PART 1 (COUNCIL DECISION 2002/813/EC)

SUMMARY NOTIFICATION INFORMATION FORMAT FOR THE RELEASE OF GENETICALLY MODIFIED ORGANISMS OTHER THAN HIGHER PLANTS IN ACCORDANCE WITH ARTICLE 11 OF DIRECTIVE 2001/18/EC

In order to tick one or several possibilities, please use crosses (meaning x or X) into the space provided as (.)

A. General information

1. Details of notification

(a)	Member State of notification	Italy
(b)	Notification number	B/IT/09/01
(c)	Date of acknowledgement of notification	26/11/2009
(d)	Title of the project **Vaccine candidate against Canine Visceral Leishmaniasis. Evaluation of efficacy by natural challenge in an experimental kennel**	
(e)	Proposed period of release	From 01/07/2010 until 31/08/2011

2. Notifier

Name of institution or company: Merial, 29, avenue Tony Garnier. 69007 Lyon - France

3. GMO characterisation

(a) Indicate whether the GMO is a:

viroid		(.)
RNA virus		(.)
DNA virus		(X)
bacterium		(.)
fungus		(.)
animal		
-	mammals	(.)
-	insect	(.)
-	fish	(.)
-	other animal	(.)

specify phylum, class …

(b) Identity of the GMO (genus and species)

vCP2350 consisting of a recombinant canarypox virus expressing the KMP 11 antigen of Leishmania, under control of the H6 promoter from the vaccinia virus.

(c) Genetic stability – according to Annex IIIa, II, A(10)

Recombinant virus vCP2350 was subjected to sequential passages (from pre-master seed to production level, *i.e.* X-1 to X+5) in chicken embryo fibroblasts. The expression analysis of the KMP11

was performed by genomic analysis via restriction endonuclease mapping and Southern blot analysis and shown to be stable on passages.

4. Is the same GMO release planned elsewhere in the Community (in conformity with Article 6(1)), by the same notifier?

   Yes (.) No (X)

   If yes, insert the country code(s) …

5. Has the same GMO been notified for release elsewhere in the Community by the same notifier?

   Yes (.) No (X)

   If yes:

   - Member State of notification …
   - Notification number B/../../…

Please use the following country codes:
Austria AT; Belgium BE; Germany DE; Denmark DK; Spain ES; Finland FI; France FR; United Kingdom GB; Greece GR; Ireland IE; Iceland IS; Italy IT; Luxembourg LU; Netherlands NL; Norway NO; Portugal PT; Sweden SE

6. Has the same GMO been notified for release or placing on the market outside the Community by the same or other notifier?

   Yes (.) No (X)

   If yes:

   - Member State of notification …
   - Notification number B/../../…

7. Summary of the potential environmental impact of the release of the GMOs.

ESTIMATION OF RISK

Consequence of hazard	**Likelihood of hazard occurring**			
	High	**Moderate**	**Low**	**Negligible**
Severe	High	High	Medium	Effectively zero
Medium	High	High	Medium/low	Effectively zero
Low	Medium/low	Low	Low	Effectively zero
Negligible	Effectively zero	Effectively zero	Effectively zero	Effectively zero

The overall risk for environment is defined as effectively zero.

B. **Information relating to the recipient or parental organism from which the GMO is derived**

1. Recipient or parental organism characterisation:

   (a) Indicate whether the recipient or parental organism is a:

   (select one only)

   viroid (.)

RNA virus (.)
DNA virus (X)
bacterium (.)
fungus (.)
animal
- mammals (.)
- insect (.)
- fish (.)
- other animal (.)
(specify phylum, class) …

other, specify …

2. Name

(i)	order and/or higher taxon (for animals)	Virus
(ii)	genus	Avipoxvirus
(iii)	species	Canarypox virus
(iv)	subspecies	…
(v)	strain	ALVAC Clone
(vi)	pathovar (biotype, ecotype, race, etc.)	…
(vii)	common name	strain of the KANAPOX vaccine (live vaccine against canarypox)

3. Geographical distribution of the organism

(a) Indigenous to, or otherwise established in, the country where the notification is made:
Yes (X) No (.) Not known (.)

Wild strain (canary pox)

(b) Indigenous to, or otherwise established in, other EC countries:
(i) Yes (X)

If yes, indicate the type of ecosystem in which it is found:

Atlantic ..
Mediteranean ..
Boreal ..
Alpine ..
Continental ..
Macaronesian ..

(ii) No (.)
(iii) Not known (.)

(c) Is it frequently used in the country where the notification is made?
Yes (.) No (X)

(d) Is it frequently kept in the country where the notification is made?
Yes (.) No (X)

4. Natural habitat of the organism

(a) If the organism is a microorganism

water	(.)
soil, free-living	(.)
soil in association with plant-root systems	(.)
in association with plant leaf/stem systems	(.)
other, specify	during infection in an animal (canary)

(b) If the organism is an animal: natural habitat or usual agroecosystem:
Not applicable

5. (a) Detection techniques
Culture on CEC (chick embryo cells), PCR and microscopy

(b) Identification techniques
IFI
PCR, probe possible
Western blot

6. Is the recipient organism classified under existing Community rules relating to the protection of human health and/or the environment?
Yes (X) No (.)
If yes, specify
Group I, Class 1. Commission of Genetic engineering (France)

7. Is the recipient organism significantly pathogenic or harmful in any other way (including its extracellular products), either living or dead?
Yes (.) No (X) it is a vaccine strain Not known (.)

If yes:

(a) to which of the following organisms:

humans	(.)
animals	(.)
plants	(.)
other	(.)

(b) give the relevant information specified under Annex III A, point II. (A)(11)(d) of Directive 2001/18/EC
…

8. Information concerning reproduction

(a) Generation time in natural ecosystems:
Not applicable

(b) Generation time in the ecosystem where the release will take place:
Not applicable

(c) Way of reproduction: Sexual .. Asexual ..
Not applicable

(c) Factors affecting reproduction:
Viral type cytoplasmic replication in permissive cells (canary, CEC) …

9. Survivability

(a) ability to form structures enhancing survival or dormancy:

(i)	endospores	(.)
(ii)	cysts	(.)
(iii)	sclerotia	(.)
(iv)	asexual spores (fungi)	(.)
(v)	sexual spores (funghi)	(.)
(vi)	eggs	(.)
(vii)	pupae	(.)
(viii)	larvae	(.)
(ix)	other, specify	…

(b) relevant factors affecting survivability:
…

10. (a) Ways of dissemination
Contact or airbone dissemination (for the wild strain)
Very limited dissemination for the vaccine strain

(b) Factors affecting dissemination
High density of canary population kept in cages

11. Previous genetic modifications of the recipient or parental organism already notified for release in the country where the notification is made (give notification numbers)
…, B/../../…

The recipient virus is not a genetically modified virus by itself (the wild strain has been attenuated by more than 200 passages on CEC). The obtained strain has been passaged four times on CEC (4th passage is called strain CP1). After four cloning cycles by isolation on plaque (plaque cloning), this clone has been called "ALVAC".

Various projects have used the ALVAC clone as a recipient:

Identity of recombinant virus	Origin of the insert	Target species	References
vCP65	Rabies virus	Cat	Registered in the USA (USDA/APHIS)
vCP258	Canine distemper virus	Dog, ferret	Registered in the USA (USDA/APHIS)
vCP97	Feline leukemia virus	Cat	Registered in the EC (centralised procedure) and in the USA (USDA/APHIS)
vCP1529/ vCP2242*, vCP1533	Equine influenza virus (Kentucky/Ohio and Newmarket strains)	Horse	Registered in the EC (centralised procedure) and in the USA
vCP2017	West Nile virus	Horse	Registered in the USA (USDA/APHIS)
vCP125	Human Immunodeficiency Virus	Human	CGBM (France) dossier No.950204
			Egan 1995, Pialoux 1995

* in Europe vCP1529 was replaced by vCP2242 to be in accordance with current OIE/WHO recommendations

C. **Information relating to the genetic modification**

1. Type of the genetic modification

(i)	insertion of genetic material	(X)
(ii)	deletion of genetic material	(X)
(iii)	base substitution	(.)
(iv)	cell fusion	(.)
(v)	others, specify …	

2. Intended outcome of the genetic modification
Insertions of a cassette containing the KMP11 antigen under control of the vaccinia-derived H6 promoter for expression and, subsequently, deletion of the canarypox virus gene selected to be the site of insertion (i.e. C5 locus)

3. (a) Has a vector been used in the process of modification?
Yes (X) No (.)

If no, go straight to question 5.

(b) If yes, is the vector wholly or partially present in the modified organism?
Yes (X) No (.)

If no, go straight to question 5.

4. If the answer to 3(b) is yes, supply the following information

(a) Type of vector

plasmid	(X)
bacteriophage	(.)
virus	(.)
cosmid	(.)
transposable element	(.)
other, specify …	

(b) Identity of the vector
pJSY1992

(c) Host range of the vector
Escherichia coli

(d) Presence in the vector of sequences giving a selectable or identifiable phenotype
Yes (X) No (.)

antibiotic resistance	(X)
other, specify …	

Indication of which antibiotic resistance gene is inserted
Ampicilline

(e) Constituent fragments of the vector

pJSY1992.1= pJSY1992 + + flanking arm C5L + H6 promoter + Glycoprotein G gene + flanking arm C5R

(f) Method for introducing the vector into the recipient organism

(i)	transformation	(.)
(ii)	electroporation	(.)
(iii)	macroinjection	(.)
(iv)	microinjection	(.)
(v)	infection	(.)
(vi)	other, specify	(X)

in vitro homologous recombination

5. If the answer to question B.3(a) and (b) is no, what was the method used in the process of modification?

Not applicable

(i)	transformation	(.)
(ii)	microinjection	(.)
(iii)	microencapsulation	(.)
(iv)	macroinjection	(.)
(v)	other, specify …	

6. Composition of the insert

(a) Composition of the insert

The insert is constituted of an expression cassette (KMP 11 antigen under control of vaccinia virus H6 promoter) surrounded by flanking arms of canarypox virus origin.

(b) Source of each constituent part of the insert

Expressed gene:

KMP 11 antigen from *Leishmania infantum*

Promoter:

H6, vaccinia origin. Reference: Perkus *et al.*, J. Virology. 1989, **63**, 3829-3836.

Flanking arms:

ALVAC origin

(c) Intended function of each constituent part of the insert in the GMO

Flanking arms: for the homologous recombination

Promoter: for the specific expression of the gene

Gene: expression of the protective immunogen

(d) Location of the insert in the host organism

-	on a free plasmid	(.)
-	integrated in the chromosome	(.)
-	other, specify	(X)

integrated into the viral genome

(e) Does the insert contain parts whose product or function are not known?

Yes (.) No (X)

If yes, specify …

D. Information on the organism(s) from which the insert is derived

For the H6 promoter:

1. Indicate whether it is a:

viroid	(.)
RNA virus	()
DNA virus	(X)
bacterium	(.)
fungus	(.)
animal	
- mammals	(.)
- insect	(.)
- fish	(.)
- other animal	(.)
(specify phylum, class)	…
other, specify	…

2. Complete name

(i)	order and/or higher taxon (for animals)	Virus
(ii)	family name for plants	Poxviridae Subfamily: Chordopoxviridae
(iii)	genus	Orthopoxvirus
(iv)	species	Vaccinia
(v)	subspecies	…
(vi)	strain	…
(vii)	cultivar/breeding line	…
(viii)	pathovar	…
(ix)	common name	…

3. Is the organism significantly pathogenic or harmful in any other way (including its extracellular products), either living or dead?

Yes (.) No (X) (vaccine strain) Not known (.)

If yes, specify the following:

(b) to which of the following organisms:

humans	(.)
animals	(.)
plants	(.)
other	..

(b) are the donated sequences involved in any way to the pathogenic or harmful properties of the organism

Yes (.) No (.) Not known (.)

If yes, give the relevant information under Annex III A, point II(A)(11)(d):

…

4. Is the donor organism classified under existing Community rules relating to the protection of human health and the environment, such as Directive 90/679/EEC on the protection of workers from risks to exposure to biological agents at work?

Yes (X) No (.)

If yes, specify Class 3

5. Do the donor and recipient organism exchange genetic material naturally?

Yes (.) No (X) Not known (.)

For the inserted gene (KMP 11)

1. Indicate whether it is a:

viroid (.)
RNA virus (.)
DNA virus (.)
bacterium (.)
fungus (.)
animal
- mammals (.)
- insect (.)
- fish (.)
- other animal (.)

(specify phylum, class) …

other, specify (X)
Parasite

Commento [CPh1]: It is not a virus but a parasite (see below)

2. Complete name

(j) order and/or higher taxon (for animals)	
(ii) family name for plants	*Trypanosomatidae*
(iii) genus	*Leishmania donovani*
(iv) species	*Leishmania donovani infantum*
(v) subspecies	…
(vi) strain	…
(vii) cultivar/breeding line	…
(viii) pathovar	…
(ix) common name	

3. Is the organism significantly pathogenic or harmful in any other way (including its extracellular products), either living or dead?

Yes (X) No (.) Not known (.)

If yes, specify the following:

(c) to which of the following organisms:

humans X
animals X

plants (.)
other ..

(b) are the donated sequences involved in any way to the pathogenic or harmful properties of the organism
Yes X No (.) Not known (.)

If yes, give the relevant information under Annex III A, point II(A)(11)(d):
The KMP-11 (Kinetoplastid Membrane Protein of 11 kDA) antigen of *Leishmania infantum* acts as an immunogen. KMP-11 is a highly conserved protein.

4. Is the donor organism classified under existing Community rules relating to the protection of human health and the environment, such as Directive 90/679/EEC on the protection of workers from risks to exposure to biological agents at work?
Yes (X) No (.)

If yes, specify Class 3

5. Do the donor and recipient organism exchange genetic material naturally?
Yes (.) No (X) Not known (.)

E. Information relating to the genetically modified organism

1. Genetic traits and phenotypic characteristics of the recipient or parental organism which have been changed as a result of the genetic modification

(a) is the GMO different from the recipient as far as survivability is concerned?
Yes (.) No (X) Not known (.)
Specify …

(b) is the GMO in any way different from the recipient as far as mode and/or rate of reproduction is concerned?
Yes (.) No (X) Unknown (.)
Specify …

(c) is the GMO in any way different from the recipient as far as dissemination is concerned?
Yes (.) No (X) Not known (.)
Specify …

(d) is the GMO in any way different from the recipient as far as pathogenicity is concerned?
Yes (.) No (.) Not known (.)
Specify …

2. Genetic stability of the genetically modified organism
Recombinant virus vCP2350 was subjected to sequential passages (from pre-master seed to production level, *i.e.* X-1 to X+5) in chicken embryo fibroblasts. The expression analysis of the

KMP11 was performed by genomic analysis via restriction endonuclease mapping and Southern blot analysis and shown to be stable on passages.

3. Is the GMO significantly pathogenic or harmful in any way (including its extracellular products), either living or dead?
Yes (.) No (X) Unknown (.)

(a) to which of the following organisms?

humans (.)
animals (.)
plants (.)
other …

(b) give the relevant information specified under Annex III A, point II(A)(11)(d) and II(C)(2)(i)
…

4. Description of identification and detection methods

(a) Techniques used to detect the GMO in the environment
Isolation on CEC (by culture), PCR and microscopy

(b) Techniques used to identify the GMO

Restriction endonuclease mapping of the viral DNA
IFI
PCR, probe possible
Western blot

F. Information relating to the release

1. Purpose of the release (including any significant potential environmental benefits that may be expected)

Study of the efficacy of the vaccine containing the GMO in target species (dogs).

2. Is the site of the release different from the natural habitat or from the ecosystem in which the recipient or parental organism is regularly used, kept or found?
Yes (X) No (.)

If yes, specify
The GMO is injected to dogs (non-permissive species, does not generally allow the replication of avipoxvirus and the canarypox virus in particular).

3. Information concerning the release and the surrounding area

(a) Geographical location (administrative region and where appropriate grid reference):
80048 Sant'anastasia Naples – Campany - Italy

(b) Size of the site (m^2): 170 m^2

(i) actual release site (m^2): … m^2
(ii) wider release site (m^2): … m^2

(c) Proximity to internationally recognised biotopes or protected areas (including drinking water reservoirs), which could be affected:
Not applicable (non-diffusible virus)

(d) Flora and fauna including crops, livestock and migratory species which may potentially interact with the GMO

None

4. Method and amount of release

(a) Quantities of GMOs to be released:

One dose (titre at $7.3\log_{10}$ CCID50 per dose) will be administered to each of the 25 dogs included in the trial

(b) Duration of the operation:

Each of the 25 dogs is administered one injection of vCP2350. The trial last 24 months but the booster injection, subject of this notification, is performed once at 12 month in the kennel.

(c) Methods and procedures to avoid and/or minimise the spread of the GMOs beyond the site of the release
- intramuscular parenteral injection (natural physical containment)
- non-permissive species (natural biological containment)
- recovery of contaminated containers and materials (destruction by incineration).

5. Short description of average environmental conditions (weather, temperature, etc.)

Release for an experimental trial in an open fenced kennel in Italy. Environmental conditions (weather, temperature, etc.) should not affect survival, multiplication and dissemination

6. Relevant data regarding previous releases carried out with the same GMO, if any, specially related to the potential environmental and human health impacts from the release.

Not applicable

G. Interactions of the GMO with the environment and potential impact on the environment, if significantly different from the recipient or parent organism

1. Name of target organism (if applicable)
(i) order and/or higher taxon (for animals)
Kingdom: Animal
Phylum: Vertebrates
Class: Mammals
Subclass:

Superorder:
Order: Carnivores
(j) Suborder:

(ii)	family name	Canidae
(iii)	genus	…
(iv)	species	Canis lupus…
(v)	subspecies	Canis lupus familiaris
(vi)	strain	…
(vii)	cultivar/breeding line	…
(viii)	pathovar	…
(ix)	common name	…

2. Anticipated mechanism and result of interaction between the released GMOs and the target organism (if applicable)

Expression of the inserted gene, without replication of the GMOs, leading to the development of a specific protective immunity.

3. Any other potentially significant interactions with other organisms in the environment

Expression without replication in mammals, non pathogenic in permissive species (canary type).

4. Is post-release selection such as increased competitiveness, increased invasiveness for the GMO likely to occur?

Yes (.) No (X) Not known (.)

Give details

…

5. Types of ecosystems to which the GMO could be disseminated from the site of release and in which it could become established

Within the scope of the experimental protocol, there is no such risk

6. Complete name of non-target organisms which (taking into account the nature of the receiving environment) may be unintentionally significantly harmed by the release of the GMO

Not applicable. Non-pathogenic vaccine strain

(i)	order and/or higher taxon (for animals)	…
(ii)	family name for plants	…
(iii)	genus	…
(iv)	species	…
(v)	subspecies	…
(vi)	strain	…
(vii)	cultivar/breeding line	…
(viii)	pathovar	…
(ix)	common name	…

7. Likelihood of genetic exchange in vivo

(a) from the GMO to other organisms in the release ecosystem:

See b)

(b) from other organisms to the GMO:

Potential for genetic transfer and exchange between poxviruses: the recombination between a canarypox-based virus (such as vCP2350) and another poxvirus is theoretically possible but this has never been observed so far for the commercial ALVAC constructs. The construction of vCP2350 itself is based upon an *in vitro* homologous recombination. However, for an *in vivo* recombination to occur under natural conditions, a simultaneous co-infection by two poxviruses (one of them being the ALVAC vector or the ALVAC-derived recombinant) with some degree of homology is necessary within the same cell. This is highly unlikely to happen in the conditions of dissemination; in particular, considering the fact that the GMO must be used in dogs, species in which poxvirus infection is extremely rare. Altogether, these elements confirm that potential for genetic transfer and exchange between poxviruses is negligible.
Potential for genetic transfer and exchange with an organism related to the donor organism: Recombination between a canarypox virus (DNA virus) and a *Leishmania* parasite is highly unlikely to happen because the parasite replication is host-restricted in dogs and humans, whereas the canarypox does not replicate in these species. Further, the cellular tropism of *Leishmania infantum* amastigotes in dogs and humans is restricted to phagolyzosomes of macrophages, neutrophils, fibroblasts and dendritic cells, none of which is readily infected by a canarypox virus injected intra-muscularly into dogs.

(c) likely consequences of gene transfer:

Not applicable (gene transfer is highly unlikely, see b.).

8. Give references to relevant results (if available) from studies of the behaviour and characteristics of the GMO and its ecological impact carried out in stimulated natural environments (e.g. microcosms, etc.):

Studies on the GMO:

In vitro, the lack of replication of the GMO in mammalian cells (of cat or dog origin) was demonstrated.
In vivo, safety was demonstrated in dogs and hamsters. Absence of shedding was demonstrated in dogs.

9. Possible environmentally significant interactions with biogeochemical processes (if different from the recipient or parental organism)

Studies in men, horses, cats, dogs, cattle, pigs, mice, guinea-pigs, rabbits, monkeys as well as in cyclophosphamide immuno-depressed mice have demonstrated the safety of this virus. Also, the impossibility of replication of the vector in mammals has been demonstrated *in vitro* on mammalian cells (equine, canine and feline) and evaluated in the mouse using an ALVAC luciferase construct.

H. Information relating to monitoring

1. Methods for monitoring the GMOs

The GMO may be isolated by culture on CEC (test for cythopathic effect and characterisation by indirect immunofluorescence or PCR). The GMO is a non replicative organism which disappears rapidly after parenteral injection. This method of monitoring will be used if necessary.

2. Methods for monitoring ecosystem effects

The experimental animals which are administered the GMO are individually identified and are monitored by a veterinarian. Both the veterinarian and the owner of the kennel are informed about the nature of the GMO. The protocol contains a description of the monitoring and data system concerning the animal and its immediate environment.

3. Methods for detecting transfer of the donated genetic material from the GMO to other organisms

Not applicable

4. Size of the monitoring area (m^2)

170 m2 (experimental kennel)

5. Duration of the monitoring

Dogs will be individually monitored throughout the protocol, i.e. over two days after booster injection and then throughout the second year of the study, monthly 12 months after the booster injection

6. Frequency of the monitoring

See above

I. Information on post-release and waste treatment

1. Post-release treatment of the site
Not applicable. The virus is injected by parenteral route (physical containment).

2. Post-release treatment of the GMOs
Not applicable. The virus disappears rapidly (because of biological and physical containments).

3. (a) Type and amount of waste generated
Glass bottle (one per dog) and material for injection (needle, syringe)

3. (b) Treatment of waste
Waste will be recovered, then destroyed by incineration under the responsibility of Merial.

J. Information on emergency response plans

1. Methods and procedures for controlling the dissemination of the GMO(s) in case of unexpected spread

The host range of the canarypox is very narrow and only canaries could be affected by an epidemy caused by the canarypox virus. The transmission between canaries requires a close contact (same or contiguous cages), probability very low in this context. In this case, classical isolation and disinfection measures would be implemented.
Canarypoxvirus does not spread among chickens and does not replicate in mammals.

2. Methods for removal of the GMO(s) of the areas potentially affected

In case of accident (e.g., broken bottles) or accidental contamination of surfaces (walls, ceilings, examination tables), disinfection is carried out with 70° ethanol. In case of contamination of the skin of the animal or persons in charge of its restrain during the injection, the contaminated area will be treated, as the injection site, with classical disinfectants (70° ethanol solution).

3. Methods for disposal or sanitation of plants, animals, soils, etc. that could be exposed during or after the spread

Not applicable

4. Plans for protecting human health and the environment in the event of an undesirable effect

Despite the negligible risk related to the use of the vCP2350, an emergency plan is established.
In case of accidental injection to man, a physician will be notified for monitoring, if necessary. Indeed, the ALVAC vector by itself or as recombinant has already been used in humans (trials, at the phase I and II) without observing any safety problem, even after several administrations. Because the GMO and the vector were proven to be safe (non replicative in mammals) and because there were no pathogenic effects caused by the products expressed by the GMO, only a medical data system (human and veterinary) will be implemented.
In all cases, the person responsible for the trial will be informed. All events will be recorded and analysed in detail as well as their monitoring if any.
In case of accidental breaking of a vial, the contaminated surface should be disinfected with ethanol 70°.
In case of an unexpected event after the booster injection, three operating phases will be implemented:

Alert phase:

. any observation which cannot be related to the normal post-vaccinal adverse reactions (transient and mild swelling at the injection site and transient lethargy) will be reported to the Investigator veterinary surgeon and to the Monitor of the trial.
. no specific measures will be taken for the involved animal as dogs are already kept closed in the kennel.

Investigation phase:
. appropriate samples will be collected and sent to the laboratory for virus isolation and identification,
. treatment of the animal will be prescribed immediately by the veterinary surgeon responsible for the kennel under the responsibility of the investigator.

Action phase:
. *the diagnosis is known before the end of the trial and the event is not related to the vaccine:*
The Investigator will start treating the involved animal.
. *the diagnosis is known before the end of the trial and the event is related to the vaccine:*
Dogs which have already been inoculated with the vaccine will be closely followed-up for at least 1 month
. *the cause of the event is not known by the end of the trial:*
If the cause of the unexpected event is not established at the end of the trial, an adverse reaction related to the vaccine cannot be eliminated. If necessary, the follow-up of all the animals included in the trial may be extended for 1 month after the end of the trial. Post-mortem necropsy performed should help in determining the cause of the event.