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# Neonatal immunity and immunisation in early age: lessons from veterinary medicine

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*The objective of this paper is to review adaptive immunity of young animals using examples from my own experience and from the literature. Trials carried out by us with a modified live and inactivated canine parvovirus vaccine in newborn puppies provide evidence of the immune capacity of these puppies.*

*With regard to transfer of immunity from mother to offspring, there is a role for transplacental and colostrum immunity. Examples of passive protection of young animals against different infections include passive protection of kittens against the feline immunodeficiency virus. However, passive immunity, though very useful at an early age, varies in duration and makes implementation of standard vaccination schedules difficult.*

*Other experiments demonstrate that, under certain conditions, it is possible to overcome residual maternally-derived antibodies and to induce post-vaccinal immunity. © 1998 Elsevier Science Ltd. All rights reserved.*

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## TRANSFER OF IMMUNITY FROM MOTHER TO OFFSPRING

Even if newborn animals are immunologically competent, they receive assistance from their mother in the form of passive antibodies transferred through placenta or colostrum. The route by which maternally-derived antibodies reach the foetus is determined by the nature of the placental barrier (*Table 1*). Since, in primates, the placenta is haemochorial, this type of placentation allows the transfer of IgG but not IgM, IgA or IgE to the foetus. Dogs and cats have an endotheliochorial placenta in which the chorionic epithelium is in contact with the endothelium of the maternal capillaries. In these species, a little IgG (5-10%) may transfer from the mother to the puppy or kitten, but most is obtained through the colostrum.

The placenta of ruminants is syndesmochorial, i.e. the chorionic epithelium is in direct contact with

uterine tissues, while the placenta of horses and pigs is epitheliochorial and the foetal chorionic epithelium is in contact with the intact uterine epithelium. In animals with both of these types of placentation, the transplacental passage of immunoglobulin molecules is totally prevented and newborn animals of these species must therefore receive antibodies through the colostrum<sup>1</sup>.

## ABSORPTION OF COLOSTRUM

Young animals that suckle soon after birth take colostrum into their intestinal tract. In these young animals, the level of proteolytic activity in the digestive tract is low and is further minimised by the presence of trypsin inhibitors in the colostrum. Therefore, colostrum proteins reach the small intestine, particularly the intact ileum where they are actively taken up by epithelial cells by pinocytosis and passed through these cells into the intestinal capillaries, reaching the systemic circulation. As a result, newborn animals obtain a massive transmission of maternal immuno-

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**Table 1** Relationship between placental type and transfer of immunoglobulins from dam to foetus/newborn via placenta or colostrum

Species	Type of placentation	Placental transfer	Colostrum transfer
Pigs, horses	epitheliochorial	0	+++
Ruminants	syndesmochorial	0	+++
Dogs and cats	endotheliochorial	+	+++
Primates	haemochorial	++	+
Rodents	haemendothelial	+++	+

From Tizard<sup>3</sup>.

globulin. The period during which the intestine is permeable to proteins varies between species and, to a degree, by immunoglobulin class. In general, permeability is highest immediately after birth and declines rapidly within 24 h because of maturation of intestinal cells and establishment of intestinal flora. For calves, the level of passive antibody is maximum 24 h after birth. The bacterial flora subsequently degrade immunoglobulins.

The immunity transmitted by colostrum is determined by the level of systemic immunity of the mother. In general, this level is similar to the dam's level but the quantity of transferred immunoglobulins depends on the quantity of colostrum ingested and on its immunoglobulin content.

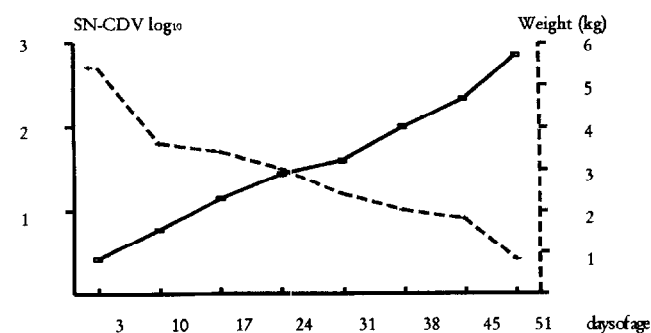
For multiparous animal species, the degree of passive immunity can be heterogeneous between litters and between the members of a same litter because of variability of colostrum absorption. This is true for piglets, puppies and kittens whose levels of passive antibodies are maximum 36–48 h after birth. Afterwards, the level of maternally-derived antibodies gradually decreases depending more on the growth rate of the animal than on catabolism. This is particularly clear in canine species where dogs belonging to rapid-growth breeds eliminate their maternally-derived antibodies more quickly than slow-growth breeds. *Figure 1* shows the kinetics of disappearance of SN-Distemper passive antibodies and the growth rate of a litter of Beagle puppies<sup>2</sup>.

The half-life of antibodies to distemper and canine infectious hepatitis is 8.4 days in dogs<sup>3</sup> and 9.5 days for antibodies to feline panleucopenia in cats<sup>4</sup>. On average, passive antibodies to distemper in puppies will have declined to insignificant levels at about 10–12 weeks. For canine parvovirus, residual maternally-derived antibodies can be detected up to 15 weeks. The sensitivity of the method of detection can play a role in the apparent time of disappearance.

The level of maternally-derived antibodies reaches (on average) 1–3% of their initial value: in 30 days for dogs and cats, 40 days for lambs, 60 days for pigs, 100 days for calves and 115 days for horses<sup>1,5</sup>.

## MATERNAL IMMUNITY AND PROTECTION AGAINST INFECTION

The passive colostrum immunity strongly protects the young animal as shown in puppies infected by virulent



**Figure 1** Decrease of CDV Ab<sub>m</sub> after birth. Growth weight *m* of puppies.

canine parvovirus<sup>6</sup>. The receptivity and susceptibility to the virus depend more on the level of residual passive antibodies than on age. Below a titre of 1/64–1/80, a puppy can be infected by the parvovirus.

In kittens, the story is similar for feline panleucopenia<sup>4</sup>. In an experiment aimed at determining passive protection against feline immunodeficiency virus by maternally-derived antibodies, 24 kittens, coming from five queens chronically infected by feline immunodeficiency virus (FIV), were all challenged at varying times with the homologous strain of FIV. *Table 2* shows the results of protection observed (no viraemia for 39 days following infection). Biomathematical analysis using a moving average method shows the classical decrease of protection with age (*Figure 2*). However, no correlation between anti-p24 antibody titres and the level of protection could be observed. In piglets, the same type of experiment was carried out to show passive protection against pseudorabies, a systemic disease. A direct correlation between SN antibodies and protection was observed<sup>7</sup>.

For an enteric viral infection such as transmissible gastroenteritis caused by a coronavirus, the protection comes from local immunity. The sow's milk is very rich in IgA. These immunoglobulins are fixed to the intestinal epithelium and neutralise the viral infection. These IgA are more resistant than IgG and IgM to proteolytic enzymes of the intestinal tract. Systemic passive immunisation presents no advantage for infections of the digestive system. Dams must be repeatedly vaccinated so that they can transfer not only colostrum but also milk as rich as possible in immunoglobulins and therefore ensure lactogenic immunity<sup>8</sup>.

## IMMUNE RESPONSE OF THE NEWBORN ANIMAL

The development of immunologic competency is incremental in all animal species. However, as a general rule, mammals with a short period of gestation have less mature immune system at birth than species with long period of gestation<sup>9</sup>.

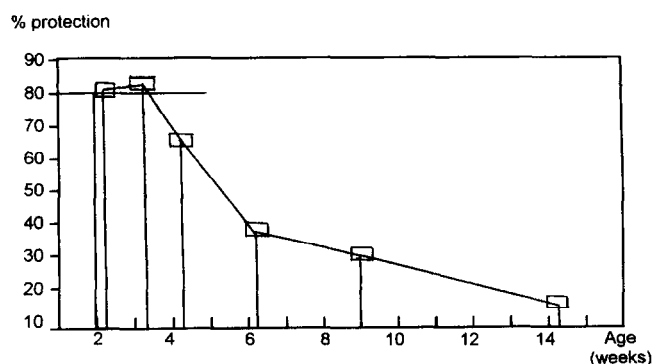
Newborn animals, coming from a protected environment, are launched into a world, rich in potentially pathogenic micro-organisms. It is classical to consider that young animals are fully capable of developing an immune response at that time. However, any immune response developed in infancy must necessarily be a primary response with both a relatively long period of latency and a low concentration of antibody produced<sup>5</sup>. As an example, 100 minimum immunising doses (10<sup>4.5</sup> TICD<sub>50</sub>) of a modified live vaccine, canine parvovirus strain Cornell, were subcutaneously inoculated to five fully susceptible specific pathogen-free (SPF) Beagle puppies at 1 day of age. No adverse reaction was reported and the post-vaccinal kinetics of antibodies (HAI) between 21 and 91 days post-vaccination was similar to older puppies in terms of level and stability, hence showing the complete maturity of the immune system (*Table 3*).

In another experiment, using five groups of five SPF Beagle puppies of different ages, between 1 day and 3 months, and fully susceptible to rabies antigens, we inoculated one dose of our killed vaccine adjuvanted

**Table 2** Passive protection against FIV by maternally-derived antibodies (24 kittens)

Age (weeks) of infection	1	2	3	4	5	7	9	21	24
Number of infected kittens	6	2	5	2	2	2	2	1	2
Protected kittens (39 dpv)	4	2	4	2	1	0	0	1	0

with aluminium hydroxide\* (NIH = 3) by the subcutaneous route. SN rabies antibodies were titred on the day of vaccination (day 0) and on day 21 and day 42 post-vaccination (Table 4) in order to evaluate the immune response of newborn animals. The statistical analysis provided evidence of a significant difference between the groups of dogs 21 days post-vaccination with the best antibody response observed in the youngest puppies (1 day old). 42 days post-vaccination, there was no significant difference between the groups. These two experiments were designed to be more for 'academic' than routine use, but certainly provide evidence of satisfactory immunocompetency of puppies within the first days of life.



**Figure 2** FIV: maternal immunity % protection *f* (age of infection) by moving average technic.

**Table 3** Safety control on newborn SPF beagle puppies. CPV<sub>2</sub>-MLV Strain Cornell (10<sup>4.5</sup> TCID<sub>50</sub>). Kinetics of active antibodies (HAI log<sub>10</sub>)

Puppy no.	Age (in days)			
	1	21	42	91
1	<1.0	2.5	2.8	2.8
2	<1.0	2.8	2.7	2.5
3	<1.0	3.1	3.0	2.9
4	<1.0	3.4	3.2	3.2
5	<1.0	3.4	3.2	3.2
m	<1.0	3.04	2.98	2.92

\*RABISIN®, MERIAL SAS.

**Table 4** Immune response of SPF puppies of different ages, rabies vaccination, SN antibodies (RFFIT)

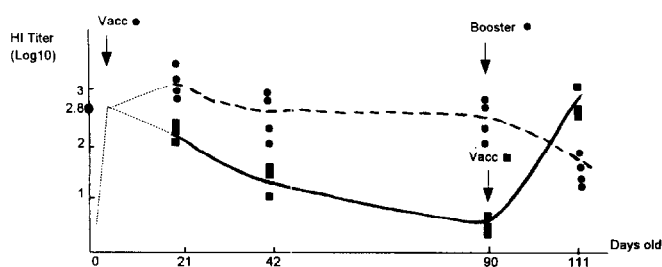
Groups (five puppies)	Mean titres of SN rabies antibodies — days post-vaccination (dpv)			
	21 (log <sub>10</sub> )	SD	42 (log <sub>10</sub> )	SD
1 day old	3.69	0.018	1.59	0.603
2 weeks old	2.13	1.287	1.59	1.323
1 month old	2.66	0.338	2.73	0.117
2 months old	2.55	0.225	1.95	0.765
3 months old	3.27	0.027	1.95	1.035

## MATERNAL IMMUNITY AND VACCINATION

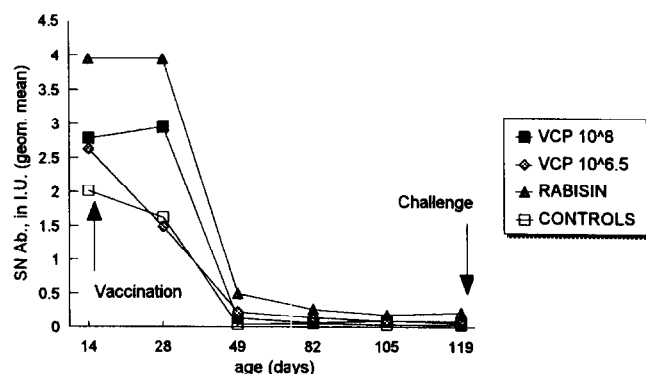
Vaccination at an early age is advisable because infectious diseases and their consequences are a great risk for young animals. All domestic species are immunocompetent at birth although additional maturation of the immune response will occur during the neonatal period. The main obstacle to successful vaccination in young animals is the presence of blocking levels of maternally-derived antibodies. The positive influence of maternal immunity with regard to protection against infection becomes negative facing vaccine inoculation. Ideally, the vaccine should allow immunisation of the animal at the age when it first becomes susceptible to infection, prior to decline of maternally-derived antibodies below protective levels. This is rarely achieved and is a major problem with canine parvovirus in an infected kennel. As maternally-derived antibodies decline, there is a critical period of time during which neonates have too much antibody to respond to a vaccine but not enough to resist a field infection. This window of susceptibility makes it difficult to raise healthy animals in heavily contaminated environment despite well-designed vaccination schedules.

Multiple strategies have been used to develop a more efficacious vaccination schedule. In a canine parvovirus experiment with attenuated live vaccine, we immunised puppies just at birth, puppies born to vaccinated or convalescent dams. The vaccine was administered by the subcutaneous route at birth, before colostrum ingestion, to three puppies of a litter of seven. Four puppies were kept as unvaccinated controls. Figure 3 illustrates the normal decrease of antibodies in unvaccinated control puppies and the antibody kinetics of the three vaccinated puppies, showing active immunisation. The second injection of vaccine, performed at the age of 90 days, had no effect on the three puppies vaccinated at birth. The control group showed a normal induction of antibodies after primary vaccination. When vaccination was performed immediately after the colostrum ingestion or on puppies coming from convalescent high titre dams, vaccination at birth was unsuccessful leading to abandon this type of vaccine indication.

To vaccinate puppies against distemper, Baker *et al.*<sup>10</sup> and Gillespie<sup>11</sup> have proposed a predictive nomogram of the minimum age for vaccination of



**Figure 3** Neonatal vaccination: MLV-canine parvovirus. Four puppies vaccinated at birth, boosted at 90 days. Three puppies non-vaccinated at birth, vaccinated at 90 days. Kinetics of antibodies.



**Figure 4** Rabies immunisation by vaccination in presence of maternal antibodies. SN antibodies kinetics.

puppies depending on the antibody titre of the dam. Given the complexity and cost of this option as well as the heterogeneity of maternally-derived passive antibodies within litters, it has never been applied in practice. The current vaccination schedule against distemper includes a first injection at 7–8 weeks of age and a second injection at 11–12 weeks of age. The first injection is intended for the immunisation of puppies having already lost their maternally-derived antibodies and the second for the immunisation of the animals which have neutralised the vaccine at the time of the first injection. It is the most practical, easiest and most efficacious schedule<sup>12</sup>.

In order to avoid any risk of interference, French regulations do not allow rabies vaccination before 3 months of age. For distemper and rabies, the vaccination schedules are acceptable insofar as contamination rarely results from close contact between a diseased excreting animal and a receptive susceptible animal.

For canine parvovirus, the situation is different. An efficacious vaccination schedule requires numerous adjustments due to the epidemiology of this disease. Since the canine parvovirus is highly stable in the environment, susceptible puppies get infected in a contaminated environment as soon as maternal immunity declines to a titre of 1/64–1/80 haemagglutination inhibiting (HAI) antibodies. With an homologous live vaccine<sup>13</sup>, titre of  $10^{5.5}$  TCID<sub>50</sub> ml<sup>-1</sup>, more than 95% of dogs with a residual antibody titre of 1/10 seroconverted to the vaccine but only 50% of dogs with a titre of 1/20 seroconverted and none seroconverted with titres greater than 1/80. The window of vulnerability, during which puppies are susceptible to infection but still are resistant to vaccination, may last 2–5 weeks depending on the vaccine used.

All the strains of modified live viruses do not have the same intrinsic immunogenicity. Some strains are over-attenuated and their 50% minimum immunising dose is high. The best possible compromise must be found between attenuation (residual pathogenicity) and immunogenicity. This observation has resulted in preparation of overdosed or high-titre vaccines for the most difficult situations<sup>14</sup>.

An experiment relating to vaccination against rabies is summarized as follows. The decrease of maternally-derived antibodies and the absence of serological response following vaccination with a killed rabies vaccine in the presence of these antibodies had previously been observed and published<sup>15</sup>. However, no virulent challenge was carried out at that time. In a recent study, four groups of four Beagle puppies born to dams boosted during pregnancy with a killed adjuvanted vaccine (RABISIN) were used. All puppies received colostral immunity. The first group constituted the unvaccinated control group. The second group was vaccinated at the age of 14 days with one dose of RABISIN vaccine (NIH = 7) by the subcutaneous route. The third group was vaccinated at the age of 14 days with a Recombinant Canary Pox Rabies vaccine<sup>16–18</sup> titrating  $10^8$  TCID<sub>50</sub> per dose (SC route). The fourth group was vaccinated at the age of 14 days with one dose of a Recombinant Canary Pox Rabies vaccine titrating  $10^{6.7}$  TCID<sub>50</sub> per dose (SC route), the minimum immunogenic dose. All of them were challenged with a field canine rabies strain (NY) at 120 days of age. The four unvaccinated control puppies were euthanased after the onset of clinical signs of rabies. All the puppies of groups 2 and 3 (RABISIN and VCP-RG  $10^8$ ) and two puppies out of four in group 4 were fully protected. The kinetics of rabies virus SN antibodies was monitored on these twelve puppies (Figure 4). On challenge day, the mean antibody titre was low, if not zero, in all the vaccinated and control groups (0.22 IU in the group vaccinated with RABISIN, 0.06 IU in the group vaccinated with VCP  $10^8$ , 0.08 IU in the group vaccinated with VCP  $10^{6.7}$ , and 0.03 IU in the control group). In such a study, even though very limited, established ideas are challenged and confirm the fact that passive antibodies of maternal origin, up to a certain limit, may be overcome by vaccines whose antigenic content is greater than traditional vaccines of excellent quality or in which a vector to which the neonate is naive may allow antigen presentation.

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