Published in : Vaccine (2001), vol. 19, n°32, pp. 4805–4815
DOI: 10.1016/s0264-410x(01)00211-0
Status : Postprint (Author's version)

KINETICS OF HUMORAL IMMUNE RESPONSE AFTER RABIES VR-G ORAL VACCINATION OF CAPTIVE FOX CUBS (*VULPES VULPES*) WITH OR WITHOUT MATERNALLY DERIVED ANTIBODIES AGAINST THE VACCINE

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KEYWORDS: mots-clés

ABSTRACT

In western Europe during the spring, the largest proportion of fox populations are cubs and the key to successful rabies oral vaccination campaigns is cub vaccination. In this paper we report on studies of the serology of 93 fox (*Vulpes vulpes*) cubs born to unvaccinated and orally vaccinated captive vixens, some of which were orally vaccinated at 30 or at 90 days of age with the vaccinia recombinant vaccine (VR-G) that expresses the rabies virus glycoprotein. The duration of cub passively acquired antibody, the development of immune responses to oral vaccination at either 30 or 90 days of age, possible interference between passive and active immunity to such vaccination and resistance to a potentially lethal rabies challenge dose when five months old were measured. The study showed that rabies neutralising antibody can be passed to their cubs by vixens orally vaccinated with VR-G during pregnancy. Maternally derived antibody titres in cubs declined with time and disappeared by 45–75 days after birth. Thirty days old cubs serologically responded to oral vaccination. No interference between antibody of maternal origin and active immunity conferred by VR-G oral vaccination or between antibody of maternal origin and protection was observed. Thus, very young cub immunisation against rabies with VR-G per os is possible whatever the immune status of their mothers. Provided a vaccine-bait suitable for such young cubs exists, oral vaccination at den entrances with VR-G is a feasibility.

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Introduction

The failure of other methods to halt the spread of rabies in the fox (*Vulpes vulpes*) populations of western Europe led to large scale field trials of fox oral vaccination. The first such trials were carried out in Switzerland in 1978, using a derivative (SAD_{Bern}) of the Street Alabama Dufferin (SAD) attenuated strain of rabies virus conjoined to chicken-head baits [1]. In time, other derivatives of SAD such as SAD_{B19} [2] and SAG_1 and SAG_2, the latter of which are escape mutants derived from monoclonal antibody neutralisation of the SAD_{Bern} virus [3], were also used in trials, as was a vaccinia virus recombinant (VR-G) capable of expressing the immunising glycoprotein [4,5] of rabies virus. SAG2 and VR-G are now the only vaccines that fulfil all the World Health Organization (WHO) requirements for anti-rabies safety for numerous target and non-target wild animal species [6–9]. In parallel with the developments in vaccinal viruses, attention was focused on the development of baits and bait delivery systems such that, by the end of the twentieth century, vast numbers of vaccine-loaded machine-made baits had been dropped by fixed-wing aircraft or helicopters over vast areas of western Europe.

Two related major hindrances to the complete eradication of rabies from western Europe were persistence of the disease in limited areas through lack of sustained vaccination campaigns and/or of sufficient bait density and resurgence of disease in areas apparently freed from infection. Both situations were exacerbated by natural increases in fox populations [10–12]. In spring, cubs are the largest group of a fox population; they are also the most difficult group to vaccinate. After spring campaigns, bait uptake ranges between 22 and 52% of cubs but reaches 75% of adults. After autumn campaigns, bait uptake reaches 70–80% of both adult and young foxes [13]. According to Breitenmoser *et al.* [14], the increase in the number of young unvaccinated foxes was the main reason of the persistence of rabies in the Swiss Jura.

To combat this problem, some measures to improve the efficiency of fox oral vaccination, particularly of cubs, have already been tested in the field. For example, vaccination campaigns have been conducted during summer to reduce the period during which susceptible fox cubs are not immunised. Briefly, the results obtained from one such trial [15] showed no significant increase in the proportion of immunised young foxes. A trial to vaccinate cubs by vaccine bait distribution at the entrances to dens was also carried out and although successful it proved difficult to organise and was expensive [16,17].

In the field, most cubs in oral vaccination areas are offspring of immunised vixens. Thus, it is possible that maternally derived antibodies might interfere with the ability of the vaccine to provoke a lasting immunological response in the cubs. Such interference has been observed in other canids (reviewed in [18,19]). In this paper we record the results of our studies of the immunisation and resistance to a rabies challenge of fox cubs orally vaccinated with VR-G in both the presence and absence of maternal antibodies. We show that cubs can respond to antigenic stimulation when they are only 30 days old and that the presence or absence of maternal antibodies had no bearing on the outcome of a rabies challenge.

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Materials and method

FOXES

The animal model used in this study was the silver fox, which belongs to the same species as the 'wild' red fox, (*Vulpes vulpes*). All silver fox cubs used were born in the experimental farm of our laboratory. Males (17) 1– 4years old and females (40) 2–9 years old, were purchased at least 1 month before the beginning of the reproductive activity of the vixens from the Norwegian Fur Breeder's Association (Oslo, Norway). On arrival, all were treated with anthelmintics (Droncit®, Bayer Pharma, France and Ivomec®, Merial SAS, France) and were vaccinated against canine distemper, viral hepatitis, parvovirus infection, infectious tracheobronchitis and leptospirosis with Canigen® CHPPi/L (Virbac, France). They were kept in individual cages, fed daily with a commercial dry food for adult dogs and water was provided ad libitum. All animals were observed daily.

Vixen health parameters and mating were conducted at our experimental farm. Vixen sexual cycles were monitored using both vaginal resistivity and keratinisation of epithelial cells in vaginal smears. Once a vixen was determined as receptive, a male was co-housed with it for 1 day. After this, detection of spermatozoa was performed by examination of vaginal smears to assess the covering. In this way it was possible to determine precisely the beginning of the gestation period and the estimated parturition time (the mean gestation period being 52 days). Covered vixens were then transferred to maternity cages and pregnancy was verified 30 days after mating by echography or trans-abdominal palpation.

When cubs of the litter were 8–9 weeks old, the dam was removed and the cubs were re-caged usually in pairs. They were fed daily with commercial dry food for young dogs with drinking water ad libitum. According to their size (and usually when 3–4 months old), young foxes were placed in individual cages. At this time they were also treated with anthelmintics (Droncit® and Ivomec®) and vaccinated with Canigen® CHPPi/L (two injections, 4 weeks apart).

RABIES VACCINE

VR-G (or VVTGgRAB oral vaccine bait (Raboral®, Merial SA, France)) is a recombinant vaccinia virus (strain Copenhagen, thermosensitive ts 26) expressing the immunising glycoprotein of the ERA strain of rabies virus [4]. It has been tested in foxes for safety and efficacy [5,7,20].

All vaccine-doses were of the same batch (80U342), stored at + 4 °C until use. Stability of the titre of the vaccine was verified by titration on the day of arrival, before the beginning of the experiment and after each set of administrations. The titre of VR-G virus was expressed in median cell culture infectious dose per dose ($CCID_{50}$/dose). Titrations were done on VERO cells (ATCC No. CCL81) in 96-well microtitre plates, 100 µl of a 2×10^5 cell/ml suspension/well. Serial (10-fold) dilutions of the virus were made and six 100-µl replicates of each dilution were distributed. After 120 h incubation at 37 °C and 5% CO_2 the cytopathic effect of the virus was observed with an inverted microscope. Reading was qualitative and a well was considered positive as soon as at least one lytic plaque was observed. The virus titre was calculated by the

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neoprobit graphic method [21]. During the experiment the titre of the VR-G varied between $10^{8.03}$ and $10^{8.44}$ $CCID_{50}$/dose.

VACCINATION

To eliminate variation in the absorbed volume of vaccine, VR-G was administered by direct instillation into the oral cavity with a needleness syringe on manually restrained alert animals. For both adult and young foxes the instilled volume corresponded to one average dose of VR-G vaccine (i.e. 2.7 ml for the batch used in this experiment).

Pregnant vixens were randomly divided into two groups. Group A had been vaccinated at 30 days of pregnancy and group B were unvaccinated. From both groups, litters were divided in three further groups, E, F, G born to vaccinated and H, J, K born to unvaccinated vixens respectively. Cubs of groups E and H were vaccinated when 30 days old and those of groups F and J were vaccinated when 90 days old. Fox cubs of groups G and K were not vaccinated.

BLOOD SAMPLING

Bleeding was by jugular venepuncture without anaesthesia. All vixens were bled before mating and again 30 days after parturition. All cubs were first bled at 30 days of age and thereafter at 2-week intervals until 5 months of age and again before euthanasia. Serum was tested for rabies neutralising antibodies by a modified version of the rapid fluorescent focus inhibition test (RFFIT) on microplate [22,23]. The titres obtained were expressed in International Units (IU)/ml by comparison with the second WHO standard for Rabies Immunoglobulin (activity of 30 IU per ampoule). The 0.5 IU/ml conventional level defined in humans as indicative of protection against rabies [24] was used as the positive threshold, as it is currently used in rabies epidemiological surveys of wildlife.

CHALLENGE

The challenge virus was first isolated from sub-maxillary salivary glands of naturally infected rabid foxes in 1986 [25]. The batch (GS-7) was then passaged three times in individual foxes [26]. A fourth passage was made in six foxes and their pooled sub-maxillary salivary glands constituted the GS-9 batch. Titre was determined by intracerebral inoculation of mice.

All young foxes were challenged when five months old. As cub birth times were spread over 1.5 months, they were divided into seven groups on the basis of age. In order to challenge the seven groups with the same virus suspension, one ampoule of GS-9 was thawed and diluted to give a final titre of 10^3 median mouse intracerebral lethal doses/ml ($MICLD_{50}$/ml). Aliquots of the diluted virus were then stored at −80°C until use and one aliquot was then thawed before each set of inoculations. Immediately after each challenge, the virus suspension was back-titrated intracerebrally in mice. These measured titres ranged between $10^{2.25}$ and $10^{2.85}$ $MICLD_{50}$/ml. Cub challenge was performed by intra- muscular inoculation of 1 ml of virus suspension into the left temporal muscle. Previous experiments had shown that a dose of 10^3 $MICLD_{50}$ induced a mortality time of 18–20 days in unvaccinated foxes [26,27]. As it was impossible to add a control litter during every challenge session, the expected mortality delay of controls was arbitrarily

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fixed to 20 days. The classical observation period for post vaccination challenge is 45 days after the death of the last dying control. This allowed the determination of the minimal observation period of surviving foxes to 20 + 45 days post challenge.

Brain material and sub-maxillary salivary glands of all young foxes dying of rabies or euthanased at the end of the observation period were examined for the presence of rabies antigen by direct immunofluorescence [28] and cell culture test [29]. For ethical reasons, it was decided to euthanase rabid animals when paralysed.

STATISTICAL ANALYSIS

Statistical analyses were performed on the $\log_{10}$ of the number of IU/ml. Differences between groups in the neutralising antibody titre were analysed by using analysis of variance and Student's *t*-test for unpaired data at a 95% confidence level.

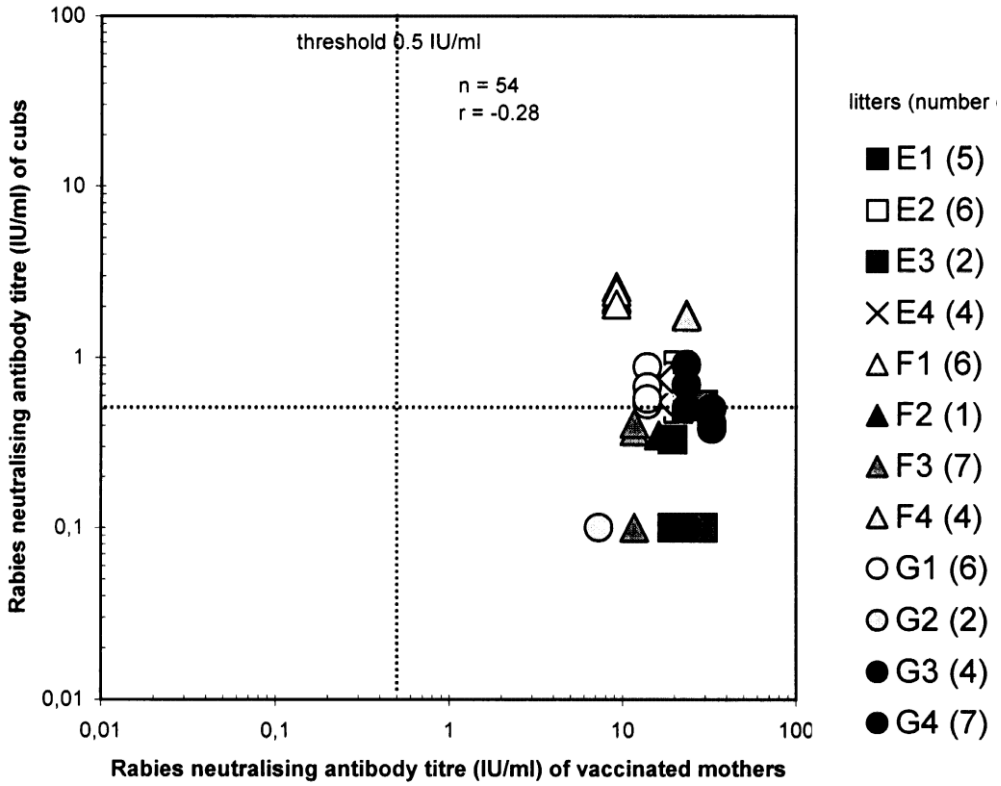

Fig. 1. Relationship between rabies neutralising antibody titres of the vaccinated vixens and titres of their 30-day-old cubs. (... threshold of positivity: 0.5 IU/ml).

Results

FOX CUBS

Births occurred from the end of March to the beginning of May. Twenty one litters with 97 cubs alive at 30 days (whelped by vixens aged from 2 to 6 years) were obtained. The number of cubs

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per litter ranged from one to seven, with an average of 4.5 (S.D.=1.88). All animals remained in good health during the experiment (before challenge) except four cubs that were euthanased because of inter-current bacterial infections.

PASSIVE IMMUNITY OF FOX CUBS

Fifty two days after oral instillation of the VR-G vaccine (i.e. 30 days after whelping), the neutralising antibody titres of the 12 vaccinated females (group A) ranged between 7.31 (log=0.86) and 32.86 IU/ml (log=1.52). None of the nine unvaccinated vixens (group B) had neutralising antibody and none of their cubs (groups H, J and K, n = 43) had antibody to rabies virus at 30 days of age. The transferred neutralising antibody titres of the 30-day-old cubs born from the 12 vaccinated vixens (groups E, F and G, n=54) ranged from 0.1 (log=−1.0) to 2.59 IU/ml (log=0.41). A very significant difference between the neutralising antibody titres of these cubs and those of cubs born from unvaccinated vixens was shown (P=10−15). The levels of neutralising antibodies detected in the 30-day-old cubs were very significantly lower than those detected in maternal sera 52 days after vaccination of the vixens (P=9×10−10). Furthermore there was no correlation between the neutralising antibody titres of the 12 vaccinated vixens and the transferred neutralising antibody titres of their respective 30-day-old cubs (groups E, F and G, n=54, r=−0.28) (Fig. 1).

Fig. 2 shows the frequency distribution of neutralising antibody titres of cubs born to vixens of group A measured at 30 days of age. The graph clearly shows three different groups: among the 54 cubs of vaccinated mothers, 14 had no detectable neutralising antibody, 32 had neutralising antibody levels around the 0.5 IU/ml (log=−0.3) cut-off value and eight had higher neutralising antibody titres, ranging from 1.58 to 2.59 IU/ml (log=0.2–0.41).

The mean neutralising antibody titre of each 30 days litter is shown in Fig. 3. A significant difference between litters of vaccinated vixens (E1, E2, E4, F1, F3, F4, G1, G3 and G4) ($F_{8/40}$=15.45, P<10^{-3}) was observed. Litters E3, F2 and G2 that had only one or two cubs were not included in this statistical analysis. Moreover, we observed that some litters showed heterogeneous antibody titres (for example litters F4, G4) whereas some others had homogenous antibody titres (for example litters F1, G1).

Fig. 4(a) demonstrates the kinetics of maternally derived antibodies decline in litters of vaccinated vixens and subsequent rise after vaccinated at 90 days (group F). The decline was related to the antibody level present at 30 days of age and was similar for all litters. Similar results were obtained with unvaccinated fox cubs born to vaccinated vixens (group G, data not shown). Duration of maternal antibodies in litter F1 (the highest mean neutralising titre at 30 days) was the longest, but

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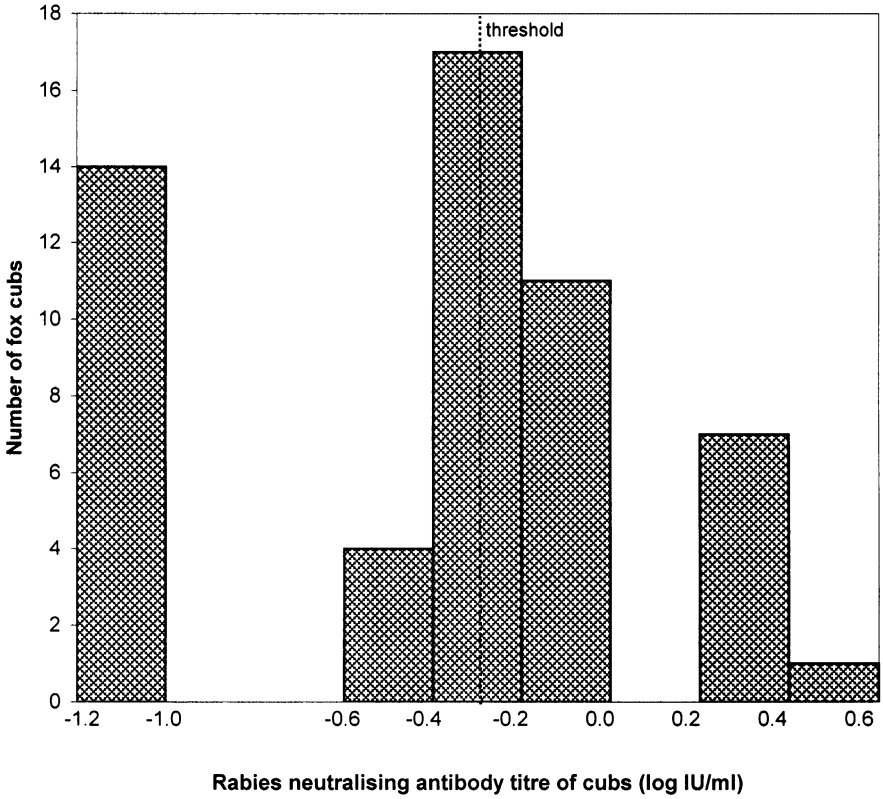

Fig. 2. Frequency distribution of rabies neutralising antibody titres of cubs born to vaccinated vixens.

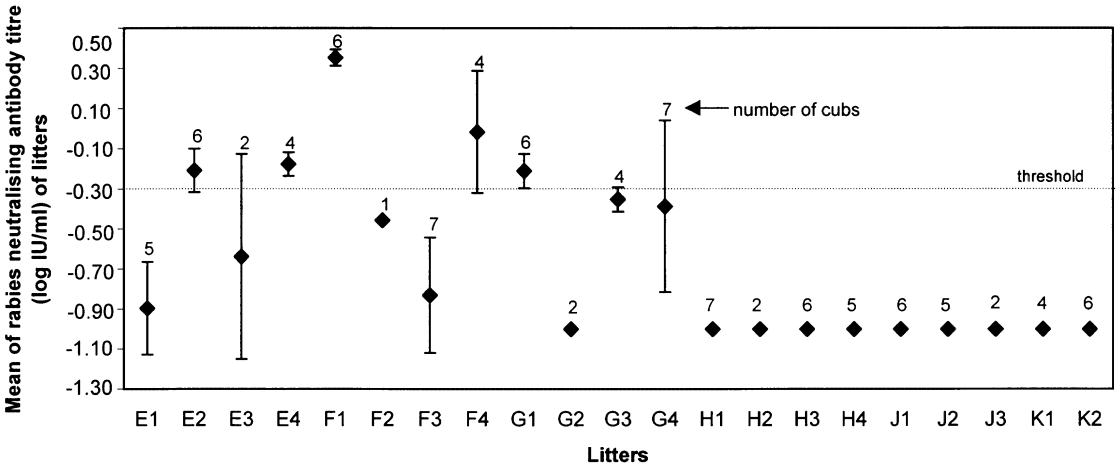

Fig. 3. Mean of the rabies neutralising antibody titres of the 30-day-old litters. Litters E, F and G born to vaccinated vixens; litters H, J and K born to non-vaccinated vixens.

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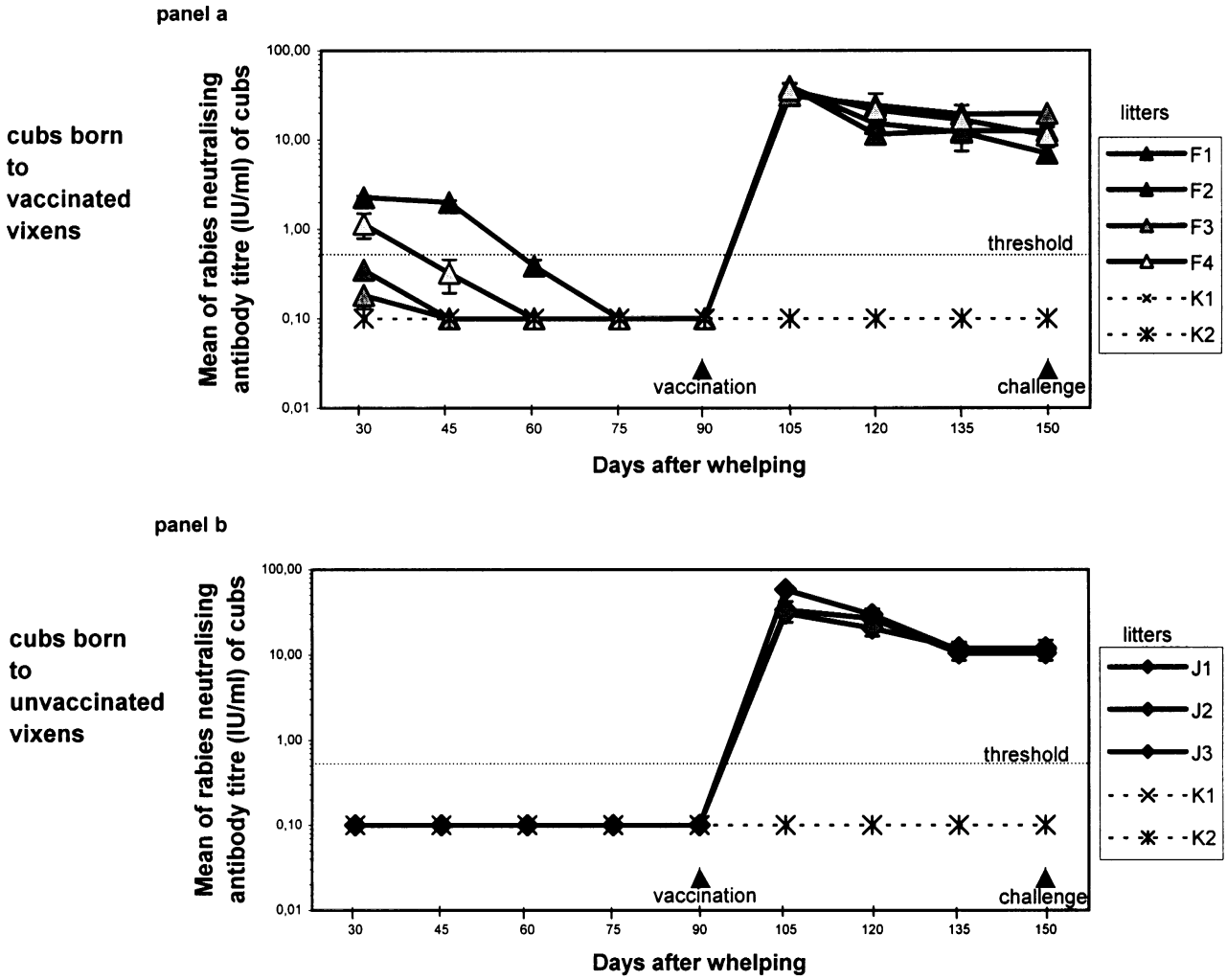

Fig. 4. Kinetics of serological response of fox cubs vaccinated at 90 days born to vaccinated (a) or non-vaccinated (b) vixens.

had disappeared by 75 days. None of the litters with a mean titre close to 0.5 IU/ml at 30 days had neutralising activity at 45 days (i.e. litter F2 Fig. 4a) and litters G3 and G4 (data not shown)) or 60 days after whelping (i.e. litter F4 Fig. 4a) and litter G1 (data not shown)). Fig. 5 shows that 15 days after vaccination, all cubs of group E (i.e. born to vaccinated vixens and vaccinated at 30 days of age) had higher rabies neutralising antibody titres. However, there was no correlation between the levels of maternally derived antibodies of these cubs at 30 days of age (which were similar at the time of the vaccination) and those obtained for these same cubs 15 days after their vaccination (Fig. 5, n=17, r=−0.02).

Fig. 6 shows the kinetics of serological response of cubs born to vaccinated vixens (group E, Fig. 6a) or to non-vaccinated vixens (group H, Fig. 6b) and vaccinated at 30 days of age. Fifteen days after oral administration of the vaccine (i.e. at 45 days of age), rabies antibody level means were all increased in all litters (mean=23.55 IU/ml (log=1.34), S.D.=9.33 for Fig. 6a; mean=33.98 (log=1.51),

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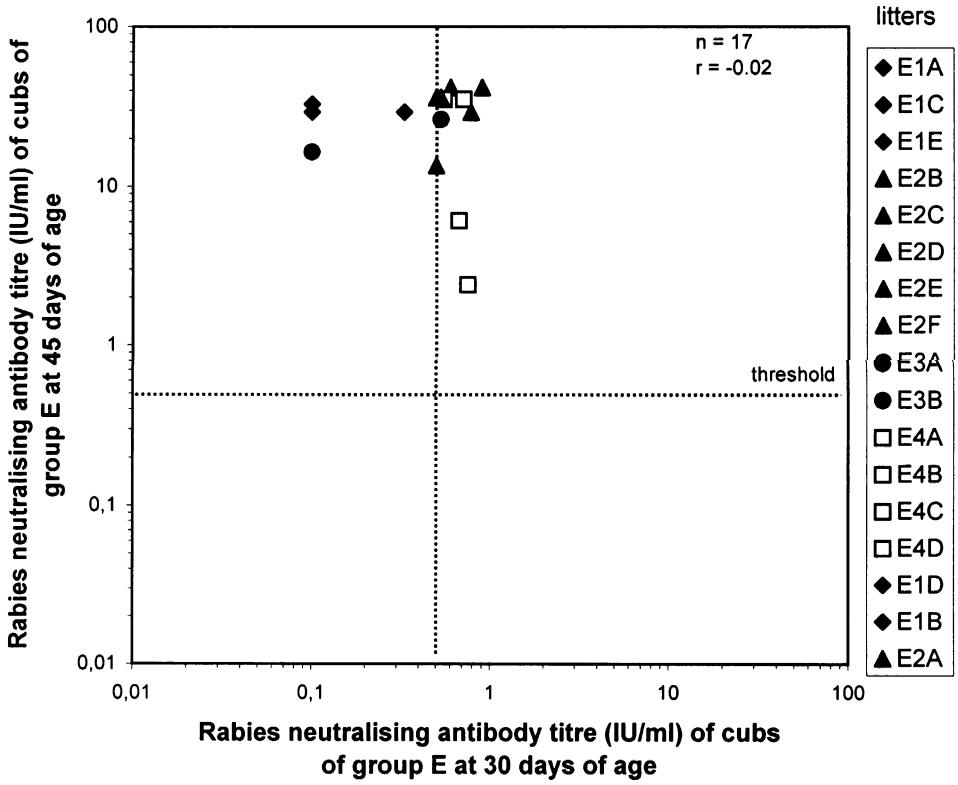

Fig. 5. Rabies neutralising antibody titres of fox cubs born to vaccinated vixens: relationship between cubs pre (i.e. at 30 days of age) and post (i.e. at 45 days of age) vaccination titres. (cubs from litters: + E1, .._ E2, • E3, D E4), (… threshold of positivity: 0.5 IU/ml).

S.D.=13.31 for Fig. 6b). After this, the antibody means slightly decreased until 90 days of age (mean= 9.45 IU/ml (log=0.95), S.D.=3.58 for Fig. 6a; mean=10.89 IU/ml (log= 1.02), S.D.=3.28 for Fig. 6b) and then remained stable until the young foxes were at least 5 months of age (mean= 6.55 IU/ml (log=0.77), S.D.=2.86 for Fig. 6a; mean=5.79 IU/ml (log=0.75), S.D.=1.39 for Fig. 6b), which corresponded to the time of challenge. Comparison of serological results of cubs either born to vaccinated vixens (group E, Fig. 6a) or born to non- vaccinated vixens (group H, Fig. 6b) shows that 15 days after oral administration (i.e. at 45 days of age), antibody titre means were not significantly different ($F_{7/30}$=2.23, P=0.06). This similarity of antibody titre means (0.059<P<0.451) in litters born to vaccinated vixens (group E, Fig. 6a) or to unvaccinated vixens (group H, Fig. 6b) was also seen throughout the study (i.e. at 60, 75, 90, 105, 120 and 150 days of age).

Fig. 4 shows similar results, with cubs born to vaccinated vixens (group F, Fig. 4a) or to non-vaccinated vixens (group J, Fig. 4b) and vaccinated at 90 days of age. Rabies antibody kinetics were similar to those described above, i.e. increased of levels 15 days after vaccination (mean=34.85 IU/ml (log=1.53), S.D.=5.65 for Fig. 4a; mean=36.93 IU/ml (log= 1.54), S.D.=17.87 for Fig. 4b) followed by a slight decrease then stability until at least 5 months of age (mean=11.5 IU/ml (log=1.03), S.D.=4.28 for Fig. 4a; mean=10.97 IU/ml (log=1.04), S.D.=0.87 for Fig. 4b). As previously observed for cubs vaccinated at 30 days of age, neutralising antibody mean titres of cubs vaccinated at 90 days were similar not only at 15 days after oral administration of the vaccine (i.e. at 105 days of age) (F6/24=0.74, P=0.62) but also thereafter

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(i.e. at 120, 135 and 150 days of age) with no significant difference (0.063<P<0.897) between litters born to vaccinated vixens (group F, Fig. 4a) or to unvaccinated vixens (group J, Fig. 4b).

Comparison of results presented in Figs. 6 and 4 shows that 15 days after vaccination and until time of the challenge, neutralising antibody titre means of cubs born to vaccinated or unvaccinated vixens and vacci- nated at 30 days (groups E and H) or at 90 days (groups F and J) did not significantly differ (0.144<P<0.935).

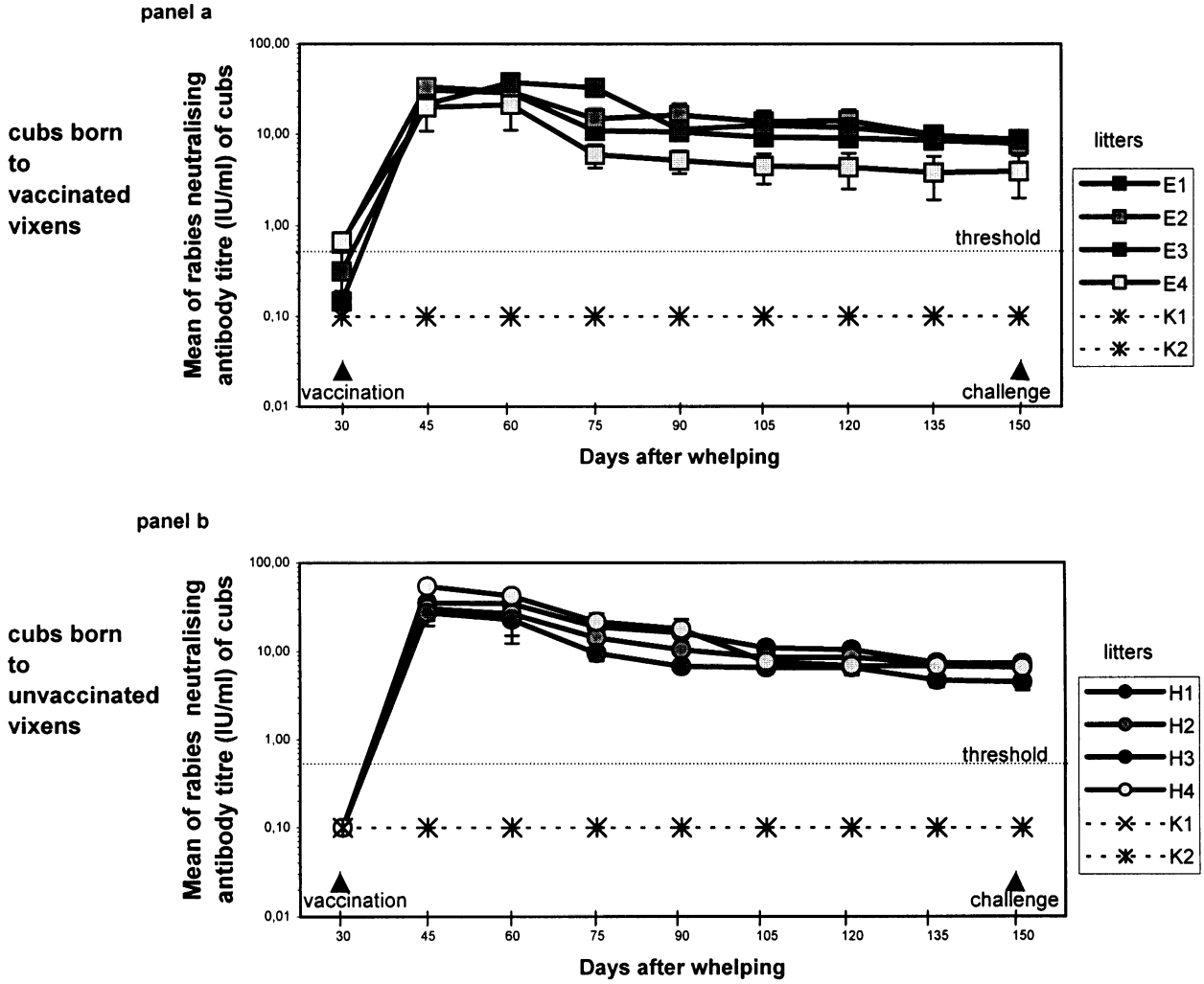

Fig. 6. Kinetics of serological response of fox cubs vaccinated at 30 days born to vaccinated (a) or non-vaccinated (b) vixens.

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RESISTANCE TO CHALLENGE

Whatever the vaccinal status of the vixens, all vaccinated young foxes (n=65) resisted a rabies challenge and all but one unvaccinated young foxes (n=28) died of rabies (Table 1). The mean period between challenge and death was 16.5 days (from 13 to 20 days). The clinical phase lasted less than 2 days. Rabies was confirmed positive by laboratory tests in non-vaccinated foxes and negative on vaccinated animals and the surviving unvaccinated cub at the end of the observation period.

Discussion

Previous findings have shown that the key to successful rabies oral vaccination in spring is to give cubs access to vaccine baits [13,14]. Several methods to improve vaccination coverage of cubs have been tested [15,16]. However, few data concerning the ability of cubs to produce rabies antibodies in response to SADB19 oral rabies vaccine [30,31] or to VR-G vaccine [32] have been reported. Furthermore, it is well known that even if newborn animals are immunocompetent at birth, an additional maturation of the immune response will occur during the neonatal period. In domestic carnivore species, transfer of maternal antibodies can protect neonates against various infections; the presence of blocking levels of those maternal antibodies may however be an obstacle to successful later vaccination (reviewed in [18,19]). In the red fox, this interference phenomenon has already been suggested [16,32] but reported in only one study that used SADB19 oral vaccine [31]. This led us to conduct an experimental trial to assess the duration of passive immunity acquired by cubs born to VR-G orally vaccinated vixens and to follow the development of immune competency of cubs in order to assess interference between passive and active immunity in cubs.

MATERNAL IMMUNITY

Few data on rabies maternal antibody transfer have been published. One study [33] of parenterally vaccinated (subcutaneous or intramuscular routes) dogs showed that rabies neutralising antibodies could not be detected in any puppies born to rabies vaccinated dams prior to their vaccination. However, two other studies [34,35] demonstrated that rabies neutralising antibodies can be transferred from dams to their pups. According to Winters *et al.* [34], in dogs transfer of rabies maternal antibodies is partly transplacental but mainly through the colostrum. The first studies showing that major transfer of maternal antibodies occurs during ingestion of colostrum by new-born pups were de- scribed for canine distemper virus [36,37]. This was also reported by Pollock *et al.* [38] in a study of maternally derived antibodies to canine parvovirus. In this latter study, it was also demonstrated that the amount of maternal antibodies that pups receive is proportional to the titre of the dam.

Our study shows that neutralising antibodies to rabies virus can be transferred to their cubs by vixens orally vaccinated with VR-G during pregnancy. Neutralising antibody mean titres in sera of these cubs were significantly lower than the titres of maternal sera. However, contrary to the studies in dogs [38] and in foxes [31], we observed no correlation between the amount of

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maternal antibodies of cubs and the titre of their respective mothers. If in foxes, as in dogs, maternally derived antibodies are transferred mainly through the colostrum, the absence of correlation might be related to poor mother care of some vixens, particularly first time or otherwise stressed vixens.

We also observed that maternal antibody titres were variable both between litters and between cubs of a litter. This finding may be due to differences in colostral absorption, as described in other multiparous species (reviewed in [19]). Moreover, 14 of the 54 observed cubs belonging to the same or different litters (i.e. cubs of litter G2 and some cubs of litters E1, E3, F3 and G4), had no detectable neutralising activity in their sera. As serum samples were not collected until cubs were 30 days old, it is possible that these cubs may have had low levels of maternal antibodies, which had disappeared in under 30 days.

The period of 30 days before cub bleeding was designed to ensure cub survival, since previous unpublished observations in our experimental station had shown that some vixens stressed within the first 30 days of parturition cannibalised their cubs or developed poor mothering behaviour. Moreover, there appeared to be no relationship between litter size and neutralising antibody titres of cubs, but as some vixens killed and cannibalised some of their cubs during the first days after birth, absence of a relationship could not be proven. Although it was shown that maternally derived antibody titres in cubs declined regularly with time (and disappeared 45–75 days after birth), we did not investigate their ability to protect against challenge.

IMMUNE-COMPETENCE OF FOX CUBS

Reports have shown that when 4–5-week-old pups born to non-immunised dams were intramuscularly vaccinated with Flury low egg passage (LEP) rabies vaccine, they are capable of producing rabies antibodies, but 10–16-week-old pups responded much better, which suggests that the immune response in older dogs is due to a maturation of their immune systems [35,39]. Our results showed that 1-month-old fox cubs born to non-vaccinated vixens can respond to VR-G oral vaccination by the production of specific rabies neutralising antibodies. When the neutralising antibody mean titres of these cubs were compared with those of older (90 days) cubs, no difference in neutralising titres was observed. This result suggests that in foxes there is no additional maturation of the immune system after 30 days of age. The capacity of 30-day-old fox cubs to respond to VR-G oral vaccination may even exist earlier, but this possibility was not examined in this study in order to ensure cubs survival.

INTERFERENCE BETWEEN MATERNAL AND ACTIVE IMMUNITY

According to Aghomo *et al.* [35] puppies born to naive bitches intramuscularly vaccinated with an attenuated Flury-LEP live virus vaccine presented rabies antibody titres significantly higher than those of puppies born to vaccinated bitches. Bernardi *et al.* [40] also demonstrated, in hamsters born to dams intraperitoneally vaccinated with a rabies inactivated PV virus vaccine that the presence of maternal antibodies, even when those antibodies were present at titres insufficient to protect against infection, inhibited the formation of an immune response in offspring.

Published in : Vaccine (2001), vol. 19, n°32, pp. 4805–4815
DOI: 10.1016/s0264-410x(01)00211-0
Status : Postprint (Author's version)

Our results contrast with these previous reports and also with those of Müller *et al.* [31] (in which results were obtained in foxes after use of oral live modified vaccine SADB19), since for all cubs vaccinated at 30 days we observed no significant difference in the magnitude of antibody response between the group of cubs born to vaccinated vixens (group E) and that born to unvaccinated vixens (group H). Moreover, whatever the age of vaccination (i.e. 30 or 90 days) and the immune status of the vixens, we also observed no significant difference in the magnitude of the antibody responses. These data demonstrated the absence of interference between passive immunity of maternal origin and active immunity conferred by VR-G oral vaccination. Since we performed vaccination with a recombinant vaccine, the type of vaccine might be the cause of this discrepancy. However, Seghaier *et al.* [41] showed that puppies born to dams subcutaneously vaccinated with an inactivated rabies vaccine (CVS strain) responded to vaccination with no significant interference between maternal and active immunity. Moreover, intranasal vaccination with a modified live canine parvovirus vaccine stimulated a good antibody response in pups with maternally derived antibodies and vaccinated in the first weeks of life [42].

RESISTANCE TO CHALLENGE

Our results, show that all vaccinated cubs challenged at 5 months of age resisted an intramuscular challenge with fox street rabies virus, whether or not the vixens have been orally vaccinated with VR-G during pregnancy. This demonstrated the absence of interference between passive immunity and protection conferred by oral vaccination with VR-G. Survival from a severe challenge of a control cub was unexpected, but this phenomenon has already been observed in other similar trials [43,44].

Our data demonstrated the very early ability of fox cubs to respond to oral vaccination with VR-G, their ability to resist a severe rabies challenge (killing nine of ten control cubs) and the absence of interference between passive antibody of maternal origin and response to oral vaccination. Therefore, fox cubs immunisation against rabies with VR-G per os is possible whatever the immune status of their mothers. Hence, oral vaccination of fox cubs at den entrances with VR-G may protect them. We are presently conducting a similar experiment with SAG2 oral vaccine. Trials in which the protective effect of maternally derived antibodies would also be determined.

In other respects, the fact that foxes can respond to antigenic stimulation when they are only 30 days old, whatever the immune status of the vixen, should be taken in account when interpreting the serological results of epidemiological studies. In such studies, the red fox is used as a sentinel for the presence of different antigens or diseases in an area because of its opportunistic behaviour. This kind of study allows spatial and temporal interpretation. The temporal aspect should now consider that at 1 month of age fox cubs may have antibody from either passive immunity or from active immunisation.

Published in : Vaccine (2001), vol. 19, n°32, pp. 4805–4815
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Status : Postprint (Author's version)

Acknowledgements

The authors are very grateful to Dr Arthur King for review of this manuscript. The authors also thank the staff of the laboratory of the AFSSA-Nancy for their excellent technical assistance. Financial support for this work was provided by the European Community Com- mission (contract FAIR CT97-3515: 'Rabies vaccination in emergency-Wildlife vaccination rabies in difficult and emergency situations and its potential im- pact on the environment').

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