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Scientific Opinion of the Scientific Panel on Animal Health and Welfare on a request from the Commission regarding an

"Assessment of the risk of rabies introduction into the UK, Ireland, Sweden, Malta, as a consequence of abandoning the serological test measuring protective antibodies to rabies."

EFSA-Q-2006-014

Adopted by the AHAW Panel on 11th of December 2006

SUMMARY

Although the rabies situation in Europe improved greatly during the last Century, the disease is still prevalent in wildlife in some EU member states and adjacent third countries. In areas where rabies occurs in wildlife a certain spill-over to domestic animals, including pets, may occur. It is therefore well justified that appropriate measures aimed at preventing pets from further spreading the disease are maintained as long as the disease persists in wildlife.

Within EU the control of rabies in animals is based on a combination of national rules and Community legislation. The non-commercial movement of pets (dog, cat, ferrets) is governed by Regulation (EU) No 998/2003¹, establishing provisions for pet movement within the Community and from third countries into the EU. Article 6 of this Regulation provides that Ireland, UK, Sweden and Malta may maintain their national provisions for a transitional period of 5 years from the entry into force of this Regulation, *i.e.* until July 2008. These derogations consist of the requirement of an individual serological test for detection of neutralising rabies-antibodies and a waiting period before entry of pet animals into their territory.

The Regulation further states that the derogations will be reviewed at the end of this transitory period of 5 years. To this end, the Commission has to submit to the European Parliament and to the Council, before the 1st of February 2007, a report on the need to maintain the serological test, and with appropriate proposals for determining the regime to be applied after this period. The report shall be based on the experience gained so far and on a risk analysis, following receipt of a scientific opinion of the European Food Safety Authority (EFSA).

As a consequence, the Commission requested EFSA to issue a scientific opinion in order to assist in proposing appropriate amendments to the above Regulation. Specifically, the opinion should address to what extent abandoning the serological test could be envisaged without increasing the risk of introducing rabies and, if the need to maintain the serological test in certain circumstances is scientifically justified, what would be the appropriate regime/protocol giving equivalent assurances for the protection of these Member States against introduction of rabies.

A number of countries have carried out independent rabies risk assessments in relation to pet movement. These assessments vary considerably in assumed control strategies and risk pathways and are therefore not directly comparable. They do, however, share most of the underlying parameter estimates used for modelling. To meet the terms of reference of the present assessment, it was judged necessary to develop risk pathways designed specifically to answer the questions raised, *i.e.* segregating the effect of testing from all other measures, using either established parameter estimates or modified according to available evidence.

The risk of transmission of rabies by pet movement is related to moving an animal incubating disease. Pre-exposure vaccination of pets confers quick and almost complete protection to subsequent exposure by contact, *e.g.* bites. On the other hand, infection prior to vaccination cannot be controlled by immunisation but will require a quarantine and observation period covering the incubation period to be revealed. Previously, quarantine was implemented by physical isolation but with the advent of efficient vaccines, an "immunological quarantine" can be implemented with much less consequence for animal welfare.

The unrestricted risk that a pet is incubating rabies at the time of primo-vaccination is equal to the prevalence of rabies-incubating pets in the population of origin. The prevalence can be estimated from the observed incidence of rabies in the population combined with an estimate of population size and the distribution of incubation times after natural infection. Following induction of protective immunity by vaccinating animal already incubating rabies will still develop clinical disease as a function of time after vaccination. Observing a vaccinated animal over a certain period

¹ OJ L 146/1, 13.06.2003, p. 1-9.

will thus gradually reduce the risk (termed type A in this opinion) that this animal incubates rabies, given that it has not developed clinical signs.

Rabies vaccines are currently authorised on the basis of challenge tests in a target species and the measurement of protective levels of antibody. For monitoring the response to vaccination, the best available correlate with protection is to demonstrate that animals have achieved a serological titre of 0.5 IU/ml.

As for any population of animals, a proportion may fail to mount an adequate serological response to vaccines and this may indicate that the individual animal may not be adequately protected against infection with the virus. If undetected, such animals may become infected after vaccination (termed type B risk in this opinion). The risk of the primo-vaccination with a single dose failing to induce an adequate serological response depends on the species, the age, the type of vaccine and the route and method of administration. Studies in published literature suggest that this risk can be effectively eliminated by administering a second vaccination at 4 to 6 weeks after the first, single vaccination thereby enhancing the chance that the desired antibody titre is achieved.

However, such recommendations are not part of the currently approved immunisation schemes for most authorised products on the EU market where efficacy is ensured by rigorous challenge studies established by the European Pharmacopoeia.

The overall risk reducing effect of applying a protocol including vaccination with or without testing and a specified waiting time has been modelled in a quantitative risk model. The major conclusions drawn from the study are:

- The primary means of protecting a pet from rabies in the population at risk is by vaccination. Inactivated rabies vaccines are highly efficient and induce rapid protective immunity that prevents infection and subsequent transmission of the disease.
- In quantitative terms, the type A risk constitutes by far the major risk. Therefore, a waiting time (defined as the time spent between vaccination and pet movement to the destined country), is the major effective measure to mitigate the risk of rabies introduction due to an animal being infected before primo-vaccination.
- The risk of infection following exposure during the waiting time (type B risk) depends on the protection induced by the vaccination in field conditions and becomes relatively more important as type A risk is reduced with extended waiting times (over 100 days). Serological testing can be used to identify seronegative pets and will therefore reduce this risk accordingly.
- Depending on the risk assessment model applied, a total risk reduction of 1.5 and 3.8, respectively, could be attributed to serological testing when the waiting time was 120 days. The same or an even better risk reduction can be obtained by replacing serological testing with a second vaccination 4 to 6 weeks after the first vaccination.
- For animals coming from countries with a negligible incidence of rabies, the best way to prevent rabies infection is simply by assuring adequate immunity after primo-vaccination before moving. In these countries, there is no rationale for including a waiting time beyond the time where protective immunity has been reached.
- The risk of transmitting rabies from populations where the annual incidence is below 1 in a million is considered negligible, even without applying a specific risk-mitigating protocol.

Vaccination against rabies, using an authorised vaccine administered according to the approved vaccination schedule should remain the key requirement for pet movement between Member States. If further risk reduction is required, the protocol should include a waiting time following primo-vaccination and the length of waiting time should reflect the objective for risk reduction. The risk of having a certain proportion of pets vaccinated under field conditions that may not be fully protected can be reduced either by carrying out a serological test to measure antibodies or by

administering a second injection of vaccine, provided that approved vaccination schedules are amended to include the option of administering a second injection where necessary.

Key words

Lyssavirus, , *Rhabdoviridae*, mammalian reservoirs, red fox , raccoon dog , transmission, incubation period, epidemiology, rabies in domestic animals, rabies in humans, control measures, surveillance and eradication programmes in wildlife, rabies vaccination, protection and seroconversion, FAVN and RFFIT tests , test specificity, risk assessment, rabies incidence, rabies prevalence, risk of infection, waiting time, risk of rabies introduction.

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ABBREVIATIONS

CNS	Central nervous system
EBL	European bat lyssavirus (type 1 or 2)
EU	European Union
FAVN	Fluorescent antibody virus neutralisation test
MS	Member States
MNT	Mouse protection assay (neutralization test)
OIE	<i>Office International des Epizooties</i> (World organisation for animal health)
OVF	Oral vaccination of foxes
PETS	UK Pet Travel Scheme
RFFIT	Rapid fluorescent focus inhibition test
SCAHAW	EU Scientific Committee on Animal Health and Animal Welfare
UK	United Kingdom
WHO	World Health Organisation

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1. TERMS OF REFERENCE

1.1. BACKGROUND

The Regulation (EU) No 998/2003² lays down the rules for the non-commercial movements of pet animals (dog, cat, ferrets) both within the Community as well as from third countries into the EU.

Article 6 of the above Regulation provides that Ireland, UK, Sweden and Malta may maintain their national provisions for a transitional period of 5 years from the entry into force of this Regulation, *i.e.* until July 2008. These derogations consist of the requirement of an individual serological test³ for detection of neutralising rabies-antibodies (Articles 6 & 15) before entry of pet animals into their territory.

The Regulation further states that the above derogations will be reviewed at the end of this transitory period of 5 years.

To this end, the Commission has to submit to the European Parliament and to the Council, before the 1st February 2007, a report on the need to maintain the serological test, and with appropriate proposals for determining the regime to be applied after this period. This report shall be based on the experience gained so far and on a risk analysis, following receipt of a scientific opinion of the European Food Safety Authority, (EFSA) (Article 23).

As a consequence, the Commission requests EFSA to issue a scientific opinion in order to assist the Commission in proposing appropriate amendments to the above Regulation that are scientifically justified.

1.2. MANDATE

In view of the above, the Commission requests EFSA, in accordance with Article 29 (1) (a) of Regulation (EC) No 178/2002⁴, to issue a scientific opinion on the risk assessment of rabies introduction into Ireland, the UK, Sweden and Malta, as a consequence of abandoning the serological test for antibody titration for rabies.

In particular, the opinion should provide scientific advice on the following questions:

- To what extent the abandoning of the individual serological test for neutralising rabies-antibodies titration could be envisaged, taking into account the different epidemiological situations with regard to rabies prevailing in the Member States other than Ireland, UK, Sweden and Malta, without increasing the risk of introducing rabies into these latter countries from the remainder of the EU territory.
- If the assessment reveals that in certain circumstances the need to maintain the serological test for antibody titration for rabies is scientifically justified (in other words, if the consequential risk is higher than negligible), what would be the appropriate regime/protocol (vaccination / serological test/ movement) to be considered as giving equivalent assurances for the protection of these Member States, taking into account the different national rules that are currently in force⁵.

1.3. SCOPE OF THE REPORT

According to art 5 of Regulation (EC) No 998/2003, movement of dogs and cats over 3 months of age between member states requires a valid anti-rabies vaccination without any waiting time before movement. In addition, the UK, Ireland, Sweden and Malta have been granted derogations

² OJ L 146/1, 13.06.2003, p.1-9.

³ Neutralising antibody titration of at least 0,5 IU/ml, carried out on a sample taken 6 months before the entry (UK, Ireland and Malta) or 4 months after vaccination (Sweden).

⁴ OJ L 31, 1.2.2002, p. 1.

⁵ See [Annex I](#)

to maintain the requirement for a serological test at a specified time after vaccination. The lapse of time between vaccination/serological testing and movement constitutes a virtual quarantine: if the animal presents no symptoms of the disease during this period the probability of having been infected before induction of vaccinal immunity is then considered negligible.

The risk of transmission of rabies by pet movement is defined as the risk of moving an animal incubating disease. Pre-exposure vaccination of pets with currently licensed vaccines is considered to confer quick and almost complete immunity to subsequent challenge by contact, *e.g.* bites. On the other hand, post-exposure vaccination is considered to confer only limited protection depending *inter alia* on a short interval between exposure and vaccination. Consequently, unknown exposure prior to vaccination cannot be controlled by immunisation but will require a quarantine and observation period covering the incubation period of the relevant species. Previously, quarantine was implemented by physical isolation but after the advent of efficient vaccines, an "immunological quarantine" that effectively prevents establishment of infection following accidental exposure can be implemented with much less consequences for animal welfare.

The risks associated with pets incubating rabies can be dealt with and will depend on various estimates under the following headings: 1. prevalence of rabies in the country of origin in pets and other contact species, 2. failures related to establishing protective immunity and maintaining immunological quarantine for a specified time period. Several sources of failure may contribute to the overall risk, *e.g.* biological variation, test specificity and non-compliance with statutory requirements. These causes should be clearly discerned in order to produce meaningful risk assessments. The final risk of rabies being transferred by pets can be expected to be proportional to the total number of pets being moved throughout member states. Unfortunately, no information is presently available on numbers of movement of pet animals through Member States with exception of movements to UK (Figure 1.1) where the increase may be seen as a consequence of the change in the legislation regarding vaccination and quarantine. The level of import is likely to stabilise. A proportion of these animals is represented by UK pets that return from abroad.

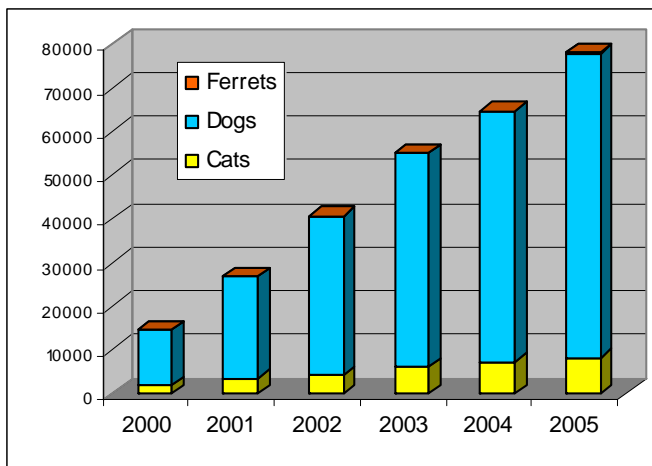


Figure 1-1 - PETS: Statistics - Number of pet cats and dogs entering the UK under PETS per year (<http://www.defra.gov.uk/animalh/quarantine/pets/procedures/stats.htm>)

Following initial discussions with the Commission it was agreed that the assessment should focus on the risk reduction contributed by serology following vaccination. It was also agreed that bat rabies should not be included in the scope of this risk evaluation. It was also suggested that the first part of the terms of reference could be answered by a quantitative risk assessment. The risk of rabies introduction will be assessed using two scenarios: with or without serological testing.

Moreover, the effect of not vaccinating was included. The determined values will be communicated to the Commission for further consideration. The second part of the mandate was proposed to be done by a qualitative approach. If possible, the assessment should lead to recommendations for a harmonised scheme for non-commercial movement of pets within EU.

2. HAZARD IDENTIFICATION AND CHARACTERISATION

2.1. DESCRIPTION OF RABIES DISEASE

Rabies is a viral zoonotic disease of mammals, including humans, which causes encephalomyelitis. Rabies is induced by neurotropic viruses of the *Lyssavirus* genus, *Rhabdoviridae* family. So far it is known that the genus *Lyssavirus* includes 7 genotypes (Bourhy *et al.*, 1993; Gould, 1998). The number of genotypes is likely to increase (Kuzmin *et al.*, 2003). The genotype 1 comprises classical rabies virus responsible for most human rabies cases and it is distributed in almost every country throughout the world. The virus is maintained in mammalian reservoirs, mainly domestic and wild carnivores as well as bats. Dogs represent the major rabies reservoir in developing countries.

2.2. TRANSMISSION AND RESERVOIR

The rabies virus is transmitted to other animals through close contact with saliva from infected animals. Bite is the main mode of virus transmission; scratching and licking on broken skin and mucous membrane can also transmit the disease. The transport of the virus from the site of virus entry occurs through the neuronal pathway into the brain which is the preferential site of virus replication. Once the virus reaches the central nervous system, it replicates massively. The virus is then transported to many tissues, such as skeletal and cardiac muscles, adrenal glands, kidneys, retina, cornea, etc, through the nerves.

Productive viral replication takes place predominantly in salivary glands, excreting virus transmissible to other mammals. The rabies virus is circulating in different susceptible populations of mammal species, which constitute the virus reservoir, allowing the maintenance of an epidemiological cycle of rabies in a certain geographical area.

In most industrialised areas (USA, Canada, Europe), the main reservoirs of rabies are wild mammals (mainly raccoon, skunk, fox and bat in the USA and Canada and the red fox and raccoon dogs in Europe). In developing countries in Africa, Asia and South America dogs represent the major rabies reservoir, transmitting rabies to other animals and to humans as well.

Different independent epidemiologic cycles exist in bats, involving different genotypes of *Lyssavirus* (in particular genotype 1 in the Americas, genotypes 5 (EBL1) and 6 (EBL2) in Europe and genotype 7 in Australia) (Cliquet and Picard-Meyer, 2004a).

2.3. PATHOGENESIS

After virus inoculation through animal bite, local replication of virus at the site of entry may occur for a long time (Fekadu, 1988). The virus enters into peripheral nerves at the infection site and replicates in non nervous tissues (muscle cells). After uptake into peripheral nerves, the virus then moves along the nerve axons to the central nervous system (CNS) (Jackson, 2002). After infection develops within the CNS, the rabies virus disseminates rapidly by centrifugal spread to peripheral nerves, which may lead to invasion of highly innervated sites of various tissues, including the salivary glands. The immune response to naturally acquired virus is slow and generally, specific rabies neutralising antibodies are not detected before onset of illness, leading to a fatal outcome in most cases (Fekadu, 1988).

2.3.1. Incubation period

The incubation period (the time from initial viral exposure at first demonstration of clinical signs) is dependent upon viral dose, route and strain as well as the site of inoculation. Concentrated inoculum of virus produces a short period of incubation and a rapid course of the disease, before spread of rabies virus throughout the brain. Limited data are available on incubation periods after natural infection. Data available for rabid cats indicate an incubation period ranging from 9 days to 6 months, but generally being between 4 and 6 weeks (Bunn, 1991). For dogs, this period ranges from a week to several months (Fekadu, 1988 and 1993). Long incubation periods

observed in naturally infected animals may be due to infection with very small amounts of virus (Fekadu, 1993).

Incubation periods have frequently been described by log-normal distributions (see Figure 2.1), indicating an underlying multifactorial process (Limpert *et al.*, 2001). Based on a combination of experimental studies (Soulebot *et al.*, 1981; Fekadu *et al.*, 1982; Trimarchi *et al.*, 1986) and data from France and UK quarantine (Advisory group on Quarantine, 1998; Committee of Inquiry on Rabies, 1971), Jones *et al.* (2005) described the distribution of incubation periods for canine and feline rabies by the use of a log-normal distribution with an expected value (mean) of 38 days and standard deviation of 45 days. In general, experimental data indicated shorter incubation periods (<70 days), whereas data from the quarantine indicated longer periods (2-234 days) (Jones *et al.*, 2005). This could be due to different infecting doses or possibly secondary infections within quarantine.

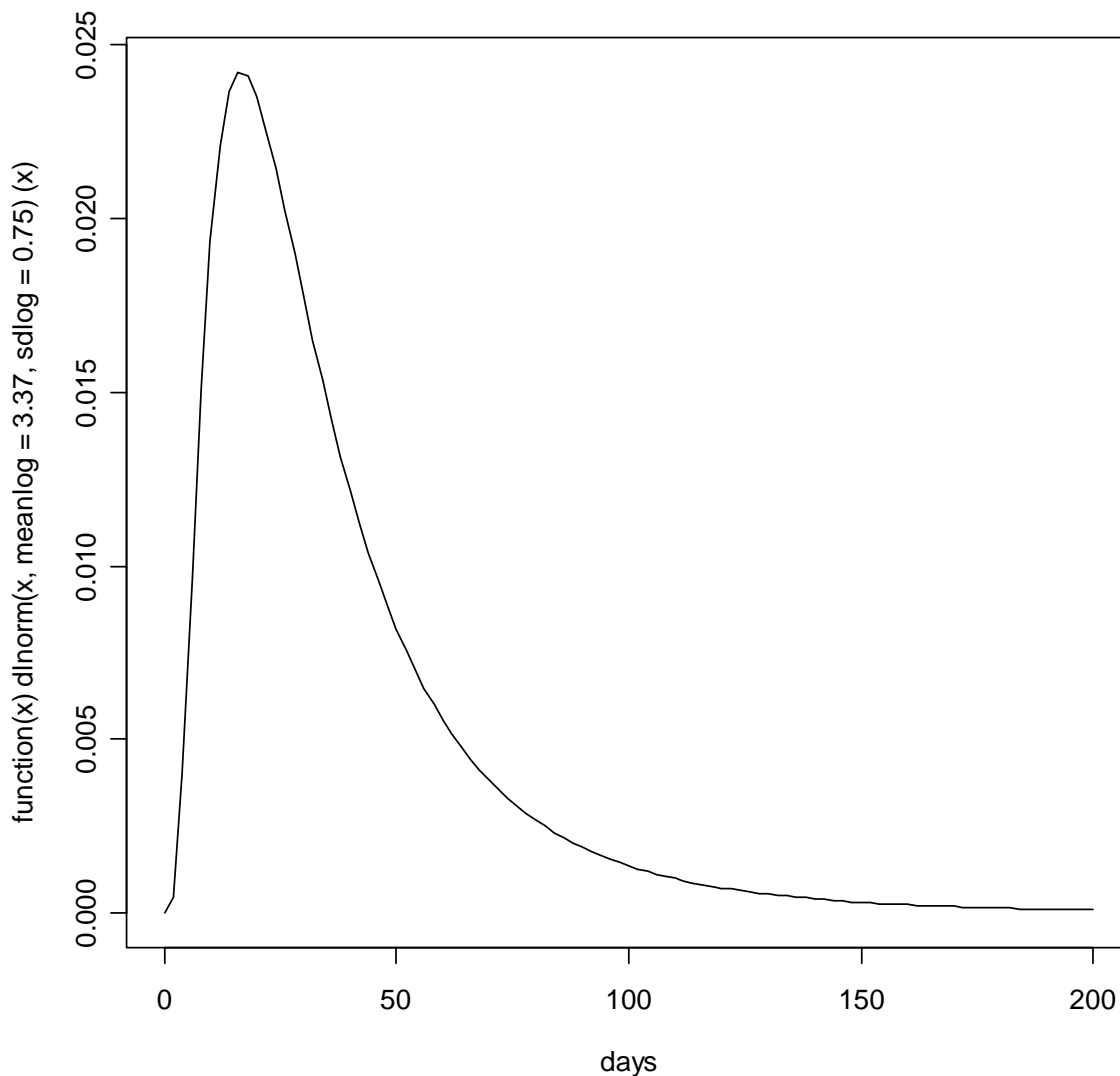


Figure 2-1 - Density plot of log-normal distribution of the length of the incubation period of rabies in dogs and cats (adapted from Jones *et al.*, 2005)

For transmission of rabies, the key factor is the presence of the virus in saliva and salivary glands in particular, before the period where detectable clinical signs are recorded. It has been documented that dogs can excrete rabies virus in the saliva up to 14 days before clinical signs appear (Fekadu, 1982). Those data are crucial since transmission of rabies may unknowingly occur and therefore no preventive measures are taken because the animal appears healthy.

2.4. CLINICAL SIGNS IN ANIMALS

The clinical signs of disease may vary as well as the duration of illness (Fekadu, 1993; Bowen Davis and Lowings, 2000). The first symptoms of rabies are generally non-specific during the prodromal (initial) stage where behavioural changes are recorded. During this stage, the animal is generally abnormally alert, restless, hyperactive and hyper sensitive to noise and light, with increased tendency for dogs to lick their owners. Animals may have fever and dilatation of pupils as well as excessive salivation. This phase lasts generally two to five days. In the acute period, which usually ends after 2 to 10 days, signs of hyperactivity (furious form) or paralysis (dumb rabies, which is more common) are recognised.

During the excitative (furious) stage, the clinical signs are more readily indicative of rabies; animals present an increase of aggression and restlessness. Sometimes the animal runs without apparent reason, bites with no provocation and eats abnormal objects in its path.

When the excitative form is very short or absent, the paralytic stage occurs and is characterised by extensive paralysis of masseter and respiratory muscles. The animal becomes timid and shy. Due to paralysis of laryngeal and pharyngeal muscles, characteristic changes in the bark or howling is observed and the animal has difficulty in swallowing, leading to drooling. Within a few days, the disease usually progresses to muscular incoordination, paralysis, coma and death.

2.5. RABIES EPIDEMIOLOGY IN EUROPE

2.5.1. Rabies in wild animals

At the end of the 19th Century, the reservoir of rabies virus was the dog (*Canis canis*) in most European countries, and the main sylvatic reservoir was the wolf (*Canis lupus*). In these areas, rabies control programmes using intensified mass parenteral vaccination campaigns and a reduction of stray populations have been effective (Pastoret *et al.*, 2004).

During the first half of the 20th Century, canine rabies progressively disappeared in most European countries and vulpine rabies appeared in the East part of Poland and spread rapidly in all directions with an annual spread of 30-60 km. With the exception of the British Isles (Great Britain, Ireland) and the Scandinavian Peninsula, most European countries became infected (for review, see Cliquet and Aubert, 2004b).

The red fox (*Vulpes vulpes*) is the main species involved in rabies epidemiology of Western and Central Europe and is at the origin of rabies transmission to other wild species and to domestic animals as well, including pets, bovines, ovines and caprines. The raccoon dog (*Nyctereutes procyonoides*) represents a major factor in the epidemiology and epizootiology of rabies in Eastern and Northern Europe (Botvinkin *et al.*, 1981). Turkey is the only European country where the domestic dog is the principal vector of rabies.

In addition, distinct epidemiological cycles occur in bats involving different rabies related viruses depending upon geographical areas.

In Europe, rabies is a notifiable disease. Available epidemiological data provided by 40 European countries currently are compiled at the WHO Collaborating Centre for Rabies Surveillance and Research (Wusterhausen, Germany) and data are available on an Internet website (<http://www.rbe.fli.bund.de>).

In most European countries, the highest incidence was observed in 1989. Rabies cases then decreased until 1997 and increased again until 2003 with a total number of 11,085 cases. In 2004,

the total number of rabies cases was 5,452. Raccoon dogs represent around 15 % of total cases and are now the second most important reservoir wildlife species after the red fox infected with rabies.

Tables 2.1 and 2.2 as well Figure 2.2 and 2.3 outline the total number of rabies cases since 2000 in the 25 EU member states in wild and domestic animals as well as in countries bordering the EU. The overall incidence of rabies has shown a slight decrease since 2003 in member states, particularly in the number of rabid wildlife (from 3,076 total wildlife cases in 2003 to 2089 in 2005).

The decrease seen over the last 10 years in rabies incidence may be explained by recent implementation of control measures in wildlife in a number of member states. Another hypothesis to consider is the dynamics of rabies spread in fox populations: when the virus is spreading, it may kill most of the foxes in the area, lowering the local density population below a certain threshold necessary for the infection to persist. These areas will be rapidly repopulated by neighbouring foxes which will also be infected. Therefore, the pattern of rabies incidence may follow waves of increases and decreases with peaks observed generally every two to three years (Schneider *et al.*, 1987).

Table 2-1 - Rabies cases in wildlife and in domestic mammals 2000-2005 in EU member states excluding cases of rabies in bats (compiled from Rabies Bulletins Europe 2000-2005 and Community summary report on zoonoses, 2004 (Malta))

EU country	2000					2001					2002					2003					2004					2005					
	Total number	Wildlife animals	Domestic animals	Dogs	Cats	Total number	Wildlife animals	Domestic animals	Dogs	Cats	Total number	Wildlife animals	Domestic animals	Dogs	Cats	Total number	Wildlife animals	Domestic animals	Dogs	Cats	Total number	Wildlife animals	Domestic animals	Dogs	Cats	Total number	Wildlife animals	Domestic animals	Dogs	Cats	
Cyprus	0		0			0	0	0	0	0	0	0	0	-	-	-	-	-	-	-	0	0	0	0	0	0	0	-	-	-	-
Denmark	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Finland	0	0	0	0	0	0	0	0	0	0	0	0	0	-	-	1*	0	0	0	0	0	0	0	0	0	0	0	-	-	-	-
France	0	0	0	0	0	1**	0	0	0	0	1**	0	1	1	0	0	0	0	0	0	0	3**	0	0	0	0	0	0	0	0	0
Malta	0					0					0					0					0					0					
Greece	0	0	0	0	0	0	0	0	0	0	0	0	0	-	-	0	0	0	-	-	0	0	0	0	0	0	0	-	-	-	-
Italy	0	0	0	0	0	0	0	0	0	0	0	0	0	-	-	0	0	0	-	-	0	0	0	0	0	0	0	-	-	-	-
Luxembourg	0	0	0	0	0	0	0	0	0	0	0	0	0	-	-	0	-	0	-	-	0	0	0	0	0	0	0	-	-	-	-
Spain	0	0	0	0	0	13	0	10***	4	0	7	0	7	4	0	0	-	0	-	-	1	0	1	1	0	1	0	1	1	1	0
Portugal	0	0	0	0	0	0	0	0	0	0	0	0	0	-	-	0	-	0	-	-	0	0	0	0	0	0	0	-	-	-	-
Sweden	0	0	0	0	0	0	0	0	0	0	0	0	0	-	-	0	-	0	-	-	0	0	0	0	0	0	0	-	-	-	-
U.K.	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	-	0	-	-	0	0	0	0	0	0	0	0	0	0	0
Netherlands	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Belgium	0	0	0	0	0	0	0	0	0	0	0	0	0	-	-	0	0	0	-	-	0	0	0	0	0	0	0	-	-	-	-
Ireland	0	0	0	0	0	0	0	0	0	0	0	0	0	-	-	0	0	0	-	-	0	0	0	0	0	0	0	-	-	-	-
Czech Republic	165	156	9	2	3	35	33	2	0	2	3	3	0	0	0	0	-	0	-	-	0	0	0	0	0	0	0	0	0	0	0
Germany	182	166	16	0	3	41	38	3	0	1	35	33	2	1	1	24	24	0	0	0	35	33	1	1	0	42	41	1	0	0	0
Austria	2	2	0	0	0	11	10	1	0	1	24	22	2	1	1	1	0	1	0	0	1	1	0	0	0	0	0	0	0	0	0
Slovenia	114	110	4	2	2	135	125	10	7	3	15	15	0	0	0	8	8	0	0	0	2	2	0	0	0	3	3	0	0	0	0
Slovakia	351	280	71	21	44	87	75	12	4	8	113	94	19	6	11	326	284	42	18	21	57	52	5	1	3	50	46	4	3	1	
Hungary	514	398	116	24	61	310	236	74	14	42	160	129	31	4	17	172	129	43	5	18	125	111	14	6	5	9	7	2	0	2	
Latvia	516	398	118	49	41	477	393	84	33	37	500	411	89	31	32	963	828	135	62	52	443	350	93	33	35	421	353	68	20	29	
Estonia	129	93	36	11	4	167	137	30	6	12	422	355	67	24	22	814	697	117	34	28	314	254	60	24	20	266	229	37	6	8	
Lithuania	854	571	283	43	58	680	489	191	34	57	932	681	251	46	70	1108	796	312	56	81	553	408	145	39	34	1652	1312	340	89	92	
Poland	2204	1854	350	60	114	2944	2565	379	97	177	1183	1030	153	31	69	382	310	72	19	27	126	103	23	4	10	138	98	36	5	7	
TOTAL	5031	4028	1003	212	330	4903	4101	796	199	340	3396	2773	623	149	223	3799	3076	722	194	227	1660	1314	342	109	107	2578	2089	489	124	139	

* : 1 equine imported, ** : dogs imported from North Africa, *** : 3 imported cases from Millila (Territory North Africa)

Table 2-2 - Total number of rabies cases in countries bordering EU member states, excluding cases of rabies in bats (compiled from Rabies Bulletins Europe 2000-2005)

Country	2000					2001					2002					2003					2004					2005				
	Total number	Wildlife animals	Domestic animals	Dogs	Cats	Total number	Wildlife animals	Domestic animals	Dogs	Cats	Total number	Wildlife animals	Domestic animals	Dogs	Cats	Total number	Wildlife animals	Domestic animals	Dogs	Cats	Total number	Wildlife animals	Domestic animals	Dogs	Cats	Total number	Wildlife animals	Domestic animals	Dogs	Cats
Russian Federation	1232	487	745	227	181	1919	694	1225	346	306	3083	1207	1876	515	357	2862	1360	1502	624	347	1536	563	973	361	282	3079	1306	1773	647	493
Belarus	306	203	103	38	33	540	373	167	63	46	809	580	229	77	81	1077	761	316	133	119	211	135	76	27	28	591	442	149	63	41
Ukraine	ND	ND	ND	ND	ND	1611	638	973	217	377	1547	658	889	239	336	2028	924	1104	369	442	906	425	481	132	221	2111	959	1152	358	470
Romania	97	53	44	20	12	386	293	93	30	23	115	70	45	18	10	95	67	28	8	5	187	119	68	28	18	530	354	176	64	61
Serbia and Montenegro	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	261	207	54	17	29	210	167	43	8	19	101	84	17	8	7
Croatia	917	870	47	15	17	489	456	33	9	4	501	475	26	7	8	633	590	43	21	14	504	471	33	13	15	557	525	32	11	14
Bosnia-Herzegovina	9	9	0	0	0	31	26	5	2	0	52	42	10	3	1	80	63	17	5	5	48	34	14	6	3	36	30	6	2	1
Albania	0	0	0	-	-	1	0	1	1	0	1	1	0	0	0	2	2	0	0	0	3	1	2	0	0	2	2	0	0	0
Macedonia	0	0	0	-	-	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0	0	0	-	-	0	0	0	0	0	ND	ND	ND	ND	ND
Bulgaria	22	22	0	0	0	62	62	0	0	0	16	16	0	0	0	19	15	4	2	0	11	4	7	0	2	8	4	4	1	0
Turkey	297	12	285	252	0	189	16	173	127	0	249	35	217	75	1	156	17	139	59	8	111	8	103	51	3	193	11	182	100	4
TOTAL	2880	1656	1224	552	243	5228	2558	2670	795	756	6373	3084	3292	934	794	7213	4006	3207	1238	969	3727	1927	1800	626	591	7208	3717	3491	1254	1091

ND: no data

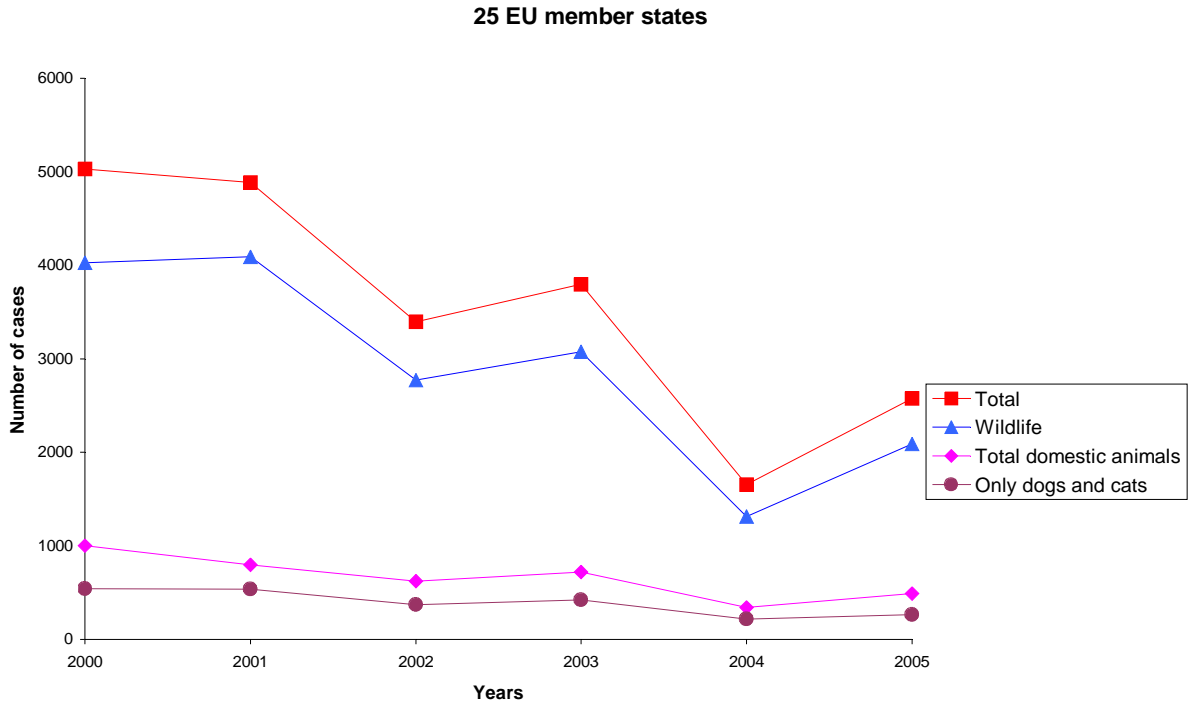


Figure 2-2 - Evolution of rabies incidence in EU member states

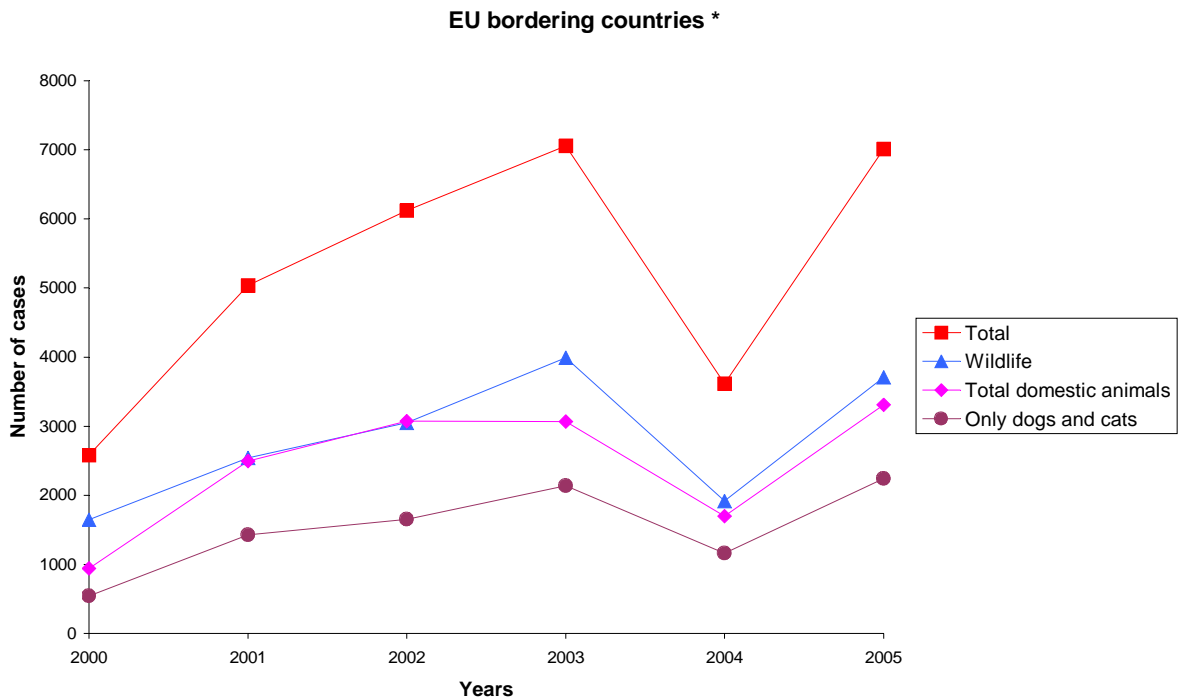


Figure 2-3 - Evolution of rabies incidence in some countries bordering EU

* Russian Federation, Belarus, Ukraine, Romania, Serbia and Montenegro, Croatia, Bosnia-Herzegovina, Albania, Macedonia, Bulgaria.

(Compiled from Rabies-Bulletin Europe 2000-2005)

2.5.2. Rabies in domestic animals

In Europe, rabies cases recorded in domestic animals are generally the result of transmission of infection from a rabid wild animal. It may also be the result of a translocation or importation of a rabid pet from endemic areas (vaccinated or unvaccinated). In this case, the pet may be either an adopted animal from the endemic area or the owner's animal that has been infected by a rabid animal during the stay in the endemic area.

The main domestic species infected are dogs and cats followed by bovines and ovine-caprine species.

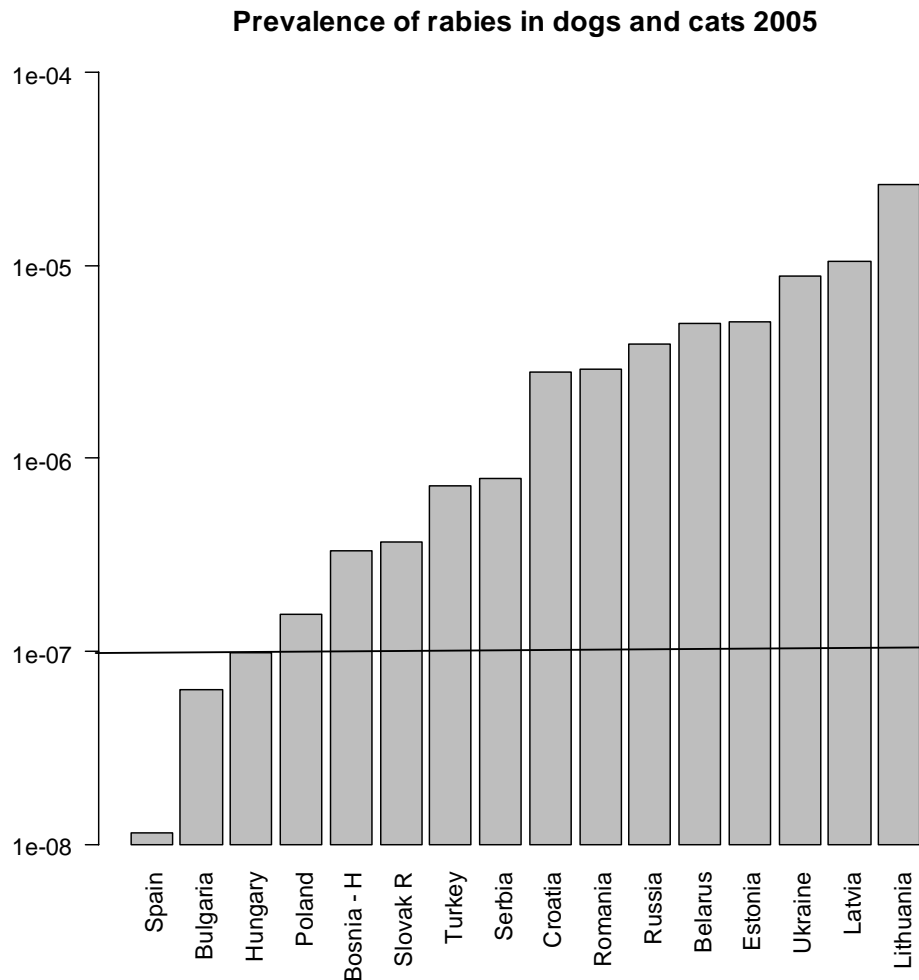


Figure 2-4 - Prevalence estimates were calculated from incidence data in tables 2.1 and 2.2 and estimating pet population from human population data (Eurostat) as described in section 4.3.2.

Between 1990 and 2004, the rabies incidence in domestic mammals in the EU 25 member states has decreased in parallel to the observed reduction in wildlife (see Table 2.1 and Figure 2.2). The proportion of rabid domestic cats and dogs to the total number of cases is more or less stable: since 2000 this ratio has varied from 11 to 13% (calculations based on data from Table 2.1). Based on estimates of pet populations (dogs and cats) as a fraction of human population, 1.0 dog and 1.1 cats per 10 people (Jones *et al.*, 2002), the prevalence of rabies in Europe in 2005 has been calculated from incidence data in Table 2.1 and 2.2 and presented in Figure 2.4. Notably, the prevalence varies almost 4 orders of magnitude with the Baltic countries and Ukraine showing highest prevalence (*ca.* 10^{-5}).

Since 2000, the rabies incidence in domestic animals in EU bordering countries has followed a different trend than in EU (Table 2.2 and Figure 2.3). In Ukraine and in the Russian Federation, an increasing number of rabies cases were diagnosed in domestic animals. The proportion of rabid cats and dogs diagnosed in EU bordering countries has apparently increased since 2000 compared with the total number of rabid animals (the relative incidence of rabid cats/dogs is 21% in 2000, 28% and 27% in 2001 and 2002, respectively, 30% in 2003 and 32% in 2004 and 2005). It is interesting to note that the number of domestic animals diagnosed with rabies is approximately similar to the number of animals diagnosed in wildlife, in contrast to what is observed in the 25 EU countries. Several possible explanations for this difference can be envisaged, *i.e.* varying efficiency of wildlife-domestic animal barrier, monitoring differences or possibly horizontal spread within domestic animals.

The results demonstrate clearly the importance of rabies in EU bordering countries, with high numbers of rabid cats and dogs diagnosed annually. The rabies surveillance in those countries may be more focused on detection and diagnosis of disease in domestic species.

2.5.3. Rabies in humans

The incidence of human rabies is limited in Europe, being either indigenous cases contracted in the country of origin by a rabid fox or domestic carnivore without appropriate post-exposure treatment or imported cases from countries where canine rabies is endemic, usually from Africa or Asia. Furthermore, organ transplantation from non-diagnosed fatalities of rabies has led to secondary cases in recipients.

Indigenous cases are mostly reported in Eastern Europe (Ukraine, Russia) while cases in rabies-free countries are mostly imported. In 2002, a human case was recorded in a bat handler in the UK following infection by a bat virus isolate (European bat lyssaviruses (EBL) are genetically distinct from those found in terrestrial mammals). Table 2.3 records the total number of human rabies cases notified in Europe since 2000.

Table 2-3 - Human rabies cases in Europe from 2000 to 2005 (compilation from Rabies Bulletin Europe).

<i>Year</i>	<i>Country</i>	<i>Indigenous cases</i>	<i>Imported cases</i>
2000	Lithuania	1	
	Romania	1	
	Russia	7	
2001	Russia	10	
	U.K.		2 (Philippines, Nigeria)
2002	Russia	5	
	Ukraine	1	
	U.K.	1*	
2003	Latvia	1	
	Russia	3	
	Ukraine	2	
	France		1 (Gabon)
2004	Lithuania	1	
	Russia	12	
	Germany		1 (India)
	Austria		1 (Morocco)
2005	Germany		1 (India) + 3 (organ graft)
	U.K.		1 (India)
	Russia	4	
Total		49	10

* EBL2 rabies cases in a bat handler.

2.6. DESCRIPTION OF IMPORTED ANIMAL CASES

European countries occasionally report imported rabies cases in cats and dogs and other domestic animals. In most of these cases, affected animals are non-vaccinated puppies or young dogs.

The risk associated with importing pets depends upon geographical location as well as patterns of tourism and exchange practices of countries. Furthermore, travelers are often not sufficiently informed of the rabies risk when importing an animal illegally from an enzootic area. In France, the risk is mainly attributed to North African countries, in particular Morocco. According to OIE data (Handistatus), rabies occurs continuously in Maghreb countries, in particular in dogs, cats, cattle and horses. In Germany, cases have been attributed to Turkey, Hungary, Nepal and Azerbaijan. For the Azerbaijan case, the puppy (one month old) was vaccinated (Suess, 2001) against rabies before leaving.

In Finland, an imported rabid horse from Estonia was reported in 2003 (OIE, 2003).

In France, from 1968 to 2000, a total of 17 imported pets (16 dogs and 1 cat, 9 cases from North Africa, Turkey, India, Ivory Coast, Pakistan and Burkina Fasso) incubating rabies have been reported (Aubert *et al.*, 1996). From 2001 to 2005, five rabies cases were diagnosed in dogs, all illegally imported to France from Morocco (Astoul *et al.*, 2004). Increased tourism may have contributed to an enhanced risk. The Maghreb countries, especially Morocco are indeed highly appreciated by French tourists traveling by car. In all cases, the isolated rabies virus was of canine origin. In 1999, an additional case was reported in an imported fruit bat (*Roussetus sp.*) infected by a rabies virus belonging to genotype 2 (Aubert, 1999).

For the last case recorded in France in August 2004, a great number of persons and animals could have been in contact with the puppy (as the owner visited many tourist places with his dog). Therefore, an international alert was launched on both veterinary and human health sides (Astoul *et al.*, 2004). A total of 187 persons received post-exposure treatment and more than 1200 animals (most of them had been found dead) were analyzed for rabies as well as 57 animals confirmed as having been in contact with the puppy. All samples examined were negative (Servas *et al.*, 2005).

An analysis of available data indicated that of these 22 cases in France (1968 till 2005) four cases led to further spread in domestic pets (7 dogs and a cat in 1969, 6 dogs in 1974, one cat in 1981 and 3 dogs in 1983) (Aubert *et al.*, 1996).

2.7. CONCLUSIONS

The 25 EU member states have greatly varying rabies situations. Some countries are free from rabies, while in others it is still prevalent at a lower or higher level.

Over the last five years rabies incidence within EU member states has been declining whereas in EU bordering countries it has been reported to be increasing. The results demonstrate clearly the importance of rabies in EU bordering countries, with high numbers of rabid cats and dogs diagnosed annually.

The red fox (*Vulpes vulpes*) is the main species involved in rabies epidemiology in Western and Central Europe and is at the origin of rabies transmission to other wild species and to domestic animals as well, including pets, bovines, ovines and caprines.

The raccoon dog (*Nyctereutes procyonoides*) represents a major factor in the epidemiology and epizootiology of rabies in Eastern and Northern Europe.

The main domestic species infected are dogs and cats, followed by bovines and ovine-caprine species.

Based on data from 2005, the epidemiological situation in EU can be summarised as follows:

- Some Countries are free from rabies or have been free for at least two years (except in bats and imported cases): United Kingdom, Malta, Sweden, Ireland, Denmark and Cyprus.

- Other countries have not had rabies cases reported for at least two years (except from bats or imported animals) but have a non-negligible probability of introduction from neighbouring areas. Several of these countries had known prior sylvatic infection which was eliminated by use of oral vaccination in wildlife, including the countries recognised as officially rabies free, in 1991 (Finland, Netherlands), 1997 (Italy), 2000 (France), 2001 (Luxembourg, Belgium) and 2005 (Czech Republic). Portugal, Spain (except the North Africa part) and Greece can be included in this group.
- Other countries have low-prevalent endemic areas, with few number of sylvatic rabies cases reported: Austria, Germany and Slovenia. All these three countries are currently conducting oral vaccination programmes in wild life.
- Finally, there are countries with a prevalence higher than 1 per 10 millions pets (corresponding to 1 new case per year of rabies in a population consisting of 1 million pets) and several cases in wildlife: Estonia, Hungary, Latvia, Lithuania, Poland, Romania, and the Slovakia.

3. CONTROL OF RABIES IN EU

Control of rabies in European countries combines risk mitigation measures in wildlife as well as prophylactic means in domestic animals and humans:

- Control of rabies in wildlife by the use of oral vaccination and population density control.
- Control of rabies in domestic animals is mainly based on parenteral compulsory vaccination against rabies, destruction of stray dogs and national legislation for surveillance of the disease.
- Human medical prophylaxis includes administration of post-exposure treatment (with or without rabies immunoglobulines) and preventive rabies vaccination of certain persons at risk (WHO Guide for post-exposure prophylaxis)

3.1. SURVEILLANCE AND ERADICATION PROGRAMMES IN WILDLIFE

Early measures to control rabies in Europe consisted of an intense fox culling using various methods to reduce vulpine population density. These measures were insufficient to stop the progression of the disease.

Oral vaccination of wildlife has proven to be the only powerful and efficient tool for controlling rabies: using an aerial distribution method, vaccine baits containing a capsule or a plastic sachet filled with an attenuated anti-rabies liquid vaccine are deposited in the fields throughout fox habitats.

Vaccine baits are generally distributed twice per year, during spring and autumn. Practical recommendations have been established by the European Commission, regarding the general strategy to use, the method of vaccination as well as the choice of the vaccine (SCAHAW, 2002).

Seven member states have become rabies-free (according to the OIE Animal Health Code, 2006) as a result of oral vaccination programmes. However, these operations are often maintained in rabies-free bordering areas depending upon the epidemiological situation in neighbouring countries. In Finland, along the border with Russia, oral vaccinations are still maintained (since 1989). In France, along the border with Germany, oral vaccination campaigns were pursued until the end of 2003 and in 2005.

Member states still infected by wildlife rabies (Austria, Estonia, Germany, Hungary, Latvia, Lithuania, Poland, Slovakia and Slovenia) have eradication programmes using oral vaccinations of foxes (see summary in Table 3.2).

Oral vaccination programmes were started several years ago in Germany (1983), Austria (1986) and Slovenia (1988) and in these countries rabies occurs only in some residual foci. Countries such as Slovakia, Hungary (start of OVF in 1992) and Poland (start of OVF in 1993) have succeeded in reducing the occurrence of rabies in the target species. The control of rabies in those countries, which are in a terminal phase of eradication, may be complicated by the epidemiological situation of neighbouring countries which have not currently adopted oral vaccination. The three Baltic countries, recording high number of rabies cases, initiated oral vaccination campaigns on a large scale recently.

3.2. VACCINATION OF PETS

3.2.1. Description of Vaccines

In 1885 Louis Pasteur developed the first crude rabies vaccine based on attenuated virus from desiccated nerve tissue. Over the next 50 years various vaccines based on nerve tissue were developed, followed by development of duck embryo vaccines in the 1950s and eventually present-day cell culture vaccines (Dreesen, 1997; Plotkin, 2000). Due to a higher cost of cell culture vaccines nerve tissue vaccines are still used in some parts of the world, despite a WHO recommendation to phase them out (WHO position paper, 2002).

Nowadays, inactivated vaccines are formulated from virus produced in cell culture, using either primary cells or continuous cell lines. Several vaccine types can be distinguished among veterinary vaccines according to the strain of rabies virus used and the characteristics of cell substrate chosen for viral replication. The seed virus/cell systems vary considerably between different manufacturers.

In Europe, rabies vaccines for parenteral veterinary use in domestic animals are inactivated vaccines, most of them including an adjuvant to enhance the immunogenic response. Rabies vaccines must be produced in cell cultures and each vaccine batch should contain at least 1 IU of antigen per dose and should not be licensed or released unless an adequately designed experiment has demonstrated that duration of immunity is at least one year in the species for which the vaccines are used. The European Pharmacopeia requires an assessment of the protective efficacy at the end of the immunity period in at least 25 vaccinated cats and dogs and at least 10 controls where, following challenge with an authorised virulent dose of rabies virus, at most 2 animals out of 25 demonstrate signs of rabies, corresponding to a vaccine efficacy (probability of protection) of 90% (confidence limits 71-100%).

Monovalent or combined inactivated vaccines are currently used for the immunization of dogs and cats. Several different antigens are incorporated in canine rabies vaccine, such as canine distemper, canine hepatitis, leptospirosis and canine parvovirus. Combined rabies vaccines for cats may include various other antigens such as feline parvo- and calicivirus. The WHO, OIE and European Pharmacopeia provides guidelines with stringent quality criteria to be followed by manufacturers before marketing (OIE Manual, 2004; WHO, 2005; European Pharmacopeia).

3.2.2. *Immunological response to the vaccine (protection and seroconversion)*

The classical response following administration of an inactivated vaccine involves induction of both antibody and cell mediated immunity (Nathanson and Gonzales-Scarano, 1991). Rabies virus glycoprotein G, which is anchored on the surface, is strongly linked to induction of humoral immunity and is the only structural protein that induces virus-neutralizing antibodies (Cox *et al.*, 1977). Therefore, determination of neutralizing antibodies in serum of vaccinated cats and dogs can be used to predict the resistance of animals to subsequent infection.

In contrast to human infection with rabies virus, post-exposure prophylaxis (active and passive immunisation) in pets has limited or no effect (Hanlon *et al.*, 2002). Current rabies vaccines for dogs and cats are approved for induction of pre-exposure immunity for 1-3 years following a single-shot primo-vaccination. Pets can be vaccinated from 3 months of age without interference from any residual maternal antibodies. Revaccination⁶ is done after 1-3 years followed by annual, biennial or triennial revaccination depending on manufacturer's claims and national rules.

Protective immunity in individual animals is normally assessed by correlating the antibody response to results of experimental challenge. According to WHO and OIE an antibody level of 0.5 IU/ml or higher is correlated with a very high probability of protection. However, two different interpretations are being applied, the more stringent requiring 0.5 IU/ml at the time of challenge (European Pharmacopoeia for non-carnivores; Swedish rules for non-commercial movement of pets), whereas the less stringent interpretation assumes a high probability of protection as long as an initial response of at least 0.5 IU/ml has been obtained (OIE animal health code; UK PETS; EU regulation 998/2003 for third countries). Aubert (1992) summarized a number of studies correlating the antibody level (in most cases at the time of challenge) with protection. He concluded that (1) animals having antibodies at the time of challenge had the highest probability of surviving and that (2) animals with no detectable antibodies at the time of challenge have a high probability of surviving if they seroconverted after vaccination

⁶ Revaccination (or Booster) is a vaccination given after a certain time interval (at least 6 months) after the primo-vaccination in order to maintain long term protection.

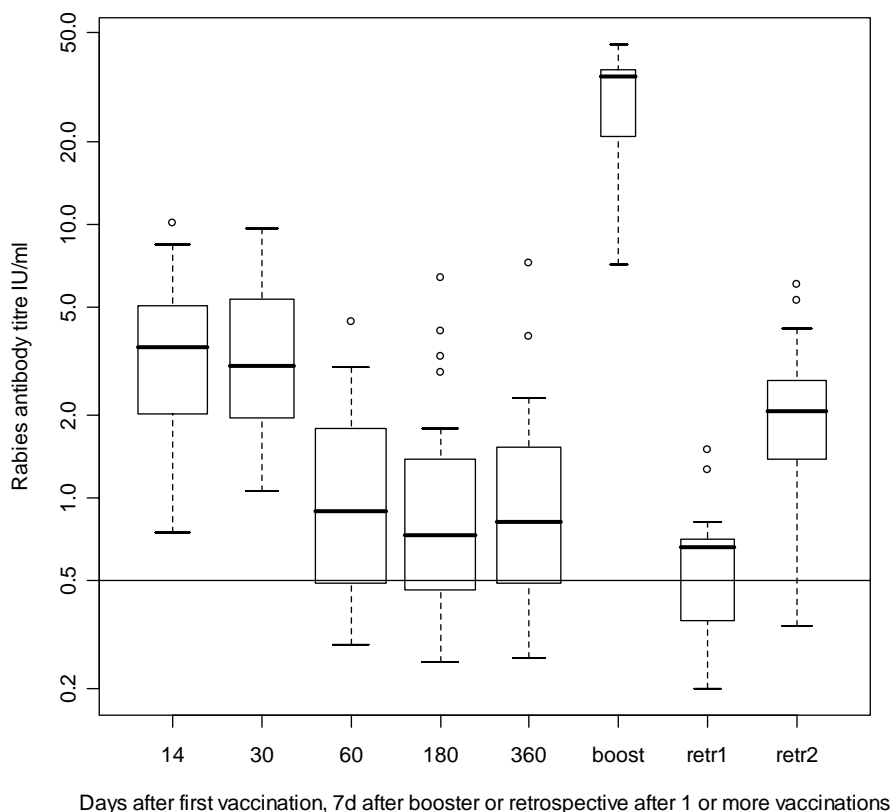


Figure 3-1 - Boxplot diagram of antibody levels in dogs after a single vaccination or a booster given 180 or 360 days after primary vaccination. Retr1 are titres following single vaccination 5-12 months earlier. Retr2 are titres following multiple vaccinations 1-12 years earlier (data from Sage *et al.*, 1993).

The humoral response following rabies vaccination shows first a short latent phase, then an exponential phase, a plateau, and at last a decrease in the antibody level (Tepsumethanon *et al.*, 1991; Sage *et al.*, 1993; Bahloul *et al.*, 2005). For a primo-vaccinated animal receiving one injection, the peak is generally reached between three and six weeks following vaccination and decreases rapidly thereafter. Figure 3.1 illustrates this, demonstrating that the antibody level drops below 0.5 IU/ml at 60 days after vaccination in a considerable fraction of primo-vaccinated dogs and that revaccination (booster) induces an anamnestic response, enabling the antibody level to remain above 0.5 IU/ml.

The main factors influencing the observed serological response of dogs following rabies vaccination are:

- the time interval between vaccination and blood testing: the peak of rabies antibodies is generally obtained about 4 weeks after vaccination and steadily drops close to or below the 0.5 IU/ml threshold;
- the number of rabies vaccine doses: pluri-vaccinated dogs have titres above 0.5 IU/ml more frequently in comparison to single-shot primary vaccinated dogs (Tepsumethanon *et al.*, 1991; Sage *et al.*, 1993; Sihvonen *et al.*, 1995, Mansfield *et al.*, 2004);

- the vaccine type: for single-shot primo-vaccination, it has been demonstrated that multivalent vaccines induce a titre equal to or higher than 0.5 IU/ml less frequently than monovalent vaccines (Cliquet *et al.*, 2003);
- the age of the animal: very young animals seem to respond less well than animals older than one year. Older dogs (above seven years old) also seem to respond less well than animals between one and seven years old.

Table 3.1 shows the antibody response in dogs following routine vaccination with different vaccines used in Europe. For vaccines 1 and 2, the serum samples are taken >120 days after vaccination. Large individual variations are observed in cats and dogs in the response levels of neutralising antibodies. In the context of routine antibody testing for international trade purposes, laboratories have obtained antibody titre responses less than 0.5 IU/ml mainly in vaccinated dogs but also in cats, ranging between 1.1 to 11.1%.

Table 3-1 - Antibody responses in dogs after vaccination with different vaccines in Europe

	≥ 0.5 IU/ml	< 0.5 IU/ml	N° of dogs	Time between vaccination and sampling	Reference
Vaccine 1	97,3%	2,7%	1975	More than 120 days	Berndtsson (unpublished data from 2005)
Vaccine 2	88,9%	11,1%	1676	More than 120 days	Berndtsson (unpublished data from 2005)
Vaccine 3	98.6%	1.4%	2714	30 days	Jones <i>et al.</i> (2002) and Sihvonen <i>et al.</i> (1995)
Vaccine 4	98.9%	1.1%	2856	30 days	Jones <i>et al.</i> (2002)
Vaccine 5	97.9%	2.1%	47	30-40 days	Sihvonen <i>et al.</i> (1995)
Several vaccines used in France	92.6 %	7.4 %	17693	variable (1-6 months)	Cliquet <i>et al.</i> (2003)

3.2.3. Description of the FAVN and RFFIT test

The FAVN and RFFIT tests are both OIE prescribed tests for international trade (OIE, 2004) allowing rabies neutralising antibody titration in sera of vaccinated animals.

The principle of these tests is the *in vitro* neutralisation of a constant amount of rabies virus inoculated to susceptible cells by rabies neutralising antibodies at different dilutions (serum under test). The serum titre is the dilution at which 100% of the virus content is neutralised in 50% of the wells.

FAVN test and RFFIT are using similar reagents and biologicals; however methodologies of FAVN test and RFFIT are different as well as the reading and the interpretation of the results. Several papers have demonstrated that both tests show good agreement when comparing results obtained on the same sera (Briggs *et al.*, 1998; Cliquet *et al.*, 1998).

The diagnostic specificity of both tests is highly satisfactory, however, several studies have demonstrated that using RFFIT, a cytotoxic effect may occur when the tested sera are contaminated, generating a risk of false positive reactions (Smith *et al.*, 1973; Kurz *et al.*, 1986; Cliquet *et al.*,

1998). A study clearly demonstrated that the FAVN test provides a significantly better distinction between positive and negative samples, due to the fact that negative samples tested by FAVN test produced lower results in comparison to those obtained with RFFIT (Cliquet *et al.*, 1998).

The diagnostic specificity of a test is the proportion of uninfected reference animals that test negative in the assay (definition from Jacobson, 1998 and 2004; OIE, 2000 and 2004); uninfected reference animals that test positive are considered to have false positive results. The specificity of the FAVN test has been evaluated by titrating 414 sera from unvaccinated dogs (Cliquet *et al.*, 1998). No false positive reactions were recorded, indicating a specificity close to 100%.

In the study of Jones *et al.* (2005) a test specificity of 0.88 was assumed. This was based on the observed difference between the number of positive tests on vaccinated animal in FAVN and mouse protection assay (MNT), respectively (Cliquet *et al.*, 1998). However, it has been clearly stated (Scientific Veterinary Committee, 1997) that the sensitivity of FAVN is higher than in MNT, thus the difference most likely reflects a difference in sensitivity in accordance with the interpretation by Cliquet *et al.* (1998).

The Community Reference Laboratory for Rabies Serology has been designated by the European Commission to carry out proficiency testing to evaluate the performances of laboratories using FAVN test and RFFIT in the context of international movements of cats and dogs. From November 1999 to October 2004, a total of 13 proficiency tests have been organised representing 315 appraisals. Statistical analysis has been conducted (data not published) for assessing different criteria (specificity, intra- and inter-laboratory consistency). For all the 315 appraisals, failures have never been attributed to the specificity criterion, which was always 100% in all approved laboratories.

3.2.4. Description of vaccination strategies for rabies in EU Member states

The main aim of vaccinating cats and dogs against rabies is to establish preexposure immunity and protect individual animals from contracting rabies, hereby preventing further spread to humans or other domestic animals.

After the spread of fox rabies started in Europe at the Russian-Polish border in the 1930s, the number of rabid dogs dramatically increased. In many European countries dog rabies was brought back under control in the late 1940s after using rabies vaccine and immunisation as well as strict sanitary measures on dogs in rabies infected regions. The infection ceased to spread and urban rabies eventually disappeared. In the areas where rabies had been introduced, the eradication programme was very successful. It is well known that if the concentration of susceptible animals is low the transmission chain of rabies infection is broken.

Vaccination programmes in pets and other domestic animals are implemented in several member states as part of an overall control programme for rabies. Table 3.2 summarizes vaccination plans in pets for the year 2004 as reported in EFSA's First Community Summary Report on Trends and Sources of Zoonoses, Zoonotic Agents and Antimicrobial resistance in the European Union in 2004 (EFSA, 2005). In most countries plans encompass both dogs and cats whereas in some countries only dogs are included.

Table 3-2 -Vaccination programmes for rabies in animals in EU and Norway, 2004⁷.

<i>Country</i>	<i>Vaccination programmes in pets</i>	<i>Vaccination programmes in wildlife</i>
Austria	-	Since 1991, oral vaccines distributed to foxes twice a year. The programme is approved and co-financed by EU (2003/849/EC ⁸).
Belgium	Compulsory vaccination of dogs and cats in the south and if staying at public campgrounds	Oral vaccines were distributed until 2003.
Cyprus	-	-
Czech Republic	Compulsory vaccination of carnivores in captivity	In 1989, oral vaccination of foxes in some districts. In 2003, covers the whole country except for rabies free districts. Since 2004, vaccination twice a year by air in selected areas, mainly along the boarder with Poland and Slovakia. The programme is approved and will be co-financed by EU (2003/849/EC).
Denmark	-	-
Estonia	Compulsory vaccination of dogs and cats	In 2004, oral vaccines were distributed twice on one island. From 2005, a vaccination programme covering half the country has been approved and will be co-financed by EU.
Finland	Vaccination in dogs and cats are recommended	Since 1991, oral vaccines distributed to foxes and raccoon dogs twice a year along the Russian border by flight. Since 2004, twice a year. The programme is approved and co-financed by EU (2003/849/EC).
France	-	Oral vaccines distributed to foxes will start again in 2005.
Germany	-	Oral vaccines distributed to foxes twice a year by flight. The programme is approved and co-financed by EU (2003/849/EC).
Greece	Compulsory vaccination of dogs	-
Hungary	Compulsory vaccination of dogs	Since 2004, oral vaccines distributed to foxes twice a year by flight. The programme started in 1997.
Ireland	-	-
Italy	-	Oral vaccines distributed to foxes in the Region Friuli Venezia Giulia.
Latvia	-	Oral vaccines distributed to foxes twice a year by flight. The programme is approved and co-financed by EU (2003/849/EC).
Lithuania	Compulsory vaccination of dogs and cats	Since 1995, oral vaccines distributed to foxes twice a year by flight.
Luxembourg	-	Oral vaccines distributed to foxes will start in 2005.
Malta	-	-
Norway	Vaccination of dogs and cats being brought in and out of the country	-
Poland	Vaccination programme for dogs since 1949	Since 2002, oral vaccines distributed to foxes twice a year by flight. The programme is approved and co-financed by EU (2003/849/EC).
Portugal	Compulsory vaccination of dogs since 1925	-

⁷ EFSA's First Community Summary Report on Trends and Sources of Zoonoses, Zoonotic Agents and Antimicrobial resistance in the European Union in 2004 (EFSA, 2005)

This table may not represent the actual situation. The data for 2005 was published on the 22nd December of 2006:
http://www.efsa.europa.eu/en/science/monitoring_zoonoses/reports/zoonoses_report_2005.html

⁸ O J L 322, 09/12/2003, p. 16-27.

<i>Country</i>	<i>Vaccination programmes in pets</i>	<i>Vaccination programmes in wildlife</i>
Slovakia	Compulsory vaccination of domestic carnivores	Oral vaccines distributed to foxes twice a year by flight. The programme is approved and co-financed by EU (2003/849/EC).
Slovenia	Compulsory vaccination of dogs since 1947	Since 1995, Oral vaccines distributed to foxes twice a year by flight. The programme is approved and co-financed by EU (2003/849/EC).
Spain	-	From 2004, compulsory surveillance according to Directive 03/99/EEC
Sweden	Vaccination of dogs and cats being brought in and out of the country	-
The Netherlands	-	-
UK	-	-

3.3. REQUIREMENTS IN RELATION TO NON-COMMERCIAL MOVEMENTS OF PET ANIMALS

Regulation (EU) No 998/2003 lays down the rules for the non-commercial movements of pet animals (dog, cat, ferrets) both within the Community as well as from third countries into the EU. Article 5 lists the requirement for animal identification and valid vaccination as certified in a passport for movement between member states. Additionally, a waiting time of at least 21 days from the time of primo-vaccination must be observed before moving the animal (Commission Decision 2005/91/EC⁹).

Article 6 of the above Regulation provides that entry into Ireland, UK, Sweden and Malta shall in addition require for a transitional period of 5 years from the entry into force of the Regulation that a test for neutralizing antibodies be carried out within the periods specified in their national rules.

Both the UK pet travel scheme (PETS) and the Swedish system require vaccination against rabies and blood testing after vaccination, demonstrating a titre of at least 0.5 IU/ml. Anti-rabies serological tests have to be conducted by laboratories approved by the European Commission, decision 2004/233/EC

Different rules exist when importing animals from rabies-infected 3rd countries (those not listed in Annex II of Regulation 998/2003) into EU and finally the OIE animal health code stipulates yet another set of criteria to be fulfilled when importing from countries not officially free from rabies (except bat rabies). All these rules have been summarised graphically in Figure 3.3.

3.3.1. Pet travel scheme procedure

Pet dogs and cats (including guide and hearing dogs) can since 2000 enter or re-enter the UK, Ireland or Malta without quarantine provided they meet the rules of the Pet Travel Scheme (PETS).(www.defra.gov.uk)

The pet must first be fitted with a microchip before any other of the procedures for PETS are carried out. The pet must then be vaccinated at the age of at least 3 months against rabies and blood tested.

⁹ OJ L 031, 04.02.2005, p. 61-61.

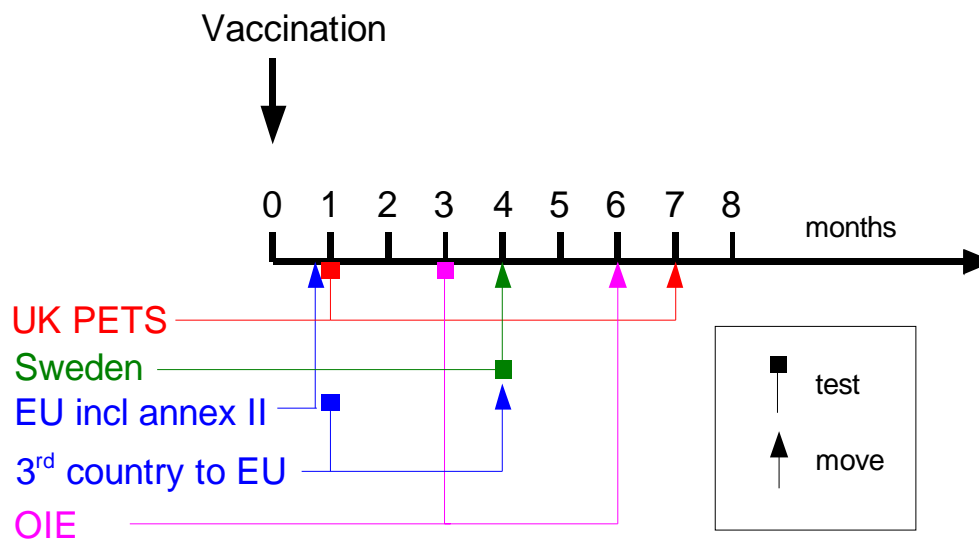


Figure 3-3 - Graphical descriptions of the different vaccination and testing regimes in Great Britain, Ireland, Malta, Sweden plus current EU and OIE rules

The blood test can be performed directly after vaccination but about 30 days after vaccination is recommended. The pet must then wait six months after blood sampling before entering UK, Ireland or Malta. Before entering it must be treated against ticks and tapeworm.

3.4. CONCLUSIONS

Rabies can be controlled by vaccination programmes in wildlife and domestic animals. Most countries in which rabies occurs have or will soon start oral vaccination programmes in wildlife. The control of rabies in many of these countries may be complicated by the epidemiological situation in neighbouring countries which have a high incidence of rabies and have not currently adopted oral vaccination.

In areas with wildlife rabies systematic vaccination in dogs and cats provide an efficient means of reducing rabies in domestic animals and human exposure.

Current rabies vaccines for dogs and cats are considered to provide immunity for 1 to 3 years following the primo-vaccination. Pets can be vaccinated initially at 3 months of age without interference from any residual maternal antibodies.

The humoral response following rabies primo-vaccination reaches a peak between three and six weeks following vaccination and decreases rapidly thereafter. Rabies neutralising antibody levels of at least 0.5 IU/ml are correlated with a high probability of protection against a subsequent challenge.

Large individual variations are observed in cats and dogs in the response levels of neutralising antibodies. In the context of routine antibody testing for international trade purposes, laboratories have obtained antibody titre responses less than 0.5 IU/ml ranging between 1.1 to 8.6 %. When the time period between vaccination and blood sampling is around 30 days, titres below 0.5 IU/ml are found in 1-2% of primo-vaccinated animals.

Field studies show that a small fraction of pets does not mount an antibody response of 0.5 IU/ml or above after first injection of vaccine. These animals will in almost all cases respond after a second injection.

A considerable fraction of animals responding to the first injection will drop below 0.5 IU/ml after 2-3 months. Although they have a high probability of being protected even without detectable antibodies, a second injection will induce a long-lasting antibody level above 0.5 IU/ml.

Animals with neutralising antibodies at the time of challenge are considered to have a higher probability of protection than those with no detectable neutralising antibodies.

Current vaccines prescribe revaccination after one, two or three years but sometimes a second injection after 4 to 6 weeks is recommended to elevate the antibody level after the first injection of the primo-vaccination.

For animals having received two injections of vaccine with an interval of 4 to 6 weeks as a primary immunisation, the risk that they will not have obtained protective immunity can be considered negligible.

The RFFIT and FAVN tests are both prescribed tests by OIE for international trade allowing rabies neutralising antibody titration in sera of vaccinated animals. The specificity of both tests is highly satisfactory; however, several studies have demonstrated that using RFFIT, a risk of false positive reactions may occur when test sera are contaminated. In proficiency testing no false positive reactions have been recorded, indicating a specificity close to 100%.

4. RISK ASSESSMENT

4.1. METHOD OF RISK ASSESSMENT

Risk assessment is recognised by the World Organisation for Animal Health (OIE) as a transparent and scientific method for determining the risk of unwanted events occurring. It is being used in particular within animal importation and food safety. The results of a risk assessment serve as a decision support for risk managers.

A full risk assessment contains the following elements:

- Hazard identification
- Release assessment
- Exposure assessment
- Consequence assessment
- Risk estimate

The hazard identification is presented in chapter 3. According to the mandate, the aim of the present risk assessment is to assess the risk of introducing rabies through non-commercial movement of pets between member states (MS) (section 2.2). Hence, focus is on the release assessment, whereas the exposure and consequence assessments are only dealt with briefly.

Risk assessments can be conducted in a qualitative or a quantitative manner. A qualitative assessment presents data in a logical way and aims at summing up the risk in words using terms like negligible, low, moderate or high without allocating exact numerical values to probabilities and costs or consequences. Contrary to this, a quantitative assessment is built upon mathematical and statistical methods and the result is presented numerically. The uncertainty of the result reflects true variation and uncertainty in our knowledge of important parameters.

The rabies risk involved in movement of pets has been addressed earlier by the Scientific Veterinary Committee in 1992 and 1997. A number of countries have also carried out independent risk assessments on pet movement and rabies (MacDiarmid and Corrin, 1997; Hawaii Department of Agriculture, 2002; Jones *et al.*, 2002; Jones *et al.*, 2005; Weng, 2004; Hallgren *et al.*, 2005 and 2006; Norwegian scientific committee for food safety, 2005). These assessments show considerable variation in the assumed control strategies and risk pathways and a variable weighting of particular aspects associated with pet movement (geographical area, legal *vs.* illegal movement, compliance issues, etc.), hence they are not directly comparable. They do, however, share most of the underlying parameter estimates used for modelling. Therefore, to meet the terms of reference of the present assessment, it was judged necessary to develop risk pathways designed specifically to answer the questions raised, *i.e.* segregating the effect of testing from all other measures, using either established parameter estimates or modified according to available evidence.

The (unrestricted) risk of transmitting rabies by pet movement is basically dependent on the prevalence of rabies within the population at risk. Prevalence estimates and the definition of population at risk may, however, be influenced by many different factors, resulting in variability and uncertainty. This risk assessment will examine the risk reducing capacity of protocols with or without application of a serological test as applied to pet movements within EU.

4.2. RISK PROFILE

The hazard of transmission of rabies by pet movement is defined as the risk of moving an animal incubating disease, irrespective of vaccination status. The population at risk can be defined as all susceptible pets within a population with a given observed incidence I and calculated prevalence of incubating disease P . Once an animal is vaccinated, a process is initiated that within 7-10 days leads to removal of this animal from the population at risk, if not already infected before vaccination. Two types of risk can be envisaged from a biological understanding of the disease development. The first kind of risk, termed type A risk in the following, relates to the risk that an animal is

already incubating rabies at the time of vaccination. The second type of risk, termed type B, is related to the failure of inducing protective immunity following vaccination and failure to correctly identify this condition. A failure to induce vaccinal immunity will effectively leave the animal within the population at risk.

In any case, the overall risk will depend on a difference of prevalence between two countries and moving an animal from one country to another with the same prevalence by definition does not pose a risk. Also, the risk may be considered two-way, because moving an animal from high-to-low compared with low-to-high presents a different risk. Therefore the two situations should be managed separately.

Three scenarios with different combinations of vaccination, serotest and waiting time W between vaccination and movement can be outlined:

- *No measures:*
 - The unrestricted risk of transmission will be equal to prevalence P (type A risk);
- *Vaccination without serotest:*
 - Type A risk: infected before vaccination - risk of transmission will decline by some function of Prevalence (P) & waiting time (WT);
 - Type B risk: vaccinated but not protected, risk depends on vaccine efficiency (Ev), (WT) and incidence rate (IR);
- *Vaccination with serotest:*
 - Type A risk: infected before vaccination - risk of transmission will decline by some function of P & WT (same as (A) above);
 - Type B risk: vaccinated but not protected with false positive test result, *i.e.* the animal declared immune although still susceptible, the risk depends on vaccine efficiency Ev, WT, (IR) and test specificity (Sp).

Note: The risk assessment model implemented in excel is available in the website together with the scientific opinion.

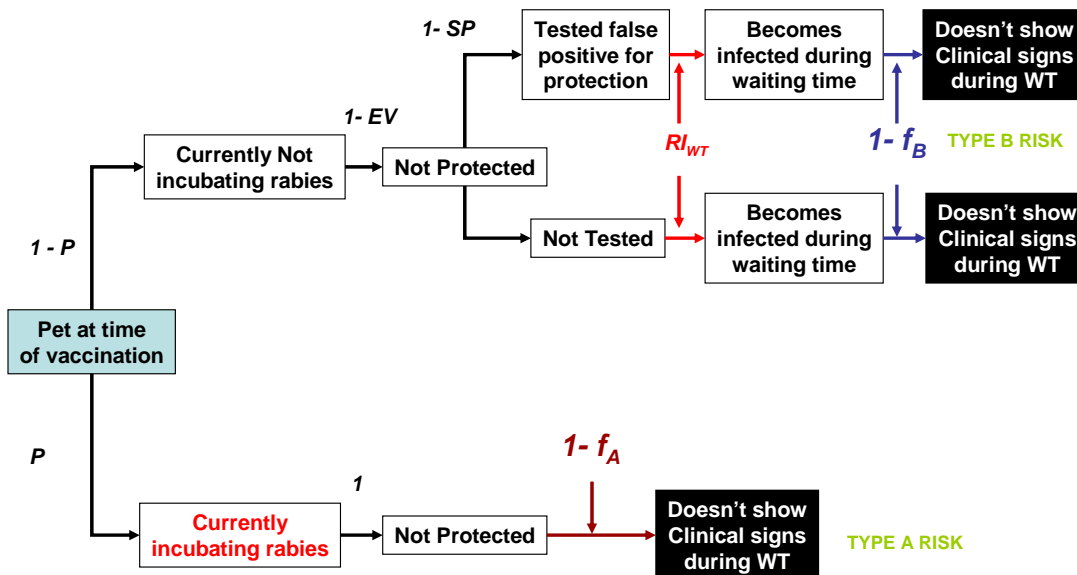


Figure 4-1 – Graphical description of risk rabies introduction associated with movement of a pet.

4.3. RISK MODEL PARAMETERS

4.3.1. Population at risk

In a rabies-infected country the population at risk can be defined as pets being eligible to trans-border movement accompanied by humans and having been susceptible to rabies infection during some interval within a period prior to the date of travel spanning the maximum incubation period of rabies. Within the population at risk there may be differences in exposure and incidence rates depending on environmental factors such as rural/urban residence, owned versus stray animals, indoor/outdoor living etc. and associated differences in the level of disease reporting. These differences are normally not known and therefore estimates will generally be based on average figures or modelled from various assumptions.

In rabies-free countries the population at risk can be defined as pets being eligible to trans-border movement accompanied by humans and being susceptible to rabies infection during some interval within a period covering the stay in an infected country. Again, differences in exposure rates may be envisaged, depending on rural/urban residence, indoor/outdoor living, etc. and additionally length of stay. For this risk category, there is no biological justification to introduce a waiting time following successful vaccination. Once immunity is established and maintained, the animal can be considered removed from the population at risk for the rest of its lifetime.

The primary means of removing an individual from the population at risk is by vaccination. From the above definitions it can be concluded that the population at risk is associated with the fraction of pets undergoing first time vaccination (primo-vaccination). Following first time vaccination most individuals will be rendered immune and at the time of revaccination, typically one year later, they are no longer considered at risk, as also reflected in current legislation.

As mentioned in section 1.3, little information is available on pet movement statistics, let alone the fraction of animals travelling following first time vaccination, and whether animals are returning to a rabies-free country or being indigenous to the infected country. This makes it difficult to calculate annual risk figures on the basis of actual movement numbers.

4.3.2. Prevalence (P)

The prevalence of animals incubating rabies can be estimated from annual incidence data of rabies in pets by assuming that a constant incidence of diagnosed cases also reflects a constant incidence of new infections. Given an expected mean incubation time of 38 days (see below) the prevalence P can then be expressed as

$$P = \text{incidence} * 38 / (\text{population at risk} * 365)$$

If the population of pets is not known, an indirect estimate based on human population data can be used, *i.e.* 1.0 dog and 1.1 cats per 10 people (Jones *et al.* 2002). Alternatively, a published formula from the American Veterinary Medical Association may be used, based on the number of households (0.58 dogs and 0.66 cats per household) (AVMA, 2002 U.S. Pet Ownership & Demographics Sourcebook). In the study of Hallgren *et al.* (2006) pet/human ratios of 0.1-0.25 were found for 6 different EU countries.

If there are no recorded cases of rabies in pets but a low incidence in wildlife, an indirect estimate of the incidence in pets may be calculated as 10% of that in wildlife (see section 2.5.2). If there are no recorded cases in domestic animals or wildlife for at least two consecutive years the prevalence can be considered negligible (OIE requirement for free country).

4.3.3. Probability of developing clinical signs before the end of the waiting time (f_A, f_B)

The geometric mean (median) and standard deviation of the log-normal distribution of incubation periods used in this risk assessment are 3.370 and 0.750 respectively (see Figure 2.1).

A waiting time beginning at the time point where initiation of new infections has been effectively blocked by either physical quarantine or immunological protection (vaccination) and ending at the day of movement will reduce the risk of animals already incubating rabies from being moved.

The probabilities of developing clinical signs before the end of the waiting time are named f_A for type A risk and f_B for type B risk.

This interval will normally not be known but can be modelled as a uniform distribution assuming a constant incidence of rabies.

To estimate f_A it is needed to calculate the residual time of incubation after vaccination as shown in Figure 4.2 and compare it with length of waiting time. In order to calculate the residual time two simulation approaches were applied:

- Approach 1 – assumes a constant incidence of rabies. The day of infection is modelled as uniform from 0 to 365 days before the vaccination. The residual time of incubation is calculated by subtracting the number of days between infection and vaccination from the incubation period sampled from the log normal distribution.
- Approach 2 – because the vaccination is applied within the incubation period so the day of vaccination is assumed uniformly distributed from 0 to the end of the incubation period which is drawn from the same log normal distribution as in approach 1. The residual time is equal to the incubation period minus number of days between infection and vaccination

To estimate f_B we need to determine the day of infection, add it to the incubation period and compare the value obtained with the waiting time (Figure 4.2). Assuming a constant incidence of rabies, the day of infection is considered as uniform from 0 to the end of waiting time.

The outputs of the two simulation approaches for f_A and for f_B are presented in Table 4.1.

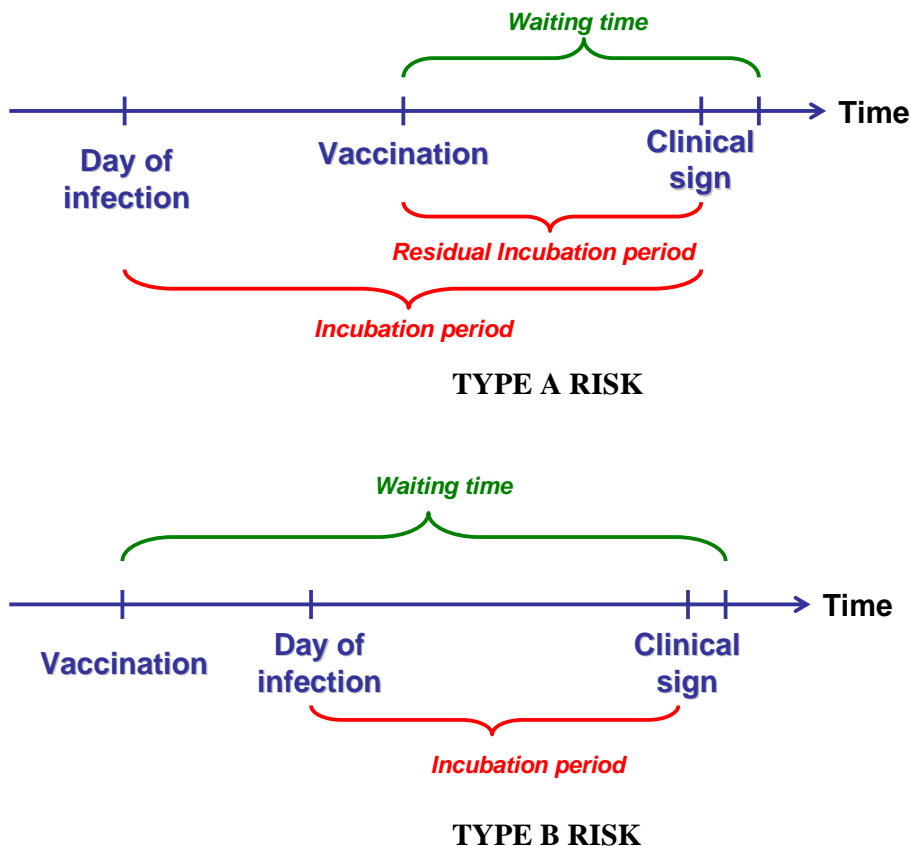


Figure 4-2 – Graphical description of modelling the effect of waiting time on the probability of developing clinical signs before the end of waiting time.

Table 4-1 – Probability of developing clinical signs before the end of the waiting time for type A (f_A) and type B (f_B) risks.

Waiting time	f_A - Approach 1	f_A - Approach 2	f_B
10	0.230	0.419	0.020
20	0.447	0.675	0.107
30	0.603	0.808	0.209
40	0.712	0.884	0.307
50	0.785	0.924	0.389
60	0.839	0.952	0.456
70	0.879	0.966	0.512
80	0.908	0.977	0.562
90	0.929	0.984	0.601
100	0.945	0.988	0.638
110	0.956	0.991	0.668
120	0.964	0.993	0.690
130	0.971	0.995	0.714
140	0.976	0.996	0.730
150	0.980	0.996	0.747
160	0.983	0.997	0.763
170	0.986	0.998	0.775
180	0.988	0.999	0.789
190	0.990	0.999	0.799
200	0.992	0.999	0.808

As an example, if waiting time is equal to 120 days, then the probability of developing clinical signs before the end of WT, is 96.4 % and 99.3% respectively for type A approach 1 and type A approach 2 and 69.0% for type B (Table 4.1). It means that if an animal is infected prior to vaccination there is a 3.6% (100% minus 96.4%) and 0.7% (100% minus 99.3%) probability of the pet developing clinical symptoms after the end of waiting time, respectively for type A approach 1 and type A approach 2. Similarly, if an animal gets infected during WT, there is a 31.0% (100%- 69.0%) probability of the pet developing clinical symptoms after the end of WT.

4.3.4. Protection induced after vaccination

Several factors influence whether protective immunity is reached following vaccination. These factors include species and individual differences in immune response, type and composition of vaccines, and whether the animal receives only a single injection for the primo-vaccination or also a second injection. Due to the inherent limitations of challenge studies, protective immunity is normally assessed indirectly by measuring the antibody response. A level of 0.5 IU/ml or above is considered indicative of an immune response conferring protection although waning levels of antibodies may not always be associated with loss of protection. It should be remembered that antibody levels following primo vaccination peak around 1 month after vaccination and may decline rapidly hereafter.

Current vaccines prescribe revaccination after one, two or three years but sometimes a second injection after 4 to 6 weeks is recommended to elevate the antibody level after the first injection of the primo-vaccination. For animals having received two injections of vaccine with an interval of 4 to 6 weeks as a primary immunisation the risk that they will not have obtained protective immunity can be considered negligible. In a study of rabies cases in dogs and cats in 1988 in the US the median age of cases was 1 year. Out of a total estimate of 33 million vaccinated pets vaccine failure was identified in 2 cats and one dog, all having received only a single dose of vaccine at the age of 3-6 months (Eng and Fishbein, 1990).

A number of field studies have been published on antibody levels following vaccination (Sihvonen *et al.*, 1995; Fooks *et al.*, 2002; Cliquet *et al.*, 2003; Mansfield *et al.*, 2004). Overall, 92.6-98.7% of

dogs had titres ≥ 0.5 IU/ml. Furthermore, [online results](#) from Hawaii Department of Agriculture on rabies pre import FAVN tests yielded 194 samples out of 11970 (1.6%) with titres less than 0.5 IU/ml (accessed September 27, 2006). Data from routine testing in Denmark in 2004 and 2005 yielded 1.4% with titres less than 0.5 IU/ml for dogs (n=11582) and 2.1% for cats (n=874) (Danish Institute for Food and Veterinary Research, Annual statistics, <http://beretning.vetinst.dk/>).

When analysed in more detail, it is clear that primo-vaccination and interval between vaccination and testing are major determinants of test outcome (Cliquet *et al.*, 2003; Mansfield *et al.*, 2004). In the most recent study (Mansfield *et al.*, 2004), 4-5% of dogs had titres below 0.5 IU/ml. Using an interval of 30 days between vaccination and testing a sufficient antibody response is detected in 98-99% of primo-vaccinates and it is therefore suggested to use a level of protection of 98% for the present risk assessment.

In the present risk assessment the following categories of animals were assumed:

1. If an animal has an antibody titre of 0.5 IU/ml at the end of a waiting period, it is considered that this animal was protected during the entire waiting time. Accordingly, the risk of becoming infected during that waiting period is considered negligible.
2. If an animal develops an antibody response of 0.5 IU/ml or above 2-4 weeks after vaccination it is considered that this animal has a high probability of being protected in a period lasting until the time of recommended revaccination. Accordingly, the risk of becoming infected during that period is considered very low to negligible.
3. If an animal does not develop an antibody response of 0.5 IU/ml or above 2-4 weeks after vaccination, it is considered that this animal has a lower probability of being protected. This assumption is not related to the efficacy of any vaccine, but rather to defining a certain level of risk. This risk stems from a lack of field data about the proportion of pets not demonstrating an antibody response of 0.5 IU/ml that may not be fully protected depending on the type of vaccine.
4. If an animal does not develop an antibody response of 0.5 IU/ml or above 2-4 weeks after vaccination, a second injection will with a high probability lead to an antibody response of 0.5 IU/ml or above 2-4 weeks after this second injection.

Very little published data are available to support (4) and the assumption is mainly based on expert advice from laboratories authorised to do RFFIT or FAVN testing. Consequently, the number of true non-responders after two injections is considered negligible.

There is a certain difference in the probability of protection between categories (1) and (2) but it is difficult to quantify due to lack of data.

4.3.5. *Test specificity (SP)*

Diagnostic test specificity determines whether truly antibody-negative individuals are correctly assigned as such or whether some individuals are erroneously classified as (false) positive. If diagnostic specificity is less than 100%, the group of false-positive individuals will contribute to type B risk. On the other hand it should be remembered that low diagnostic sensitivity would lead to false-negatives which would not add to risk because test-negative animals cannot be moved.

According to the Community reference laboratory for rabies serology the specificity of the FAVN test is generally found to be high, being between 99.7% and 100% (95% confidence interval) (see section 4.2.3). A tentative value of 99% is therefore being used in the following.

4.3.6. *Risk of infection during the waiting time (RIWT)*

The risk of infection during the waiting time can be estimated from annual incidence data of rabies in pets by assuming that a constant incidence of diagnosed cases also reflects a constant incidence of new infections. For a given a waiting time the risk is calculated as

$$\text{RIWT} = \text{Incidence} * \text{WT} / (\text{population at risk} * 365)$$

4.4. RESULTS

Table 4.2 compiles all the risk estimates in relation to waiting time and application of serological testing based on a number of imported pets of 5000 from a country with a prevalence of 1 per million (1.0E-6).

The relationship between type A and type B risk is illustrated in figure 4.3. It can be seen that type A risk is the main source of risk up to waiting times of 90 or 150 days, depending on the approach used to model the effect of waiting time on the type A risk. Inclusion of a serotest as a means of reducing type B risk will therefore only contribute significantly to the overall risk reduction for waiting times above 90 or 150 days (figure 4.4).

However, the overall risk interpreted in terms of number of years between introductions of rabies for a prevalence of 1.0E-6 is higher than 3000 years if the waiting time is higher than 100 days whether using the test or not (figure 4.5).

The probability of rabies introduction is proportional to the prevalence of rabies in the country of origin and the number of pets moved. As an example if the prevalence is changed from 1 per million (1.0E-6) to 1 per 10 millions, one can use the estimated risk from Table 4.2 and divide them by 10. The same principle can be applied if one change the number of pets moved. Following this logic, the risk from a country with a prevalence equal to or less than 1 per 10 million, corresponds to a high number of years between importation (higher than 3000 years), whether a waiting time is applied or not.

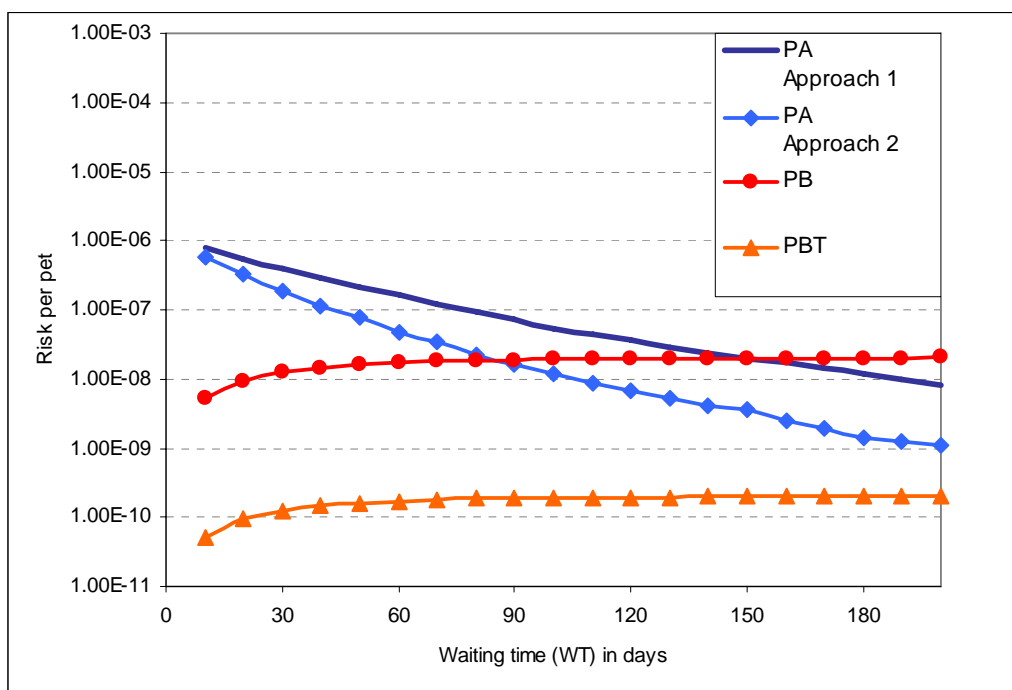


Figure 4-3 - Type A and type B risk when moving one pet from a country with a prevalence of 1.0E-6 as a function of the length of waiting period and the use of serological test.

PA- the risk of introducing an animal that was infected before vaccination

PB- the risk of introducing an animal that is infected after vaccination

PBT - the risk of introducing an animal that is infected after vaccination and testing positive

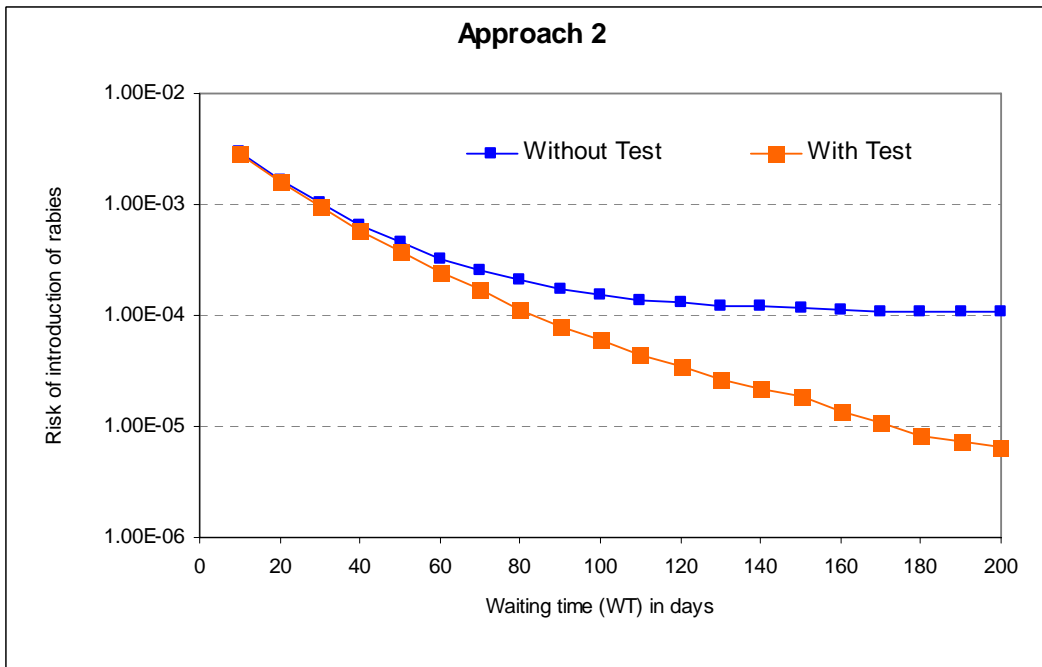
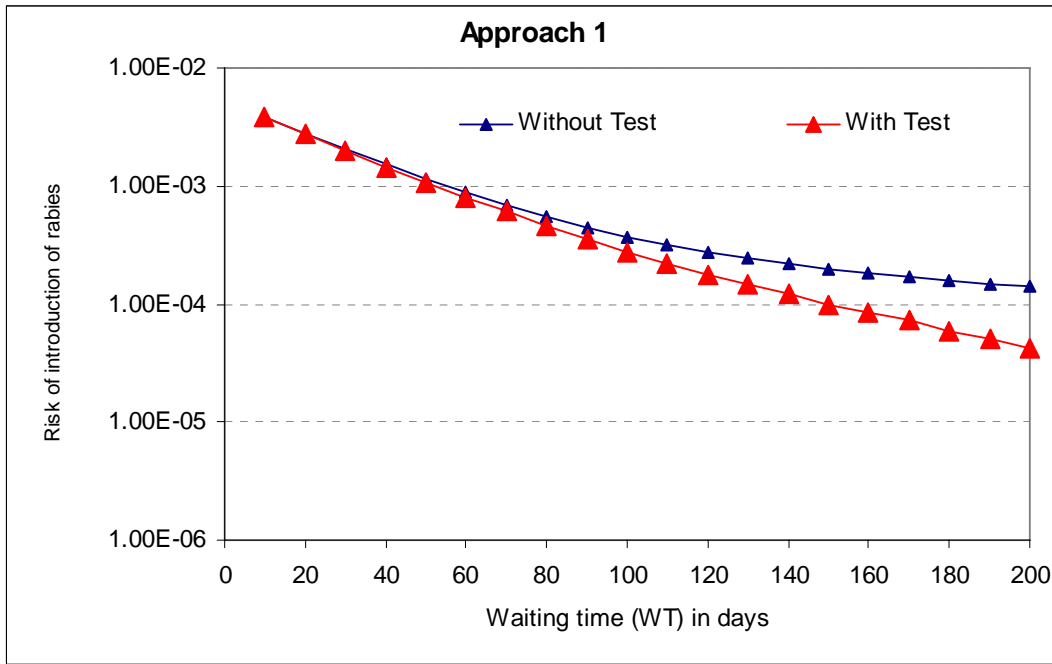


Figure 4-4 - Risk of introduction of rabies based on a number of imported pets of 5000 from a country with a prevalence of 1 per million (1.0E-6), in relation to waiting time and use of serological test.

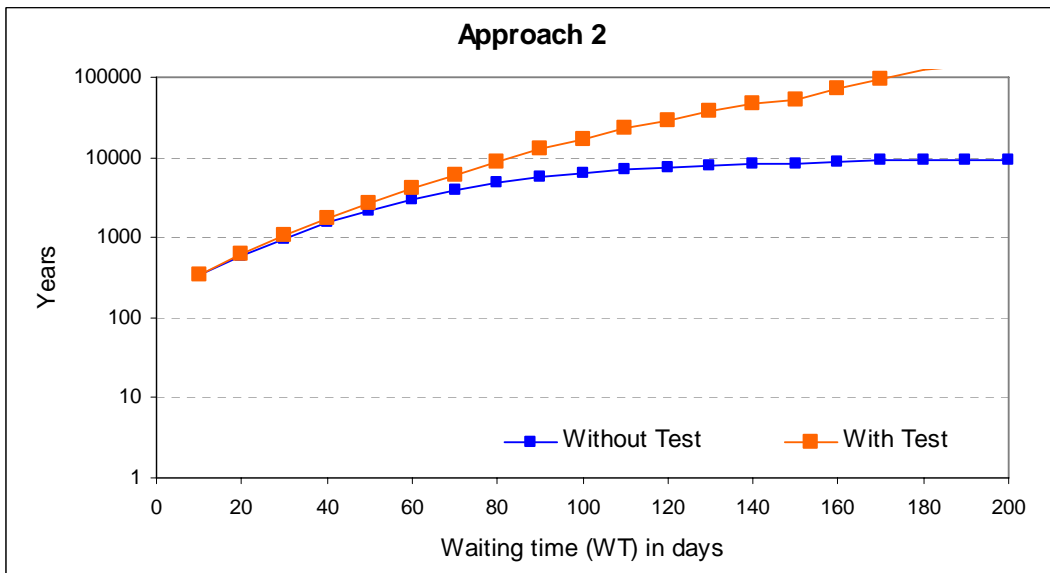
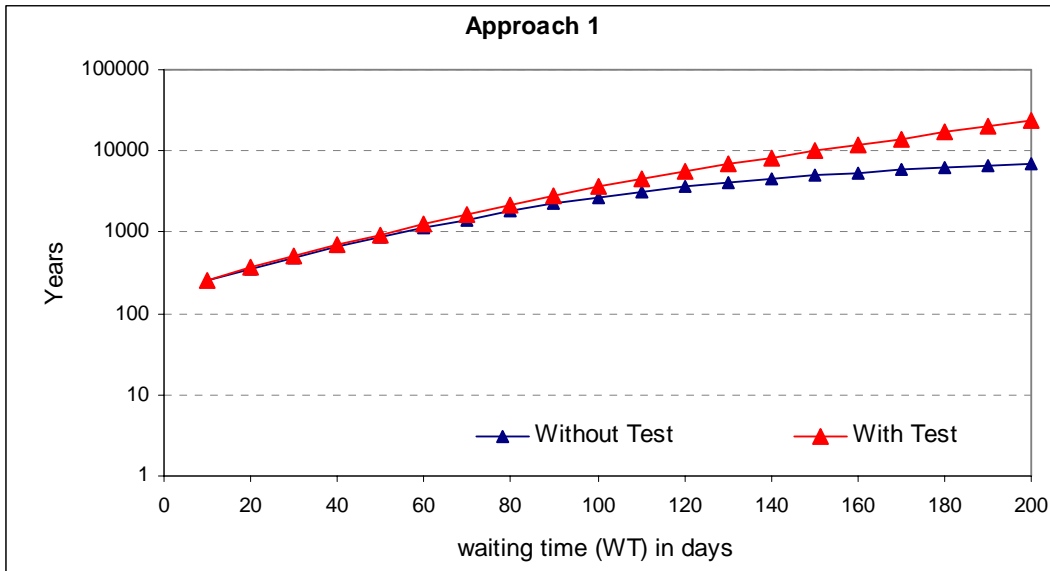


Figure 4-5 - Number of years between introduction of rabies based on a number of imported pets of 5000 from a country with a prevalence of 1 per million (1.0E-6), in relation to waiting time and use of serological test.

Table 4-2 – Risk of rabies introduction for a prevalence equal to 1.0E-6 in relation to waiting time and application of serological testing based on a vaccination efficiency of 0.98, serological test specificity of 0.99 and a number of imported pets of 5000.

WT	P _A Approach 1	P _A Approach 2	P _B	P _{BT}	Risk of rabies introduction without test Approach 1	Risk of rabies introduction without test Approach 2	Risk of rabies introduction with test Approach 1	Risk of rabies introduction with test Approach 2	Test effect Approach 1	Test effect Approach 2
10	7.70E-07	5.81E-07	5.16E-09	5.16E-11	3.87E-03	2.93E-03	3.84E-03	2.90E-03	1.01	1.01
20	5.53E-07	3.25E-07	9.40E-09	9.40E-11	2.81E-03	1.67E-03	2.76E-03	1.62E-03	1.02	1.03
30	3.97E-07	1.92E-07	1.25E-08	1.25E-10	2.05E-03	1.02E-03	1.99E-03	9.58E-04	1.03	1.06
40	2.88E-07	1.16E-07	1.46E-08	1.46E-10	1.51E-03	6.53E-04	1.44E-03	5.81E-04	1.05	1.12
50	2.15E-07	7.56E-08	1.61E-08	1.61E-10	1.16E-03	4.58E-04	1.08E-03	3.79E-04	1.07	1.21
60	1.61E-07	4.84E-08	1.72E-08	1.72E-10	8.88E-04	3.28E-04	8.03E-04	2.43E-04	1.11	1.35
70	1.21E-07	3.37E-08	1.80E-08	1.80E-10	6.97E-04	2.58E-04	6.08E-04	1.69E-04	1.15	1.53
80	9.24E-08	2.27E-08	1.84E-08	1.84E-10	5.54E-04	2.06E-04	4.63E-04	1.15E-04	1.20	1.80
90	7.11E-08	1.58E-08	1.89E-08	1.89E-10	4.50E-04	1.73E-04	3.57E-04	7.98E-05	1.26	2.17
100	5.51E-08	1.17E-08	1.91E-08	1.91E-10	3.71E-04	1.54E-04	2.77E-04	5.92E-05	1.34	2.59
110	4.40E-08	8.58E-09	1.92E-08	1.92E-10	3.16E-04	1.39E-04	2.21E-04	4.39E-05	1.43	3.17
120	3.58E-08	6.70E-09	1.96E-08	1.96E-10	2.77E-04	1.31E-04	1.80E-04	3.45E-05	1.54	3.81
130	2.92E-08	5.12E-09	1.96E-08	1.96E-10	2.44E-04	1.24E-04	1.47E-04	2.66E-05	1.66	4.65
140	2.42E-08	4.09E-09	1.99E-08	1.99E-10	2.20E-04	1.20E-04	1.22E-04	2.14E-05	1.81	5.58
150	1.98E-08	3.54E-09	2.00E-08	2.00E-10	1.99E-04	1.18E-04	9.98E-05	1.87E-05	1.99	6.30
160	1.69E-08	2.55E-09	1.99E-08	1.99E-10	1.84E-04	1.12E-04	8.53E-05	1.37E-05	2.16	8.17
170	1.45E-08	1.92E-09	2.01E-08	2.01E-10	1.73E-04	1.10E-04	7.33E-05	1.06E-05	2.36	10.39
180	1.17E-08	1.43E-09	2.00E-08	2.00E-10	1.58E-04	1.07E-04	5.93E-05	8.15E-06	2.67	13.16
190	9.83E-09	1.26E-09	2.01E-08	2.01E-10	1.50E-04	1.07E-04	5.02E-05	7.30E-06	2.98	14.61
200	8.29E-09	1.08E-09	2.03E-08	2.03E-10	1.43E-04	1.07E-04	4.25E-05	6.41E-06	3.36	16.64

4.5. DISCUSSION

A number of risk assessments have been undertaken to study the risk associated with movement of pets to and from countries infected with rabies (MacDiarmid and Corrin, 1997; Hawaii Department of Agriculture, 2002; Jones *et al.*, 2002; Jones *et al.*, 2005; Weng, 2004; Hallgren *et al.*, 2005 and 2006; Norwegian scientific committee for food safety, 2005). These studies have each included defined risk pathways covering various combinations of biological, epidemiological, legal and compliance issues in an attempt to estimate rabies risk associated with pet movement.

The present risk assessment has focused primarily on biological and epidemiological aspects in an attempt to clarify how these elements are contributing to risk or risk reduction. The model was built in order to separate the effect of waiting time and the effect of vaccination and testing. Compliance issues are considered secondary to these aspects and can be assessed indirectly by the modulating effect on each biological effect. For example, non-compliance in terms of vaccination will increase the type B risk by a given non-compliance factor.

The results of this risk assessment describe clearly the interrelationship between the effects of prevalence, waiting time, vaccination and testing on reducing the overall risk of rabies introduction.

Within EU, the highest rabies prevalence in pets is found in the Baltic States. Taken together, the prevalence of pet rabies in these countries was about 15 per million or $1.5 \cdot 10^{-5}$ in 2005. In Poland, Hungary and Slovakia the prevalence in 2005 was around 10^{-7} (2 orders of magnitude lower than in the Baltic States) while in the remainder of member countries the prevalence was nil. If, as an example, a risk of 10^{-6} is considered as the acceptable risk limit for importing a rabies-infected pet, this would mean that risk reduction would only be needed for pets coming from the Baltic countries, *e.g.* by including a waiting time for pets undergoing first time vaccination. The need to maintain the serological testing depends on the actual prevalence and waiting time chosen by the MS. For countries with a prevalence higher than 1 per million the first step to be taken is the implementation of the waiting time. The higher the actual prevalence, the longer should be the waiting time required in order to reach an acceptable level of risk.

The implementation of serological testing may be considered when the required waiting time exceeds 100 days. Depending on the risk assessment model applied, a risk reduction of 1.5 and 3.8, respectively, could be attributed to serological testing when the waiting time was 120 days. This is in good agreement with the results of the Swedish assessment (Hallgren, 2006) which found that testing would reduce the risk by a factor two at a waiting time of 120 days. The same or an even better risk reduction can be obtained by replacing serological testing with a second vaccination 4 to 6 weeks after the first vaccination.

For animals coming from countries with a negligible prevalence of rabies and going to countries with non-negligible prevalence of rabies, the best way to prevent rabies infection is simply by assuring adequate immunity after primo-vaccination (by serotest or a second injection) before moving. There is no rationale for including a waiting time beyond the time where protective immunity has been reached for this group.

4.6. CONCLUSIONS

As a result of the present risk assessment, two main groups of countries were identified:

- Countries with a negligible incidence of rabies in pets (lower than 1 per million pets per year);
 - Countries with a non negligible incidence of rabies in pets (higher or equal to 1 per million pets per year).
- If the incidence is negligible, there is no absolute effect of any risk mitigating measures, although vaccination with authorised rabies vaccines and animal identification is still considered as a basic measure in relation to pet movement.
 - There is no rationale for including a waiting time beyond the time where protective immunity has been reached for animals coming from countries with negligible incidence of rabies.

- Risk mitigation measures should be implemented with respect to movement of pets to and from countries with non-negligible prevalence.
- The primary means of removing an individual from the population at risk is by vaccination. Inactivated rabies vaccines are highly efficient and induce rapid protective immunity that prevents infection and subsequent transmission. Due to biological variation a small fraction may remain non-protected following primo-vaccination, especially in animals younger than 1 year.
- Rabies vaccines are currently authorised on the basis of challenge tests in a target species and the measurement of protective levels of antibody. For monitoring the response to vaccination, the best available correlate with protection is to demonstrate that animals have achieved a serological titre of 0.5 IU/ml.
- A small fraction of pets do not mount an antibody response of 0.5 IU/ml or above after first injection of vaccine. The risk of having a certain proportion of pets vaccinated in field conditions which are not fully protected is reduced either by carrying out a serological test to measure present antibodies or by a second injection of an authorised vaccine.
- Vaccination after infection has little or no effect on subsequent development of disease. There are no methods available for detection or identification of animals incubating rabies.
- Control of rabies transmission by pet movement is based on inducing protective immunity by vaccination before movement in order to remove the animal from the susceptible population. Removal from the population at risk will only become effective when incubation can be excluded.
- A waiting time (defined as the time spent between vaccination and pet movement), is an effective measure to mitigate the risk of rabies introduction due to an animal being infected before vaccination (type A risk).
- The risk of infection during the waiting time (type B risk) depends on the level of protection induced by the vaccination in field conditions and becomes relatively more important as type A risk is reduced with extended waiting times (over 100 days). Serological testing can be used to identify seronegative pets and will therefore reduce this risk accordingly.
- Depending on the risk assessment model applied, a total risk reduction of 1.5 and 3.8, respectively, could be attributed to serological testing when the waiting time was 120 days. The same or an even better risk reduction can be obtained by replacing serological testing with a second vaccination 4 to 6 weeks after the first vaccination.
- For animals coming from countries with a negligible incidence and going to countries with non-negligible incidence of rabies, the best way to prevent rabies infection and thus eliminating the risk of being infected upon return is simply by assuring adequate immunity after primo-vaccination.
- The risk that seronegative pets contracts rabies during a visit to a country with a non-negligible incidence can be described with the same approach as for type B risk, replacing waiting time with length of visit.

4.7. RECOMMENDATIONS

A rabies vaccination using an authorised vaccine and applied according to the approved claims should remain the key requirement for pet movement between member states.

Further requirements should be based on whether rabies occurs in the pet population or not. If rabies occurs in the pet population where pets reside before primo-vaccination, a waiting time following primo-vaccination is necessary in order to reduce the risk of importing rabies-infected pets. The waiting time should be applied if the incidence of pets incubating rabies exceeds the level of acceptable unrestricted risk (1 case per million pets per year). Pets originating from countries with a negligible incidence can visit countries with non-negligible incidence of rabies (pets and/or wildlife) without risk once protective immunity has been established and is maintained.

The following recommendations can therefore be given:

a) Requirements in relation to animal movement out of an area with non-negligible risk should be based on:

- Identification;
- Vaccination (followed by testing or second injection whenever a waiting time of more than 100 days is required);
- Apply waiting time according to level of risk reduction needed.

b) Requirements in relation to animal movement into an area with non-negligible risk should be based on:

- Identification;
- Vaccination (followed by testing or second injection).

The risk of having a certain proportion of pets vaccinated under field conditions which are not fully protected is reduced either by carrying out a serological test to measure antibodies or by a second injection of an authorised vaccine. These recommendations are based on general good practice and on general published data and are not directly related to data from studies using vaccines currently available on the EU market. For this reason, in order to implement the recommendations in this opinion, it would be necessary to ask the manufacturers to conduct the necessary research studies to support the administration of two doses of vaccine. Alternatively, manufacturers could be asked to supply data on the proportion of animals which do not reach a titre of 0.5 IU/ml after a single vaccination and which are also not protected against challenge in order to determine with more certainty the level of risk after a single vaccination.

For countries with an incidence higher than 1 per million pets per year, the implementation of a waiting time is recommended as the most efficient risk-reducing measure. The higher is the actual prevalence, the longer should be the waiting time required in order to reach an acceptable level of risk. The implementation of serological testing or other risk-reducing measures may be considered when the required waiting time exceeds 100 days.

Some of the monographs for the safety and potency testing of inactivated rabies vaccines have required that animals be allowed to die in the control and challenge groups. The Panel would recommend that any monograph that requires death as an endpoint is reviewed, to ensure that monographs are in line with legislation regulating animal research, in regard to the implementing the Three Rs of humane experimentation, and not causing more animal suffering than is necessary to achieve the scientific objective.

4.8. RECOMMENDATIONS FOR FUTURE RESEARCH

The estimate of incubation period distribution is based on a combination of data from the British quarantine prior to 1970 and experimental data. In general, the quarantine data shows longer incubation periods than the experimental data, however, it is unknown to which degree secondary infection during quarantine occurred prior to 1970, where pets were not vaccinated when allowed into quarantine. It is therefore recommended to collect further data on the distribution of incubation periods following natural infection. Additional studies would be necessary to support the administration of two doses of vaccine as the primary vaccination course (see above).

More research on new serological tests could lead to improvements in the monitoring of the effect of vaccination and its correlation with protection

5. ANNEX I

5.1. ADDITIONAL INFORMATION

I Rules applicable to the movement between continental MS and the UK, Ireland and Malta:

- 1) Anti-rabies vaccination plus antibody estimation on a blood sample collected at least six months prior to travel.
- 2) Treatment (Praziquantel) for tapeworm and external treatment for ticks 24 to 48 hours prior to travel.

Website: <http://www.defra.gov.uk/animalh/quarantine/pets/procedures/owners.htm>

II Rules applicable to the movement between continental MS and Sweden:

- 1) Anti-rabies vaccination plus antibody estimation on a blood sample collected after 120 days and less than one year after vaccination.
- 2) Treatment for tapeworm during the 10 days before the movement (Finland also requires this treatment for pets entering its territory).

Website: <http://www.sjv.se/home/arnesomraden/animalhealthwelfare/importexportofliveanimals/dogsandcats>

III Situation of rabies in Europe:

Website: <http://www.who-rabies-bulletin.org>

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