pigs in which maternal antibodies would have deteriorated are refractory to clinical EEEV infection (Pursell et al., 1972; Elvinger et al., 1994a).

Maternally derived antibodies that were transferred via colostrum to the pigs were protective in an experimental challenge study. None of the pigs from vaccinated sows suffered clinical disease, although virus was isolated sporadically from blood and oropharyngeal swabs from 2 of the 6 pigs, and from the tonsils of one pig after necropsy 5 days after experimental inoculation. Amounts of circulating virus appeared to be very low since virus could not be titrated and also could not be reisolated after freezing of the specimens. Conversely, all 6 pigs from non-vaccinated sows showed EEE related disease signs of varying severity, and significant virus titers were found in blood. Titers in pigs from non-vaccinated sows could be sufficient for mosquitoes to acquire infectious doses of EEEV (Scott and Weaver, 1989; Mitchell et al., 1993).

Vaccination of sows would reduce circulating virus in pigs, and prevent the potential infection of biologic vectors or transmission of virus by mechanical means. Reduction of amplification of EEEV in pigs could contribute to prevention of EEEV infection of other farm animals or farm labor.

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