2. 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 — Ireland…
(b) Notification number — B/IE/12/02
(c) Date of acknowledgement of notification — 14/December/2012
(d) Title of the project: 'A blinded, placebo controlled, clinical and efficacy study of Equilis RhodE in horses in Ireland'.

(e) Proposed period of release — From 13/03/2013 until 30/09/2016

2. Notifier

Name of institution or company: Intervet International B.V., Wim de Korverstraat 35, NL - 5831 AN Boxmeer, the Netherlands.

3. GMO characterisation

(a) Indicate whether the GMO is a:

viroid (.)

RNA virus (.)

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

(b) Identity of the GMO (genus and species)
Genus: *Rhodococcus*
Species: *Rhodococcus equi (*Deletion mutant of Rhodococcus equi strain RG2837)

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

Genetic transfer is limited to exchange of plasmids. The main factor for this is close contact between different *R. equi* organisms and the receiving organism should not already contain a $VapA^+$ plasmid. Analysis of the available literature does not provide any reason to assume that transduction and transformation play an important role in the natural environment of *R. equi*. Furthermore natural competence has not been reported for any *Rhodococci*.

4. Is the same GMO release planned elsewhere in the Community (in conformity with Article 6(1)), by the same notifier?
   Yes (X) No ()

   If yes, insert the country code(s) NL and DE

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

Yes (X) No (.)

If yes:

- Member State of notification : NL and DE
- Notification number B/NL/09/004 and BVL 107/2012/4

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.

Rhodococcus equi is a common soil bacterium that also can colonize the gut and nasal passages of animals, especially herbivores, and is the cause of severe pneumonia in foals. The GMO (vaccine strain) is shed in the environment with the manure of vaccinated animals during a short period following vaccination (is detectable for at least 4 weeks post vaccination). The GMO is an unmarked deletion mutant of *Rhodococcus equi*. Because of this deletion the bacterium is less able to survive in macrophages (in contrast to the wild type) and therefore safe for foals (in contrast to the wild type). The deletions do not provide any competitive benefits outside the vaccinated animals, compared to wild type *R. equi.* The attenuation (deletion of the *ipdAB1* and *ipdAB2* genes from the chromosome and therefore less able to survive in macrophages), does not play a role outside the animal. It must be assumed that the vaccine strain will be shed into the environment, where it will behave the same in soil as wild type *R. equi*. This was confirmed by spiking experiments where the vaccine strain and the parent strain both survived for more than a year in soil and water and no

difference between the two strains were apparent. However, the attenuation will reduce the spreading by horses or other animals. Except for a hampered macrophage survival no differences in survivability between vaccine strain and wild type have been observed, so it is not clear whether the deletions have any negative competitive effect in certain environments (e.g. environments enriched with steroids). The most negative assumption is that outside the host there is no difference in survivability between vaccine strain and wildtype. Given the nature of the vaccine strain (unmarked deletion mutant, with no additional genes introduced into the environment), environmental impact of release of the GMO is judged 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 (.)

bacterium (X)

fungus (.)

animal

- mammals (.)
- insect (.)
- fish (.)
- other animal (.)
  (specify phylum, class) …

other, specify …

2. Name

(i)	order and/or higher taxon (for animals)	…
(ii)	genus	*Rhodococcus*
(iii)	species	*Rhodococcus equi*
(iv)	subspecies	
(v)	strain	RE1
(vi)	pathovar (biotype, ecotype, race, etc.)	…
(vii)	common name	…

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 (.)

(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: The bacterium occurs world-wide in soil and surface water, especially where herbivores graze. In these animals it colonizes nasal cavities and gut. In foals colonization of airways can lead to pneumonia.

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	(X)
soil, free-living	(X)
soil in association with plant-root systems	(.)

in association with plant leaf/stem systems (.)
other, specify …

If the organism is an animal:

5. (a+b) Detection and identification techniques

Isolation on selective agar and PCR followed by bacteriological determination and PCR

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 EC class 2 organism (EC 2000/54/EG)

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

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

If yes:

(a) to which of the following organisms:

humans (X) only immunocompromised humans
animals (X) pneumonia in foals
plants (.)
other (.)

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

The parent organism is a facultatively pathogenic soil saprophyte. The live wildtype *Rhodococcus equi* can cause pneumonia in foals and can cause infections in immunocompromised humans (e.g. AIDS patients). *R equi* rarely infects immunocompetent humans. *R. equi* strains are also isolated from pigs, where they can cause tuberculosis-like lesions, but can also be found in submandibular lymph nodes and tonsils of healthy animals. *R. equi* also cause tuberculosis-like lesions in lymph nodes of cattle and in the livers of young goats. Other animals occasionally infected by *R. equi* are sheep, llama, cats and dogs. Of all species, disease caused by *R. equi* infection in foals is by far the most devastating. *R. equi*, which normally is found in various environments from soil and ground water, infects the foal by inhalation of aerosolized dust contaminated with these bacteria, invades, survives and multiplies in alveolar macrophages by arresting the normal pathway of phagosome maturation. Neutrophilic leucocytosis and hyperfibrinogenaemia

are common findings, associated with abscessation and pulmonary changes. Experimental data suggest that *R equi* is capable of inhibiting oxidative bactericidal functions of polymorphonuclear cells. Electron microscopy of *R equi* in equine macrophages demonstrates that the organisms appear to avoid being killed by interfering with phagosome-lysosome fusion. Most of the information about the pathogenesis of *R equi* infections is derived from animal isolates. However, the infection in humans seems to differ from that in foals. A 15- to 17-kd virulence-associated protein antigen (VapA), is highly associated with virulence in foals. Nearly all isolates from pigs have a 20-kd virulence-associated protein antigen (VapB). In human beings, only about 20-25% of isolates have been reported to express either VapA, or VapB. The rest does not have Vap or VapB encoding genes. There are no reports about toxigenicity, allergenicity or vectors.*R. equi* infections of foals occur worldwide. Increased incidences of *R. equi* pneumonia is associated with large farm size, high density and population size of foals, high numbers of airborne virulent *R. equi*, low soil moisture, high temperatures and a poor pasture grass cover. Farms with endemic *R. equi* pneumonia are heavily contaminated with virulent *R. equi*. However avirulent *R. equi* are frequently found in environment and faeces on every farm.

In the first weeks of a foal's life, ingestion of *R. equi* often leads to colonization of the intestines. Foals shed large quantities of *R. equi* as compared with adults, but the number of bacteria in faeces declines after 7 weeks of age. Ingestion of *R. equi* does not usually result in disease, but in immunization. As a result of this process, older foals and adult animals have antibodies against *R. equi* and rarely get infected.

Virulence of *R. equi* is associated with the possession of Virulence Associated Proteins (VAPs) that are encoded by the virulence plasmid. The plasmid is essential for multiplication in macrophages, prolonged inhibition of phagosome maturation and it enhances cytotoxicity. Isogenic strains from which the plasmid has been removed are avirulent in foals and mice and do not multiply in macrophages.

So far, three types of VAPs have been identified, two of which have been sequenced and further investigated. Whereas possession of certain VAPs seems to be specific for strains infecting foals (VapA+), pigs (VapB+) or cattle (VapAB-). By contrast all three plasmid types could be found in *R. equi* strains from humans, a host in which the infection is opportunistic and associated with immunosuppression. Additionally, strains devoid of virulence plasmids are regarded as non-pathogenic for foals and mice have also been isolated in immunocompromised humans. Immunocompetent humans are rarely affected by *R. equi*, while a compromised cell mediated immunity predisposes one to *R. equi* infection. As with other immunocompromised individuals, infection mostly results in pneumonia with fever, cough, and chest pain, but can also

spread to other organs and cause bacteraemia. The fact that human isolates from pathological conditions have all types of plasmid categories, including plasmid less strains, indicates that the immunocompromised human host is susceptible to a variety of *R. equi* strains and emphasises the opportunistic nature of *R. equi* in this host.

The minimum infective dose under natural conditions is not known for any species, including humans because it never has been determined. In the artificial intratracheal challenge model, doses from 10^4 CFU and higher appear infectious.

8. Information concerning reproduction

(a) Generation time in natural ecosystems:
Depending on conditions 30 min to days

(b) Generation time in the ecosystem where the release will take place:
See previous

(c) Way of reproduction: ~~Sexual~~ ..
Asexual: cell division

(c) Factors affecting reproduction:
temperature, nutrients

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:
temperature, pH, availability of nutrients

10. (a) Ways of dissemination
Wind (attached to dust particles), animals (nasal and or gut colonization)

(b) Factors affecting dissemination
Presence of herbivores, housing practices and weather conditions

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)

Not applicable

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

1. Type of the genetic modification

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

2. Intended outcome of the genetic modification
Unable to survive in macrophages (in contrast to wildtype)

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 ()

Only 2x6 nucleotides (GATATC) and (AGATCT) remain at the two ligation sites. These nucleotides are remnants of the plasmid used for gene deletion.

.

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

(b) Identity of the vector
…

(c) Host range of the vector
…

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

antibiotic resistance (.)
other, specify …

Indication of which antibiotic resistance gene is inserted
…

(e) Constituent fragments of the vector
…

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

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

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

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

6. Composition of the insert

Not applicable (deletion mutant).

(a) Composition of the insert
…

(b) Source of each constituent part of the insert
…

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

(d) Location of the insert in the host organism

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

(e) Does the insert contain parts whose product or function are not known?
Yes (.) No (.)

If yes, specify …

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

1. Indicate whether it is a:

viroid (.)

RNA virus (.)

DNA virus (.)

bacterium (.)

fungus (.)

animal

- mammals (.)
- insect (.)
- fish (.)
- other animal (.)
  (specify phylum, class) …

other, specify …

2. Complete name

(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 …

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

If yes, specify …

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

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

Specify

*Yes: the GMO is less able to survive in macrophage. Invasion and growth of the

alveolar macrophages is essential for the development of pneumonia.

**No: there appears to be no difference in survival in the environment.

(b) is the GMO in any way different from the recipient as far as mode and/or rate of reproduction is concerned?

Yes (X)* No (X)** Unknown (.)

Specify

*Yes: the GMO is less able to survive and reproduce in macrophage. Invasion and growth of the alveolar macrophages is essential for the development of pneumonia.

**No: there appears to be no difference in growth in the environment.

(c) is the GMO in any way different from the recipient as far as dissemination is concerned?

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

Specify

*Yes: as the GMO is less able to grow in the lungs there will be less dissemination.

**No: there appears to be no difference in growth in the gut and therefore rectal

excretion will be similar.

(d) is the GMO in any way different from the recipient as far as pathogenicity is concerned?

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

Specify

The GMO is less able to survive and grow in alveolar macrophage, the site of infection that is central in the development of R. equi pneumonia in foals.

2. Genetic stability of the genetically modified organism
See section A.3.c

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
The GMO cannot be monitored directly in the environment. Indirect monitoring can be done by taking samples and plate them out on selective agar. Positive

identification will follow from R. equi and GMO specific PCR's.

(b) Techniques used to identify the GMO
Selective agar and PCR's based on the genome region that has been modified.

F. Information relating to the release

1. Purpose of the release (including any significant potential environmental benefits that may be expected)
Study the efficacy of the vaccine under field conditions

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 (.) No (X)

If yes, specify …

3. Information concerning the release and the surrounding area

(a) Geographical location (administrative region and where appropriate grid reference):
The vaccine will be tested in foals on Belmont Stud Farm, Belmont, Co. Offaly
Grid reference: N53° 15.072 W007°53.887

(b) Size of the site (m^2): 52.4 Ha.
(i) actual release site (m^2): 1.11 Ha.
(ii) wider release site (m^2): 52.4 Ha

(c) Proximity to internationally recognised biotopes or protected areas (including drinking water reservoirs), which could be affected:

The farm is located in an agricultural area. The area is characterised by a clay soil covered with permanent pasture. The nearest Special Area of Conservation (SAC) is Clara Bog, located 22 km from the release site and therefore not affected by the release of the vaccine strain.

Groundwater is the principle source of water in Offaly. 72% of the people of Offaly receive water from schemes with groundwater sources, compared with a national average of less than 25%. A groundwater protection scheme has been adopted for all public and group water supply sources in Offaly. Groundwater, which is the source for a large proportion of the drinking water in Co. Offaly, receives a precautionary dose of chlorine to provide disinfection. Surface water is the source for 4 drinking water supplies in Co. Offaly, namely. The River Shannon is the closest supply source for Belmont through the Banagher Regional Water Supply. A tributary of the Shannon is adjacent to the Front Field of Belmont farm and the horses use it for drinking. The closest point of entry to the Shannon is approximately 8 km form the release site. The Shannon river sources receive full physio-chemical treatment, consisting of coagulation, flocculation, sedimentation, filtration, pH correction and disinfection. The release of this organism will have no impact on water quality. As *R. equi* is widespread in the environment on all horse farms in the hinterland and the vaccine strain does not introduce any new genes or plasmids into the environment, the risk is effectively zero.

(d) Flora and fauna including crops, livestock and migratory species which may potentially interact with the GMO
Rodents and birds can come into contact with pasture. Neighbouring farms contain cattle.

4. Method and amount of release

(a) Quantities of GMOs to be released:
Vaccine dose consists of approximately 0.5 -9 x 10^{10} CFU per dose. Up to 300 doses will be used.

(b) Duration of the operation:
Field trial on one farm; duration per location approximately 12-24 months.

(c) Methods and procedures to avoid and/or minimise the spread of the GMOs beyond the site of the release

The foals will be physically contained on the release site, in stables and/or (fenced) pasture, and remain there for at least 6 weeks after last vaccination (peak shedding is expected the first few days after rectal vaccination). The straw and litter of the foals during the first week after each vaccination, the period of peak shedding, will be removed mechanically into closed containers. The straw and litter will be heat-inactivated by a specialised and approved company. Each year the stables on the release site will be cleaned and disinfected.

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

The weather conditions are as usually found in Ireland

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.

None available

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)	Animals / Vertebrates / Mammals / Equidae
(ii)	family name for plants	
(iii)	genus	Equus
(iv)	species	ferus
(v)	subspecies	caballus
(vi)	strain	…
(vii)	cultivar/breeding line	all breeds
(viii)	pathovar	…
(ix)	common name	…

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

Vaccine strain will be present transiently in the intestine and interact with the local lymph nodes and thereby inducing a protective immune response

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

Outside the animal host the vaccine strain will behave similar to the wild 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
   In the soil (pasture) where the horses graze.

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
   None known

(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:
   Unlikely

   (b) from other organisms to the GMO:
   Unlikely

      (a) likely consequences of gene transfer:
      Consequences of genes transfer will be unlikely. Occurrence of gene transfer is not more likely than for wildtype R. equi.

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.):
   Not available

9. Possible environmentally significant interactions with biogeochemical processes (if different from the recipient or parental organism)
   None

H. Information relating to monitoring

1. Methods for monitoring the GMOs
   Isolation on selective agar and further identification by PCR.

2. Methods for monitoring ecosystem effects
   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. It is a deletion mutant.

4. Size of the monitoring area (m^2)
   1.11 Ha.
   This corresponds to the area of all buildings where vaccine administration will take place and the pastures where the foals will be held for up to one week after vaccine administration. Peak shedding is expected to occur during this period.

5. Duration of the monitoring
   Up to three consecutive foaling seasons: from 1st vaccination until the last vaccinated foals reaches the age of 6 months.

6. Frequency of the monitoring
   During the 14 days after each vaccination the animals will be monitored daily. Later the foals will be examined every 2 weeks and also the environment will be monitored with 14 day intervals.

I. Information on post-release and waste treatment

1. Post-release treatment of the site

Each year after the last study animals have been vacinated, the release site will be cleaned as follows. After the complete removal the straw and litter, the stable, the total concrete floor before the stable and all equipment used will be cleaned with water and disinfected. No post-release treatment of the paddocks is necessary; it is an attenuated deletion mutant.

2. Post-release treatment of the GMOs
   See above.

3. (a) Type and amount of waste generated
Vials, syringes, applicators and up to 1000 m^3 of straw and litter

3. (b) Treatment of waste
By heat inactivation by an approved company or immersion in an appropriate disinfectant

J. Information on emergency response plans

1. Methods and procedures for controlling the dissemination of the GMO(s) in case of unexpected spread
   Controlling spread is not necessary since it is an attenuated deletion mutant, there is not more risk than the already present wildtype Rhodococcus equi. See section J4

2. Methods for removal of the GMO(s) of the areas potentially affected
   Not applicable (see above).

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

Not applicable (see above).

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 vaccine strain RG2837, an emergency

plan has been established in which three operating phases are implemented.

1. Alert phase

Any observation which cannot be related to normal post vaccination reactions must be reported to the investigator and to the monitor of the trial.

2. Investigation phase

Appropriate samples are collected and sent to the laboratory for isolation and identification. If present, diseased animals will be treated with antibiotics. Dead animals will be destroyed. In the unlikely case that humans are affected they also will be treated with antibiotics.

3. Action phase

The study will be cancelled and the unit will be cleaned and decontaminated by using an approved disinfectant. The animals will remain in isolation until a decision has been taken by the applicant in consultation with the responsible authorities concerning the consequences for the animals. This may, for instance, consist of antibiotic treatment, monitoring of shedding or a combination of measures.