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EXPERIMENTAL INFECTION OF EUROPEAN WILD SWINE *(SUS SCROFA* L.) WITH PSEUDORABIES VIRUS

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Abstract: Experimental infection with pseudorabies virus was carried out by oral exposure of four young wild swine held in contact with two unexposed controls. No disease was observed but virological procedures indicated that the virus was shed in saliva and, in one case, in the nasal discharge, with subsequent infection of the control animals. After slaughter the virus was reisolated from the tonsils but not from lungs and brain. Virus reisolation from the tonsils was obtained in two animals after the throat swabs became negative. Virus neutralizing antibodies were detected.

INTRODUCTION

Pseudorabies, or Aujeszky's disease, is a viral infection occurring naturally in several species of domestic and wild mammals in different geographic areas (Friend and Trainer, 1970; Gustafson, 1975; Kirkpatrick, et al., 1980; Lautie, 1969; Ryu, 1975). The disease causes death among young domestic swine and abortion or macerated fetuses in pregnant sows. Domestic swine are considered to be the principal reservoir of the disease (Shope, 1935; McFerran and Dow, 1964). Little is found in the literature on the susceptibility of wild swine *(Sus scrofa* L.) to pseudorabies virus. A 1913 report gave information on a case of the disease occurring naturally in wild swine (Lautie, 1969).

The objectives of this experiment were to determine the behaviour of young European wild swine when infected with pseudorabies virus.

MATERIALS AND METHODS

Six 16-wk old wild swine were used, this being the age when these animals in our region (Tuscany) are trapped and transplanted in other areas. The animals were trapped in a game reserve and held in an isolation room (4 × 6 m) in our experimental facilities. Water was provided by automatic devices and food was supplied directly on the floor.

The virus used was isolated from a domestic pig naturally infected by pseudorabies and was used after two passages on pig kidney (PK15) cell line cultures. Rabbits inoculated subcutaneously with 10^3 $TCID_{50}$ experienced intense itching and died within 48 hr.

The PK15 cell cultures were grown at 37 C in glass flasks (20 cm^2) with Eagles Minimum Essential Medium containing 7% newborn calf serum, 2.5 mcgr of gentamycin sulfate and 50 U of mycostatin, with the pH adjusted to 7.6 with $NaHCO_3$.

Prior to experimental infection the sera of the animals were examined via the serum neutralization test; none had pseudorabies neutralizing antibodies.

The infection of the wild swine was carried out by introducing 2.0 ml of virus suspension into the oral cavity of each of four animals, the inoculum containing different amounts of virus (Table 1). Two other animals were used as uninfected controls and were in contact with the infected animals. All animals were observed daily for signs of the disease. At intervals, throat, nasal and conjunctival swabs and blood samples were collected until time of slaughter, when the follow-

TABLE 1. Results of virus isolation and serologic testing on wild swine exposed to pseudorabies virus.

Animal No.	Inoculum ($TCID_{50}$)[a]	Test[b]	Days after inoculation 3	5	7	12	16	21	30	42
1	10^5	TS	+	+	—	—				
		NS	—	—	—	—				
		CS	—	—	—	—				
		TO				+				
		SN	4			16				
2	10^6	TS	+	+		—	—			
		NS	—	—		—	—			
		CS	—	—		—	—			
		TO					+			
		SN					16			
3	10^6	TS		+	+	—		—		—
		NS		—	+	—		—		—
		CS		—	—	—		—		—
		TO								—
		SN				16		64		64
4	$10^{6.3}$	TS	+		+	+	—	—	—	
		NS			—	—	—	—	—	
		CS			+	—	—	—	—	
		TO							—	
		SN			4			32		
5	0	TS			—	—	—	+		
		NS			—	—	—	—		
		CS			—	—	—	—		
		TO						+		
		SN					0	4		
6	0	TS	—		—	+	—	—		—
		NS	—		—	—	—	—		—
		CS	—		—	—	—	—		—
		TO								—
		SN					4			16

[a] $TCID_{50}$ = tissue culture infective dose — 50%

[b] TS = throat swab; NS = nasal swab; CS = conjunctival swab; TO = tonsil at time of slaughter; SN = reciprocal of serum neutralization titer

ing organs were examined for virus: cerebrum, cerebellum, tonsil, parotid and submaxillary lymph nodes, parotid and submaxillary salivary glands, lungs, liver, spleen, kidneys, bronchial and mediastinal lymph nodes, mesenteric lymph nodes. Cultures of PK15 cells were used for virus isolation from the organs and from the throat, nasal and conjunctival swabs.

Specimens from some organs were examined using fluorescent antibody procedures. Sera were tested for specific pseudorabies antibodies with two-fold dilutions of test serum added to equal volumes of virus suspension containing approximately 100-200 $TCID_{50}$ of virus. The neutralizing titer of serum was taken as the highest dilution of the serum where no cytopathic effect was observed.

RESULTS

No signs of pseudorabies developed in the six animals during the course of the experiment, that is up to 42 days from the time the animals were inoculated. They continued to eat normally and to be highly aggressive. When a wild pig is handled the body temperature rises to 41 or 42 C. The detection of a febrile state is, therefore, quite difficult.

The results of virus isolation are presented in Table 1. From each animal used in the experiment, the virus was recovered from the throat swabs. In one animal, virus was isolated also in the nasal swab and in another in the conjunctival swab. The shedding of virus lasted 5-12 days. In the two control swine the oral shedding lasted for a shorter period. Virus was reisolated from the tonsils of three animals, when oral shedding was positive (animal no. 5) and then 3 days (animal no. 1) and 9 days (animal no. 2) after the throat swabs became negative. Virus was not demonstrated in the slaughtered animals indicating that after about 15-30 days from the end of the oral shedding period, the virus could not be recovered from the tonsils. Attempts to reisolate viruses from the lungs, brain and lymph nodes were unsuccessful. This agreed with the results of the immunofluorescence tests in which antigen was demonstrated only in sections of tonsil. Serum neutralization tests showed a progressive increase in specific antibody during the experiment in all the wild swine, as a further demonstration of virus multiplication.

DISCUSSION

Virulent pseudorabies virus, inoculated into the oral cavity of four 16-wk old wild swine, caused asymptomatic infection with shedding and transmission of the virus to other wild swine. The absence of signs of disease may be related to multiplication of the virus in the oropharynx, without involvement of other organs.

The virological procedures demonstrated that oral virus shedding lasts about 1-2 wk and the disappearance of the virus from the oral discharge coincided with the appearance of neutralizing antibodies in the blood, as reported in domestic swine by other workers (McFerran and Dow, 1964; 1965). The subsequent recovery of virus from the tonsils of the animals, after the throat swabs became negative, suggests the possibility of a carrier state in wild swine, as indicated by a number of other investigators (Kirkpatrick et al., 1980; McFerran and Dow, 1964; 1965). If the shedding of virus and its prolonged presence in the tonsils occurs naturally in wild swine, these animals might be a potential danger for domestic swine and dogs in relation to local customs of commerce and hunting. In the past we have had cases of pseudorabies in dogs with histories of recent bites by wild boars during hunting. The persistence of the virus in the tonsils or in other tissues, might result in later shedding of virus as a result of stress associated with trapping or transportation by truck, as is the custom in our region.

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