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Practical significance of rabies antibodies in cats and dogs

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Summary: *Doubt has sometimes been cast upon the protective effect of rabies antibodies in serum. Animals and humans suffering from fatal rabies often produce high antibody titres, while rabies cases are also observed in vaccinated animals. Cellular immunity is also largely involved in protection. Nevertheless, a large number of laboratory experiments and field observations clearly demonstrate that cats and dogs which develop antibodies after vaccination and before challenge have a very high probability of surviving any challenge, no matter how strong the dose and which virus strain was used.*

Rabies antibody titration can, therefore, afford a strong additional guarantee to the vaccination certificates accompanying domestic carnivores during transportation between countries. Quarantine rules should also be adapted to the epidemiological features in the exporting country, e.g. statistics of vaccination failure in cats and dogs and host-virus adaptation of the rabies strains circulating in these countries.

KEYWORDS: Antibodies Cats - Dogs - Rabies - Survival to challenge.

INTRODUCTION

Cats and dogs can introduce rabies into disease free countries if they are incubating the disease and are transported during the pre-symptomatic phase. To prevent such introduction, vaccination is recommended. The present article reviews publications dealing with rabies protection afforded to cats and dogs by vaccination.

Only the parenteral route of vaccination will be considered, as the oral route is employed only for wandering and non-restrained carnivores; extensive results for individual cats and dogs are unavailable. Also, since oral vaccination could mobilise immunity pathways other than those obtained parenterally, the results with one procedure may be not transposable to the other.

Furthermore, no consideration will be given here to the results of vaccination after exposure, which does little, if anything, to alter disease (20).

Emphasis will be given to the most common method for measuring rabies immunisation: assays for **rabies virus neutralising antibodies in serum** (henceforth referred to as "neutralising antibodies"). The practical significance and consequences of rabies virus neutralising antibodies in cats and dogs are considered; namely, to what extent do neutralising antibody titres confer protection against subsequent challenge?

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No consideration will be given to the question of whether real protection against challenge is provided by neutralising antibodies and/or other immunity factors. Titres of neutralising antibodies in serum are simply viewed as the easiest means of evaluating the likelihood that a cat or dog will not contract rabies following exposure.

STUDIES IN DOGS

NEUTRALISING ANTIBODIES AFTER VACCINATION

General considerations

The kinetics obtained for neutralising antibodies after vaccination have been thoroughly described in the literature. The curve of neutralising antibodies after vaccination and boosters follows the pattern generally observed with other antigens: seroconversion and rapid rise of the level of neutralising antibodies after first vaccination, followed by a slow decrease, a new rise after booster to reach a higher level than previously observed, then a new decrease leading to a stabilised higher level (Fig. 1) (8, 51, 63). The decrease of neutralising antibody levels has been evaluated

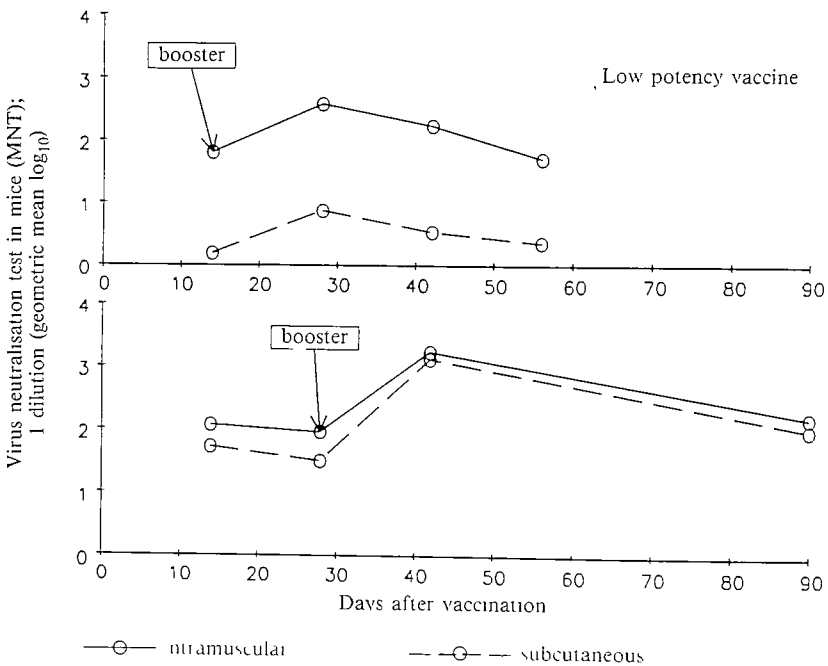

FIG. 1

Kinetics of rabies neutralising antibodies in sera of laboratory dogs vaccinated with a tissue culture vaccine
Variations according to vaccination route and antigenic value of the vaccine as measured by the NIH test
(63, 64)

in domestic populations of owned dogs in several countries: in Canada, titres of neutralising antibodies in the sera of dogs showed a clear division between, on the one hand, dogs vaccinated or revaccinated one year before and, on the other hand, dogs revaccinated three weeks before (33). Data from Thailand and Java show that the neutralising antibody titre decreases very rapidly after 60 to 120 days to levels 5 to 25 fold less than the highest point reached during the kinetics (Fig. 2) (40, 65). The higher level of neutralising antibodies obtained when owned dogs are vaccinated several times has been described by Sasaki and colleagues (55, 56).

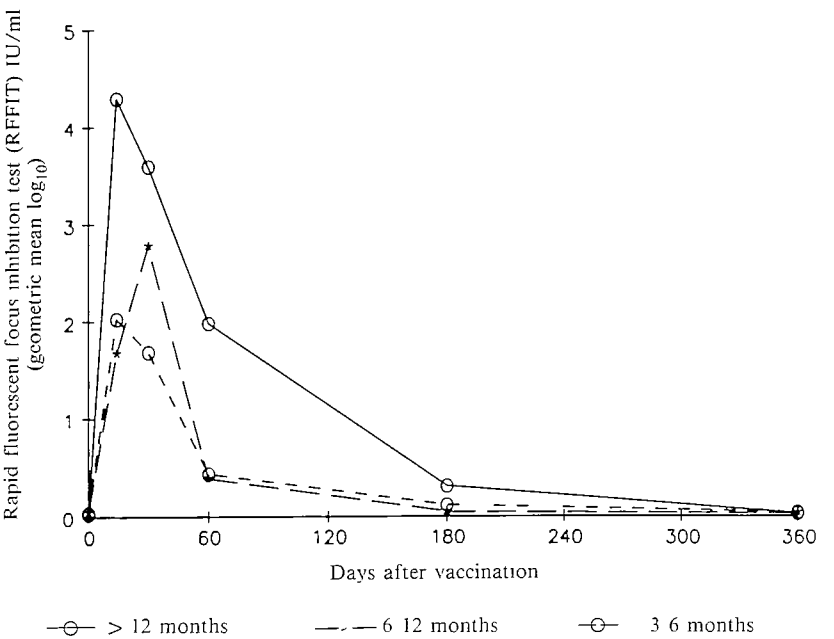

FIG. 2

Kinetics of rabies neutralising antibodies in sera of owned dogs of various ages in Thailand after one subcutaneous vaccination with a tissue culture vaccine

The number of dogs sampled was
54 at day 0 and 31 at day 360
(65)

With regard to the production of neutralising antibodies and the relation of these antibodies to protection against challenge, a clear distinction must be made between live virus vaccines and inactivated virus vaccines. These two types of vaccine cannot be directly compared. The best relation between antibody production and protection has always been obtained with inactivated virus vaccines and it is, therefore, the latter which will be considered in greater detail, especially as they currently represent the only type of vaccine authorised in a great many countries.

High individual variability

In laboratory dogs bred and kept under the same conditions and in comparable health, neutralising antibody titres obtained after the same form of vaccination commonly range from zero to twenty international units (IU) or more per ml (19, 51, 53).

Influence of vaccine types and potency

The first complete study of live virus vaccines was published by Dean and colleagues (32). This study established a correlation between antibody production and resistance to challenge, which was confirmed by later studies (see below). As far as inactivated virus vaccines are concerned, besides individual variation, the level of neutralising antibodies in serum correlates positively with the antigenic value of the vaccine as determined by the American National Institutes of Health (NIH) test. This observation is common in the course of vaccine production and control on laboratory dogs (Fig. 1) (47, 63). The influence of the antigenic value of the vaccine on the level of neutralising antibodies has also been demonstrated in domestic populations of owned dogs; in Switzerland, Engels and colleagues (35) showed that in owned dogs, higher titres were generally obtained with inactivated vaccines than with live (and less potent) vaccines.

However, when inactivated virus vaccines with an antigenic value (as measured by the NIH test) equal to or greater than 1.0 IU per dose are employed, no correlation can be shown between the level of neutralising antibodies in individual dogs and the titre of the vaccine. This result was first demonstrated in owned dogs to in France by Blancou and colleagues (13). In this experiment, dogs were sampled randomly from populations living under various conditions and were vaccinated with a range of commercially available vaccines. Chappuis and colleagues (30) and Lazarowicz and colleagues (45) used laboratory dogs to investigate whether the administration of vaccines from the same producer would entail a correlation between the NIH titre of vaccines and the level of neutralising antibody response. Even under standardised conditions, no correlation was found. The same conclusion can be drawn from the results of Barth and colleagues (7).

In summary, a significant variation of neutralising antibody response can be shown only under a broad range of vaccine potencies (61). When the potencies of commercial inactivated virus vaccines are fairly high, the neutralising antibody response will be related only to the immune responses of individual dogs.

Influence of the route of vaccination

Since Pasteur, the route of vaccination has been subcutaneous (s.c.). Fuenzalida in 1967 demonstrated that the intramuscular (i.m.) route resulted in higher neutralising antibody titres in sera of dogs (37). Apart from Merry (46), who found no clear advantage for the i.m. over the s.c. route, the results obtained by Fuenzalida were largely confirmed (22, 25, 63). However, the advantage of the i.m. route diminishes with high potency vaccines (Fig. 1) (63) and the use of adjuvanted vaccines renders the i.m. route excessively painful. Adjuvants confer a longer lasting immunity, which can be obtained with a smaller quantity of antigen, as first demonstrated on laboratory dogs (52), then on owned dogs (43, 68, 69). Despite the use of smaller quantities of antigen and a reduced vaccination schedule (less frequent boosters), the neutralising antibody levels reached after one, two or three years with adjuvanted vaccines were

equivalent to those reached with non-adjuvanted vaccines given according to the usual schedule (two injections of vaccine the first year, with annual boosters).

The importance of the vaccination route was clearly demonstrated with intradermal injection of vaccine in dogs (68). Unfortunately, the advantages of an enhanced response obtained with a minute dose of vaccine (2×0.1 ml) were offset by the fact that intradermal injection must be performed on the inside of the ear and, hence, this procedure must be conducted dangerously near the mouth of the animal.

Influence of age

It has been shown that dogs 11-16 weeks of age respond better to Flury low egg passage (LEP) or high egg passage (HEP) vaccine than dogs 5-10 weeks of age (81% vs. 38% protection from challenge, respectively) (41). The relationship between the age of animals and protection from challenge was confirmed in a laboratory study by Bunn in three- to five-month-old pups. Three months after vaccination with Flury LEP vaccine, ten of forty pups had antibody titres below 1/5 (24, 25).

A survey on owned dogs in France showed that even beyond three months of age, older dogs produced higher titres (Fig. 3) (13).

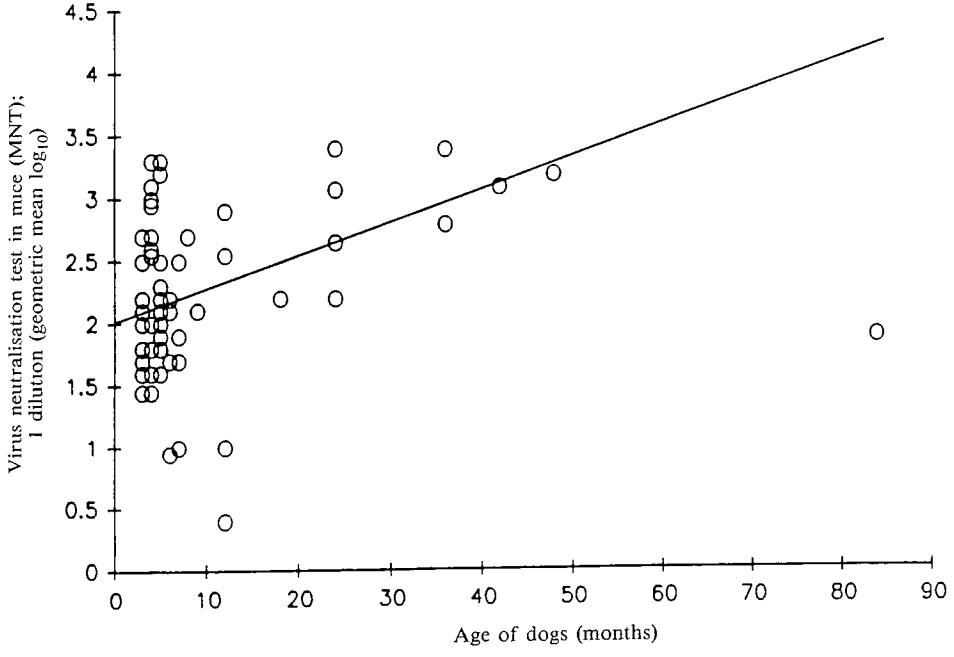

FIG. 3

Correlation between rabies antibody level reached after one vaccine injection and age of dogs
Study conducted on 66 owned dogs in France
(13)

The influence of age on the neutralising antibody response in dogs was also clearly demonstrated on owned dogs in Thailand by Teepsumethanon and colleagues (65). These authors described the kinetics of neutralising antibodies in three age groups: 3 weeks to 3 months, 6 to 12 months, and more than 12 months. Whenever the mean level of neutralising antibodies was evaluated after vaccination, the older dogs had the highest levels of response. Given the difficult conditions prevailing in Thailand, the superior response of older dogs could also be related to the increased life expectancy of dogs with a more powerful immune system (Fig. 2).

The presence of specific neutralising antibodies transmitted to puppies via colostrum impedes development of active immunity. The interference between passive neutralising antibodies of maternal origin and active immunisation has been studied by Précausta (52, 53). Puppies of non immune bitches vaccinated at the age of one month respond with the same neutralising antibody level as puppies vaccinated at seven months of age. Puppies of immune bitches vaccinated at one month of age show neutralising antibody levels which decrease according to the same kinetics as unvaccinated members of the same litter.

After ten weeks (44) to twelve weeks (52), no traces of maternal neutralising antibodies remain. Surveys in pet dog populations where systematic vaccination of adult dogs is practised (in France and elsewhere) have confirmed that no further interference between active and passive immunity occurs beyond this age (53).

Influence of the health and breeding status of dogs

Blancou and colleagues (19) compared the proportion of individuals developing neutralising antibodies in 64 dogs after the administration of adjuvanted or non-adjuvanted vaccines. This rate may vary considerably depending on the category of dog (bred for laboratories, belonging to individuals in France or uncontrolled in Tunisia). The rate drops from 100% to 59% in the case of semi-stray dogs as compared to laboratory dogs (Fig. 4). Urban dogs in Lima (Peru) exhibited better rates than in Tunisia, but the rates were still lower than in dogs kept under laboratory conditions (31). Although the health status of these populations had not been measured in the previous studies, this status is probably responsible for the differences observed by Teepsumethanon and colleagues (65) in Thailand: Thai pet dogs which had received one s.c. dose of rabies vaccine exhibited a better neutralising antibody response when they did not suffer from anaemia (Fig. 5). In 440 pets under quarantine in Hawaii, Sasaki and colleagues (56) demonstrated that those with internal parasites had significantly lower levels of neutralising antibodies than those without parasites.

LEVEL OF NEUTRALISING ANTIBODIES IN SERA AND RESULTS OF CHALLENGE

Challenge under laboratory conditions

In view of the serious problem posed by rabies, challenge of previously vaccinated dogs has often been performed even when a large proportion of the dogs under experiment exhibited a seroconversion. Moreover, such challenges are performed in response to doubts which have sometimes been cast on the significance of neutralising antibodies to rabies, due to the fact that high titres have been measured in human beings and animals dying of rabies. In fact, very few diseases show so clear a correlation as in rabies between seroconversion **before** challenge and protection from challenge.

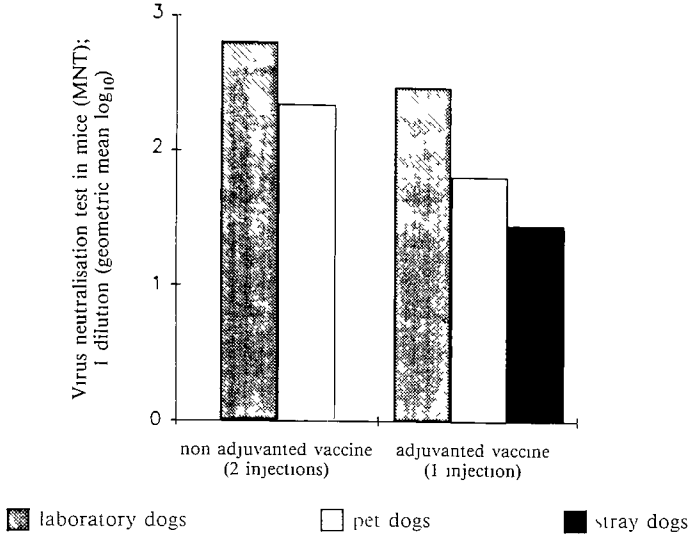

FIG. 4

Influence of the breeding standards of dogs on the level of rabies antibody reached one year after vaccination

Comparison of laboratory dogs, pet dogs in France and stray dogs in Tunisia

(19)

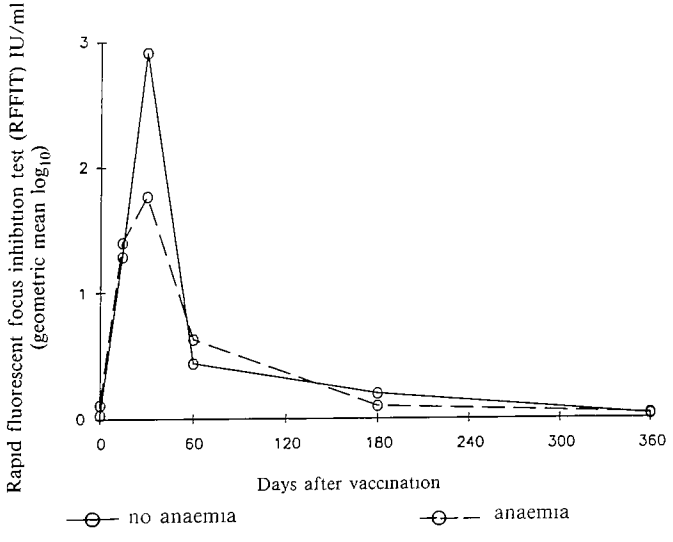

FIG. 5

Influence of the health status of Thai dogs on the level of rabies antibody reached after one vaccination

Comparison of dogs with or without anaemia

(65)

In the context of movement of dogs between countries, it is possible to check the efficiency of previous vaccinations. A large number of reports can be summarised by the simple comparison of the proportion of dogs surviving challenge vs. the proportion of dogs with detectable neutralising antibodies in serum just before challenge (i.e. theoretically when the neutralising antibody level is lowest). These summaries are given in Tables I to VIII.

Sikes and colleagues (62) employed several types of vaccine on dogs and challenged them one or three years after vaccination (Tables I and II). Sikes (61) commented on the three-year experiment as follows: "In this study, as in many others, presence of neutralising antibodies to rabies at the time of challenge did not indicate protection for all of the animals. Likewise, absence of neutralising antibodies in serum at the time of challenge did not mean the animals were unprotected. However, there was strong statistical significance ($P < 0.1$) that animals with neutralising antibodies at the time of challenge were better protected than those with no detectable neutralising antibodies."

TABLE I

Laboratory dogs: one intramuscular vaccination with various vaccines, challenge with rabies virus NYC-Ga strain one year after vaccination

(61)

Vaccine	Dogs with antibodies just before challenge (%)	Dogs surviving challenge
Experiment 1		
LEP tissue culture	88	9/10
LEP tissue culture	73	10/10
ERA tissue culture	73	10/10
LEP chicken embryo	70	10/10
HEP tissue culture	63	10/10
CVS adjuvanted	13	7/10
None	0	0/10
Experiment 2		
Suckling mouse brain	95	10/10
Suckling mouse brain	67	10/10
None	0	0/10

LEP: low egg passage
HEP: high egg passage
ERA Elizabeth (Gaynor) Rokitniki Abelseth
CVS: challenge virus strain

Sikes employed the NYC-Ga (New York City-Georgia) dog salivary gland strain of rabies virus. The same strain has also been used for challenge in other experiments (Tables III to VI) and the results confirm each point of the statements made by Sikes (60) regarding vaccination of dogs:

a) generally, groups of dogs with a high percentage of seroconversion will have the highest probability of surviving challenge

TABLE II

Laboratory dogs: one intramuscular vaccination with various vaccines, challenge with rabies virus NYC-Ga strain three years after vaccination
(61)

Vaccine	Dogs with antibodies just before challenge (%)	Dogs surviving challenge
LEP tissue culture	87	29/30
LEP tissue culture	69	26/29
ERA tissue culture	57	27/30
LEP chicken embryo	54	28/30
Suckling mouse brain	48	27/27
HEP tissue culture	42	27/29
Suckling mouse brain	28	23/29
CVS adjuvanted	0	17/29
None	0	3/30

Results of challenge	Antibodies before challenge yes	no
Rabid	3 *	26
Surviving	157	47

* titres of 1/2, 1/3 and 1/5 (endpoint neutralising dilutions of the serum)

TABLE III

Laboratory dogs: one vaccination with HEP vaccine, challenge with rabies virus NYC-Ga strain three years after vaccination
(22)

Vaccination	Dogs with detectable antibodies just before challenge	Dogs surviving challenge
Intramuscular injection		
Undiluted	29/30	30/30
Diluted 1/10	6/10	10/10
Diluted 1/100	4/10	9/10
Subcutaneous injection		
Undiluted	4/29	17/29
Diluted 1/10	0/9	2/9
Diluted 1/100	0/8	2/8
None	0/30	0/30

TABLE IV

Laboratory dogs: subcutaneous vaccination with tissue culture vaccine, challenge with rabies virus NYC-Ga strain twenty-seven months after vaccination

(8)

Antigenic value of vaccine *	Dogs with detectable antibodies 12 months after vaccination	Dogs surviving challenge	
		Vaccinated	Controls
0.6	8/8	8/8	0/7
1.7	19/20	17/18 **	3/12
4.6	10/10	9/9	
2.3	6/9	8/9 ***	4/12

* measured by the NIH test, expressed in IU/dose
** The dog which died of rabies had always had the lowest antibody titre in the group
*** The dog which died of rabies had never seroconverted

TABLE V

Laboratory dogs: subcutaneous vaccination with tissue culture vaccine, challenge with rabies virus NYC-Ga strain three years after vaccination

(52)

Vaccination	Dogs with serum antibodies >0.5 IU/ml just before challenge	Dogs surviving challenge	
		Vaccinated	Controls
One injection of adjuvanted vaccine	29/30	29/30	0/20

TABLE VI

Laboratory dogs: intramuscular vaccination with tissue culture adjuvanted vaccine, challenge with rabies virus NYC-Ga strain three years after vaccination

(59)

Vaccination	Dogs with detectable antibodies just before challenge	Dogs surviving challenge
Yes	14/25	23/25 *
No	0/10	2/10

* One of the two dogs which died following challenge was seronegative, the other had a titre of 1/4 (endpoint neutralising dilution of the serum)

b) on an individual basis:

a dog with neutralising antibodies just **before challenge** will have the best chance of surviving a severe challenge

– a dog with no detectable neutralising antibodies just before challenge will have a high chance of surviving a severe challenge if it seroconverted **after vaccination**

some dogs will not survive a severe challenge even if they have detectable neutralising antibody titres **before challenge**; generally these titres are the lowest of the group.

In studies of fox strains of rabies virus (Tables VII and VIII), the possibility of procuring a strong immunity as long as four to five years after vaccination, and of enhancing protection by the use of adjuvanted vaccines, has been demonstrated. These studies also confirmed the correlation between neutralising antibodies and protection against a fox strain.

TABLE VII

Laboratory dogs: intramuscular vaccination with ERA vaccine, challenge with rabies virus fox strain four or five years after vaccination
(44)

Vaccination	Dogs with detectable antibodies just before challenge	Dogs surviving challenge
Four years before challenge		
Yes	5/10	7/10
No	0/9	0/9
Five years before challenge		
Yes	7/14	13/14
No	0/14	5/14

TABLE VIII

Laboratory dogs: subcutaneous vaccination with adjuvanted tissue culture vaccine, challenge with rabies virus fox strain two years after booster vaccination
(38)

Antigenic value of vaccine *	Dogs with detectable antibodies just before challenge	Dogs surviving challenge	
		Vaccinated	Controls
4.2	9/10 **	10/10	0/5

* measured by the NIH test, expressed in IU/dose
** two dogs with an antibody titre <0.5 IU/ml

Bunn and colleagues (28, 29) gathered pre-challenge neutralising antibody titres and challenge results obtained on dogs by the United States National Veterinary Services Laboratories and by vaccine manufacturers. Most of the dogs were challenged with the NYC-Ga strain, but results obtained with fox or skunk strains were also added. Sera were titrated either by the virus neutralisation test in mice (MNT) (5) or the rapid fluorescent focus inhibition test (RFFIT) (62). Data on neutralising antibodies originally expressed in arithmetical dilutions by Bunn (26, 27) have been converted into IU in Figure 6. Beyond 0.03 IU/ml with the MNT or 0.05 IU/ml with the RFFIT, the expected survival to challenge by a dog strain reaches 95%. With 288 dogs having RFFIT titres above 0.1 IU/ml, a 100% survival rate was obtained. The maximum survival rate observed among animals with the highest neutralising antibody titres measured by MNT was 99.5%.

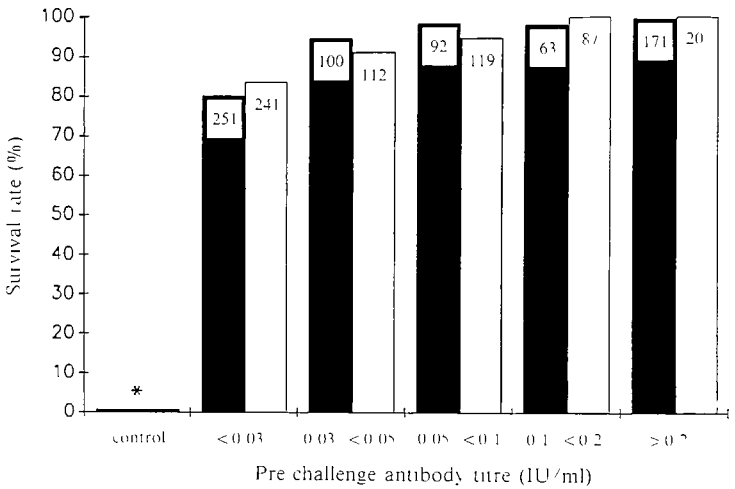

FIG. 6

Survival rate after challenge of laboratory dogs correlated with the level of rabies antibody reached before challenge

Dogs were vaccinated with various vaccines and challenged one year after vaccination with NYC Ga, fox or skunk strains; the number of dogs in each class is written at the top of the bars
(27)

Given the higher susceptibility of dogs to dog strains (e.g. NYC Ga), which was proven by cross challenge of dogs with homologous and heterologous (fox) strains (15, 17), the challenge with fox strains could be expected to be less severe. Unfortunately, the data are too scarce to permit a definitive conclusion.

Natural infection of vaccinated dogs

The number of vaccinated dogs which become naturally infected is related to several factors other than vaccine potency, such as probability of encountering an infected animal, severity of bites, health status and immune efficiency of the vaccinated dogs, and host virus adaptation. Such considerations could explain why vaccinated dogs suffer rabies more often in the course of dog rabies enzootics than during fox rabies enzootics. In Thailand, 9% of the dogs found positive upon laboratory diagnosis had been vaccinated within the previous two years (39). In Nigeria, a survey of 2,500 dogs vaccinated over two years, showed that at least four died of rabies three to eight months after vaccination (1, 2).

The following reasons (16) for the failure of immunity may be suggested:

inappropriate vaccination with inadequately stored or improperly injected vaccine

– vaccination during the incubation of rabies or before the onset of an immunological response

– a heavy challenge overwhelming host defences

– intrinsic incapacity in the host.

Whatever the origins of rabies cases recorded in vaccinated dogs, their number seems relatively low in areas contaminated with fox rabies (e.g. in Europe) (20). In France, only ten cases of so-called vaccination failures in dogs (and four among cats) have been registered over a period of twenty-three years (6). This number should be compared with the 4,250,000 cats and dogs vaccinated annually in France (this figure is based on the annual number of vaccine doses sold for domestic carnivores). The probability of a cat or dog becoming rabid if vaccinated can be estimated as $14/(23 \times 4{,}250{,}000)$, which is less than 1/6,980,000. In France, dogs in contact with a rabid animal in an enzootic area are not sacrificed and can be kept alive if, prior to contamination, they have been properly vaccinated (with certificate and identification). In such cases, the animals are immediately revaccinated. A study of more than 3,500 dogs which had close contacts (bites in 36% of cases) with foxes (mainly) or other carnivores which were diagnosed as rabid by laboratory examination, revealed that only three dogs developed rabies (50). The failure rate in animals which were **definitely contaminated** can be estimated as 3/3,500, given that injection of vaccine after contamination has been shown to provide no protection (20). It must be emphasised that these failures were recorded before 1984 and that failure is now less probable, given the generalisation of adjuvanted vaccines for dogs. In the United States of America, four rabies vaccine failures were recorded in cats and dogs in 1988 with 33,182,575 vaccinated domestic carnivores the same year (rate – 1/8,296,000) (34).

Such evaluations could be useful in comparing the risks of vaccination with those of quarantine. For even when they are strictly managed, quarantines still entail a risk. For instance, in many countries, the quarantine period is six months. However, longer incubation periods have been reported in dogs (8.5 months after challenge) (67) and in other carnivores (12 months or more for foxes) (57). According to Sasaki and colleagues (55), Beynon determined that a quarantine period of nine months would be necessary to detect all cases of incubating rabies with a 95% degree of confidence.

STUDIES IN CATS

NEUTRALISING ANTIBODIES AFTER VACCINATION

Although fewer studies have been conducted on vaccination of cats against rabies, several of the characteristics observed in dogs were also observed in cats:

the kinetics of neutralising antibodies follow the same profile in the two species (8, 23, 64)

the relationship between the potency of vaccines and the level of neutralising antibodies: Lawson and colleagues (44) have shown that the less diluted modified live vaccines induced the highest rate of seroconversion in vaccinated cats (Table IX) but Lazarowicz and colleagues (45) obtained no correlation of the antigenic value of inactivated virus vaccines as determined by the NIH test and mean neutralising antibody titres in vaccinated cats; however, as for dogs, it is necessary to take account of the fact that the production of antibodies (and protection against challenge) obtained after administration of live virus vaccine (44) and inactivated virus vaccine (45) can show great divergence and are not readily comparable

the greater efficacy of intramuscular vaccination (59) (Table X).

TABLE IX

Laboratory cats: intramuscular vaccination with various vaccines, challenge with rabies virus fox strain five weeks and four years after vaccination
(44)

Vaccination	Cats with detectable antibodies just before challenge	Cats surviving challenge
Five weeks before challenge		
ERA undiluted or diluted 1/10	19/19	19/19
Inactivated virus vaccine undiluted or diluted 1/10	20/20	20/20
ERA diluted 1/100 or 1/1,000	5/20	12/20
HEP diluted 1/1,000	1/40	11/40
Inactivated virus vaccine diluted 1/1,000	0/5	2/5
None	0/11	3/11
Four years before challenge		
ERA	7/8	8/8
None	0/8	1/10

ERA: Elizabeth (Gaynor) Rokitniki Abelseth
HEP: high egg passage

Concerning the influence of age, Précausta and colleagues (53) described the good neutralising antibody response achieved by three month-old kittens, even those born of immune queens.

To our knowledge, Blancou and colleagues (18, 19) are the only authors who have tested vaccination results on owned cat populations. Pet cats appeared to respond well to the administration of an adjuvanted vaccine. In contrast, the same authors

TABLE X

Laboratory cats: intramuscular vaccination with tissue culture adjuvanted vaccine, challenge with rabies virus NYC-Ga strain one to three years after vaccination
(59)

Vaccination	Cats with detectable antibodies just before challenge	Cats surviving challenge
One year before challenge		
Yes (subcutaneous)	5/5	5/5
No	0/4	0/4
Three years before challenge		
Yes (intramuscular)	25/25	24/25 *
No	0/10	1/10

* Prior to challenge, the cat which died of rabies had an antibody titre of 1/2 (endpoint neutralising dilution)

obtained mild or zero neutralising antibody responses when vaccinating cats sampled from stray populations in France. With stray cats, the worst result was obtained with non-adjuvanted vaccines: 5 of 9 individuals did not respond (Fig. 7).

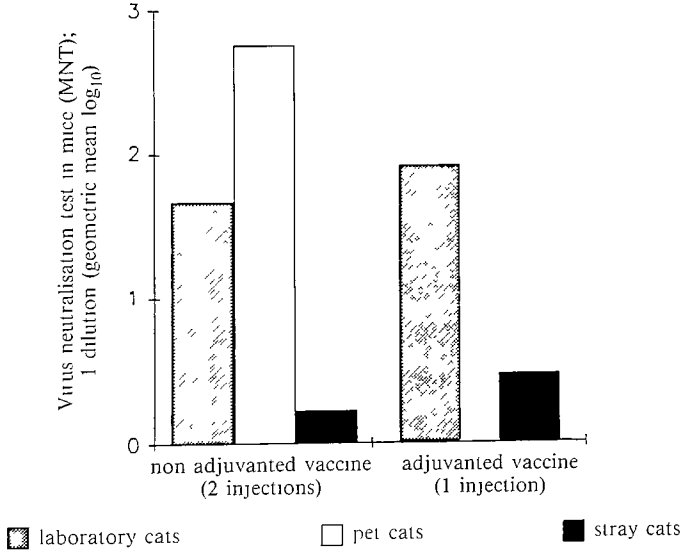

FIG. 7

Influence of the breeding standards of cats on the level of rabies antibody reached one year after vaccination
Comparison between laboratory cats, pet cats in France and stray cats in Tunisia
(19)

LEVEL OF NEUTRALISING ANTIBODIES AND RESULT OF CHALLENGE

Challenge under laboratory conditions

Although there are few field studies on the immunity of pet or stray cat populations, there are numerous laboratory studies on challenge of vaccinated cats (Tables IX to XII).

TABLE XI

Laboratory cats: subcutaneous vaccination with tissue culture vaccine, challenge with rabies virus NYC-Ga strain 3.4 to 3.7 years after vaccination
(64)

Vaccination	Cats with antibodies >0.5 IU/ml 0-6 months before challenge	Cats surviving challenge	
		Vaccinated	Controls
Non-adjuvanted vaccine	8/8	8/8	0/5
Adjuvanted vaccine	5/5	5/5	0/5
Adjuvanted vaccine	8/11	10/10	1/10

TABLE XII

Laboratory cats: challenged with rabies virus NYC-Ga strain four to six-and-a-half months after vaccination
(42)

Vaccination	Cats with antibodies >0.5 IU/ml just before challenge	Cats surviving challenge	
		Vaccinated	Controls
Inactivated virus in cell culture			
antigenic value 0.9 [a]	8/8	7/8 [c]	
antigenic value 1.8 [a]	5/8	8/8	0/16
Modified live virus			
ERA	2/8	3/8 [b,c]	

[a] measured by the NIH test, expressed in IU/dose
[c] The cat which died of rabies had a pre-challenge titre of 5.34 IU/ml
[b,c] The cats which died of rabies had the lowest antibody titres

ERA. Elizabeth (Gaynor) Rokitniki Abelseth

Challenge was performed with a dog strain (NYC-Ga) mimicking the situation of canine street rabies (42, 61, 64) or, in other experiment, a fox strain mimicking the situation of sylvatic fox rabies in continental Europe (38, 44). With both strains, the general conclusion was the same as for dogs: the probability of a cat surviving challenge can be predicted by the level of neutralising antibodies. Of course, unexpected deaths can occur: Kihm and colleagues (42) reported a rabies death in

a cat which had a pre-challenge titre of 5.34 IU and Blancou and colleagues (18) in another cat with a pre challenge titre of 0.87 IU/ml.

The cumulative challenge results on cats reported by Bunn (26, 27) are described in Figure 8 and Tables XIII and XIV. With a neutralising antibody level of more than 0.1 IU (measured by MNT) or more than 0.2 IU (measured by RFFIT), all of the cats survived challenge.

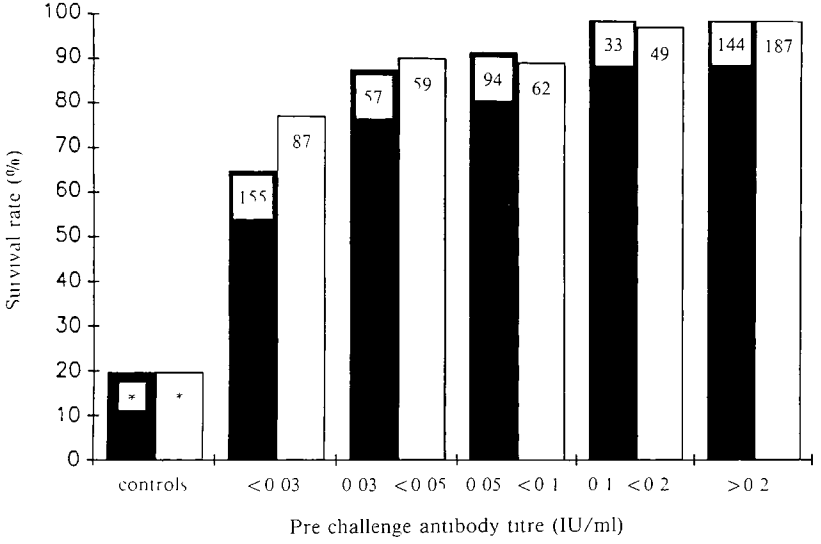

FIG. 8

Survival rate after challenge of laboratory cats correlated with the level of rabies antibody reached before challenge

Cats were vaccinated with various vaccines and challenged one year after vaccination with NYC-Ga, fox or skunk strains; the number of cats in each class is written at the top of the bars
(27)

Natural infection of vaccinated cats

The safety problem associated with the receptivity of cats to live virus vaccines such as Flury LEP and HEP or Street Alabama Dufferin (SAD) strain vaccines will not be reviewed here (11). But it should be remembered that while cats are the species with the largest number of rabies cases directly induced by the inoculation of live modified virus strains, other species such as dogs and foxes are also receptive (72).

TABLE XIII

Challenge results from rabies immunogenicity tests conducted in dogs and cats with vaccines approved for use in the United States of America
(27)

Vaccine	Antibody titre *				
	<5	5-9	10-19	20-39	⩾40
Flury modified live vaccine	1/50 **	0/16	0/26	0/15	0/40
SAD modified live vaccine	5/55	3/36	1/41	0/35	1/188
SAD inactivated virus, tissue culture origin	21/156	2/63	1/116	0/79	0/150
Pasteur inactivated virus, tissue culture origin	5/44	1/45	0/38	0/32	0/76
Pasteur inactivated virus, nervous tissue origin	13/133	2/62	5/73	1/34	0/164

* antibody titres expressed as 50% endpoint neutralising dilutions established by either the virus neutralisation test in mice (MNT) or the rapid fluorescent focus inhibition test (RFFIT)
** dead/challenged

SAD: Street Alabama Dufferin strain

TABLE XIV

Neutralising antibody titres in dogs and cats and protection from challenge with rabies virus
(26)

Animals	Antibody test	Antibody titre *				
		<5	5-9	10-19	20-39	⩾40
Dogs	MNT	56/251 **	9/100	9/92	1/63	0/171
	RFFIT	84/241	13/112	9/119	0/87	0/201
	Total	140/492	22/212	18/211	1/150	0/372
Cats	MNT	25/155	5/57	5/94	0/33	0/144
	RFFIT	17/87	3/59	1/62	1/49	1/187
	Total	42/242	8/116	6/156	1/82	1/331

* antibody titres expressed as 50% endpoint neutralising dilutions established by either the virus neutralisation test in mice (MNT) or the rapid fluorescent focus inhibition test (RFFIT)
** dead/challenged

Inactivated virus vaccines are employed on cats as they are more efficient in protecting the species against natural challenge. However, considering the results of challenge experiments on vaccinated cats, natural infection among vaccinated pet cats is suspected to be as frequent as for vaccinated dogs. But investigations on rabies cases in vaccinated cats are scarce: apart from the four cases reported in France (6) there appear to be no other reports. This discrepancy is due to the fact that dogs have been studied considerably more than cats.

THE SIGNIFICANCE OF NEUTRALISING ANTIBODIES IN NON-VACCINATED CARNIVORES

Non-specific and specific neutralising factors

Sekine and colleagues (58) found that sera of normal rabbits and guinea-pigs contained non-specific inhibitors capable of neutralising the virus in the presence of complement. In a well-conducted seroneutralisation on mice, inactivation of sera is performed for 30 minutes at 56°C. Virus inhibition by other substances was described in infected skunks and foxes (74). Infection by mycobacteria, e.g. Bacillus Calmette-Guérin (BCG), can also induce the production of rabies neutralising antibodies in mice and provide protection against rabies in a number of animals (70). Since more specific immunological tests (such as enzyme-linked immunosorbent assay: ELISA) have become widespread, non-specific neutralising factors have not generated further scientific reports.

In endemic areas, serosurveys in wild carnivores demonstrated a high proportion of apparently healthy individuals with neutralising antibodies in serum (54, 71) and it has been suggested that these antibodies may have been produced following contact with virus from other species and were, therefore, immunising but rarely fatal (12). However, the same observations have also been reported for dog populations in areas where dog rabies is endemic: in Thailand, in areas where no canine vaccination programme has ever been conducted, 15-20% of dogs had neutralising antibodies, yet remained perfectly normal when observed for prolonged periods (75); similar results had also been reported previously in other countries of Asia and in Africa (3, 36). These observations correlate with the high probability of inter-individual contamination within the reservoir species, which is not the case for pet populations in areas where rabies is endemic. The possibility of non fatal contamination of dogs by non-canine strains (e.g. those from wild animals living in the region) has also been proposed (20). Several questions thus arise regarding:

a) the specificity of serum titrations and the threshold level for protection against rabies

b) the possibility of rabies outbreaks in naturally seroconverted dogs, and the interval between seroconversion and the onset of clinical symptoms.

Rabies infections

The viral infection triggers the production of neutralising antibodies. When a high dose of rabies virus reaches the central nervous system, neutralising antibodies are not detectable before or at the onset of clinical signs; they are usually induced by longer incubation periods. This phenomenon has been studied mainly in laboratory rodents, which supply the chief model of rabies immunopathology (49, 73). Unfortunately (but not surprisingly, considering the difficulty of handling rabid carnivores), there appears to be no literature on the frequency and intensity of neutralising antibody production in non-vaccinated infected cats and dogs. Some data can be found in articles by Artois and colleagues (4), Blancou and colleagues (17) and Fekadu (36) regarding latent or abortive rabies.

Bell and colleagues (10) proved that dogs which recovered from rabies after intracerebral inoculation of homologous strains, had high titres of neutralising antibody in the cerebrospinal fluid as well as in serum and retained these titres for

several months, whereas vaccinated dogs did not have high cerebrospinal fluid titres. Murphy and colleagues (48) demonstrated the same phenomenon in cats.

Bell and colleagues (9) were the first to apply cerebrospinal fluid titration for an epidemiological survey. Of 120 dogs sampled in an area where rabies was enzootic (Buenos Aires), none was found to be positive; thus, it cannot be concluded that non fatal rabies is common.

Blenden and colleagues (21) have suggested that the kinetics of antibody levels in blood and cerebrospinal fluid should be compared, to determine whether specific antibodies have been produced by infection or by immunisation. Without a booster after a first blood and cerebrospinal sampling, the antibody level should remain stable in cases of immunisation, or increase in cases of infection. In fact, such procedures have never been routinely used anywhere. Indeed, given the variability of the titration test, the constancy of an antibody titre over time is difficult to verify even in a vaccinated animal.

Given the lack of easily-performed experimental methods, the only basis for considering that an individual dog or cat possessing rabies neutralising antibodies has been vaccinated is good individual identification and certification.

DISCUSSION

Laboratory conditions described in the challenge of vaccinated cats and dogs generally appear more severe than natural conditions of challenge in the field. In normal practice, experimenters use extremely long intervals between vaccination and challenge (three to five years) and high virus doses involving 100% mortality in controls. In areas contaminated by fox rabies, natural challenge is not as severe for dogs and this could compensate for the fact that the health status of pets may be lower than that of dogs bred in the laboratory. Epidemiological observation is by far the more important evidence; in continental Europe, rabies vaccination of cats and dogs is so efficient that where the annual risk of a fatal case of rabies has been evaluated for a vaccinated pet, this risk is minute (1/6,980,000). It is also noteworthy that in continental Europe, fox rabies has never been propagated by domestic animals from an enzootic area to a free one even if administrative rules concerning compulsory confinment, leashing or vaccination have sometimes been broken either deliberately or by the simple fact that rabid pets have escaped from their owners.

If a neutralising antibody titration was required for certifying the immunological capacity of vaccinated animals, two questions would arise regarding:

a) the choice of techniques for antibody titration

b) the definition and acceptance of a minimum antibody titre considered as providing protection against rabies.

A general analysis of challenge experiments leads to the conclusion that neutralising antibody titres enable prediction of survival more often on a qualitative basis (i.e. Do the animals have detectable neutralising antibodies or not?) than on a quantitative basis. This fact becomes apparent when one tries to determine a "protective" threshold. For this purpose, either method of seroneutralisation (RFFIT

or MNT) can be employed, provided a correlation between the two methods has been demonstrated in the same laboratory (14, 66).

Agreements on the international transfer of dogs and cats could be formulated, therefore, based on a designated minimum level of neutralising antibodies, and could be proposed as an alternative to quarantine measures. The designated threshold could be based on the results presented in this study. The security of the protection constituted by this threshold would be increased by the extent to which it excedes the level recognised as effective against experimental challenge in cats and dogs (0.1 IU/ml and 0.2 IU/ml, respectively, measured by RFFIT).

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SIGNIFICATION PRATIQUE DES ANTICORPS RABIQUES CHEZ LE CHAT ET LE CHIEN. M.F.A. Aubert.

Résumé : *Le rôle protecteur des anticorps sériques neutralisant le virus rabique a parfois été mis en doute. Mais même si les patients ou les animaux qui meurent de rage peuvent produire des anticorps à des titres élevés, même si des cas de rage ont été décrits chez des animaux préalablement vaccinés et même si la composante cellulaire de l'immunité peut participer pour une bonne part à la protection, un très grand nombre d'expériences de laboratoire et d'observations de terrain prouvent que les chats et les chiens qui ont produit des anticorps neutralisants spécifiques après la vaccination et avant l'épreuve virulente ont une probabilité très élevée de survivre à une épreuve virulente, quelle que soit la dose ou la souche virale utilisée.*

En conséquence, le titrage des anticorps neutralisant le virus rabique peut constituer une garantie supplémentaire au certificat de vaccination des carnivores domestiques lors des transferts internationaux. Les modalités de quarantaine devraient aussi être adaptées aux données épidémiologiques de la rage qui sévit dans le pays d'origine de ces carnivores. Ces données peuvent être, par exemple, les statistiques des échecs vaccinaux chez ces espèces ainsi que le caractère d'adaptation aux différentes espèces hôtes, des souches de virus rabique qui circulent dans ces pays.

MOTS-CLÉS : Anticorps neutralisant Chat Chien Rage - Survie à l'épreuve.

SIGNIFICADO PRÁCTICO DE LOS ANTICUERPOS ANTIRRÁBICOS EN EL GATO Y EL PERRO. M.F.A. Aubert.

Resumen: *Los anticuerpos antirrábicos seroneutralizantes han sido a menudo sospechados de no conferir una buena protección contra la rabia. Es cierto que en los casos humanos y animales de muerte por rabia, los sujetos pueden producir*

altos índices de anticuerpos; se han observado también casos de rabia en animales que habían sido previamente vacunados; enfín, la inmunidad celular puede efectivamente actuar sobre la protección. Sin embargo, a pesar de estos hechos, numerosas pruebas de laboratorio y observaciones de campo han demostrado que los gatos y perros que producen anticuerpos neutralizantes específicos después de haber sido vacunados y antes de ser sometidos a la prueba virulenta, tienen probabilidades muy altas de sobrevivir a dicha prueba, cualquiera sea la dosis o la cepa viral empleada.

Por lo tanto, además del certificado de vacunación, la indicación de los títulos de anticuerpos neutralizantes antirrábicos puede constituir una garantía adicional durante el transporte internacional de carnívoros domésticos. Las medidas de cuarentena igualmente deben ser adaptadas a la situación epidemiológica de la rabia en el país de origen de los animales. Los datos epidemiológicos relevantes son, por ejemplo, las estadísticas relativas a los fracasos de la vacunación en dichas especies, así como las características de adaptación de las cepas de virus rábico existentes en esos países a diferentes especies huéspedes.

PALABRAS CLAVE: Anticuerpos neutralizantes - Gato Perro - Rabia - Resistencia a la prueba.

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