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ANTIBODIES TO BERNE VIRUS IN HORSES AND OTHER ANIMALS

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ABSTRACT

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After inoculation into 2 foals, Berne virus induced neutralizing antibody, but did not cause clinical symptoms. In a horizontal study of seropositive mares and their offspring, a decline of maternal antibodies and a sudden synchronous seroconversion in all foals were observed, again without clinical symptoms. The virus is widespread in the Swiss horse population and has been so during the last decade; rises in antibody titers were noted in 9% of paired sera sampled at random. Positive reactions were also obtained in serum neutralization tests and ELISA using small numbers of horse sera from Germany, France and the U.S.A.

The results of neutralization tests and ELISA were correlated in 83% of random samples tested; 13% were neutralization-positive and ELISA-negative and in 4% the inverse was observed. Neutralizing activity was found in the sera of other ungulates (cattle, goat, sheep and pig), laboratory rabbits and 2 species of wild mice (*Clethrionomys glareolus* and *Apodemus sylvaticus*). Inconclusive results were obtained with feline and human sera; those from dogs and foxes (*Vulpes vulpes*) were consistently negative. The probable occurrence of antigenic variants in Berne-type viruses is discussed.

INTRODUCTION

The purification and partial characterization of a new enveloped RNA virus isolated during routine diagnostic work from a horse in Berne, Switzerland has been reported (Weiss et al., 1983). 'Berne virus' measures 120—140 nm in its largest diameter and consists of a peplomer-bearing envelope and an elongated core which is usually bent into an open torus within the membrane. The core is tubular in appearance and has a morphology indicative of helical nucleocapsid symmetry. The virus possesses an RNA genome since its growth is not affected by DNA nucleotide analogues;

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actinomycin D and alpha-amanitin on the other hand do inhibit replication, as does UV irradiation of the cells before infection. A unique pattern of viral polypeptides was reported (Horzinek et al., 1984).

Berne virus was shown to be serologically unrelated to known equine viruses as well as to representatives of the 3 main antigenic clusters of the *Coronaviridae* family (infectious bronchitis, mouse hepatitis and transmissible gastroenteritis viruses), which it resembles superficially in negatively stained preparations. However, an antigenic relationship was detected with the Breda group of viruses recently discovered by workers in Ames, IA (Woode et al., 1982). In this paper, results of seroepidemiological studies in horses and other animal species are presented, with the aim of showing the distribution and possible significance of this virus in the domestic and wild animal population.

MATERIALS AND METHODS

Cell culture

Berne virus (strain P138/72) was originally isolated in secondary horse kidney cells and our neutralization tests early in this study (before 1978) were also performed in these cells. Later on, virus propagation and assay was done in embryonic mule skin cells (EMS line) grown in Eagle's minimum essential medium supplemented with non-essential amino acids (1%), L-glutamine (200 mM), sodium bicarbonate, antibiotics and 2—10% foetal calf serum; the serum had been pre-screened for the absence of antibody against Berne virus by neutralization tests.

Origin of sera

A total of 273 randomly selected adult horses from various regions of Switzerland were sampled two times, in order to show seroconversion. The period between the bleedings ranged between 3 and 45 days, with about 70% of the second samples taken 14—25 days after the first ones. Additional ungrouped random serum samples were obtained from Berlin (Germany), the Lyon area (France) and from various regions of the USA. In the Swiss "Haras Federal Avenches" 20 mares and their foals were followed serologically over a period of 13 months (Table I).

A limited serosurvey was attempted using random samples from cattle, goats, sheep, pigs, dogs, cats and laboratory rabbits; most sera were from Switzerland, supplemented with some cat and rabbit specimens from The Netherlands. In addition, 46 red fox (*Vulpes vulpes*) sera were tested. From the same source, mouse sera from wild-caught specimens of the species *Clethrionomys glareolus*, *Apodemus sylvaticus* and *A. flavicollis* were obtained.

Infectivity assay

Infectious doses (ID_{50}) were routinely determined in flat bottom microtiter trays (Greiner and Soehne, Nuertingen, Germany) by adding 100 μl volumes of serial 10-fold virus dilutions to wells containing a monolayer of 3×10^4 EMS cells. The Spearman-Kaerber formula was applied for the calculation of infectivity titers after reading the plates 5 days after infection.

Neutralization assay

Neutralization titers were determined after reacting serial 2-fold dilutions of the inactivated serum with 100 ID_{50} units of native Berne virus for 60 min at 37°C. Subsequently, 4 EMS cell monolayers (in microtiter wells) per dilution were inoculated and the plates were examined for CPE 5 days after infection.

Enzyme linked immunosorbent assay (ELISA)

An indirect ELISA procedure (Engvall and Perlmann, 1972) was used for monitoring antibody in sera from horses, cattle and goats. Antigen semipurified by ammonium sulphate precipitation and subsequent interphase centrifugation (15/50% sucrose; Weiss et al., 1983) was adsorbed to polystyrene Dynatech Immulon flat bottom micro-ELISA plates in the presence of a 0.1 M carbonate/bicarbonate buffer, pH 9.6. Prediluted horse sera (1/50 or 1/80) and an anti-horse total IgG preparation conjugated to horse radish peroxidase (Sigma, St. Louis, MO, U.S.A.) by a 2-step glutaraldehyde coupling procedure (Avrameas et al., 1978) were employed. In the substrate mixture 2.2′-azinodi-(3-ethyl-benzthiazolin-sulphonate(6)) (ABTS, Boehringer, Mannheim West Germany) was used at a concentration of 2 mM in 0.1 M acetate buffer, pH 4.0, containing 50 mM NaH_2PO_4 and 25 mM H_2O_2 as described previously (Horzinek et al., 1982). Absorption measurements were made at 405 nm using a Titertek Multiscan recording photometer (Flow Labs., Glasgow, U.K.).

Experimental infection

Since Berne virus had been isolated from a horse with pseudomembranenous enteritis and miliary granulomas with necrosis in the liver (Weiss et al., 1983), the pathogenic properties of the agent were studied in 2 foals. The animals, which were about 12 months old, received approximately 10^7 ID_{50} units of P138/72 material by the intravenous route. They were examined daily for disease symptoms.

RESULTS

Inoculation experiments

Serum samples taken from the foals before inoculation of Berne virus had neutralization titers of ⩽20 (Fig. 1). A distinct rise in titer could be detected on Days 5 and 8 post-infection (p.i.) and maximum values were reached at Days 9 and 11 p.i., respectively; afterwards the titers tended to decline. Neither clinical signs nor abnormal laboratory data were observed in the animals during the whole experiment.

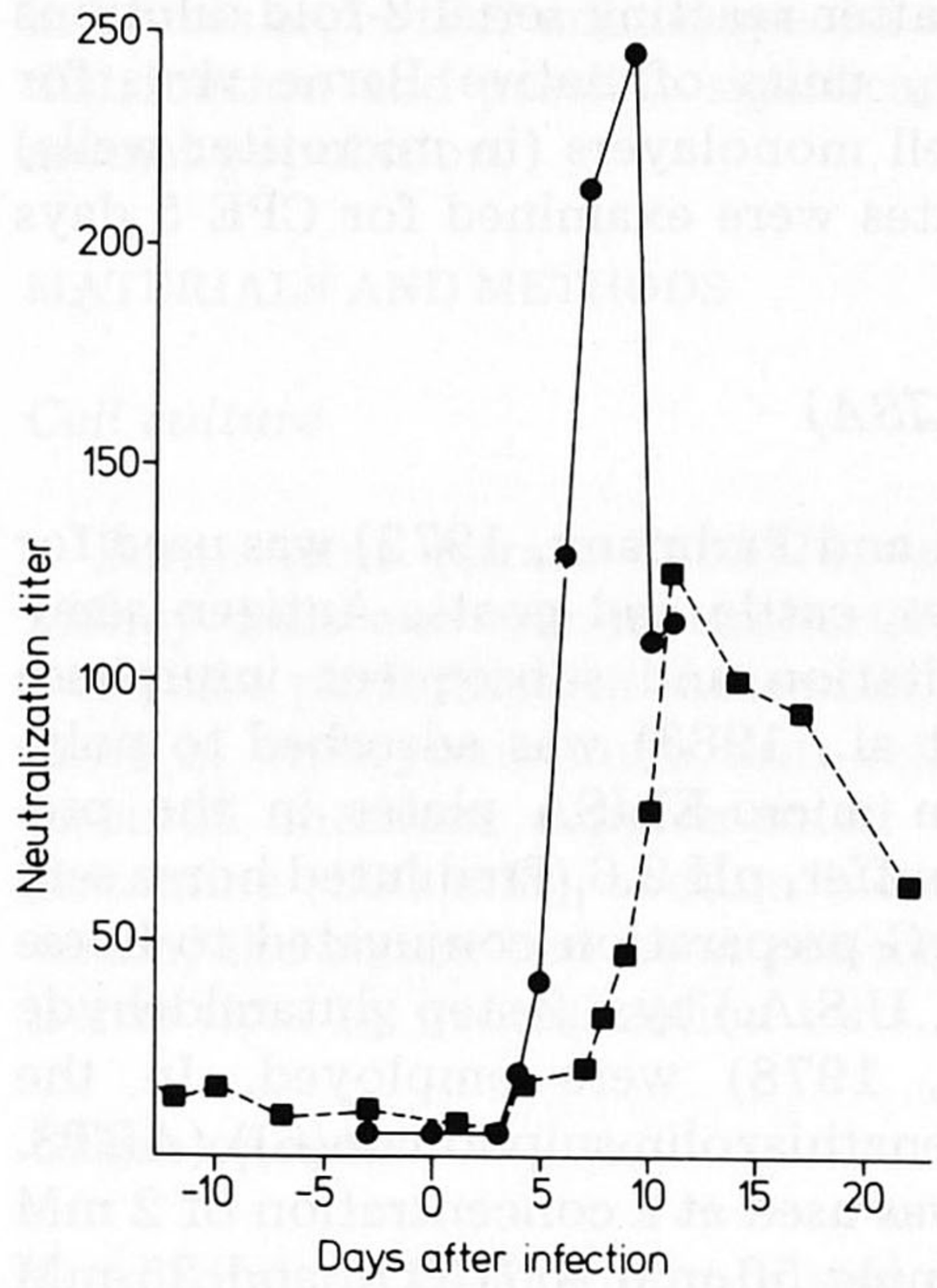

Fig. 1. Neutralizing antibody development in 2 foals infected experimentally with Berne virus on Day 0.

Neutralizing antibodies in herds

It can be seen in Table I that the antibody titers in foals initially reflected those of their mothers with whom they shared separate boxes; when they were 11 to 23 weeks old the titers had dropped below detection level. In December—January, when the animals were between 9 and 12 months of age a sudden seroconversion was noted in the whole herd which had been housed together in one stable 2 months previously (beginning of October). Endemic infection had obviously taken place; however, enteric disease symptoms were not reported for this period. The animals remained seropositive at 2 subsequent tests.

The activity of Berne virus in the Swiss adult horse population was eval-

TABLE I

Neutralizing antibody titers in mares and their foals (Haras Federal Avenches)

Name [a]		Mare	Foal								
			Days after birth (sample no.)								
			102(1)	135(2)	163(3)	205(4)	254(5)	295(6)	359(7)	374(8)	393(9)
ALI	(1)	230	20	5	<5	<5	<5	<5	40	70	90
ORL	(2)	30	10	<5	<5	<5	<5	<5	30	50	50
RIT	(4)	250	10	10	<5	<5	<5	<5	40	60	40
INS	(16)	160	10	10	<5	<5	<5	<5	40	60	60
SLA	(26)	110	50	10	10	<5	<5	<5	80	60	80
OTT	(27)	110	<5	<5	<5	<5	<5	<5	80	160	110
KLA	(28)	110	40	10	<5	<5	<5	<5	70	80	110
ALE	(29)	90	10	10	<5	<5	<5	<5	40	40	40
WUD	(30)	10	5	<5	<5	<5	<5	<5	60	80	80
AVE	(46)	130	10	10	<5	<5	<5	<5	40	30	60
HAI	(55)	360	10	10	<5	<5	<5	<5	30	60	60
JOR	(58)	80	10	10	<5	—	—	—	—	—	—
KOR	(64)	110	40	10	<5	<5	<5	<5	10	10	60
ODA	(64)	230	40	20	10	<5	<5	<5	20	80	20
OND	(66)	70	20	10	<5	<5	<5	<5	70	—	120
WOH	(66)	270	60	30	10	10	<5	<5	60	60	70
ONA	(66)	230	110	20	10	10	<5	<5	40	40	40
QUI	(72)	100	20	10	<5	<5	<5	<5	80	90	50
POL	(76)	>640	190	80	30	10	<5	<5	40	90	100
NAD	(79)	230	80	30	10	10	<5	<5	40	80	90

[a] The age of the foals (in parentheses) and the dates of blood sampling were calculated starting with Day 1, on which foal ALI was born.

TABLE II

Percentages of horse sera showing neutralization titers in different sampling periods

Period	No. of samples	Neutralization titer				
		<10	10—50	50—100	100—200	>200
1974/76	346	22	28	16	15	19
1982/83	161	11	32	17	15	24

uated by testing paired serum samples. Of the 273 animals under study, 9% were neutralization-negative at the first and second tests; another 9% showed a significant (more than 4-fold) rise in neutralization titer at the second test. Seroconversions from <5 to >640 were noted. Anamnestic responses were seen, with titers in the first serum samples around 100 and rises to >640. Second samples from this collection were also used for making the comparison listed in Table II. It is obvious that Berne virus has been active in the Swiss equine population during the last decade. A comparable distribution of titers was noted in the period of 1974 to 1976 and 1982/83, respectively.

Positive reactions in serum neutralization of small numbers of randomly-collected equine sera from Germany (8/11) and the USA (24/38) and in ELISA of samples from Southern France (10/28) and the USA (12/16) were recorded.

Antibodies measured by ELISA

The correspondence between the results of the serum neutralization test and ELISA was studied. One hundred randomly-selected horse sera were assayed in both tests and correlation of the results was obtained in 83 samples. In 13 cases, the neutralization test was positive (titers approaching 200) whereas ELISA was negative. The inverse was observed in 4 instances where neutralization-negative sera were weakly (3 cases) or distinctly positive (1 case) by ELISA (Table III).

TABLE III

Results of seroneutralization (SN) and ELISA performed in parallel with 100 horse sera selected at random

SN	ELISA		
	Positive	Negative	Total
Positive	74	13	87
Negative	4	9	13
Total	78	22	100

TABLE IV

Percentages of animals classified according to serum neutralization titer to Berne virus

	No. of samples	Titer range				
		<10	10—50	50—100	100—200	>200
Ungulates						
Horses	507	19	29	16	15	21
Cattle	129	14	29	25	18	14
Goat	124	31	31	15	13	10
Sheep	101	66	23	6	3	2
Pig	112	19	52	12	10	7
Carnivores						
Dog	46	100				
Fox	46	100				
Cat [a]	107	98	2			
Lagomorphs						
Rabbit	80	63	17			
Rodents						
Mouse	26	20	65	15		
Primates						
Man [a]	84	100				

[a] See text.

Antibodies in other species

As shown in Table IV, high percentages of serum samples with elevated neutralization titers were encountered in all ungulates studied (horse, cattle, goat, sheep and pig). No antibodies were detected in dogs and foxes; in cats, one sample from the Netherlands and one from Switzerland reproducibly showed titers of 10 and 14, respectively. Low, but reproducible titers, were also obtained with sera from laboratory rabbits. All 7 sera of the wild mouse species *Apodemus flavicollis* had titers <10 whereas the (2) *Clethrionomys* samples had titers of 46 and 94, respectively. Of the 17 samples from *A. sylvaticus*, only one had a titer value below 10. Testing of the human sera at low dilutions resulted in neutralization values of <10 in all 84 samples; 63 of these samples showed values of <2 upon re-examination. Erratic inhibition of virus growth was observed with 8 sera (calculated "titers" between 2 and 7).

DISCUSSION

Our study shows that: a high percentage of the adult horses in Switzerland has experienced an infection with Berne virus; in the very few experimental and natural cases observed by us, the infection was not accompanied by overt clinical symptoms; Berne virus is not "new" since antibody incidence has not changed significantly during the last 7 years; Berne virus is present in other European countries and in North America. However, it is obvious that the horse, from which the virus was originally isolated, is not the only host species of Berne virus or antigenically related viruses. The high prevalence of antibody in other ungulates and the previously demonstrated serological relationship of Berne virus with the Breda viruses and Lyon-4 virus (Weiss et al., 1983) demonstrated in cattle with diarrhea (Woode et al., 1982) indicate that agents of this new taxonomic cluster may be of significance as pathogens. We have preliminary evidence (from neutralization tests using 31 randomly collected cattle sera) that a Berne-related virus is active also in the Dutch cattle population; in only 3 samples titers were <10 and in 10 sera values of >50 were recorded. Also, cattle sera from Austria (10/10) and the U.S.A. (36/49) showed neutralization in a titer range of 10 to >200 (M. Weiss and M.C. Horzinek, 1983). Earlier, Steck et al. (1980) have reported similar results from Switzerland.

Virus neutralization is characterized by its high sensitivity and specificity. These properties should be kept in mind when interpreting findings of antibody in different species of animals. In ruminants and the pig where high neutralization titers occur, the causative viruses are expected to be closely related to our equine isolate. The marginal values and erratic inhibitions obtained with feline, rabbit and human sera may reflect low affinity antibody induced by a more distant serotype; murine sera would assume an intermediate position.

Using hemagglutination-inhibition tests, Woode et al. (1983) compared 2 isolates (Iowa 1 and 2) with another strain from Ohio (Saif et al., 1981) and arrived at the conclusion that 2 serotypes exist in Breda virus. In its exclusivity (recognition of determinants on the virion membrane surface), hemagglutination-inhibition is similar to neutralization; ELISA, however, would also detect internal (nucleocapsid) antigens which tend to be evolutionary more conserved and broadly cross-reactive. The discrepancies observed (Table III) in the sense that ELISA-negative horse sera gave positive results in neutralization are explained by the superior sensitivity of the latter test. It can be assumed that in those cases where neutralization-negative sera have been found ELISA-positive, internal virion antigens are detected. Experiments are under way to examine sera from different animal species using ELISA and radioimmune precipitation.

Berne virus did not induce overt disease symptoms in the horse in the few experimental and natural infections observed. It cannot be excluded, however, that the virus may cause inconspicuous or chronic degenerative changes after an apparent primary infection. The pathogenic significance of related viruses has been established in cattle (Saif et al., 1981; Woode et al., 1982) and electron microscopic evidence of coronavirus-like particles in association with enteric disease (see e.g., Pass et al., 1982) should alert the investigator to this new family of viruses (Weiss et al., 1983; Horzinek et al., 1984). For the time being, however, Berne virus in horses must be considered as a "virus in search of disease".

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