EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

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CVMP assessment report

Feline endogenous retrovirus RD114 in some live attenuated vaccines commercially available in the EU for use in animals

Procedure no: EMEA/V/A/058

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1. Background information on the procedure

1.1. Background

In April 2010, the European Medicines Agency (the Agency) was made aware of the publication of an article (Miyazawa T et.al., Isolation of an infectious endogenous retrovirus in a proportion of live attenuated vaccines for pets. J Virol. 2010 Apr;84(7):3690-4) which presents new information regarding the detection of feline replication-competent endogenous retrovirus RD114 in some live attenuated vaccines commercially available in the EU (UK) and in Japan for use in cats and dogs.

In this study, highly sensitive methods of detection based on new molecular technologies were used by teams of researchers in two laboratories in Scotland and Japan to test, independently, anonymised samples of 6 vaccines made commercially available in the UK from 5 different manufacturers and 11 vaccines made commercially available in Japan from 7 different manufacturers, respectively. Based on the information available so far, some batches of the above mentioned products were reported to be positive for replication-competent RD114. At present, the identity of the vaccines testing positive for RD114 is unknown to the Agency.

It should be noted that already in January 2008, the Agency's Committee for Medicinal Products for Veterinary Use (CVMP) was informed of a finding of a putatively replication competent feline retrovirus RD114 detected in certain vaccines for cats. This information had initially been brought to the attention of a company by the Japanese competent authority (JMAFF). The information available was limited, which prevented an appropriate risk assessment to be performed. As a result, the CVMP and its working parties agreed to assist the European Regulatory Network. Using the best available expertise protocols for testing by manufacturers of relevant vaccines were developed, endorsed and implemented in collaboration with industry. Major companies that market vaccines for cats in the EU were approached and provided initial information. The study methodology was mutually endorsed by CVMP and concerned companies in June 2008 and since then these companies are investigating their feline cell lines which are used for manufacturing of veterinary vaccines, for presence of retrovirus RD114 in the concerned cell lines.

1.2. Executive Director request

In April 2010, the Executive Director of the Agency considered that the new published findings require a review of the ongoing activities carried out by industry and that the ongoing activities, and future ones, take place within the context of drawing up a formal opinion of the Committee.

In view of the above, the Executive Director of the Agency made a request to the Committee for Medicinal Products for Veterinary Use under Article 30(3) of Regulation (EC) No 726/2004 of the European Parliament and of the Council to give a scientific opinion on the following questions:

1. In light of published information reporting that replication-competent retrovirus RD114 has been detected in some unidentified commercially available live attenuated vaccines used for dogs and cats in the EU, is there currently a public or animal health concern caused by such finished vaccines, or their raw materials?

2. If any concerns are identified, is there currently sufficient information to consider that the overall benefit-risk balance of the products is affected such as to warrant prompt regulatory action?

3. If further information is required before a re-assessment of the benefit-risk balance can be completed, can the Committee propose what additional information is required and how it might be obtained?

4. The published paper describes the application of novel molecular methods for the detection of extraneous agents, and sub-components thereof, in vaccines and/or their substrates. Can the CVMP comment on the suitability of such methods for routine use for regulatory purposes and indicate if there is a need to update existing guidance related to the testing and elimination of such substances in the context of development and/or testing of live attenuated vaccines.

5. Depending on the outcome of these investigations, the Committee should consider the need for appropriate guidance for other vaccines.

This report outlines the conclusions of the Committee, including recommendations for regulatory action.

1.3. Steps taken during the procedure

- During the May 2010 CVMP meeting, the following was agreed:
  - Jean-Claude Rouby was appointed rapporteur.
  - Frederic Descamps was appointed co-rapporteur.
  - The procedure under Article 30 started on 20 May 2010.
  - No marketing authorisation holders were involved in the procedure, as no individual products had been identified.
- The rapporteur's assessment report was circulated to all CVMP members on 28 June 2010, including amendments following prior circulation to the co-rapporteur.
- On 28 June 2010 the rapporteur's assessment report was (to be) forwarded to IFAH-Europe, including an invitation to be consulted during the July 2010 plenary meeting of the Committee.
- On 13 July 2010 IFAH-Europe was consulted by the CVMP during its plenary meeting on the draft assessment.
- A revised rapporteur's assessment report was circulated to all CVMP members on <insert date>, including amendments following prior circulation to the co-rapporteur.
- On 15 September 2010, the CVMP adopted an opinion including conclusions and recommendations on basis of this assessment report.

2. Scientific discussion

2.1. Introduction

The new article (Miyazawa T et.al., Isolation of an infectious endogenous retrovirus in a proportion of live attenuated vaccines for pets. J Virol. 2010 Apr;84(7):3690-4) presents new information regarding the detection of feline replication-competent endogenous retrovirus RD114 in some live attenuated vaccines commercially available in the EU (UK) and in Japan for use in cats and dogs. In this study, highly sensitive methods of detection based on new molecular technologies were used by teams of researchers in two laboratories in Scotland and Japan to test, independently, anonymised samples of 6 vaccines made commercially available in the UK from 5 different manufacturers and 11 vaccines made commercially available in Japan from 7 different manufacturers, respectively. Based on the information available so far, some batches of the above mentioned products were reported to be positive for replication-competent RD114. At present, the identity of the vaccines testing positive for RD114 is unknown to the Agency.

In this context, the Executive Director of the Agency has made a request to the Committee for Medicinal Products for Veterinary Use under Article 30(3) of Regulation (EC) No 726/2004 of the European Parliament and of the Council to give a scientific opinion on the questions listed in section 1.2.

2.2. Critical evaluation

The five questions are addressed individually below.

Question 1 – Assessment of animal or public health concern

Question: In light of published information reporting that replication-competent retrovirus RD114 has been detected in some unidentified commercially available live attenuated vaccines used for dogs and cats in the EU, is there currently a public or animal health concern caused by such finished vaccines, or their raw materials?

Presence of retrovirus RD114, pathogenicity of retrovirus RD114, and animal/human exposure to retrovirus RD114 need to be examined, in order to identify any public or animal health concern.

Presence of retrovirus RD114

- in cat population

At present, there is reported evidence for global presence of retrovirus RD114 in the genome of domestic cats[1]. The cat genome has recently been sequenced[2], finding up to 20 copies of endogenous retrovirus RD114, of which at least one is infectious. Retrovirus RD114 is likely to be present in the genome of all cats, although some conflicting information is available in public literature on this point[3].

Therefore retrovirus RD114 can be considered as an endogenous retrovirus of cats.

As retrovirus RD114 is present in the genome of all domestic cats, then the whole global cat population, including all breeds, is likely to be affected, whether vaccinated or not.

Therefore the prevalence of retrovirus RD114 in the genome of the general cat population can be considered as very high.

- in big Felidae populations

Big felidae - e.g. lions, tigers, jaguars, leopards, cheetahs - are naïve for retrovirus RD114 in their genome. There is therefore at least a theoretical risk that retrovirus RD114 can integrate into their genome.

- in dogs

Retrovirus RD114 is not present in the dog genome, but canine cells can be infected by it[3-4]. It is not known whether these cells would be competent to produce replicative viral particles.

- in other domestic animals

The potential for retrovirus RD114 to infect other domestic species such as cattle, sheep, swine or horses is not known.

- in human beings

The presence of retrovirus RD114 in the human genome has not been reported, but human cells can be infected by it. It is not known whether these cells would be competent to produce replicative viral particles.

It should be noted that RD114 and endogenous type-C baboon retroviruses are related in many respects. Moreover, retrovirus RD114-related nucleic acid sequences are found in the genomes of all Old World Monkey and ape tissues[5]. These observations have led to the suggestion that retrovirus RD114 evolved from an endogenous primate type-C virus that infected and became endogenous in a recent ancestor of domestic cats.

In general, canine and human cell lines are more sensitive than feline cell fines to retrovirus RD114 infection in vitro.

All these points taken together, the risk that retrovirus RD114 can integrate into the genome of humans, dogs or big felidae, although theoretical at this stage, cannot be ruled out.

Pathogenicity of retrovirus RD114

Under natural conditions, retrovirus RD114 is a xenotropic virus of domestic cats: it does not productively infect or replicate in cat cells. Moreover, sera from healthy cats and cats with various diseases do not contain antibodies to retrovirus RD114 viral proteins. This indicates that these antigens are rarely expressed and/or that cats are immunologically tolerant to retrovirus RD114 proteins[3]. Under natural conditions, retrovirus RD114 is not known to cause diseases, whatever the species (including human beings).

However, injection through vaccines of retrovirus RD114 live replicative particles, even at very low level, does not mimic natural conditions of contact between retrovirus RD114 and animals or humans.

The consequences of inoculating replicative retrovirus RD114 to animals or humans are not foreseeable.

As retrovirus RD114 belongs to the retroviridae, this virus is able to integrate its RT-DNA product into the genome of a permissive host cell. Depending on the location of the integration, the function of a gene can be disrupted, including regulatory ones. This could potentially lead to the appearance of tumors at the injection site, but also elsewhere.

An issue of particular concern in cats at the present time is the appearance of fibrosarcomas at sites of injection of veterinary medicinal products. To date the aetiology of this condition is obscure and it is therefore appropriate to consider any potential role that retroviruses may play. On the other hand, it is important to note that, contrary to some other retroviruses, RD114 is not reported to contain any oncogene in its genome. This is further supported by the fact that no epidemiological data available to date could establish a cause-effect relationship between retrovirus RD114 and the development of fibrosarcomas at the sites of vaccination in cats. In this context, reference can be made on the "Advisory Notice to veterinary surgeons regarding the development of fibrosarcomas at sites of injection of veterinary medicinal products in cats" (EMEA/CVMP/205/03-FINAL): epidemiological studies have so far failed to identify specific products, or type of products, that may represent an increased risk for the development of such fibrosarcomas which are probably linked to multifactorial causes. Nevertheless further specific studies would be necessary for a relationship to be unequivocally ruled out.

While naïve cats do not exist as all have retrovirus RD114 in their genome, the consequences of surinfection due to iatrogenic inoculation of virus particles are unknown. The minimal infectious dose and the impact of repeated inoculations due to annual booster injections, is not known. However, the more the number of injections, the higher the risk of breaking threshold of tolerance.

For dogs and big cats the situation is further complicated as there is a greater potential for insertion of retroviral sequences into their genomes, these species being naïve for retrovirus RD114 by nature. For dogs, literature suggests that much lower occurrence of retroviral sequences in dog genome may point at protective mechanism against retroviral integration[7].

Some retroviruses are known to induce immunodepression. It is not known whether retrovirus RD114 shares this property too.

In conclusion, retrovirus RD114 might present a theoretical pathogenicity, especially when injected. However it has to be acknowledged that to date, no study supports this assertion.

Exposure to retrovirus RD114

Practically, only cats, big Felidae, dogs and human beings might be affected by this issue. Indeed, retrovirus RD114 will be introduced into vaccine batches by means of raw material of feline origin. Raw material of feline origin are used for manufacturing of cat vaccines (mainly through cell, virus and bacteria (intracellular) Master Seeds), and may be used for manufacturing of dog vaccines (mainly through cell Master Seeds). It is very unlikely that raw material of feline origin will be used for vaccines intended to other species.

Because there is currently a lack of authorised vaccines specifically developed for big Felidae, these might be exposed to retrovirus RD114 when vaccines authorised for domestic cats are administered to them.

Due to self-injections of cat or dogs vaccines, humans may be exposed, but to a much lesser extent as self injections occur only on an ad hoc basis and as it is very likely that a full dose will not be injected. Moreover, human beings are likely to have been exposed for a prolonged period to retrovirus RD114 through the long-standing domestication of cats, potentially with systemic exposure to replicative virus in the case of scratches and bites. It is likely that suspicions of a link between disease and exposure to cats would have been identified if RD114 was pathogenic for man.

The theoretical possibility for RD114 of vaccine origin to be excreted by a vaccinated animal to other animal species or the environment is not documented.

In conclusion, the hazard represented by replicative retrovirus RD114 cannot currently be defined. Consequently, the risk of its presence in cat and dog vaccines is not quantifiable, however the impact of this risk is probably low on animal health and extremely low on human health.

This conclusion is based on the observations that millions of doses of attenuated vaccines have been used for decades in cats (including big Felidae) and dogs without there being any observed association to date with safety issues that could be linked to infection with retrovirus RD114.

Question 2 – Considerations on benefit risk balance

Question: If any concerns are identified, is there currently sufficient information to consider that the overall benefit risk balance of the products is affected such as to warrant prompt regulatory action?

In general, there is currently insufficient data to enable a complete risk assessment to be carried out on the significance of the presence of replicative retrovirus RD114 particles in vaccines for cats and dogs. There is currently insufficient information to accurately predict the threat that vaccines containing infectious retrovirus RD114 particles might represent.

However, vaccines for dogs and cats were developed to fight against major diseases in these species, some of them being lethal or inducing severe after-effects. Moreover, in the publication at the origin of this referral, only a few batches were tested (from UK and Japan), neither representative of all the cat and dog vaccines available on the EU market, nor on all batches of a given tested vaccine. No general picture can be drawn from the data available. The only conclusion which can be drawn is that replicative retrovirus RD114 particles can be present in live attenuated vaccines for dogs and cats.

It should also be stressed that the recent published report of identification of replicative retrovirus RD114 in several batches of finished products does not represent any change in the safety profile of the vaccines concerned which have been used in some cases for decades without any apparent relationship to a disease or syndrome that could be linked to the presence within them of a retrovirus. The change lies within the fact that the technology to detect retroviruses has improved and therefore the ability to conduct a risk assessment on the potential presence of previously unsuspected agents in live attenuated vaccines.

Hence, the benefits of vaccination against cat and dog diseases - which are known and represent a concrete daily threat - clearly outweigh the potential risk that is linked to the presence of replicative retrovirus RD114 in vaccines. Retrovirus RD114 is probably not systematically present in each batch and, if present, at a relatively low amount. Consequently, the threat represented by retrovirus RD114 is currently theoretical.

In conclusion, no prompt regulatory action is warranted, although corrective actions need to be undertaken over years to come.

Question 3 - Information needed for completion of assessment

Question: If further information is required before a re-assessment of the benefit risk balance can be completed, can the Committee propose what additional information is required and how it might be obtained?

Further information about pathogenicity of retrovirus RD114 is clearly desirable, however almost impossible to generate in a short to medium time schedule, as it is part of fundamental research on retroviridae in general. Much remains unknown in this area.

Due to the non-specific nature of the disease signs to be detected including tumours/neoplasia and immunodepression, and the potentially long time lag before these signs might be seen, it would be very difficult to use adverse reaction databases to investigate a causal relationship between attenuated vaccines potentially containing retrovirus RD114 and the induction of subsequent disease in animals.

Besides, testing of feline cells used in the manufacture of cat and dog vaccines to detect the presence of retrovirus RD114 is currently ongoing, under the responsibility of concerned companies. Some results are already available, but a final conclusion can only be reached once all results become available.

On the other hand, it is not considered acceptable to have vaccine batches on the market containing unwanted live virus particles, without trying to investigate and correct this issue. This will be achieved, either by replacing the cell lines used, and/or by introducing some manufacturing step allowing clearance of the virus. Inactivation of retrovirus RD114 might also be considered. Implementation of such corrective measures will take a long time, and implies close collaborative studies between authorities and industry (*action 1*). In particular, the procedure for cell line replacement in such circumstances needs to be agreed upon, both from an administrative and a scientific point of view (*action 2*).

Question 4 - Novel molecular methods in relation to regulatory purposes

Question: The published paper describes the application of novel molecular methods for the detection of extraneous agents, and sub-components thereof, in vaccines and/or their substrates. Can the CVMP comment on the suitability of such methods for routine use for regulatory purposes and indicate if there is a need to update existing guidance related to the testing and elimination of such substances in the context of development and/or testing of live attenuated vaccines?

No golden standard technique to detect retroviruses in veterinary vaccines or in raw material of biological origin is currently available.

The publication which is at the origin of this procedure, aims to detect retrovirus RD114 in finished products. It should be emphasized that very little is known about the technique implemented by the authors, especially with regard to its validation (reproducibility, repeatability, accuracy, precision, limit of detection). It is not certain whether all details about this protocol might become publicly available, to allow validation by other scientific bodies. It is not clear either if this protocol would be applicable to all canine and feline live veterinary vaccines as e.g. some components of the finished product could interfere with the test.

On the other hand, industry and CVMP, taking into account recommendations from its scientific advisory group on this topic, agreed upon a protocol to be implemented to test the feline cell lines used by the EU marketing authorization holders.

It is suggested for the Agency to liaise with European Directorate for the Quality of Medicines (EDQM), to look to which extent collaborative studies with various Official Medicines Control Laboratories (OMCLs) are feasible to validate the protocol mentioned above. Should the outcome of the validation study either be satisfactory as such, or lead to amendments of the protocol, this (amended) protocol could then become an Ph. Eur. monograph to be implemented systematically for future feline cell Master Seeds to be used for manufacturing of veterinary vaccines (*action3*).

This would be a significant step forward, although it is not clear at this stage to which extent this protocol could be implemented to test potential contamination of Master Seeds of feline viruses or of feline intracellular bacteria that could also convey retrovirus RD114.

Question 5 - Considerations on need for guidance

Question: Depending on the outcome of these investigations, the Committee should consider the need for appropriate guidance for other vaccines.

The best way to avoid the presence of retrovirus RD114 in vaccines is to start the manufacturing process with starting materials of biological origin free of retrovirus RD114. Retrovirus RD114 can be found in various cells, some of them being used as a starting material for the manufacturing of vaccines. It can also be conveyed by feline Master Seeds (of feline viruses or of intracellular bacteria). Although retrovirus RD114 could theoretically be transmitted by other raw materials of feline origin, such ways of transmission should not occur in practice as, to our knowledge, no other raw material of feline origin is used during the manufacturing process of veterinary vaccines.

Should a protocol be available, that is

1° able to test for the presence of retrovirus RD114 (knowing that feline cell lines that are non-productive for retrovirus RD114 do exist),

2° usable to test all relevant Master Seeds (cell, virus, intracellular bacteria), and

3° giving satisfactory validation results,

and provided that any positive cell line would be excluded from manufacture of vaccines, the retrovirus RD114 issue would be circumscribed well upstream of the finished product, whatever its indication or target species. In this regard, the implementation of reverse transcriptase assay to be carried out on cell banks, as required for cell substrates for the production of human vaccines, could be considered. However, cell seeds testing, taking place early in the manufacturing process, could prove to be insufficient if silent proviral RD114 or low level of infective virions undetectable in the seeds would

further awaken or multiply during the upstream process. For this reason, testing of end of production cells could also be advisable.

Besides, the following set of fundamental studies could be recommended:

- in vitro studies : studies on cell lines of the target species and human to determine the infectious potential of RD114 and its ability to generate new infectious particles or to integrate as a provirus, …
- basic pathogenicity studies in the target animal species (dogs and cats). Besides, determination of antibody and/or T-cell responses to retrovirus RD114 proteins in animals that have received multiple annual booster vaccinations. These data would be particularly relevant for animals presenting non-specific clinical signs (tumours/neoplasia, immunodepression).
- dissemination and spreading studies in the target animal species
- tumorigenicity studies

From a more general point of view, it is not excluded that with the development of new technologies, notably metagenomics using next generation sequencing and related technologies, nucleic acid of viral origin will be found in immunological veterinary medicinal products (IVMP), as recently reported in human vaccines[6]. Addressing these issues afterwards could possibly impact the animal health and will be time and money consuming.

Hence, in addition to RD114 ad hoc measures (RT assay on cell seeds, testing of End of Production cells…), the proactive development of general guidance addressing the detection of adventitious genomic fragments or virus particles in IVMP would be advisable. In this regard, it should be underlined that the CHMP currently considers the drafting of a guideline on this topic. The development of a joint CVMP/CHMP guidance could thus be considered by the parties. This process needs however to be undertaken with caution, as the benefit-risk balance will probably be quite different between human and veterinary vaccines.

3. Overall summary of the scientific evaluation

In summary, the risk that retrovirus RD114 can integrate into the genome of humans, dogs or big felidae, although theoretical at this stage, cannot be ruled out. Retrovirus RD114 might present a theoretical pathogenicity, especially when injected. However it has to be acknowledged that to date, no study supports this presumption. The hazard represented by replicative retrovirus RD114 cannot currently be defined. Consequently, the risk of its presence in cat and dog vaccines is not quantifiable, however the impact of this risk is probably low on animal health and extremely low on human health.

The benefits of vaccination against cat and dog diseases clearly outweigh the potential risk linked to the presence of replicative retrovirus RD114 in vaccines. Retrovirus RD114 is probably not systematically present in each batch and, if present, at a relatively low amount. Consequently, the threat represented by retrovirus RD114 is currently theoretical. No prompt regulatory action is warranted, although corrective actions need to be undertaken over the coming years.

Additional information about pathogenicity of retrovirus RD114 is clearly desirable, however is impossible to generate in a short to medium time schedule, as it is part of fundamental research on retroviridae in general. Therefore much remains unknown in this area. Additional information is awaited from the ongoing testing of feline cells used in the manufacture of cat and dog vaccines to detect the presence of retrovirus RD114. These data will be necessary to complete the assessment of the benefit-risk balance and to agree on risk management measures, as needed.

On the other hand, it is considered not acceptable to have vaccine batches on the market containing unwanted live virus particles, without trying to investigate and correct this issue. Elimination of these particles will be achieved either by replacing the cell lines used and/or by introducing some manufacturing step allowing clearance of the virus. Inactivation of retrovirus RD114 might also be considered. Implementation of such corrective measures will take a long time, and implies close collaborative studies between authorities and industry (*action 1*).

It is suggested for the Agency to liaise with EDQM, to look to which extent collaborative studies with various OMCLs are feasible to validate the protocol that has been agreed earlier with industry and which is currently used by some companies to test for the presence of retrovirus RD114 in the cell lines of feline origin which are used for manufacturing of vaccines for use in animals. Should the outcome of the validation study either be satisfactory as such, or lead to amendments of the protocol, this (amended) protocol could then become an Ph. Eur. monograph to be implemented systematically for future feline cell Master Seeds to be used for manufacturing of veterinary vaccines (*action 3*).

The procedure for cell Master Seed replacement in such circumstances needs to be agreed upon, both from an administrative and a scientific point of view (*action 2*).

Additionally, the proactive development of general guidance addressing the detection of adventitious genomic fragments or virus particles in immunological veterinary medicinal products would be advisable. In this regard, it should be underlined that the CHMP are currently considering the drafting of a guideline on this topic. The development of a joint CVMP/CHMP guidance could therefore be considered by the parties, keeping in mind that the benefit-risk balance might differ considerably between human and veterinary situations.

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