anses
French agency for food, environmental and occupational health & safety

Investigate, evaluate, protect

Risk of introduction of lumpy skin disease into France

ANSES opinion
Collective expert report

March 2017 Scientific Edition

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The Director General

Maisons-Alfort, 14 March 2017

OPINION
of the French Agency for Food, Environmental and Occupational Health & Safety

on the "risk of introduction of lumpy skin disease into France"

ANSES undertakes independent and pluralistic scientific expert assessments.
ANSES's public health mission involves ensuring environmental, occupational and food safety as well as assessing the potential health risks they may entail.
It also contributes to the protection of the health and welfare of animals, the protection of plant health and the evaluation of the nutritional characteristics of food.

It provides the competent authorities with the necessary information concerning these risks as well as the requisite expertise and technical support for drafting legislative and statutory provisions and implementing risk management strategies (Article L.1313-1 of the French Public Health Code).

Its opinions are published on its website.
This opinion is a translation of the original French version. In the event of any discrepancy or ambiguity the French language text dated 14 March 2017 shall prevail.

On 18 May 2016, ANSES received a formal request from the DGAL to conduct an expert appraisal on the risk of introduction into France of lumpy skin disease.

1. BACKGROUND AND PURPOSE OF THE REQUEST

On 18 May 2016, ANSES received a formal request from the Directorate General for Food (DGAL) to conduct an expert appraisal on the risk of introduction into France of lumpy skin disease (LSD).

Since August 2015, several outbreaks of LSD have been declared in Greece, probably following introduction of the disease from Turkey, which has been suffering from an animal epidemic for the past few years. In April 2016, outbreaks were declared in Bulgaria and the Former Yugoslav Republic of Macedonia (FYROM). Since then, the epidemic has spread considerably, with numerous outbreaks in Serbia, Kosovo, Albania and Montenegro. France is officially free of this infection.

LSD affects cattle and is caused by a virus belonging to the genus *Capripoxvirus*, of the family *Poxviridae*. It is included in the list of diseases of the World Organisation for Animal Health (OIE), and is a notifiable disease that must be reported to the European Commission and the Member States (Directive 82/894/EEC[1], Decision 89/162/EEC[2]). It has also been classified as a Category 1

[1] Council Directive 82/894/EEC of 21 December 1982 on the notification of animal diseases within the Community
[2] 89/162/EEC: Commission Decision of 10 February 1989 supplementing the annexes to Council Directive 82/894/EEC on the notification of animal diseases within the Community

health hazard in France (Ministerial Order of 29 July 2013 on the definition of Category 1 and 2 health hazards for animal species).

The increase in the number of outbreaks in the European Union (EU) raises the question about the risk of introduction of the infection into France.

For this reason, through this formal request, ANSES is being asked to:

1. assess the risk of contamination for France taking into account the different risk factors regarding its introduction;
2. estimate the appropriate size for a vaccine (or antigen) bank, to manage an emergency vaccination campaign in the event that the disease were introduced.

2. ORGANISATION OF THE EXPERT APPRAISAL

The expert appraisal was carried out in accordance with French Standard NF X 50-110 "Quality in Expert Appraisals – General Requirements of Competence for Expert Appraisals (May 2003)".

The formal request falls within the sphere of competence of the Expert Committee on "Animal health and welfare" (CES SABA). ANSES entrusted examination of this formal request to the "LSD" Working Group, reporting to the CES SABA.

The LSD WG was made up of eight experts, who met on eight occasions between 23 June 2016 and 18 January 2017. The methodological and scientific aspects of this group's work were submitted to the CES on 13 September, 11 October and 6 December 2016, and 10 January 2017. The report was presented to the CES for validation on 7 February 2017. The expert appraisal report issued by the LSD WG takes into account the comments and additional information provided by the members of the CES. These analyses and conclusions are derived from collegial expert appraisal work conducted within a group of experts with complementary skills. The expert appraisal was coordinated by the Unit for the assessment of food and animal health-related risks (UERSABA), which was assisted by an ANSES expert from the French Agency for Veterinary Medicinal Products (ANMV).

ANSES analyses the links of interest declared by the experts prior to their appointment and throughout the work, in order to avoid potential conflicts of interest with regard to the matters dealt with as part of the expert appraisal.

The experts' declarations of interests are made public via the ANSES website (www.anses.fr).

The assessment was conducted with the help of:

- information extracted from the TRACES (TRAde Control and Expert System) database provided by the DGAL concerning imports of live cattle;
- information extracted from the Eurostat database concerning products of animal origin;
- data on notification of LSD cases from the ESA Platform (National Epidemiological Surveillance Platform for Animal Health) and the FAO (Empress-i; Global Animal Disease Information System). The number of cases was last updated on 29 November 2016;
- densities of cattle in France, calculated from data taken from semi-final annual agricultural statistics for 2014 and 2015 produced by Agreste;

- the regulatory texts cited throughout the WG's report in the form of footnotes;
- reports of meetings, internships, etc. or other information published in the press, most often available online, mentioned in footnotes;
- hearings with international specialists on LSD;
- the scientific publications listed in the references section at the end of the WG's report.

For the first question in the formal request, the experts examined the probability of the first outbreak of LSD occurring in France. To do this, they took into account the probability of the virus being introduced into France and the probability of exposure of a native bovine animal to this virus. In this report, the experts did not assess the consequences that might arise from the occurrence of this first outbreak in France.

For the second question, the experts examined the number of doses of vaccine needed to halt the spread of the disease if it were introduced into France, without taking into account the efficacy and safety of the vaccines available, or the management measures associated with this possible vaccination.

3. ANALYSIS AND CONCLUSIONS OF THE CES SABA AND THE LSD WG

3.1. Lumpy skin disease – the virus

➢ Background

Lumpy skin disease (LSD) is a viral disease of cattle caused by a virus belonging to the family *Poxviridae*, of the genus *Capripoxvirus*. It is characterised by the appearance of numerous nodules on the skin and the internal mucous membranes.

Following the first observation in Zambia in 1929, the continuous spread of LSD has been observed in most countries on the African continent, as well as in Madagascar. The transmissibility of the infective agent was first demonstrated in 1945, and the virus was first isolated in cell culture in 1957.

➢ Characteristics of the virus

The structure of the LSD virus (LSDV) is consistent with the usual standard for poxviruses. While it is antigenically similar to the other known Capripoxviruses, the LSDV is nevertheless distinct. Antigenic variability is very low within the species, and just one antigenic type of LSDV has been identified. For an enveloped virus, this virus is relatively resistant to physical and chemical agents. This is particularly true in organic matter and at low temperatures. This virus grows well *in vitro*, mainly in ruminant cells.

➢ Pathogenesis

Following transcutaneous inoculation of the LSDV, the infection spreads through the body via the lymph vessels and then the blood vessels (transient viraemia), before reaching the mucocutaneous tissue and certain internal organs (mainly the digestive mucosa, kidneys, testes). Viral titres are low in saliva, nasal discharge and semen, and are highest in the skin nodules. It should be noted that experimental infections indicate that the intravenous route is best suited to achieving generalised infection.

- **Clinical manifestation**

The classic clinical form includes a prodromal phase (hyperthermia, adenitis, mucous membrane effects) followed by a rash phase and then a necrosis phase. Mild and severe forms can also be observed. The macroscopic lesions correspond to skin, subcutaneous and other tissue nodules (respiratory tract, digestive tract, lymph nodes, etc.).

In the epidermis, microscopic lesions are mainly manifested as extensive necrosis. In the dermis, a vascular necrosis can be observed. This causes vessel thrombosis, which in turn is responsible for tissue necrosis. Phenomena of acanthosis, parakeratosis and hyperkeratosis are also observed in the epidermis and the mucous membranes.

- **Epidemiological characteristics**

Natural infection associated with the development of clinical forms is only observed in cattle, zebus and water buffaloes. Due to the frequency at which they have been affected in all the countries recognised as infected, and their sensitivity, cattle are the primary source and host of the LSDV. According to some natural and experimental observations, domestic small ruminants and various species of wild ruminants are considered to be susceptible species. However, they do not seem likely to play a significant role in the epidemiology of the disease. Nonetheless, studies are needed to determine their actual role.

LSDV is detectable in many different cattle products and by-products, such as hide, milk and semen, and can be found on vehicles and materials that have been in contact with infected cattle.

The literature provides very little information on the European arthropod vectors currently involved in spreading the LSD virus in Eastern Europe, whether regarding the species involved or the mechanisms of transmission. Moreover, to date, only the mechanical transmission capacity of LSDV vectors has been studied, and the biological transmission capacity of the LSDV among vectors is unknown. All cattle-biting arthropods found in Europe (*Stomoxys*, horse-flies, mosquitoes and ticks) can potentially play a role in the transmission of the LSDV from one bovine animal to another.

In addition to the virus being dispersed by the vectors, it could also be spread passively, over longer distances, by movements of vehicles that may be contaminated when transporting potentially-infected animals, or products or by-products of bovine origin.

3.2. Diagnosis and control of the disease

The tests used most frequently to identify the LSDV are molecular tests based on PCR, conventional or in real time, although this virus can also be identified using immunohistochemistry or immunofluorescence. The reference serological test for *Capripoxvirus* is the virus neutralisation test. An ELISA test to detect the LSDV is not yet commercially available.

In the current, fairly limited, state of knowledge on the efficacy and safety of vaccines against LSD, the choice of the Neethling strain as the vaccine strain seems to be the only option available for the moment. The concept of efficacy expresses the sum of the therapeutic indications claimed by the manufacturer, validated after primary vaccination until the booster, and demonstrated by laboratory

and field studies. Regarding vaccines against LSD, there are no such studies available, since no marketing authorisation (MA) application has been submitted, either in France or to the European Medicines Agency. No experimental data on the safety and efficacy of the vaccines, as recommended by Directive 2009/9/EC, are currently available.

The efficacy of vaccination within the EU is not well documented.

The degree of attenuation of the strain is an essential parameter: if it is excessively attenuated, it will be relatively ineffective; if it is insufficiently attenuated, the frequency and intensity of the adverse effects will be increased. In every case, the degree of attenuation should be the result of a compromise between safety and efficacy. Overall, in the available studies, there are very few data from which to determine the degree of attenuation constituting the best compromise. Information available on the safety of vaccines against LSD used in the EU, from pharmacovigilance reports, remains very fragmentary. The main adverse effects noted are in fact those of LSD, namely: a fall in milk production, fever, nodular skin lesions, abortion and death. The incidence of adverse effects is around 0.1%.

While vaccination is not recommended in the disease-free zone, it is found to be the only effective means of controlling the spread of the disease in an epidemic situation, advocated by the EU as long as the vaccine provides adequate guarantees of safety and efficacy. The EU has authorised vaccination in the Member States concerned, for example in Bulgaria in 2016[3], with some vaccines that demonstrated their efficacy in countries outside the EU (Israel and South Africa). Although these vaccines have no MA application dossier in the countries of the EU, this option is provided for by the European regulations in the event of a serious animal epidemic[4]. All the infected countries and some of their neighbours have established vaccine protocols.

The slaughter of susceptible species in an outbreak and the establishment of zones to regulate the transport of susceptible species are indispensable but, with a few exceptions, are insufficient to limit the spread of the virus. The use of insecticides is probably useful for controlling the disease, although the mechanical vectors are still poorly understood, especially in Europe.

3.3. Assessment of the probability of a first outbreak of LSD occurring in France

In order to respond to the first question of the formal request regarding the risk of introduction of LSD into France, and given the time available, **the experts assessed "only" the probability of a first outbreak of LSD on French territory for a year, based on the epidemiological situation in January 2017, the European regulations existing on this same date, and data on trade for 2016**. They did not take into account either the dissemination from the first outbreak, or the consequences of introduction of the LSDV.

The probability of a first outbreak of LSD occurring in France results from combining the probability of the virus being introduced into France with the probability that domestic cattle or wild ruminants are then exposed to this virus on French territory. The expert group, taking into account all the scientific and commercial data at its disposal, conducted an assessment of the risk of a first outbreak of LSD occurring in France, depending on the different virus sources and the possible

[3] Commission Implementing Decision (EU) 2016/1183 of 14 July 2016 approving the emergency vaccination programme against lumpy skin disease of bovine animals in Bulgaria and amending the Annex to Implementing Decision (EU) 2016/645

[4] Article 8 of Directive 2001/82/EC of the European Parliament and of the Council of 6 November 2001 on the Community code relating to veterinary medicinal products

ways in which they could be introduced (by live animals and their products - semen and embryos, by vectors, by inert media, etc.).

On the date the report was written, none of the countries bordering France had reported any infection with LSD. The experts defined a risk zone for the purpose of the analysis: a zone from which live cattle or products can be traded and in which there is a probability that certain animals are infected, without the disease having been declared. This concerns disease-free regions of European countries recognised as infected (as of 1 January 2017: Greece, Bulgaria, FYROM, Kosovo, Serbia, Albania, Montenegro) and disease-free countries bordering a country where LSD has been notified (as of 1 January 2017: Romania, Croatia, Hungary, Ukraine, Bosnia & Herzegovina).

The risk assessment was carried out according to a quantitative approach for the methods of introduction regarded by the experts as most likely (movements of animals, movements of arthropod vectors). The characteristics of the model are developed in the LSD WG's report. In the other cases, the approach was qualitative.

The values of the variables used in the model developed for the quantitative risk assessment can easily be modified later, depending on the evolution of the epidemiological situation in Europe, data relating to trade between the various Member States, and also advances in knowledge, in particular, on the vectors or the methods of transmission of the LSDV. The model could also be modified to incorporate vaccination.

The probability of a first outbreak of LSD occurring was studied according to the different possible virus sources.

The paragraphs below only mention the cases of introduction of LSD by live cattle and by infectious vectors from the LSD WG's report. The other means of introduction and their associated probabilities are shown in the summary table at the end of this section (Table 1) and are developed in the WG's report.

- **Probability of LSD being introduced by live animals**

Only animals from the EU countries belonging to the risk zone (Greece, Bulgaria, Romania, Croatia, Hungary) were taken into account in the analysis because they are the only ones in the risk zone that can trade live cattle with France. The probability of LSD being introduced by live animals is limited to the risk of introduction by cattle. The quantitative model used to calculate the probability of a first outbreak of LSD occurring took into account the probability of the LSDV being introduced into France by an infected live bovine animal and the probability of exposure of a native bovine animal to this infected live animal.

The probability of a first outbreak of LSD in France, following the introduction of infected live cattle intended for rearing, is estimated to be extremely low to low (probability between 0.004% and 0.32%, with a confidence interval of 95%) for a year, based on the epidemiological situation in January 2017, the European regulations existing on this same date, and data on trade for 2016.

Currently there are no cattle intended for the slaughterhouse being introduced from the risk zone. The probability of a first outbreak of LSD in France following the introduction of infected live cattle intended for the slaughterhouse is therefore estimated to be nil.

The experts considered, however, that if there were as many cattle intended for the slaughterhouse introduced into France as the number introduced for rearing, the probability would be nearly nil to minute (probability between 0.2 10^{-6} and 47 10^{-6}, with a confidence interval of 95%)

for a year, based on the epidemiological situation in January 2017, the European regulations existing on this same date, and data on trade for 2016.

➢ Probabilities of an outbreak of LSD occurring following the introduction of infectious vectors

The risk of LSD being introduced by the long-distance road transport of vectors is limited to the risk of introduction by *Stomoxys* (the role of horse-flies was estimated to be null in these conditions because they do not get inside the vehicles, and the *Aedes* present in our regions are essentially anthropophilic).

The probability of a first outbreak of LSD in France, following the introduction of infectious vectors transported with cattle intended for rearing, is estimated to be extremely low to low (probability between 0.002% and 0.44%, with a confidence interval of 95%) for a year, based on the epidemiological situation in January 2017, the European regulations existing on this same date, and data on trade for 2016.

Currently there are no cattle intended for the slaughterhouse being introduced from the risk zone. The probability of a first outbreak of LSD in France following the introduction of infectious vectors transported with live cattle intended for the slaughterhouse is therefore estimated to be nil.

The experts considered, however, that if there were as many cattle intended for the slaughterhouse introduced into France as the number introduced for rearing, the probability would be nearly nil to minute (probability between 0.1 10^{-6} and 27 10^{-6}, with a confidence interval of 95%) for a year, based on the epidemiological situation in January 2017, the European regulations existing on this same date, and data on trade for 2016.

The probability of a first outbreak of LSD in France following the introduction of infectious vectors transported with horses is estimated to be nearly nil (probability between 0.01 10^{-6} and 1.66 10^{-6}, with a confidence interval of 95%) for a year, based on the epidemiological situation in January 2017, the European regulations existing on this same date, and data on trade for 2016.

➢ Conclusion on the risk of LSD being introduced into France

Table 1: Summary of the probabilities of a first outbreak of LSD occurring in France

Methods of introduction of the LSDV	**Assessment of the probability of a first outbreak of LSD occurring** **Scale from 0 (nil) to 9 (very high) (AFSSA, 2008)** **(quantitative equivalent of qualitative scores, AFSSA, 2008)**
By infected live cattle intended for rearing	**[3 to 5]** (extremely low to low) (quantitative probability between 0.004% and 0.32% with a confidence interval of 95%, for one year, based on the epidemiological situation in January 2017, the European regulations existing on the same date and data on trade for 2016)

Methods of introduction of the LSDV	Assessment of the probability of a first outbreak of LSD occurring Scale from 0 (nil) to 9 (very high) (AFSSA, 2008) (quantitative equivalent of qualitative scores, AFSSA, 2008)
By *Stomoxys* that travelled with the cattle intended for rearing (according to the assumptions made by the experts: no unloading and no insect eradication)	**[3 to 5]** (extremely low to low) (quantitative probability between 0.002% and 0.44% with a confidence interval of 95%, for one year, based on the epidemiological situation in January 2017, the European regulations existing on the same date and data on trade for 2016)
By infected live cattle intended for slaughter (method not confirmed in 2016, scenario using the same introduction data as those for rearing)	**[1 to 2]** (nearly nil to minute) (quantitative probability between 0.2 10^{-6} and 47 10^{-6} with a confidence interval of 95%, for one year, based on the epidemiological situation at the beginning of 2017, the European regulations existing on the same date and data on trade for 2016)
By *Stomoxys* that travelled with the cattle intended for slaughter (method not confirmed in 2016, scenario using the same introduction data and the same assumptions as those for rearing)	**[1 to 2]** (nearly nil to minute) (quantitative probability between 0.1 10^{-6} and 27 10^{-6} with a confidence interval of 95%, for one year, based on the epidemiological situation in January 2017, the European regulations existing on the same date, and data on trade for 2016)
By fresh semen, or frozen pre-stored ova or embryos (methods not confirmed in 2016, scenario simulating a low number of introductions from the risk zone)	**[1 to 2]** (nearly nil to minute)
By non-frozen ova or embryos (methods not confirmed in 2016, scenario simulating a low number of introductions from the risk zone)	**[1 to 2]** (nearly nil to minute)
By transport vehicles that have been in contact with infected cattle	**[1 to 2]** (nearly nil to minute)
By frozen semen stored for at least 30 days after collection and before shipping	**[1]** (nearly nil)
By *Stomoxys* that have travelled with horses intended for a mixed herd (cattle/equines) or arriving in a stud farm with a herd of cattle nearby (according to the same assumptions as those for rearing)	**[1]** (nearly nil) (probability between 0.01 10^{-6} and 1.6 10^{-6} with a confidence interval of 95%, for one year, based on the epidemiological situation in January 2017, the European regulations existing on the same date, and data on trade for 2016)

Methods of introduction of the LSDV	Assessment of the probability of a first outbreak of LSD occurring Scale from 0 (nil) to 9 (very high) (AFSSA, 2008) (quantitative equivalent of qualitative scores, AFSSA, 2008)
By transport vehicles that have been in contact with infected hides	**[0 to 1]** (nil to nearly nil)
By live domestic small ruminants	**[0 to 1]** (nil to nearly nil)
By live wild ruminants, animals from zoos or circuses	**[0 to 1]** (nil to nearly nil)
By the milk of infected cattle or buffaloes	**[0 to 1]** (nil to nearly nil)
By illegal imports of live animals or animal by-products	**[0 to 1]** (nil to nearly nil)
By fresh hides of infected cattle	**[0 to 1]** (nil to nearly nil)
By transhumance or other animal husbandry practices	**[0]** (nil)
By the meat of infected cattle	**[0]** (nil)
By the use of a live attenuated vaccine	**[0]** (nil)

3.4. Estimate of the size of a vaccine bank

For this estimate, the experts did not take into account the efficacy and safety of the vaccine, or the management measures associated with this possible vaccination.

Taking into account the speed at which the infection travels (7.3 km/week), the time needed to obtain satisfactory immunisation coverage of the population in question (the assumption being that the vaccination is applied to all cattle in the zone in a single injection), and the density of cattle in the region or *département* where the first outbreak may be located (the assumption being that the risk of the first outbreak occurring is directly proportional to the number of cattle in each *département*), the experts estimated, through simulations, the size of the stock of vaccines to be established.

Considering a period of 7 weeks (5 weeks before detection of the disease and 2 weeks to vaccinate the entire population) between the occurrence of the first outbreak and the end of the vaccination period, the experts calculated that 626,204 vaccine doses would be sufficient in 75% of simulations, and 798,128 doses in 95% of simulations, to vaccinate the exposed population taking into account the speed of viral spread following the discovery of an index outbreak (excluding long-distance spread, involving "leapfrogging"). If the speed of dissemination of the LSDV varies during the disease's spread in Europe, the number of vaccine doses needed may evolve. A new estimate

of the number of doses required can be calculated by introducing the new speed of viral spread into the model.

For the French *département* with the highest density of cattle (Mayenne), the experts calculated that 945,456 doses would be necessary in 95% of simulations.

3.5. Recommendations

The experts are able to make several recommendations following this assessment, not only regarding research, but also recommendations that focus more on preventing infection by the LSDV (the recommendations listed in the paragraphs below are not classified by order of importance).

➢ Research recommendations

- Concerning the vectors, the experts believe it is necessary to develop knowledge on:
  - the epidemiological role of *Stomoxys* and horse-flies:
    - the infectious dose;
    - the survival time of the LSDV in the vector, with a reduction in the time unit (measurements in hours and not in days);
    - dispersion of *Stomoxys* (active and passive) and trapping methods;
  - the epidemiological role of ticks:
    - the infectious dose;
    - transmission methods (mechanical versus biological) and associated issues (survival time in the vector or viral multiplication within the vector, transtadial or transovarial transmission, etc.).
  - host/pathogen interactions and in particular the effect of the vector's saliva in transmission of the LSDV;
  - *Stomoxys*, as well as European ticks, Culicoides and Culicidae, in particular regarding vector density in farms and the methods of assessing vector densities;
  - vector control in farms: insecticide treatment with its limitations and alternative control methods to be investigated (trapping, repellents, growth regulators, Hymenoptera parasitoids of *Stomoxys*, etc.).
- Concerning vaccines against the LSDV, it is important to:
  - have access to data on the safety and clinical and virological efficacy of the available vaccines;
  - develop a DIVA vaccine conferring a higher level of protection and without any residual pathogenicity, which would enable improved control;
- Concerning the LSDV, studies are still needed in order to:
  - develop an improved experimental model of infection integrating direct infection or vector-borne transmission, as well as the minimum infectious dose;
  - better understand the epidemiological role of small ruminants, and that of native wildlife in the countries currently infected;
  - identify the determinants of natural resistance;
  - assess the actual role of artificial insemination and embryo transfer in the transmission of LSD.

➢ **Recommendations concerning prevention of the disease and surveillance**

Most of the recommendations listed below are those typically given in the context of emerging diseases.

It appears important to:

- include LSD in the list of diseases to be screened for in the framework of artificial insemination or embryo transfer from countries at risk;
- improve and validate diagnostic methods, in particular ELISA serological methods for the detection of antibodies, in view of using them when animals are introduced from the risk zone;
- use the applicable serological and molecular diagnostic tools in the framework of a DIVA vaccination strategy (to differentiate infected from vaccinated animals);
- extend LSD surveillance, in the infected zone, to small ruminants and ruminants from circuses and zoos;
- maintain awareness among stakeholders in the sectors concerned;
- ensure the correct implementation and control of the application of insecticides and repellents in the trucks transporting livestock;
- develop a website devoted to monitoring the epidemiological situation of LSD in the EU, with a map, as is done for bluetongue[5];
- improve the traceability of live animal movements, in particular for animals from third countries;
- implement monitoring of feedback from the field regarding the situation in the Balkans and the vaccination implemented (study under way at EFSA).

[5] https://ec.europa.eu/food/sites/food/files/animals/docs/ad_control-measures_bt_restrictedzones-map.jpg

4. AGENCY CONCLUSIONS AND RECOMMENDATIONS

The French Agency for Food, Environmental and Occupational Health & Safety endorses the conclusions and recommendations of the CES SABA on the risk of introduction of lumpy skin disease into France.

DR ROGER GENET

KEYWORDS

Bovin, dermatose nodulaire contagieuse, appréciation de risque, transmission, stomoxes

Cattle, lumpy skin disease, risk assessment, transmission, *Stomoxys*

ANNEX 1: REFERENCES

AFSSA. 2008. Une méthode qualitative d'estimation du risque en santé animale [A qualitative method for estimating animal health risks]. Maisons-Alfort, France.

ANNEX 2

Presentation of participants

PREAMBLE: The expert members of the Expert Committees and Working Groups or designated rapporteurs are all appointed in a personal capacity*, intuitu personae,* according to their area of expertise*,* and do not represent their parent organisation.

WORKING GROUP

Chairman

Mr Jordi CASAL – Universitat Autonoma de Barcelona (Spain) – expertise in zoonoses, quantitative epidemiology, exotic animal diseases, quantitative risk analysis.

Members

Mr Stéphane BERTAGNOLI – ENV Toulouse – expertise in virology, in particular, poxvirus, contagious diseases.

Mr Philippe CAUFOUR – CIRAD – expertise in virology, in particular, poxvirus, exotic animal diseases.

Mr Kris DE CLERCQ – CODA-CERVA – expertise in virology, vaccinology, exotic animal diseases.

Mr Jean-Pierre GANIÈRE – ONIRIS Nantes – expertise in contagious diseases, regulations, zoonoses, qualitative risk analysis.

Mr Philippe JACQUIET – ENV Toulouse – expertise in parasitology, vectors, exotic animal diseases.

Mr Gilles MEYER – ENV Toulouse – expertise in ruminant pathology, virology, vaccinology.

Mr Claude SAEGERMAN – Faculty of Veterinary Medicine of Liège (Belgium) – expertise in epidemiology, contagious diseases, emerging diseases, quantitative risk analysis.

EXPERT COMMITTEE

The work that is the subject of this report was monitored and adopted by the following CES:

- CES SANT on 7 February 2017

Chairman

Mr Etienne THIRY – Faculty of Veterinary Medicine of Liège (Belgium) – expertise in virology, immunology.

Members

Ms Suzanne BASTIAN – ONIRIS Nantes – expertise in epidemiology, bacteriology, parasitology.

Ms Catherine BELLOC – ONIRIS Nantes – expertise in medicine of farmed animals, monogastric animals.

Mr Alain BOISSY – INRA – expertise in ethology, animal welfare, ruminants, zootechnics.

Mr Jordi CASAL – Universitat Autonoma de Barcelona (Spain) – expertise in zoonoses, quantitative epidemiology, exotic animal diseases, quantitative risk analysis.

Mr Christophe CHARTIER – ONIRIS Nantes – expertise in parasitology, pathology of small ruminants, farming techniques, epidemiology.

Mr Eric COLLIN – Veterinary Practitioner – expertise in ruminant pathology.

Mr Frédéric DELBAC – CNRS – expertise in bees, epidemiology, parasitology, microbiology.

Mr Christian DUCROT – INRA – expertise in quantitative epidemiology, prions, antibiotic resistance, ecopathology.

Ms Barbara DUFOUR – ENV Alfort – expertise in epidemiology, infectious diseases, ruminant pathology.

Mr Guillaume FOURNIÉ – Royal Veterinary College (UK) – expertise in quantitative and qualitative risk assessment, modelling, epidemiology.

Mr Jean-Pierre GANIÈRE – ONIRIS Nantes – expertise in contagious diseases, regulations, zoonoses.

Mr Dominique GAUTHIER – Hautes-Alpes departmental laboratory – expertise in wildlife, lagomorphs, diagnostic methods.

Mr Etienne GIRAUD – INRA – expertise in antibiotic resistance, environment, comprehensive approach to animal health.

Mr Jacques GODFROID – Arctic University of Norway – expertise in risk assessment, zoonoses, epidemiology, tuberculosis, bacteriology, marine wildlife.

Mr Jean-Luc GUÉRIN – ENVT – expertise in pathology of poultry and lagomorphs, immunology, virology, zoonoses and public health.

Mr Jean GUILLOTIN – Nord departmental laboratory – general practitioner, expertise in diagnostic methods, pigs, wildlife.

Ms Nadia HADDAD – ANSES UMR BIPAR, ENV Alfort – expertise in microbiology, epidemiology, contagious diseases.

Mr Jean HARS – National Office for Hunting and Wildlife – expertise in pathology of free wildlife, epidemiology.

Ms Véronique JESTIN – expertise in avian virology, avian parasitology, crossing of the species barrier.

Ms Elsa JOURDAIN – INRA – expertise in zoonoses, quantitative epidemiology, wildlife.

Ms Claire LAUGIER – ANSES Dozulé – expertise in equine pathology, laboratory diagnosis.

Ms Monique L'HOSTIS – ONIRIS – general practitioner, expertise in parasitology, bees, wildlife.

Ms Coralie LUPO – IFREMER – expertise in epidemiology, avian pathologies and aquaculture.

Mr Gilles MEYER – ENV Toulouse – expertise in ruminant pathology, virology.

Mr Pierre MORMÈDE – INRA Toulouse – expertise in genetics of stress, endocrinology, animal welfare.

Ms Carine PARAUD – ANSES – expertise in statistics, small ruminant pathology, field parasitology.

Ms Claire PONSART – ANSES – expertise in epidemiology, bacteriology, statistics, virology, reproductive pathology.

Ms Nathalie RUVOEN – ONIRIS Nantes – expertise in contagious diseases, zoonoses, regulations.

Mr Claude SAEGERMAN – Faculty of Veterinary Medicine of Liège – expertise in epidemiology, contagious diseases, emerging diseases.

Mr Stéphan ZIENTARA – ANSES Maisons-Alfort Laboratory for Animal Health – expertise in virology.

ANSES PARTICIPATION

Scientific coordination

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Ms Florence ÉTORÉ – Deputy Head of the UERSABA Unit – ANSES

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Request for an expert appraisal on the risk of introduction of lumpy skin disease into France

Request No 2016-SA-0120 – LSD

Collective Expert
REPORT

"Expert Committee on Animal Health and Welfare"
"LSD WG"

February 2017

Key words

Bovin, dermatose nodulaire contagieuse, appréciation de risque, transmission, stomoxes

Cattle, lumpy skin disease, risk assessment, transmission, *Stomoxys*

Collective expert appraisal: summary of justification and conclusion

In an epidemiological context in which lumpy skin disease (LSD) is emerging in the European Union (EU), the DGAL formally requested ANSES to assess the risk of introduction of LSD into France and to estimate the appropriate size for a vaccine bank, to manage an emergency vaccination campaign in the event that the disease were introduced.

Lumpy skin disease – the virus

Lumpy skin disease (LSD) is a viral disease of cattle caused by a virus belonging to the family *Poxviridae*, of the genus *Capripoxvirus*. It is characterised by the appearance of numerous nodules on the skin and the internal mucous membranes.

Following the first observation in Zambia in 1929, the continuous spread of LSD has been observed in most countries on the African continent, as well as in Madagascar. The transmissibility of the infective agent was first demonstrated in 1945, and the virus was first isolated in cell culture in 1957.

The structure of the LSD virus (LSDV) is consistent with the usual standard for poxviruses. While it is antigenically similar to the other known *Capripoxvirus*, the LSDV is nevertheless distinct. Antigenic variability is very low within the species, and the attenuation of the Neethling vaccine strain is related to numerous mutations spread across virtually the entire genome. For an enveloped virus, this virus is relatively resistant to physical and chemical agents. This is particularly true in organic matter and at low temperatures. This virus grows well *in vitro,* mainly in ruminant cells.

Following transcutaneous inoculation of the LSDV, the infection spreads through the body via the lymph vessels and then the blood vessels (transient viraemia), before reaching the mucocutaneous tissue and certain internal organs (mainly the digestive mucosa, kidneys, testes). Viral titres are low in saliva, nasal discharge and semen, and are highest in the skin nodules. It should be noted that experimental infections indicate that the intravenous route is best suited to achieving generalised infection.

The classic clinical form includes a prodromal phase (hyperthermia, adenitis, mucous membrane effects) followed by a rash phase and then a necrosis phase. Mild and severe forms can also be observed. The macroscopic lesions correspond to skin, subcutaneous and underlying tissue nodules (respiratory tract, digestive tract, lymph nodes, etc.).

In the epidermis, microscopic lesions are mainly manifested as extensive necrosis. In the dermis, a vascular necrosis can be observed. This causes vessel thrombosis, which in turn is responsible for tissue necrosis. Phenomena of acanthosis, parakeratosis and hyperkeratosis are also observed in the epidermis and the mucous membranes.

Natural infection associated with the development of clinical forms is only observed in cattle, zebus and water buffaloes. Due to the frequency at which they have been affected in all the countries recognised as infected, and their sensitivity, cattle are the primary source and host of the LSDV. According to some natural and experimental observations, domestic small ruminants and various species of wild ruminants are considered to be susceptible species. However, they do not seem likely to play a significant role in the epidemiology of the disease. Nonetheless, studies are needed to determine their actual role. The LSDV is detectable in many different cattle products and by-products, such as hide, milk and semen, and can be found on vehicles and materials that have

been in contact with infected cattle. The literature provides very little information on the European arthropod vectors currently involved in spreading the LSD virus in Eastern Europe, whether regarding the species involved or the mechanisms of transmission. Moreover, to date, only the mechanical transmission capacity of LSDV vectors has been studied, and the biological transmission capacity of the LSDV among vectors is unknown. All cattle-biting arthropods found in Europe (*Stomoxys*, horse-flies, mosquitoes and ticks) can potentially play a role in the transmission of the LSDV from one bovine animal to another. In addition to the virus being dispersed by the vectors, it could also be spread passively, over longer distances, by movements of vehicles that may be contaminated when transporting potentially-infected animals, or products or by-products of bovine origin.

Spatio-temporal distribution

Identified for the first time in sub-Saharan Africa in 1929, LSD then spread across and subsequently outside the African continent, affecting Israel in 1989. Since then, outbreaks have been confirmed in the Arabian Peninsula and the Middle East. Turkey was first affected in 2013. Since 2014, LSD has affected various European countries, including Russia, Greece, Bulgaria, the Former Yugoslav Republic of Macedonia (FYROM), Serbia, Kosovo, Albania and Montenegro (see Figure 1 page 36).

Diagnosis and control of the disease

The tests used most frequently to identify the LSDV are molecular tests based on PCR, conventional or in real time, although this virus can also be identified using immunohistochemistry or immunofluorescence. The reference serological test for *Capripoxvirus* is the virus neutralisation test. An ELISA test to detect the LSDV is not yet commercially available.

In the current, fairly limited, state of knowledge on the efficacy and safety of vaccines against LSD, the choice of the Neethling strain as the vaccine strain seems to be the only option available for the moment. The concept of efficacy is a term that is generally used, but which expresses the sum of the therapeutic indications claimed by the manufacturer, validated after primary vaccination until the booster, and demonstrated by laboratory and field studies. Regarding vaccines against LSD, there are no such studies available, since no marketing authorisation (MA) application has been submitted, either in France or to the European Medicines Agency. No experimental data on the safety and efficacy of the vaccines, as recommended by Directive 2009/9/EC, are currently available.

The degree of attenuation of the strain is an essential parameter: if it is excessively attenuated, it will be relatively ineffective; if it is insufficiently attenuated, the frequency and intensity of the adverse effects will be increased. In every case, the degree of attenuation should be the result of a compromise between safety and efficacy. Overall, in the available studies, there are very few data from which to determine the degree of attenuation constituting the best compromise. Information available on the safety of vaccines against LSD used in the EU, from pharmacovigilance reports, remains very fragmentary. The main adverse effects noted are in fact those of LSD, namely: a fall in milk production, fever, nodular skin lesions, abortion and death. The incidence of adverse effects is around 0.1%.

The efficacy of vaccination within the EU is not well documented.

While vaccination is not recommended in the disease-free zone, it is found to be the only effective means of controlling the spread of the disease in an epidemic situation, advocated by the EU as long as the vaccine provides adequate guarantees of safety and efficacy. For this reason, the EU has authorised vaccination in the Member States concerned. All the infected countries and some of their neighbours have established vaccine protocols.

The slaughter of susceptible species in an outbreak and the establishment of zones to regulate the transport of susceptible species are indispensable but, with a few exceptions, are insufficient to limit the spread of the virus. The use of insecticides is probably useful for controlling the disease, although the mechanical vectors are still poorly understood, especially in Europe.

Assessment of the probability of a first outbreak of LSD occurring in France

In order to respond to the first question of the formal request regarding the risk of introduction of LSD into France, and given the time available, **the experts assessed "only" the probability of a first outbreak of LSD on French territory for a year, based on the epidemiological situation in January 2017, the European regulations existing on this same date, and data on trade for 2016**. They did not take into account either the dissemination from the first outbreak, or the consequences of introduction of the LSDV.

The probability of a first outbreak of LSD occurring in France results from combining the probability of the virus being introduced into France with the probability that domestic cattle or wild ruminants are then exposed to this virus on French territory. The expert group, taking into account all the scientific and commercial data at its disposal, conducted an assessment of the risk of a first outbreak of LSD occurring in France, depending on the different virus sources and the possible ways in which they could be introduced (by live animals and their products - semen and embryos, by vectors, by inert media, etc.).

On the date the report was written, none of the countries bordering France had declared any infection with LSD. The experts defined an at-risk area for the purpose of the analysis: a zone from which live cattle or products can be traded and in which there is a probability that certain animals are infected, without the disease having been declared. This concerns disease-free regions of European countries recognised as infected (as of 1 January 2017: Greece, Bulgaria, FYROM, Kosovo, Serbia, Albania, Montenegro) and disease-free countries bordering a country where LSD has been notified (as of 1 January 2017: Romania, Croatia, Hungary, Ukraine, Bosnia & Herzegovina).

The risk assessment was carried out according to a quantitative approach for the methods of introduction regarded by the experts as most likely (movements of animals, movements of arthropod vectors). In the other cases, the approach was qualitative.

The variables used in the model developed for the quantitative risk assessment can easily be modified, depending on the evolution of the epidemiological situation in Europe, data relating to trade between the various Member States, and also advances in knowledge, in particular, on the vectors or the methods of transmission of the LSDV. Vaccination could also be integrated in this model.

- *Probability of LSD being introduced by live animals*

Only animals from the EU countries belonging to the at-risk area (Greece, Bulgaria, Romania, Croatia, Hungary) were taken into account in the analysis. The probability of LSD being introduced by live animals is limited to the risk of introduction by live cattle.

The quantitative probability of a first outbreak of LSD occurring in France following the introduction of infected live cattle intended for rearing is estimated to be between 0.004% and 0.32%, with a confidence interval of 95%, which corresponds to an "extremely low to low" qualitative probability (3 to 5 on AFSSA's 2008 scale, which goes from 0 to 9).

Currently there are no cattle intended for the slaughterhouse being introduced from the at-risk area. The probability of a first outbreak of LSD in France following the introduction of infected live cattle intended for the slaughterhouse is therefore estimated to be nil. The experts estimated, however, that if there were as many cattle intended for the slaughterhouse introduced into France as the number introduced for rearing, the quantitative probability would be between 0.2 10^{-6} and 47 10^{-6}, with a confidence interval of 95%. This corresponds to a "nearly nil to minute" qualitative probability (1 to 2 on a scale from 0 to 9).

- *Probabilities of an outbreak of LSD occurring following the introduction of infective vectors*

The risk of LSD being introduced by the long-distance road transport of vectors is limited to the risk of introduction by *Stomoxys*. The quantitative probability of a first outbreak of LSD occurring in France following the introduction of infective vectors transported with live cattle intended for rearing is therefore estimated to be between 0.002% and 0.44%, with a confidence interval of 95%. This corresponds to an "extremely low to low" qualitative probability (3 to 5 on a scale from 0 to 9).

Currently there are no cattle intended for the slaughterhouse being introduced from the at-risk area. The probability of a first outbreak of LSD in France following the introduction of infective vectors transported with live cattle intended for the slaughterhouse is therefore estimated to be nil. The experts estimated, however, that if there were as many cattle intended for the slaughterhouse introduced into France as the number introduced for rearing, the quantitative probability of a first outbreak of LSD occurring in France would be between 0.1 10^{-6} and 27 10^{-6}, with a confidence interval of 95%. This corresponds to a "nearly nil to minute" qualitative probability (1 to 2 on a scale from 0 to 9).

The quantitative probability of a first outbreak of LSD occurring in France following the introduction of infective vectors transported with horses is estimated to be between 0.01 10^{-6} and 1.66 10^{-6}, with a confidence interval of 95%. This corresponds to a "nearly nil" qualitative probability (1 on a scale from 0 to 9).

- *Probabilities of a first outbreak of LSD occurring following the introduction by other modes of transmission*

As previously, the risk assessment only examined introductions from the countries in the at-risk area. The only cases of introduction of LSD described below are those for which the probability of occurrence of a first outbreak is estimated to be equal to or greater than 1 (nearly nil). The other means of introduction and their associated probabilities are shown in the summary table in the conclusion of the report (Table 23, page 95).

The probability of an outbreak of LSD occurring through transport vehicles that have been in contact with infected cattle is estimated to be nearly nil to minute (1 to 2 on a scale of 0 to 9) and, through transport vehicles that have been in contact with infected hides, this probability is estimated to be nil to nearly nil (0 to 1 on a scale of 0 to 9).

The probability of the LSDV being introduced into France in a consignment of fresh hides from infected cattle is estimated to be nearly nil to minute (1 to 2 on a scale of 0 to 9). However, the probability of native cattle being exposed to the LSDV following this introduction is estimated to be nil to nearly nil. Therefore, the occurrence of an outbreak of LSD following the introduction into France of a consignment of fresh hides shipped from an establishment located in an undeclared LSD at-risk area or one handling cattle hides from such a zone can be estimated as nil to nearly nil (0 to 1 on a scale of 0 to 9).

The probability of an outbreak through insemination or embryo transfer after use of semen, oocytes or embryos shipped from an at-risk area (in 2016, the volumes traded were very low for these products), which results from combining the probabilities of introduction and exposure, can be estimated as nearly nil (1 on a scale of 0 to 9) for frozen semen stored for at least 30 days after collection and before shipping, and nearly nil to minute (1 to 2 on a scale of 0 to 9) for fresh semen, or frozen pre-stored oocytes or embryos, as well as for non-frozen oocytes or embryos.

Estimate of the size of a vaccine bank

For this estimate, the experts did not take into account the efficacy and safety of the vaccine, or the management measures associated with this possible vaccination.

Taking into account the speed at which the infection travels (7.3 km/week), the time needed to obtain satisfactory immunisation coverage of the population in question (the assumption being that the vaccination is applied to all cattle in the zone in a single injection), and the density of cattle in the region or *département* where the first outbreak may be located (the assumption being that the risk of the first outbreak occurring is directly proportional to the number of cattle in each *département*), the experts estimated, through simulations, the size of the stock of vaccines to be established.

Considering a period of 7 weeks (5 weeks before detection of the disease and 2 weeks to vaccinate the entire population) between the occurrence of the first outbreak and the end of the vaccination period, the experts calculated that 626,204 vaccine doses would be sufficient in 75% of simulations, and 798,128 doses in 95% of simulations, to vaccinate the exposed population taking into account the speed of viral spread following the discovery of an index outbreak (excluding long-distance spread, involving "leapfrogging").

For the French *département* with the highest density of cattle (Mayenne), the experts calculated that 945,456 doses would be necessary in 95% of simulations.

If the speed of dissemination of the LSDV should vary during the disease's spread in Europe, the number of vaccine doses needed may evolve. A new estimate of the number of doses required can be calculated by introducing the new assessed speed of viral spread into the model.

Recommendations

A number of recommendations were made by the experts following this risk assessment: research recommendations (on the LSDV, its potential vectors and vaccines) but also recommendations that focus more on preventing infection by the LSDV, as well as its surveillance.

Presentation of participants

PREAMBLE: The expert members of the Expert Committees and Working Groups or designated rapporteurs are all appointed in a personal capacity*, intuitu personae,* according to their area of expertise*,* and do not represent their parent organisation.

WORKING GROUP

Chairman

Mr Jordi CASAL – Universitat Autonoma de Barcelona (Spain) – expertise in zoonoses, quantitative epidemiology, exotic animal diseases, quantitative risk analysis.

Members

Mr Stéphane BERTAGNOLI – ENV Toulouse – expertise in virology, in particular, poxvirus, contagious diseases.

Mr Philippe CAUFOUR – CIRAD – expertise in virology, in particular, poxvirus, exotic animal diseases.

Mr Kris DE CLERCQ – CODA-CERVA – expertise in virology, vaccinology, exotic animal diseases.

Mr Jean-Pierre GANIÈRE – ONIRIS Nantes – expertise in contagious diseases, regulations, zoonoses, qualitative risk analysis.

Mr Philippe JACQUIET – ENV Toulouse – expertise in parasitology, vectors, exotic animal diseases.

Mr Gilles MEYER – ENV Toulouse – expertise in ruminant pathology, virology, vaccinology.

Mr Claude SAEGERMAN – Faculty of Veterinary Medicine of Liège (Belgium) – expertise in epidemiology, contagious diseases, emerging diseases, quantitative risk analysis.

EXPERT COMMITTEE

The work that is the subject of this report was monitored and adopted by the following CES:

- CES SABA on 7 February 2017

Chairman

Mr Etienne THIRY – Faculty of Veterinary Medicine of Liège (Belgium) – expertise in virology, immunology.

Members

Ms Suzanne BASTIAN – ONIRIS Nantes – expertise in epidemiology, bacteriology, parasitology.

Ms Catherine BELLOC – ONIRIS Nantes – expertise in medicine of farmed animals, monogastric animals.

Mr Alain BOISSY – INRA – expertise in ethology, animal welfare, ruminants, zootechnics.

Mr Jordi CASAL – Universitat Autonoma de Barcelona (Spain) – expertise in zoonoses, quantitative epidemiology, exotic animal diseases, quantitative risk analysis.

Mr Christophe CHARTIER – ONIRIS Nantes – expertise in parasitology, pathology of small ruminants, farming techniques, epidemiology.

Mr Eric COLLIN – Veterinary Practitioner – expertise in ruminant pathology.

Mr Frédéric DELBAC – CNRS – expertise in bees, epidemiology, parasitology, microbiology.

Mr Christian DUCROT – INRA – expertise in quantitative epidemiology, prions, antibiotic resistance, ecopathology.

Ms Barbara DUFOUR – ENV Alfort – expertise in epidemiology, infectious diseases, ruminant pathology.

Mr Guillaume FOURNIÉ – Royal Veterinary College (UK) – expertise in quantitative and qualitative risk assessment, modelling, epidemiology.

Mr Jean-Pierre GANIÈRE – ONIRIS Nantes – expertise in contagious diseases, regulations, zoonoses.

Mr Dominique GAUTHIER – Hautes-Alpes departmental laboratory – expertise in wildlife, lagomorphs, diagnostic methods.

Mr Etienne GIRAUD – INRA – expertise in antibiotic resistance, environment, comprehensive approach to animal health.

Mr Jacques GODFROID – Arctic University of Norway – expertise in risk assessment, zoonoses, epidemiology, tuberculosis, bacteriology, marine wildlife.

Mr Jean-Luc GUÉRIN – ENVT – expertise in pathology of poultry and lagomorphs, immunology, virology, zoonoses and public health.

Mr Jean GUILLOTIN – Nord departmental laboratory – general practitioner, expertise in diagnostic methods, pigs, wildlife.

Ms Nadia HADDAD – ANSES UMR BIPAR, ENV Alfort – expertise in microbiology, epidemiology, contagious diseases.

Mr Jean HARS – National Office for Hunting and Wildlife – expertise in pathology of free wildlife, epidemiology.

Ms Véronique JESTIN – ANSES Ploufragan – expertise in avian virology, avian parasitology, crossing of the species barrier.

Ms Elsa JOURDAIN – INRA – expertise in zoonoses, quantitative epidemiology, wildlife.

Ms Claire LAUGIER – ANSES Dozulé – expertise in equine pathology, laboratory diagnosis.

Ms Monique L'HOSTIS – ONIRIS – general practitioner, expertise in parasitology, bees, wildlife.

Ms Coralie LUPO – IFREMER – expertise in epidemiology, avian pathologies and aquaculture.

Mr Gilles MEYER – ENV Toulouse – expertise in ruminant pathology, virology.

Mr Pierre MORMÈDE – INRA Toulouse – expertise in genetics of stress, endocrinology, animal welfare.

Ms Carine PARAUD – ANSES – expertise in statistics, small ruminant pathology, field parasitology.

Ms Claire PONSART – ANSES – expertise in epidemiology, bacteriology, statistics, virology, reproductive pathology.

Ms Nathalie RUVOEN – ONIRIS Nantes – expertise in contagious diseases, zoonoses, regulations.

Mr Claude SAEGERMAN – Faculty of Veterinary Medicine of Liège – expertise in epidemiology, contagious diseases, emerging diseases.

Mr Stéphan ZIENTARA – ANSES Maisons-Alfort Laboratory for Animal Health – expertise in virology.

ANSES PARTICIPATION

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Ms Florence ÉTORÉ – Deputy Head of the UERSABA Unit – ANSES

Ms Claire HAUTEFEUILLE – UERSABA Scientific Coordinator – ANSES

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Ms Eeva TUPPURAINEN, Veterinarian, Specialist Consultant in LSD, United Kingdom

Mr Louis MAARTENS, Veterinarian, Research & Development Department, Deltamune Ltd, South Africa

Ms Sotiria-Eleni ANTONIOU, Official Veterinarian, Department of Infectious and Parasitic Diseases, Directorate General of Sustainable Animal Production and Veterinary Medicine, Ministry of Rural Development and Food, Greece

Mr Eyal KLEMENT, Assistant Professor, Koret School of Veterinary Medicine, Robert H. Smith Faculty of Agriculture, Food and Environment, Hebrew University, Israel

Mr Georgi CHOBANOV, Director of the Animal Health, Welfare and Feed Controls Directorate, Bulgarian Food Safety Agency, Bulgaria

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Acronyms and abbreviations

aa: Amino acids
DNA: Deoxyribonucleic acid
MA: Marketing authorisation
ANMV: French Agency for Veterinary Medicinal Products
ANSES: French Agency for Food, Environmental and Occupational Health & Safety
FYROM: Former Yugoslav Republic of Macedonia
TAU: Temporary authorisation for use
BTV: Bluetongue virus
EC: European Commission
CEV: Cell-associated enveloped virus
CES: ANSES Expert Committee
CVO: Chief Veterinary Officer
DIVA: Differentiating infected from vaccinated animals
DGAL: French Directorate General for Food
DG SANTE: Directorate General for Health and Food Safety (EU)
ID: Infective dose
ID_{50}: Infective dose 50%
LD_{50}: Lethal dose 50%
LSD: Lumpy skin disease
BLSD: Bovine lumpy skin disease
LSDV: Lumpy skin disease virus
EFSA: European Food Safety Authority
ELISA: Enzyme-Linked Immunosorbent Assay
EV: Enveloped virus
EEV: Extracellular enveloped virus
FAO: Food and Agricultural Organisation of the United Nations
GPCR: G protein-coupled receptor
WG: Working Group
GTPV: Goat pox virus
IETS: International Embryo Transfer Society
IEV: Intracellular enveloped virus
IMV: Intracellular Mature Virion
ID: Intradermal
IV: Intravenous
Kbp: Kilo base pairs
KSGP: Kenyan sheep and goat pox virus
MV: Mature virus
NVI: Ethiopian National Veterinary Institute

OBP: Onderstepoort Biological Products
OIE: World Organisation for Animal Health
PCR: Polymerase chain reaction
PI: Post-infection
Russia: Russian Federation
SC: Subcutaneous
SPPV: Sheep pox virus
$TCID_{50}$: Median tissue culture infectious dose
TRACES: TRAde Control and Expert System
EU: European Union
PFU: Plaque-forming unit
VacV: Vaccinia virus

1. BACKGROUND AND PURPOSE OF THE REQUEST

On 18 May 2016, ANSES received a formal request from the Directorate General for Food (DGAL) to conduct an expert appraisal on the risk of introduction into France of lumpy skin disease (Annex 1).

Since August 2015, several outbreaks of lumpy skin disease (LSD) have been declared in Greece, probably following introduction of the disease from Turkey, which has been suffering from an animal epidemic for the past few years. In April 2016, outbreaks were declared in Bulgaria and the Former Yugoslav Republic of Macedonia (FYROM). Since then, the epidemic has spread considerably, with numerous outbreaks in Serbia, Kosovo[1], Albania and Montenegro. France is officially free of this infection.

LSD affects cattle and is caused by a virus belonging to the genus *Capripoxvirus,* of the family Poxviridae. It is included in the list of diseases of the World Organisation for Animal Health (OIE), and is a notifiable disease that must be reported to the European Commission and the Member States (Directive 82/894/EEC[2], Decision 89/162/EEC[3]). It has also been classified as a Category 1 health hazard (Ministerial Order of 29 July 2013 on the definition of Category 1 and 2 health hazards for animal species).

The increase in the number of outbreaks in the European Union raises the question about the risk of introduction of the infection into France.

For this reason, through this formal request, ANSES is being asked to:

1) assess the risk of contamination for France taking into account the different risk factors regarding its introduction;
2) estimate the appropriate size for a vaccine (or antigen) bank, to manage an emergency vaccination campaign in the event that the disease were introduced.

2. ORGANISATION OF THE EXPERT APPRAISAL

The expert appraisal was carried out in accordance with French Standard NF X 50-110 "Quality in Expert Appraisals – General Requirements of Competence for Expert Appraisals (May 2003)".

The formal request falls within the sphere of competence of the Expert Committee on "Animal Health and Welfare" (CES SABA). ANSES entrusted examination of this formal request to the "LSD" Working Group, reporting to the CES SABA. The methodological and scientific aspects of this group's work were submitted to the CES on 13 September, 11 October and 6 December 2016, and 10 January 2017. The report was presented to the CES for validation on 7 February 2017. The expert appraisal report issued by the LSD WG takes into account the comments and additional information provided by the members of the CES.

These analyses and conclusions are derived from collegial expert appraisal work conducted within a group of experts with complementary skills. The LSD WG was made up of eight experts, who

[1] This designation is without prejudice to the positions on the status, and is in accordance with Resolution 1244 of the United Nations Security Council and the opinion of the International Court of Justice on Kosovo's declaration of independence.
[2] Council Directive 82/894/EEC of 21 December 1982 on the notification of animal diseases within the Community
[3] 89/162/EEC:Commission Decision of 10 February 1989 supplementing the annexes to Council Directive 82/894/EEC on the notification of animal diseases within the Community

met on eight occasions between 23 June 2016 and 18 January 2017. The expert appraisal was coordinated by the Unit for the assessment of food and animal health-related risks (UERSABA), which was assisted by an ANSES expert from the French Agency for Veterinary Medicinal Products (ANMV).

ANSES analyses the links of interest declared by the experts prior to their appointment and throughout the work, in order to avoid potential conflicts of interest with regard to the matters dealt with as part of the expert appraisal.

The experts' declarations of interests are made public *via* the ANSES website (www.anses.fr).

The assessment was conducted with the help of:

- information extracted from the TRACES (TRAde Control and Expert System) database provided by the DGAL concerning imports of live cattle;
- information extracted from the Eurostat database concerning products of animal origin;
- data on notification of LSD cases from the ESA Platform (National Epidemiological Surveillance Platform for Animal Health) and the FAO (Empres-i; Global Animal Disease Information System). The number of cases was last updated on 29 November 2016. The reader can find more recent data by using the source links mentioned;
- densities of cattle in France, calculated from data taken from semi-final annual agricultural statistics for 2014 and 2015 produced by Agreste;
- the regulatory texts cited throughout the report in the form of footnotes;
- reports of meetings, internships, etc. or other information published in the press, most often available online, mentioned in footnotes;
- hearings with international specialists on LSD;
- the scientific publications listed in the references section at the end of the report.

Literature search method

At their first meeting, all the members of the WG validated the literature search method, which took place as follows:

- the experts determined the key words based on the literature search profile proposed by ANSES (Annex 2);
- with the help of these key words, the coordinators queried Scopus®, which led to an initial selection of 138 articles;
- these 138 articles were divided among the various experts, who initially read the abstracts. The experts then completed the literature grid drawn up by the coordinators (Annex 3), indicating the relevance of the articles and their areas of interest for responding to the formal request;
- the literature grids were merged by the coordinators and made available to all the WG experts. PDF versions of the articles of interest were made available to the WG experts on the Extranet.

A diagram based on the PRISMA diagram is shown in Annex 4 and traces the approach used for the literature search.

Organisation of the hearings with international specialists on LSD

Objective: These hearings were organised to validate the assumptions made by the WG experts to establish the models, in order to assess the risk of the introduction of LSD into France by infected live cattle or infective vectors.

Selection of interviewees: The WG experts selected six international specialists on LSD to ensure a homogeneous representation of managers and researchers. These experts came from countries formerly or recently infected by LSD. The only person not working directly in an infected country is recognised as a long-standing European specialist in LSD. She has invested great efforts as a consultant in the recently infected European countries.

Organisation of the hearings: An expert from the WG initially contacted these specialists by email, to inform them of the formal request and the possibility of a hearing. Then, the coordinators sent these specialists a questionnaire, accompanied by a short explanation of the work carried out by the WG experts, the arguments used in the two models, and the assumptions to be validated. This email explained that the answers had to be substantiated, and stated the response time and the possibility of contacting the Chairman of the WG in the event of any question.

The specialists' responses were then discussed by the WG and the values of each probability were modified, when deemed necessary. The final values were validated by all the WG experts. The new data thus obtained were then used in the model and the arguments were corrected.

3. SCOPE AND LIMITATIONS OF THE EXPERT ASSESSMENT

For the first question in the formal request, the experts examined the probability of the first outbreak of LSD occurring in France. To do this, they took into account the probability of the virus being introduced into France and the probability of exposure of a native bovine animal to this virus. In this report, the experts did not assess the consequences that might arise from the occurrence of this first outbreak in France.

For the second question, the experts examined the number of doses of vaccine needed to halt the spread of the disease if it were introduced into France, without taking into account the efficacy and safety of the vaccines available, or the management measures associated with this possible vaccination.

4. ANALYSIS AND CONCLUSIONS OF THE LSD WG AND THE CES SABA

4.1. Bovine lumpy skin disease – summary of knowledge

4.1.1. Background

In 1929, long before the aetiology of LSD was known, a skin disease of cattle, then known as "pseudo-urticaria" was observed in Northern Rhodesia (now Zambia) (Morris 1931, MacDonald 1931). The lesions were initially thought to be caused by an allergic reaction to insect bites and were later ascribed to poisoning by plants (Le Roux 1945). It was only in 1943 that the infectious nature of LSD became apparent, when an epizootic outbreak occurred in Ngamiland, located in the north of Botswana (Von Backström 1945). Towards the end of 1944, the disease was reported for the first time in South Africa, under the name of "knopvelsiekte" (the Afrikaans word for LSD), in the Transvaal (Thomas and Maré 1945), a region from which it then spread throughout South Africa, despite the control measures in place. Although, originally, the spread of the disease in South Africa was believed to be associated with the transport of cattle, its very rapid dissemination across low-altitude regions was then attributed to transmission by insects (Hunter and Wallace 2001). During this period, it was estimated that more than 8 million cattle were affected (Hunter and Wallace 2001).

Although von Backström was the first to reach a conclusion about the infectious nature of the disease (von Backström 1945), the work by Thomas *et al.* demonstrated for the first time the transmissibility of the infectious agent, by inoculation of a suspension of skin nodules (Thomas, Robinson, and Alexander 1945). The LSD virus (LSDV) was isolated on cell culture for the first time by Alexander *et al.* (Alexander, Plowright, and Haig 1957).

In 1957, LSD was observed for the first time in Kenya, and at the time its introduction was attributed to a herd of sheep showing clinical signs of smallpox (MacOwan 1959). LSD was subsequently observed in Central and East Africa, and then spread northwards, westwards and also towards Madagascar (Odend'hal 1983, Fenner *et al.* 1987), occurring as major animal epidemics followed by intermediate periods during which the disease was rarely reported.

Following the first observation in Zambia in 1929, the continuous spread of LSD has been observed in most countries on the African continent, as well as in Madagascar. The transmissibility of the infective agent was first demonstrated in 1945, and the virus was first isolated in cell culture in 1957.

4.1.2. Characteristics of the virus

4.1.2.1. Classification/morphology of virions/phylogeny

The LSDV is a double-stranded DNA virus belonging to the genus *Capripoxvirus*, within the sub-family *Chordopoxvirinae* and the family *Poxviridae*. In addition to the LSDV, the genus *Capripoxvirus* also contains the sheep pox (SPPV) and goat pox (GTPV) viruses. The prototype virus of LSD is the Neethling strain, which was isolated in South Africa (Alexander, Plowright, and Haig 1957).

The morphology of the LSDV meets the criteria for the family of *Poxviridae*. The poxviruses are among the largest and most complex viruses known. The average size of the viral particles has

been estimated at 320 x 260 nm (Ghaboussi 1978). As a general rule, within the genus *Capripoxvirus*, the estimated average size of the viral particles (SPPV, GTPV and LSDV), depending on the authors, ranges from 294 to 350 nm long and 260 to 300 nm wide. The mature virions (MVs, formerly known as intracellular mature virions - IMVs) of LSDV are oval in shape and, seen in cross section, have wider lateral bodies than those of virions of the genus *Orthopoxvirus* (Munz and Owen 1966).

Three structures can be distinguished within the virion: the core, the lateral bodies and the envelope(s).

The core is a thick internal shell, bounded by a protein layer 5 nm thick. It contains the viral genome and the viral proteins that are essential to the first steps of replication. The lateral bodies are lens-shaped protein structures located in the concavities of the core. The envelopes are derived from cell membranes (phospholipid type).

Depending on the type and number of envelopes, several viral forms can be distinguished, each with different structural, antigenic and functional characteristics: MVs (or IMVs), wrapped virions (WVs, formerly known as intracellular enveloped virions - IEVs), and extracellular virions (EVs) comprising the extracellular enveloped virions (EEVs) and cell-associated enveloped viruses (CEVs) (Moss 2006, Condit, Moussatche and Traktman 2006). MVs and EVs are the infectious forms. MVs correspond to the singly enveloped form of the virus and are only released at a late stage of the lysis of the host cell. This form is regarded as essential to inter-host transmission, and constitutes the vast majority of the viral progeny (70 to 99%). Some of the MVs wrap themselves in an additional double envelope (from the Golgi apparatus and the endosomes) to constitute the WVs. The WVs then migrate to the plasma membrane, merge with it and are released: they are now EEVs (form with two envelopes, and between four and six original surface proteins). The CEVs form part of the EEVs, retained on the outer side of the cell membrane (McFadden 2005). The EVs are responsible for dissemination in the host.

The genome of the *Poxviridae* is formed of linear double-stranded DNA whose ends are covalently bound by terminal hairpin loops (Weiss 1968). The size of the poxvirus DNA varies between 130 and 375 Kbp; that of the LSDV (Neethling 2490 strain) contains approximately 151 Kbp (156 reading frames, including 146 retained within the sub-family *Chordopoxvirinae*) (Tulman *et al.* 2001). The genome is organised in a central region containing highly conserved genes (enzymes, structural proteins) and inverted terminal repeat sequences of around 2.5 Kbp encoding non-essential genes (pathogenicity factors). The composition of the genome is 73-74% adenine and thymine.

The genomes of *Capripoxvirus* (SPPV, GTPV and LSDV) present 96-97% identity: nine LSDV genes encoding virulence and host spectrum factors are inactivated on the genomes of GTPV and SPPV (Tulman *et al.* 2002).

Molecular studies have demonstrated that the LSDV, the SPPV and the GTPV are phylogenetically distinct (Tulman *et al.* 2002, Tulman *et al.* 2001, Stram *et al.* 2008, Le Goff *et al.* 2009, Lamien, Le Goff*, et al.* 2011, Hosamani *et al.* 2004). More specifically, studies targeting the terminal regions of the genome (Stram *et al.* 2008) or the genes encoding the GPCR (G protein-coupled receptor) (Le Goff *et al.* 2009), RPO30 (30 kDa RNA polymerase subunit) (Lamien, Le Goff*, et al.* 2011) and P32 (envelope protein) proteins (Hosamani *et al.* 2004), have identified a consolidation of SPPV, GTPV and LSDV into clusters grouping together the strains according to the host species infected (sheep, goat and cattle). By demonstrating that the GTPV and LSDV are phylogenetically closer than the SPPV, these analyses support the hypothesis that the GTPV and LSDV could both be derived from a common ancestor close to the SPPV. Additional genomic analyses conducted on a larger

number of field isolates remain necessary to confirm these results and enable the determinants of virulence, host specificity and geographic distribution to be identified.

Within the LSDVs, a comparison of the genomes of three strains of the LSDV (South African Neethling vaccine strain, virulent South African Neethling Warmbaths isolate, virulent Kenyan Neethling 2490 strain) indicates that between the virulent strains, only 38 amino acids (aa) are modified in 29 out of 156 genes, whereas there are 438 aa substitutions between the vaccine strain and the virulent South African strain spread across 114 genes (especially in the terminal regions of the genome) (Kara *et al.* 2003).

In Israel, the circulating strain is different to the vaccine strain (Neethling). For the moment, there is no information available on the strain circulating in Europe, although Greece and Israel are working with CODA-CERVA (Brussels, Belgium) and the strains circulating in Greece and Israel are currently being sequenced.

However, the limited information available suggests to the experts that there is a fairly high probability that the strain circulating in Israel is the same as the one circulating in Turkey and in Europe.

To summarise, the structure of the LSD virus is consistent with the usual standard for poxviruses. The attenuation of the Neethling vaccine strain is related to numerous mutations spread across virtually the entire genome.

4.1.2.2. Resistance and survival of the virus (Weiss 1968, OIE 2013b)

- **Resistance to physical agents**

The LSDV, like other *Capripoxvirus*, is characterised by a fairly good resistance to variations in temperature, i.e.:

- the loss of infectivity in 80 days at 20°C;
- the loss of infectivity in 8-10 days at 37°C;
- the loss of infectivity in less than 2 h at 50-55°C and in less than 30 min at 65°C.

In the nodules on the animal, the virus remains viable for less than 42 days: it is found after 33 days in the necrotic and desiccated lesions, for up to 35 days in the dry scabs, and for at least 18 days at room temperature in air-dried hides and samples removed from the superficial and deep parts of the animal's lesions.

It has excellent resistance to cold (titres preserved for 6 months in culture medium at 4°C).

The virus is very sensitive to light: the conservation time of lesion samples at room temperature in complete darkness increases from 18 to 36 days. The virus can persist in the dark in livestock buildings for several months.

- **Resistance to chemical agents**

✓ *Resistance to pH variations:*

The virus is more stable at neutral pH (no variation in titre for pH 6.6 to 8.6, at 37°C for 5 days). However, resistance to the pH also depends on the temperature (Polson and Turner 1954):

- after 14 days at 4°C, infectious titre is maintained for pH levels between 2 and 10;

- after 4 hours at 37°C, infectious titre is reduced by half at pH 2, and is maintained for pH levels between 4 and 10;
- For pH levels of 1 or 11.8, inactivation is virtually complete regardless of the temperature conditions.

✓ *Resistance to chemical products and disinfectants:*

The virus is susceptible to ether (20%), chloroform, formaldehyde (1%) and detergents (it is destroyed by sodium dodecyl sulphate in a few minutes); it is susceptible to phenol (2%, 15 min), sodium hypochlorite (2-3%), iodine compounds (dilution 1:33), Virkon® (2%) and quaternary ammonium compounds (0.5%).

To summarise, for an enveloped virus, this virus is relatively resistant to physical and chemical agents. This is particularly true in organic matter and at low temperatures.

4.1.2.3. Biological properties: antigenicity and *in vitro* culture

■ **A single antigenic type of LSDV**

The cross-serum neutralisation technique has been used to demonstrate the antigenic identity of all the isolates collected thus far. It should be noted that no serological reaction can be used to differentiate the different *Capripoxvirus* from each other (Davies and Otema 1981).

Western-blot experiments on purified virions have shown that anti-KS1 hyperimmune sera reacted with viral proteins with molecular weights of 67, 32, 26, 19 and 17 kDa (Chand, Kitching and Black 1994). More specifically, the p32 protein, the homolog of the Vaccinia virus H3L protein, a major immunodominant membrane protein of MV particles, contains major antigens that are shared within the genus *Capripoxvirus*. A study of the recombinant structural proteins produced in *E. coli* also identified the ORFs 028 (F13 of the Vaccinia virus - VacV, EEV envelope protein), 057 (G7L of VacV, core protein), 095 (A4L of VacV, core protein), and 103 (A12L of VacV, core protein) as antigens recognised in ELISA by the sera of ruminants (cattle, sheep, goats) infected by various *Capripoxvirus* (Bowden *et al.* 2009).

The neutralising antibodies persist for at least two years (2 to 5 years depending on the authors) after natural infection. Their titre is highest one month after infection, and then decreases until the 6th month, where it remains at a plateau until the 18th month (Weiss *et al.* 1963).

■ **Culturing the virus in the laboratory**

The virus can be grown in cell culture, mainly in primary bovine or ovine cells (especially from lambs), particularly kidney and testicular cells (culture is also possible in primary rabbit cells, or chicken embryo fibroblasts). Currently, the use of OA3.Ts, ovine testis cell lines, is preferred (Babiuk *et al.* 2007). Culturing in the cell lines of other animal species has also been carried out (AVK58 'Vero' monkey kidney, and BHK21 hamster kidney).

The titres obtained after cell culture generally vary between 10^6 and 10^8 $TCID_{50}$/mL.

The LSDV also grows on embryonated chicken eggs. After inoculation, the appearance of "*pocks*" (well-delimited vesicular lesions) can be observed on the chorioallantoic membrane, but the lesions are more frequently diffuse (oedema, congestion) (optimal culture conditions: 5-7 day-old eggs, incubation from 4 to 6 days at 33.5 - 35°C) (Van Rooyen, Munz, and Weiss 1969).

In summary, a single antigenic type of LSDV has been identified. This virus grows well *in vitro,* mainly in ruminant cells. While it is antigenically similar to the other known *Capripoxvirus*, the LSDV is nevertheless distinct.

4.1.3. Pathogenesis

During experimental infections by the subcutaneous (SC) or intradermal (ID) routes, swelling develops in 4 to 7 days at the point of inoculation, preceding a hypertrophy of the draining lymph node. Generalisation of the rash (nodules), observed in these cases for half of the subjects, most often takes place between 7 and 19 days post-infection (PI). The intravenous (IV) route enables better reproduction of the disease (more generalised lesions and more severe symptoms) (Carn and Kitching 1995).

The classic dissemination pattern in the body is then as follows: after local multiplication, the virus passes into the draining lymph node, viraemia and viral distribution in the skin and mucous membranes, and towards certain internal organs and various secretions (saliva, nasal discharge, semen).

More specifically, experimental reproduction of the disease by the IV route has helped clarify distribution and viral loads, as well as shedding levels and durations. Thus, after infection of 4 to 6 month-old calves with the Neethling strain, the presence of the viral genome was detected in blood by qPCR as early as 6 days PI and up to 15 days PI, intermittently and in fairly small quantities (Babiuk, Bowden, Parkyn*, et al.* 2008). Viral isolation in cell culture was only positive 9 days PI for one animal (Babiuk, Bowden, Parkyn*, et al.* 2008). Another study conducted on bulls confirmed the intermittent nature of the viraemia, with isolation in culture having been possible this time between 8 and 22 days (for the most affected animals) (Annandale *et al.* 2010). Tuppurainen *et al.* reported having detected the virus in the blood of inoculated cattle, for 1 to 12 days by viral isolation and for 4 to 11 days by PCR, and up to 16 days post-inoculation (Tuppurainen, Venter and Coetzer 2005). Osuagwuh *et al.* isolated the virus from blood in cattle at between 9 and 23 days PI (Osuagwuh *et al.* 2007). This duration is not correlated with the intensity of the symptoms; viraemia can be observed in subjects with no skin lesions. Studies of tissues from infected animals have revealed widespread viral distribution, but with variable loads and durations. The skin nodules are the preferred sites for viral multiplication (high viral titres and numbers of genomic copies, for up to 42 days), whereas among the internal tissues, the highest titres are observed in the nasal mucosa and the abomasum (Babiuk, Bowden, Parkyn*, et al.* 2008). From the lymph nodes, in particular mesenteric and inguinal, isolation is difficult (15 days), although the PCR signals are quite strong. The virus has also been isolated from the rumen and kidneys at 15 days PI (low titres). PCR results on the other organs are erratic and intermittent, while urine and rectal swabs have remained negative. Nasal and oral swabs have been found to be very slightly positive in isolation (nasal excretion from 12 days to 18 days, and oral from 15 days to 18 days). The analyses performed on semen and testes (Irons, Tuppurainen and Venter 2005, Annandale *et al.* 2010) confirm the presence of the virus (erratic and intermittent) in semen (isolation from 8 days to 42 days; PCR positive from 6 days to 156 days), and in the testes and epididymides (isolation at 28 days).

To summarise, following transcutaneous inoculation, the infection spreads through the body via the lymph vessels and then the blood vessels (transient viraemia), before reaching the mucocutaneous

tissue and certain internal organs (mainly the digestive mucosa, kidneys, testes). Viral titres are low in saliva, nasal discharge and semen, and are highest in the nodules. It should be noted that experimental infections indicate that the intravenous route is best suited to achieving generalised infection.

4.1.4. Clinical manifestations of BLSD

Clinical signs

The clinical signs in animals infected naturally or experimentally have been widely described (Lefevre and Gourreau 2003, Barnard *et al.* 1994, Arsevska *et al.* 2016, OIE 2016, Weiss 1968). The disease includes an initial phase with hyperthermia, followed by a rash phase and then a necrosis phase. Mild and severe forms can also be observed. On the basis of these descriptions, a summary is proposed in a box in **Annex 5** of this report.

Macroscopic and microscopic lesions

In addition to the clinical signs observed in infected animals, an autopsy can provide additional information that helps with diagnosis.

After natural (especially) or experimental infection, the main macroscopic lesions are:

- in the skin and subcutaneous tissue: nodules occupying the entire thickness of the skin, and an inflammatory oedema;

- In the underlying tissue: nodules in the muscles, the nasopharynx, trachea, bronchi, lungs, rumen, abomasum, renal cortex, testes and uterus, a generalised lymphadenopathy (with lymphoid hyperplasia and oedema).

The microscopic lesions are dominated by an extensive necrosis of the epidermis. In areas remaining intact, ballooning degeneration of the squamous epithelial cells is observed, with the presence of intracytoplasmic inclusion bodies. Extensive lesions of vascular necrosis with cellular debris and major diffuse infiltration of inflammatory cells have been observed in the upper and deep dermis (Prozesky and Barnard 1982). These lesions cause thrombosis of the dermal and subcutaneous vessels, which in turn are responsible for tissue necrosis. The cells infiltrating the lesions are mainly epithelioid cells. Within the lesions, intracytoplasmic eosinophilic inclusions are also observed in inflammatory cells and endothelial cells. Lastly, phenomena of acanthosis, parakeratosis and hyperkeratosis are also observed in the epidermis. The lesions in the mucous membranes are identical.

To summarise, the classic clinical form includes a prodromal phase (hyperthermia, adenitis, mucous membrane effects) followed by a rash phase and then a necrosis phase. Mild and severe forms can also be observed. The macroscopic lesions correspond to skin, subcutaneous and underlying tissue nodules (respiratory tract, digestive tract, lymph nodes, etc.).

In the epidermis, microscopic lesions mainly appear as extensive necrosis. In the dermis, a vascular necrosis can be observed. This causes vessel thrombosis, which in turn is responsible for tissue necrosis. Phenomena of acanthosis, parakeratosis and hyperkeratosis are also observed in the epidermis and the mucous membranes.

4.1.5. Epidemiological characteristics

4.1.5.1. Sources

In view of the data available on LSD, two groups of potential contamination sources will be presented and analysed: firstly, domestic and wild ruminants, in which some species have been recognised as receptive under natural and/or experimental conditions, and secondly, the other

sources represented by certain products and by-products of animal origin, and contaminated inert objects.

Domestic and wild ruminants

✓ Domestic ruminants

The domestic ruminants usually affected by LSD under natural conditions are Bovinae: cattle, zebus and domestic buffaloes. The role of small ruminants as a potential source of contamination and spread of LSD should also be mentioned.

- Cattle (*Bos taurus*), zebus (*Bos taurus indicus*) and domestic buffaloes (*Bubalus bubalis*)

Depending on the authors, either all bovine breeds seem equally susceptible (Weiss, 1968), or differences have been identified: breeds with thin skin, such as "Friesian" cattle in the article by Barnard *et al.*, have a higher susceptibility to infection compared with indigenous African breeds with thick skin, including the Afrikaner cattle and hybrid Afrikaner breeds (Le Roux 1945, Barnard *et al.* 1994, Coetzer 2004). Cattle breeds such as Jersey and Guernsey appear more severely clinically affected (Ayre-Smith 1960). Cattle breeds referred to as "Friesian" and "Ayrshire" in the article by Davies also have high susceptibility (Davies 1991). Cattle (*Bos taurus*) have a susceptibility linked to clinical expression that is higher than zebus (*Bos taurus indicus*) and crossed zebus (Gari *et al.* 2011, Davies 1991).

Lastly, all age categories are equally susceptible, although in certain observed situations, cows were only moderately clinically affected, while their calves had developed, 24 to 48 h previously, lesions characteristic of LSD (Le Roux 1945).

The factors governing the severity of the disease have not been identified. Field studies report that very young calves, lactating cows and animals suffering from malnutrition typically develop more severe clinical profiles, probably related to compromised cellular immunity (Hunter and Wallace 2001). The high temperatures associated with farming practices designed to achieve high milk production yields are regarded as generators of stress for the animals, and contribute to the clinical severity observed in cattle of the Holstein-Friesian breed (Tageldin *et al.* 2014).

In addition to cattle and zebus, natural infections have been described in water buffalo (*Bubalus bubalis*) in Egypt, in a less severe form than in cattle (El-Nahas *et al.* 2011, Ali *et al.* 1990, Ali *et al.* 2012, Sharawi and Abd El-Rahim 2011). The LSDV strains isolated in water buffalo are genetically very similar to those isolated in cattle, suggesting transmission from cattle infected with LSD (El-Tholoth and El-Kenawy 2015).

- Small ruminants

In Kenya, analysis of the first outbreaks of LSD suggested introduction by sheep infected naturally and showing clinical signs of smallpox, without the virus responsible being identified (Capstick 1959), and cases have been reported in sheep and goats during certain epizootic outbreaks (Chamoiseau 1985, Davies 1976). In the Kenyan epizootic outbreaks, where sheep and goats concomitantly and comparably expressed clinical signs of smallpox, a viral isolate (strain KSPGV 0240) was later characterised as being a strain of LSDV (Davies 1976).

Different experimental studies attest to the ability of the LSDV strains to replicate in small ruminants. Sheep and goats infected experimentally (ID route) develop a local reaction (swelling

and redness) at the injection site (Weiss 1968). The LSDV's replication ability in sheep has been demonstrated by viral isolation on sheep infected experimentally (ID or SC route) with different field isolates. These sheep had erythematous swellings at the injection site and hypertrophy of the draining lymph nodes (Barnard *et al.* 1994). Additional studies have demonstrated that goats and sheep infected experimentally (ID route) with the Kenyan Londiani strain of the LSDV developed lesions similar to those induced in cattle (Capstick 1959). These results contrast with the absence of isolation of LSDV in the field from small ruminants in South Africa, where natural infection has only been observed in cattle (Hunter and Wallace 2001). Moreover, no recent publications have reported observing cases of LSD in sheep and goat populations in Europe and the Middle East.

However, all these data are insufficient for being able to rule on the role of small ruminants in the dispersal of the LSDV, and additional work, including field studies and experimental infections under controlled conditions, is necessary.

- ✓ Wild ruminants (wildlife or animals in captivity)

Few data are available today concerning LSD in wild ruminants. Certain species, whether free or maintained in captivity in parks, zoos or circuses, can potentially contribute to sustaining or spreading the virus in the event of infection.

First of all, to date, no confirmed epizootic outbreaks involving wildlife with expression of clinical signs have been observed in the field (Babiuk *et al.* 2008). It would still be advisable, however, to determine whether the lack of reports may be attributed firstly, to the difficulty in observing skin lesions in wildlife, especially in concealed cases, and secondly, to predators singling out animals developing LSD (Barnard 1997).

One case of LSD was reported in an Arabian oryx (*Oryx leucoryx*) in a group of 90 animals in captivity in Saudi Arabia (Greth *et al.* 1992). The virus was then observed by electron microscopy on skin nodules, attesting to the presence of a poxvirus, and neutralising antibodies against *Capripoxvirus* were identified by serum-neutralisation on paired sera. In the framework of this study, serological monitoring was carried out on the 90 animals (196 sera in total) and showed a neutralising antibody response in only two animals.

In an experimental framework, clinical signs characteristic of LSD were observed in one or more impalas (*Aepyceros melampus*) and giraffes (*Giraffa camelopardalis*) after inoculation of the LSDV Neethling strain (Young, Bassoon, and Weiss 1970). Virological analyses on tissue samples confirmed the presence of the virus, while histopathological analyses led to the identification of microscopic lesions identical to those observed on cattle. In contrast, in this same study, neither black wildebeest (*Connochaetes gnou*) nor African buffaloes (*Syncerus caffer*), also inoculated with the LSDV Neethling strain, developed clinical signs or antibody responses.

Recently, the presence of LSDV nucleic acid was reported in hide samples collected from springbok (*Antidorcas marsupialis*) in South Africa (Le Goff *et al.* 2009).

On the basis of the presence of antibodies, a few species of wild ruminants are also considered to be receptive to the virus: blue wildebeest (*Connochaetes taurinus*), black wildebeest (*Connochaetes gnou*), common eland (*Taurotragus oryx*), African buffalo (*Syncerus caffer*), springbok (*Antidorcas marsupialis*) and sable antelope (*Hippotragus niger*) (Barnard 1997). Another serological investigation on sera collected over 19 years from large numbers of animals (3445 sera, 44 species) showed low levels of antibodies only in six species: greater kudu (*Tragelaphus strepsiceros*), two species of waterbuck (*Kobus ellipsiprymnus* and *Kobus defassa*), reedbuck (*Redunca arundinum*), impala (*Aepyceros melampus*), springbok (*Antidorcas*

marsupialis) and giraffe (*Giraffa camelopardalis*) (Hedger and Hamblin 1983). In this study, neither the African buffalo nor the wildebeest presented neutralising antibodies. More recently, the seroprevalence of LSD in African buffalo in two Kenyan animal parks was evaluated at 28.2% (248 animals tested by ELISA) and 7.6% (66 sera tested by viral neutralisation) (Fagbo, Coetzer and Venter 2014). However, the epidemiological role of the African buffalo has not been clearly established and in several studies conducted on wildlife, these animals showed no signs of infection by the LSDV (Hedger and Hamblin 1983, Barnard 1997, Hamblin *et al.* 1990).

A meaningful analysis of the results of these serological surveys would require several points to be taken into account. Although the presence of antibodies in an animal does indicate its exposure to the virus and its potential for involvement in the epidemiological cycle (Barnard 1997), it does not necessarily imply that it could constitute a source of the virus. Lastly, because a concealed or asymptomatic clinical infection is not always accompanied by a level of antibodies that can be detected by serum-neutralisation, serological investigations using serum-neutralisation very likely underestimate the actual number of infected animals among wildlife.

There are currently no data on the receptivity and susceptibility of wild ruminant species present in Europe, such as Cervidae.

To summarise, natural infection associated with the development of clinical forms is only observed in cattle, zebus and water buffaloes. Due to the frequency at which they have been affected in all the countries recognised as infected, and their sensitivity, cattle are the primary source and host of the LSDV.

According to some natural and experimental observations, domestic small ruminants and various species of wild ruminants are considered to be susceptible species. However, they do not seem likely to play a significant role in the epidemiology of the disease. Nonetheless, studies are needed to determine their actual role.

■ Sources of the virus other than infected live ruminant animals

Infected cattle, whether or not they are clinically affected, constitute the main source of the virus. As described in the "Pathogenesis" section, the virus can be found in these animals in many tissues and organs, especially the skin, in particular in the skin nodules. The virus is also found in various secretions and excretions; however, it is not detected in the urine and faeces of sick animals (Babiuk *et al.* 2008).

These data suggest that certain products and by-products of bovine origin, such as hides, semen, milk and meat, could be secondary sources liable to contribute to the spread of the virus. The same is true for certain contaminated inert media or objects in contact with infected animals or contaminated products.

✓ Products of animal origin

The products and by-products concerned are mainly those derived from infected cattle, and possibly from domestic buffaloes or zebus. Other sources may nevertheless be considered, as shown by the study that helped identify LSDV nucleic acid in hides from wild ruminants (Le Goff *et al.* 2009).

- Carcasses and meat

The majority of experimentally-infected animals present detectable viraemia (by PCR or viral isolation). Apart from the skin (which represents the primary target tissue), the virus can also be found, inconsistently and in far lower quantities, in various tissues or organs: lymph nodes, lungs, spleen, kidneys, liver, cardiac tissue, ovaries, etc. The available data come essentially from experimental infections, including via the IV route, which seems best suited to achieving generalised infection (Babiuk, Bowden, Parkyn *et al.* 2008).

As far as the experts are aware, there is no study available on screening for the LSDV in the meat of infected animals. Nevertheless the viraemia in experimentally-infected animals, which can be detected by PCR or viral isolation, suggests the presence of the virus, at low titres, in these products. Moreover, no data are available on the survival of the virus in meat.

- Hides

The highest and most regular viral concentrations are detected in the skin lesions that develop at the beginning of the hyperthermia phase and persist for 2 to 4 weeks. After experimental infection, the virus is detected at titres of 5.1 to 5.3 $\log_{10}$ of plaque-forming units (PFU)/mg at 13 and 15 days PI (Babiuk *et al.* 2008). In this same study, genetic material was still detectable by PCR at 42 days PI, although no infectious virus was isolated. In contrast, the virus was not detectable in the skin outside the skin lesions, except sometimes by PCR at low titres. Weiss showed that healthy skin sampled from sick animals was virulent (Weiss 1968), and viral titres in samples of healthy skin, collected from cattle infected experimentally and exhibiting lesions, can be very high (De Clercq, personal communication).

The virus can be isolated in skin lesions of convalescent cattle for up to 39 days PI, and the genetic material is detected up to 92 days PI (Tuppurainen, Venter and Coetzer 2005).

The virus remains stable in the dried skin lesions and persists for at least 33 days at normal temperature (Weiss 1968).

- Semen, embryos, oocytes

The LSDV is shed in the semen of bulls infected experimentally (Irons, Tuppurainen and Venter 2005, Osuagwuh *et al.* 2007, Tuppurainen, Venter and Coetzer 2005, Weiss 1968, Annandale *et al.* 2010). It can be isolated from certain bulls 4 to 5 days after the onset of fever and skin lesions, and for at least 37 days. By PCR, viral nucleic acid can be detected in semen for up to 5 months PI (Irons, Tuppurainen, and Venter 2005, Tuppurainen, Venter and Coetzer 2005). Screened for by PCR, the viral material is detected in all the fractions of the ejaculate, and in the testes and epididymis (Annandale *et al.* 2010). The virus is more easily and regularly isolated in the semen of severely affected bulls, but it can also be detected by PCR in the semen of bulls not developing dermal effects (Osuagwuh *et al.* 2007). Nevertheless, in all these experiments, the virus present could not be quantified in semen.

The risk associated with artificial insemination has clearly been recognised. The virus is present in 9% of the sperm, and the blastocyst contains viral DNA (Irons 2008).

The experts are unaware of any publications concerning the effectiveness of standard procedures for processing and washing embryos to eliminate the virus. However, the IETS (International Embryo Transfer Society) has classified the LSDV in Category 4, which means that, based on

studies completed or under way, the risk of embryo transmission may not be negligible even if the embryos are handled according to the recommendations of the IETS (IETS 2015).

By inseminating heifers with experimentally-contaminated sperm (1 mL of virus suspension with a titre of 5.5 $\log_{10}$ $TCID_{50}$/mL), it is possible to reproduce the disease and demonstrate the possibility of transmitting the infection by this route, including in the embryos of inseminated females (Annandale *et al.* 2014). However, the high dose of the inoculum used means that it is impossible to conclude as to the real risk associated with the contamination of semen of naturally-infected animals.

Following experimental infection by the IV route, the absence of shedding has been demonstrated in the semen of bulls previously immunised with the attenuated Neethling vaccine strain (unlike in that of non-vaccinated control subjects) (Osuagwuh *et al.* 2007).

Despite the absence of literature and field data, it is not possible to rule out transmission by oocytes and embryos.

- Milk

The virus can be detected in the milk of females affected by LSD (Sharawi and Abd El-Rahim 2011, Weiss 1968). This can be as a result of possible viral shedding in milk but also contamination from the skin lesions during milking. In water buffalo affected by LSD in Egypt, the genetic material was detected by PCR in six samples of milk from 10 affected animals (Sharawi and Abd El-Rahim 2011). The fact that it was impossible to isolate the virus (culture on embryonated egg) from samples indicates low titres in the tested milk. In addition, as seen above, the virus is inactivated in 2 h at 56°C and 30 min at 65°C (OIE 2013b).

✓ <u>Contaminated inert media</u>

Because of the high virus levels in the skin lesions, skin flakes and scabs from sick animals are certainly the primary sources likely to lead to contamination of the animals' environment. This environment can also be contaminated, during the septicaemic phase, by saliva, nasal secretions, milk, or any other emunctory from infected animals, as well as by fresh hides or any other tissues from these animals.

Like the other *Capripoxvirus*, the LSD virus is fairly stable in the external environment (Weiss 1968, OIE 2013b, Lefèvre and Gourreau 2003). As studied in the section on the resistance of this virus (page 21), the LSDV is inactivated after several days at room temperature but resists longer in the cold (80 days at 20°C, 8 to 10 days at 37°C, 6 months at 4°C). In addition, the LSDV is sensitive to UV radiation but, in the dark, especially in the scabs, it can persist for several months (OIE 2013b).

Indirect contamination of animals by contaminated inert media (interiors of animal transport vehicles, for example) cannot therefore be ruled out, even if this has never been demonstrated. Transmission by contaminated drinking water is also regarded as possible. However, this has not been observed in tests of transmission by direct or indirect contact (shared watering) between sick and healthy subjects kept in the same premises (Carn and Kitching 1995).

In conclusion, LSDV is detectable in many different cattle products and by-products, such as hide, milk and semen, and can be found on vehicles and materials that have been in contact with infected cattle.

4.1.5.2. Methods of transmission and dissemination

Vector-borne transmission and dissemination

Although some observations suggest the possibility of direct transmission between individuals, vector-borne transmission is the predominant cause of contagion. It also partly explains the spread between farms and over a given territory. To date, only the mechanical transmission capacity of LSDV vectors has been studied, and the biological transmission capacity of the LSDV among vectors is unknown.

Two major groups of vectors are concerned, Diptera insects and *Ixodina* mites (hard-bodied ticks).

✓ Transmission by Diptera insects

Transmission by Muscidae:

The cosmopolitan species *Stomoxys calcitrans* can transmit the SPPV and GTPV (Mellor, Kitching and Wilkinson 1987). *Stomoxys* have been described as possessing a crop-like organ, suspected of serving as the pathogen "reservoir", as in mosquitoes (Coronado *et al.* 2004).

The results obtained with the LSDV are more ambiguous. During transmission tests in the laboratory, 200 *Stomoxys* fed at 24 h intervals on a viraemic bovine and then on a healthy animal did not transmit the LSDV (Chihota *et al.* 2003). PCR analyses on the *Stomoxys* fed on the viraemic cattle showed positive results immediately after the blood meal and 24 h after this meal, but all analyses were negative from 2 to 20 days post-contaminated meal. This suggests that the virus may not persist for more than 24 h in the body of the *Stomoxys*. However, this test was not repeated. Lastly, the 24 h period between the blood meals on the viraemic animal and the healthy animal was perhaps too long to allow the transfer of the viral infection. For this reason, additional experiments should be conducted in which the interrupted meals of *Stomoxys* on an infective bovine are supplemented immediately by a meal on a healthy animal.

The viral load in blood is usually low, often making it difficult to isolate the virus, particularly in clinically moderate forms. Its detection in blood is intermittent, including by PCR (Carn and Kitching 1995, Osuagwuh *et al.* 2007, Babiuk, Bowden, Parkyn *et al.* 2008). In fact, as far as the experts are aware, no data have been published on blood virus titres in viraemic animals.

In addition, *Stomoxys* may be transported by the winds over long distances and transmit the virus at their point of arrival (Klausner, Fattal and Klement 2015). The long-distance movement of *Stomoxys* (several hundred kilometres) has indeed been reported, particularly in Florida (Hogsette and Ruff 1987). However, this does not always seem to be the rule. In fact, less than 5% of captured-marked *Stomoxys* are recaptured more than 5 km from their release site (Taylor *et al.* 2010).

These observations led to the role of *Stomoxys* being suspected when the first cases of LSD appeared in Israel in August 1989. The *Stomoxys* may have been transported by the winds from Egypt, which was infected (Yeruham *et al.* 1994). In this case, it was the main assumption regarding introduction of the disease, because no new individual had recently been introduced into the infected herds. In addition, *Stomoxys* spp. are the predominant blood-sucking insects in Israel (Yeruham *et al.* 1994). On the other hand, observations in outbreaks of besnoitiosis, another emerging disease of cattle in Europe transmitted mechanically by *Stomoxys*, have shown that disease-free farms, surrounded by affected farms, retained their disease-free status for several successive years (Jacquiet *et al.* 2013). Thus, the exact role of *Stomoxys* in transmitting the LSD virus over long distances or within the more restricted boundaries of a farm remains to be clarified.

Another species of Muscidae, *Haematobia irritans*, has been implicated in the transmission of LSD in Israel but without any formal evidence being provided (Kahana-Sutin *et al.* 2016). This suspicion arises solely from the concomitant observation of abundant populations of this fly and the first cases of LSD on suckler cows at pasture.

Transmission by tabanids:

While the *Tabanidae* (4400 species described in the world, around a hundred in metropolitan France) are often implicated in the mechanical transmission of viruses (equine infectious anaemia, enzootic bovine leucosis, etc.), no mention has been made in the literature of the possible transmission of the LSDV by tabanids (Baldacchino *et al.* 2014). The "horse-fly" model is more difficult to work with in experimental conditions than the Culicidae or *Stomoxys*, which perhaps explains the absence of any specific study on LSD. Among the tabanids, completion of a blood meal interrupted by a defensive movement of the bitten animal is immediate. It takes place either on the same animal or on another bovine in the vicinity of the first. This radius may extend up to 25 metres, but it is most often less than 5 metres (Barros and Foil 2007). This trophic behaviour tends to support the intense mechanical transmission of a pathogen in the immediate vicinity of the infected cattle.

Transmission by Culicidae:

The species *Aedes aegypti* is capable of transmitting the virus in experimental conditions from viraemic cattle to a recipient animal (Chihota *et al.* 2001). Transmission takes place even if the period between the meal on the viraemic animal and the meal on the healthy animal is 6 days, and this raises the question about the persistence of the virus in the mosquito's body, for example in the crop. Indeed, the Dipteran crop (ventral diverticulum that comes after the oesophagus and extends until the fourth abdominal segment) is a pouch whose function is to store blood at the time of the meal on the host. As it lacks any enzymatic equipment, the blood stored here is not degraded. This only occurs when the crop is emptied little by little into the midgut. This possible persistence of the virus in the mosquito's body without it replicating could have major epidemiological implications because it would theoretically allow longer-distance contamination (in particular between herds) than if the virus were rapidly destroyed in the vector.

The clinical signs observed in recipient cattle during transmission by *Aedes aegypti* were mild, which led the authors to make the assumption of a moderate quantity of inoculum transported by these mosquitoes (Chihota *et al.* 2001).

Aedes aegypti is not present in metropolitan France but a species of the same genus, *Aedes albopictus,* is now very widespread there. There are no data on the possible transmission of LSD by *A. albopictus* but this should be studied.

Tests of transmission by *Anopheles stephensi* and *Culex quinquefasciatus* have proved unsuccessful (Chihota *et al.* 2003).

Transmission by Culicoides:

Two studies provide data on the potential role of *Culicoides* in the transmission of LSD. The first, published in 2003, indicates that the LSD virus is detected by PCR and viral isolation from female specimens of *C. nubeculosus,* immediately after the blood meal on an infected bovine. However,

these two tests (viral isolation and PCR) were negative in these insects from 24 h up to 20 days after the infective meal, which seems to rule out replication of the virus in this species (Chihota *et al.* 2003). In contrast, the second study reported that viral replication may take place in females of the species *C. punctatus,* although the vector role of this species was not formally demonstrated in this article (Şevik and Doğan 2016). In the absence of additional data, it is difficult at the present time to have a precise idea of the potential role of *Culicoides* in the transmission of the LSD virus.

- ✓ Transmission by ticks

Transmission of the LSD virus by ticks is an assumption made by teams of the Pirbright Institute (UK) and Onderstepoort (South Africa) (Tuppurainen *et al.* 2011).

The species in question are *Rhipicephalus appendiculatus*, a species found in upland wooded savannas in Eastern and Southern Africa, *Rhipicephalus* (formerly *Boophilus) decoloratus*, a hygrophilic species from sub-Saharan Africa, and *Amblyomma hebraeum*, a species from southern Africa (Zimbabwe, Botswana, Mozambique and South Africa).

Transmission can be intrastadial. This means that a male or female tick interrupts its meal on the first host, changes host and ends its meal on a second host. This behaviour is natural and very common in males, but is less frequent in females (it occurs, however, in the event of the host's death or very pronounced grooming behaviour). In this case, the tick acts as a mechanical vector (Lubinga *et al.* 2015). This is the natural mode of transmission of *Anaplasma marginale* by *Boophilus microplus* or *decoloratus* (F. Stachurski, CIRAD, personal communication).

Transmission can also be transtadial (from the nymph to the adult, for example) but also transovarial (the female transmits the virus to her progeny, and the larvae from this female will be able to transmit the virus) (Lubinga *et al.* 2014a, b). These two modes of transmission (transtadial and transovarial) have been demonstrated experimentally for the three species mentioned above. There does not appear to be any viral multiplication but rather a viral persistence over a long period of time, although this point remains obscure for the moment (Lubinga *et al.* 2014b).

Viral DNA has been found in homogenate of ticks collected in the field, for the three species in Egypt (*R. decoloratus*) and South Africa (*R. appendicaulatus* and *A. hebraeum*) (Tuppurainen *et al.* 2015).

None of these three species is, however, found in Europe. In France, the species commonly found in cattle are *Ixodes ricinus*, *Dermacentor marginatus*, *Dermacentor reticulatus* and *Haemaphysalis punctata,* about which no information is available with regard to their capacity to transmit the LSD virus.

The literature provides very little information on the European arthropod vectors currently involved in spreading the LSDV in Eastern Europe, whether regarding the species involved or the mechanisms of transmission. However, because virus transmission by vectors is very likely to be purely mechanical, all cattle-biting arthropods found in Europe (*Stomoxys*, horse-flies, mosquitoes and ticks) can potentially play a role in the transmission of the LSDV from one bovine animal to another.

Non-vector-borne transmission and dissemination

The LSDV is generally transmitted between farms as much by the introduction of vectors as by infected live animals.

In a study carried out in Ethiopia in 2007-2008, livestock movements were the main factor associated with the clinical form of LSD, with an odds ratio of 8.5 (95% confidence interval: 6-11; p <0.001) (Gari *et al.* 2010).

In the same country, the animal production systems and marketing chain appeared to play an important role in the introduction or reintroduction of LSD in zones free of this disease. Thus, on the basis of a risk analysis, it was demonstrated that bulls from the area of Borena (a pastoral area of Ethiopia where LSD is enzootic) constituted a high risk factor for the introduction of LSD in animal fattening stations, mainly due to the absence of clinical inspection carried out by veterinarians or implementation of any laboratory diagnostic test before the animals were moved into these fattening stations (Alemayehu, Zewde and Admassu 2013).

During a workshop held in May 2016 and organised by EFSA and DG SANTE (EC), a summary of information was presented from a questionnaire completed before the meeting by the authorities of the countries recently affected by LSD in Europe and in the Middle East, in order to share their experiences regarding this disease. Among other information, the infected countries were asked to list the routes of introduction of the disease into their territories. In five out of six countries, vectors were mentioned. It therefore seems that vectors have been the main route of introduction of the disease into these countries. However, other routes of introduction were also mentioned, such as movements of vehicles (for two countries), animals (for one country) and people (for one country) (EFSA 2016a). Illegal movements of live animals were highlighted as a problem in Bulgaria (FAO 2016).

In the countries affected by LSD, dispersion of the disease between remote areas has also been associated with movements of animals (usually illegal movements of clinically or asymptomatically infected animals) (EFSA 2016a). In Turkey, the outbreaks that appeared in the provinces of Sivas and Konya, located respectively more than 400 km to the north and 500 km to the north-west of the previous epizootic outbreak, were also attributed to animal movements (EFSA 2015).

Livestock movements were analysed as one of the most important risk factors in the occurrence of an epizootic outbreak in Switzerland. For regulatory reasons, each livestock movement must be reported in Switzerland, and this information is kept in a database of the movements of Swiss livestock[4]. The authors thus estimated that taking into account an incubation period of 28 days for LSD, a total of a little less than 20 million cattle transfers considered to be at risk were counted during the period from 1 January 2011 to 30 January 2012 (Hässig *et al.* 2015).

In conclusion, in addition to the virus being dispersed by the vectors, it could also be spread passively, over longer distances, by movements of vehicles that may be contaminated when transporting potentially-infected animals, or products or by-products of bovine origin.

[4] https://www.agate.ch/portal/fr/web/agate/die-tierverkehrsdatenbank-tvd (consulted on 25 November 2016)

4.1.6. Spatio-temporal distribution

LSD is widespread in the African countries where it is enzootic. The American and Australian continents are free with regard to all *Capripoxvirus*.

Identified for the first time in 1929 in sub-Saharan Africa, where it originated, LSD then spread over the following decades towards both the north and the south of the African continent (Woods 1988). In 1988, the first outbreaks were observed in Egypt (House *et al.* 1990) and then, for the first time outside the African continent, in Israel in 1989 (Yeruham *et al.* 1995).

Since then, outbreaks confirmed in the laboratory have been observed in the Arabian Peninsula and in the Middle East: Azerbaijan, Cyprus (North), Iran, Iraq, Israel, Jordan, Kuwait, Lebanon, the Palestinian Autonomous Territories, and Turkey in November 2013. Outbreaks of LSD were identified in Russia in May and September 2015, and then in May 2016, on the border with Azerbaijan and Georgia. Azerbaijan declared sixteen outbreaks in 2014 (none in 2015 or 2016). The epidemic has continued its progress west, affecting Greece in August 2015, Bulgaria and the Former Yugoslav Republic of Macedonia (FYROM) in April 2016, Serbia in May 2016, Albania in June 2016, and Montenegro in July 2016 (it has also progressed toward the east: Armenia, Kazakhstan, Russia and Georgia, but the available data are fragmentary) (Arsevska *et al.* 2016).

Table 1: Outbreaks of LSD in Europe in domestic cattle, situation at 29 November 2016 (source: ESA Platform, European Commission, December 2016)

Country	Date of the first outbreaks	Number of outbreaks	Measures put in place
Montenegro	21/07/2016	64	- vaccination campaign under way - delivery of 25,000 vaccine doses on 31/07/2016 and request for 70,000 additional doses to vaccinate the entire territory
Albania	28/06/2016	218	- vaccination campaign under way - delivery of 25,000 vaccine doses on 25/07/16
Kosovo	20/06/2016	46	- vaccination campaign under way - delivery of 25,000 vaccine doses on 01/07/16 and 50,000 in December 2016
Serbia	08/06/2016	221	- vaccination campaign for the entire country under way - delivery of 400,000 vaccine doses in the week of 27/06/16
Former Yugoslav Republic of Macedonia (FYROM)	22/04/2016	113	- vaccination campaign for the entire country under way - delivery of 50,000 vaccine doses on 22/05/2016

Country	Date of the first outbreaks	Number of outbreaks	Measures put in place
Bulgaria	14/04/2016	217	- vaccination campaign for the entire country completed on 15/07/16 - delivery of 150,000 vaccine doses in April 2016 and 50,000 more in December 2016
Greece	21/08/2015	221	- vaccination campaign in the north-east in 2015 and then vaccination in 2016 extended to the west - delivery of 50,000 vaccine doses on 27/04/16, and 50,000 additional doses on 24/07/16 and 50,000 additional doses in December 2016

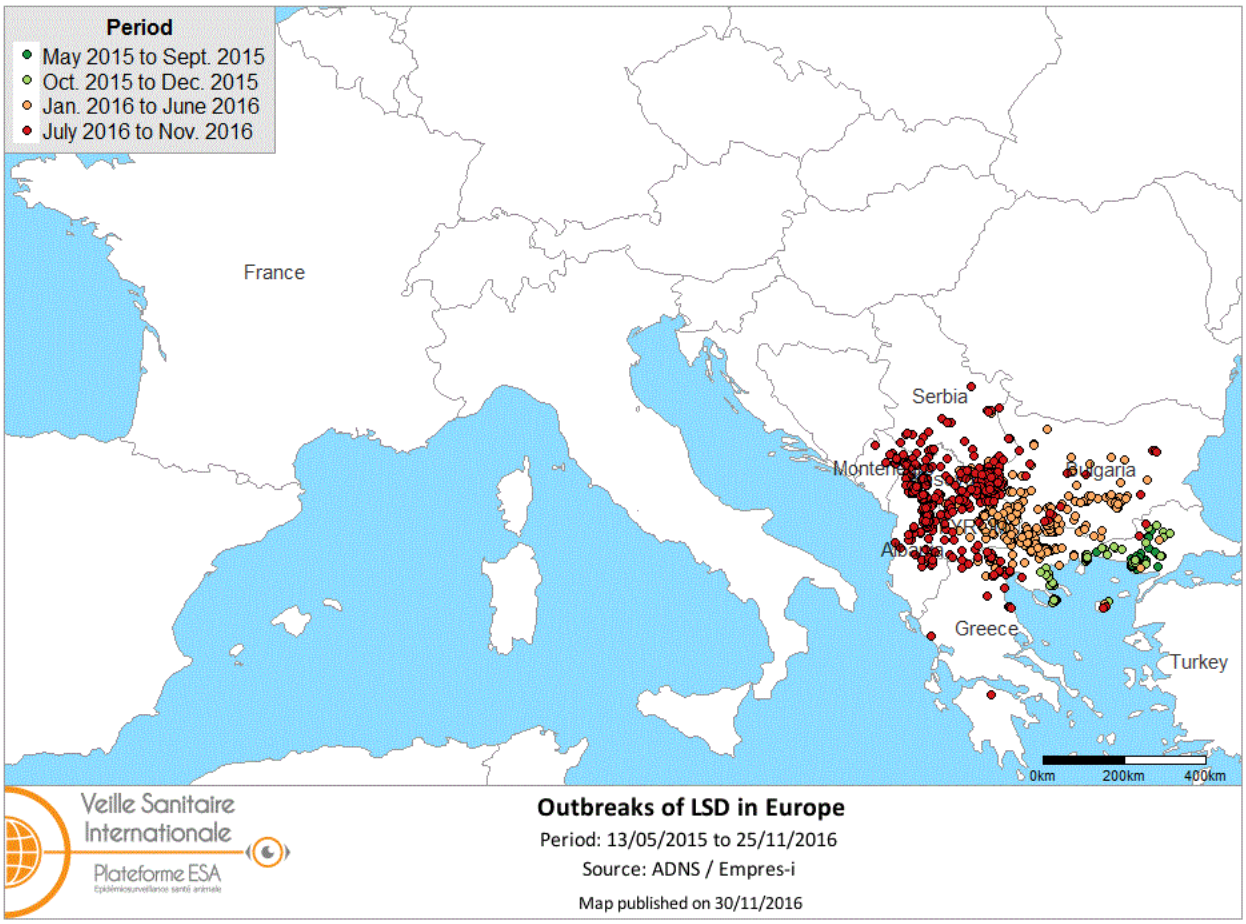

Figure 1: Spatial distribution of BLSD since its introduction into the European part of Turkey in May 2015. Situation as of 29 November 2016 (source: ADNS/Empres-I).

Identified for the first time in Zambia in 1929, LSD then spread widely across the African continent and subsequently, from 1989, outside Africa, affecting Israel and the countries of the Middle East. Since 2015, it has affected various European countries, including Russia, Greece, Bulgaria, the Former Yugoslav Republic of Macedonia (FYROM), Serbia, Kosovo, Albania and Montenegro.

4.1.7. Diagnosis and control of the disease

4.1.7.1. Diagnosis

Because of the difficulties encountered in carrying out a differential diagnosis under certain circumstances, the clinical diagnosis of LSD must be confirmed by a fast and precise laboratory diagnosis, to enable the immediate implementation of appropriate control measures.

- **Identification of the agent**

Samples should preferably be taken from the skin nodules, biological secretions (conjunctival, nasal, oral), whole blood (EDTA tube) and biopsies collected post-mortem from lung or lymph node lesions. Histological studies of tissue samples can identify histopathological characteristics suggestive of infection by the LSDV. Electron microscopy analyses can also be performed to observe the characteristic morphology of viral particles of *Capripoxvirus* present in most of the damaged tissues (Munz and Owen 1966, Davies *et al.* 1971). The antigen may also be detected by immunofluorescence. The use of this technique is restricted, however, due to a number of limitations, including its cumbersome implementation and the expertise required by the operator (OIE 2016).

The tests most commonly used today for first-line identification of the LSDV are still molecular tests based on PCR, conventional or in real time. Thus, several conventional PCR tests (Ireland and Binepal 1998, Heine *et al.* 1999, Tuppurainen, Venter and Coetzer 2005, Stram *et al.* 2008) and real-time PCR tests (Balinsky *et al.* 2008, Bowden *et al.* 2008, Lamien, Lelenta *et al.* 2011, Stubbs *et al.* 2012, Haegeman *et al.* 2013) have been described, and the performance of some of them (specificity and sensitivity) has been characterised. Real-time PCR allows the simultaneous detection, quantification and differentiation of different *Capripoxvirus* (Lamien, Lelenta*, et al.* 2011). In addition, approaches based on the Loop-mediated isothermal AMPlification (LAMP) technique also enable detection of *Capripoxvirus* genomes with a sensitivity and specificity comparable to the real-time PCR tests, and have the advantage of a simpler and less expensive technology (Das, Babiuk and McIntosh 2012, Murray *et al.* 2013). Lastly, cell lines may also be utilised to isolate the LSDV. As mentioned above, the LSDV replicates *in vitro* in a wide range of cells, including primary ruminant cells or cell lines (Binepal, Ongadi and Chepkwony 2001, Babiuk *et al.* 2007). Around ten days are needed to show a cytopathic effect (Plowright and Witcomb 1959, Davies *et al.* 1971) and specific identification of the virus is then carried out by immunohistochemistry, immunofluorescence or PCR.

To summarise, the tests used most frequently to identify the LSDV are molecular tests based on PCR, conventional or in real time, although this virus can also be identified using immunohistochemistry or immunofluorescence.

- **Serological tests**

Due to the fact that they are of the same serotype, the LSDV cannot be distinguished serologically from the sheep pox virus and goat pox virus. Although various methods can be used in the framework of serological investigations carried out on outbreaks, the reference serological test for *Capripoxvirus* remains the viral neutralisation test. The constant-serum-dilution/variable-viral-titre test, based on the calculation of the neutralisation index, is recommended (OIE 2016). An

immunofluorescence test is also described. Its use is however restricted by various limitations, including its cumbersome implementation, the experience required by the operator and the existence of serological cross-reactions (Weiss 1968, Davies and Otema 1981, Gari *et al.* 2008). An ELISA test is not yet commercially available despite the efforts undertaken by many teams (Babiuk *et al.* 2009, Bhanot *et al.* 2009, Bowden *et al.* 2009, Carn *et al.* 1994, Heine *et al.* 1999). Various candidate tests using different recombinant proteins are currently undergoing development and validation.

To summarise, the reference serological test for *Capripoxvirus* is the virus neutralisation test. An ELISA test to detect the LSDV is not yet commercially available.

4.1.7.2. Medical prophylaxis

■ **Characteristics of the vaccines available against LSD**

✓ Commercially-available vaccines

The vaccines that have been employed against LSD all use live attenuated strains of *Capripoxvirus*, either homologous: a strain of LSDV from Kenya (KSGP O-240) or a strain of LSDV from South Africa (Neethling strain), or heterologous: a Yugoslavian RM-65 strain of SPPV (Ramyar strain) or a Romanian strain of SPPV or a Gorgan strain of goat pox (GTPV) (OIE 2016).

The main vaccines available against the LSD virus are (Kreindel *et al.* 2016):

- Attenuated homologous virus vaccines[5]:

Vaccines used within the EU:

- the Lumpy Skin Disease Vaccine for Cattle® from Onderstepoort Biological Products (OBP), South Africa (Neethling strain[6]),
- Lumpyvax® – MSD Animal Health, South Africa (attenuated field strain, SIS type[7]).

Vaccines not used in the EU:

- Herbivac LS® – Deltamune, South Africa (Neethling strain);
- vaccines using the KSGP (Kenya sheep and goat pox virus) strains O-240 and O-180 (LSD virus) from different laboratories, for example, for the O-240, Kenyavac® – JOVAC (Jordan Bio-Industries Center) (Jordan).

- Attenuated heterologous virus vaccines:
  - SPPV RM-65 Jovivac® – JOVAC (Jordan) and ABIC (Israel) (at 10 times the dose used in sheep);
  - Bakirköy SPPV strain – PoxvacTM®, Vetal Animal Health Products, in Turkey (3 to 4 times the dose used in sheep);
  - Caprivac® GTPV strain (JOVAC); Jordan.

[5] The attenuated homologous strains all differ as to their degree of attenuation.
[6] The first isolate of the LSD strain in South Africa was named "Neethling". Subsequently, all isolated strains presenting very similar antigenic characteristics (very low percentage of nucleotides differing from one strain to another) have been called "prototype strain Neethling".
[7] This is a virus isolated from an affected cow within a herd of the SIS Farming Group in South Africa. Genetically, it is a "prototype strain Neethling".

Thanks to the homology and cross protection between the sheep and goat pox viruses and the LSDV, it is possible to use these viruses to vaccinate against LSD (Kitching 1983). However, the use of these vaccines should be limited to countries where sheep and/or goat pox is enzootic.

The KSGP (Kenyan sheep and goat pox virus) O-240 strain is in reality a strain of LSDV (Tuppurainen *et al.* 2014) that retains significant residual pathogenicity with regard to dairy cows (Israeli Holstein breed, Yeruham *et al.* 1994).

A Romanian strain of SPPV can also be used to vaccinate cattle against the LSDV (Tuppurainen *et al.* 2014), as was the case during the 1989-90 outbreak in Egypt, although the experts did not find a commercial form of this vaccine (Brenner *et al.* 2009).

✓ Assessment of the efficacy of vaccines

Very few studies on the efficacy of vaccines against LSD are available. Most of these studies focus on field data obtained after vaccination with heterologous vaccines and leading to a comparison between the different vaccines used.

- Attenuated homologous virus vaccine:

Lumpy Skin Disease Vaccine for Cattle® from OBP (Neethling strain):

A recent study conducted in Israel compared the efficacy of OBP's Neethling strain vaccine and the SPPV RM65 vaccine (Ben-Gera *et al.* 2015). It was conducted in parallel with a large-scale vaccination campaign in the country to stop the epidemic of 2012-2013. The study focused on 15 dairy herds located in a region of Israel where the disease had not previously been described, which had been vaccinated 2 to 5 months before the study with the SPPV RM-65 Jovivac® vaccine at the dose of $10^{2.5}TCID_{50}$/ml. The cows in all the herds were vaccinated with one or the other of the vaccines. The animals aged less than 24 months were all immunised with the Neethling vaccine ($10^{2.5}TCID_{50}$/ml) in 7 herds, and with the SPPV RM-65 vaccine in 8 herds ($10^{3.5}TCID_{50}$/ml, or 10 times the dose used in sheep). A case of LSD was defined as an animal with at least five lesions typical of LSD. A herd was declared infected if it had at least one case. A severe case of LSD was defined when a fever accompanied the presence of nodules or, for dairy cows, a 20% decrease in milk production compared to the average production from the previous two days. Of the 15 vaccinated herds, eight were declared infected with LSD (declaration on the basis of clinical signs). Morbidity in the affected farms was between 0.3 and 5.7%. Only six vaccinated animals aged less than 24 months presented clinical signs of LSD, while 76 vaccinated cows had them. The incidence of LSD in cows vaccinated with the SPPV RM-65 was 2.99% and was 1.95% in those vaccinated with the Neethling strain. The Mantel-Haenszel relative risk calculated for morbidity at least 15 days after vaccination between the SPPV RM-65 vaccine and the Neethling vaccine was 1.5 (95% CI=0.9-2.4), and 3.65 (1.6-8.3) for severe cases. This Mantel-Haenszel relative risk was 4.3 (95% CI=1.6-11.5) for laboratory-confirmed cases (Ben-Gera *et al.* 2015). The authors therefore concluded that the Neethling vaccine from OBP was significantly more effective than the SPPV RM-65 Jovivac® vaccine.

Lumpyvax® - MSD Animal Health, South Africa (attenuated field strain, SIS type[8]):

According to the assessment report[9] on the efficacy of the vaccine, out of the 10 vaccinated animals, eight showed a clear humoral immune response, including one that presented a cellular immune response.

In addition, another study, described in this same report, focused on the efficacy of this vaccine. It examined 20 animals that were separated into four groups: one group vaccinated with OBP's Lumpy Skin Disease Vaccine for Cattle®, one vaccinated with the Lumpyvax® vaccine from MSD at the commercial dose, one vaccinated with the Lumpyvax® vaccine from MSD at 10 times the commercial dose, and a control group. Four weeks after vaccination, a dose of LSDV was administered intradermally using a series of dilutions. By observing the size of the lesions over 6 days, the study concluded that the Lumpyvax® is at least as effective as the Lumpy Skin Disease Vaccine for Cattle®.

Herbivac LS® – Deltamune, South Africa (Neethling strain):

Certain information from the registration dossier for this vaccine, in particular concerning its efficacy, was disclosed confidentially to the WG experts.

In addition, a recent study compared the genotype of the strain used in this vaccine and those used in the Lumpyvax® (MSD Animal Health) and in the Lumpy Skin Disease Vaccine for Cattle® (OBP) (Mathijs *et al.* 2016). This study concluded that the complete genomes of the two strains have 99.9% nucleotide identity.

Vaccines using the KSGP (Kenya sheep and goat pox virus) O-240 and O-180 strains (LSD virus):

The efficacy of these two vaccine strains at combating the LSDV has not yet been demonstrated (Kreindel *et al.* 2016).

A study conducted in Ethiopia focused on a vaccine using an attenuated KS1-O180 strain supplied by the Ethiopian National Veterinary Institute (Gelaye *et al.* 2015). This vaccine was used to vaccinate small ruminants and cattle in order to combat *Capripoxvirus*. The study focused on 13 outbreaks suspected of having been concerned by *Capripoxvirus* between 2008 and 2012, some in which the animals had been vaccinated and others not. Skin nodules were collected from sheep, goats and cattle and were analysed by PCR. The study shows a distinction between the field strain and the vaccine strain and proves that the vaccine strain was not responsible for the outbreaks. However, in view of the low impact of vaccination on the maintenance and dissemination of the disease in the country, the authors noted the poor performance of this vaccine, compounded by the low immunisation coverage in the country.

- Attenuated heterologous virus vaccine:

SPPV RM-65 Jovivac® – JOVAC (Jordan) and ABIC (Israel):

The vaccine using an attenuated SPPV RM-65 Jovivac® from JOVAC seems to be effective against LSD. Indeed, a retrospective study based on epidemiological data obtained during an

[8] This is a virus isolated from an affected cow within a herd of the SIS Farming Group in South Africa. Genetically, it is a "prototype strain Neethling".

[9] Design Biologix CC/Vision Pharmaceutical (PTY) LTD, Lumpy Skin Disease Dossier, Intervet SA (PTY) LTD, 2003-2004.

episode of LSD in Jordan on 84 farms that vaccinated and 13 that did not vaccinate their cattle shows that the morbidity related to natural infection to the LSD virus (presence of skin lesions) was lower in the vaccinated herds (5%) compared to the non-vaccinated herds (43%) (Abutarbush 2014). In this study the mortality was also reduced, since it was 10% in the non-vaccinated herds and 1% in the vaccinated herds. Moreover, in a retrospective epidemiological study conducted in Israel at the time of the 2006-2007 outbreak on 4607 cows located on 11 farms, 11% of cattle vaccinated with this vaccine and then naturally exposed to the LSD virus exhibited typical lesions of LSD, whereas this proportion reached 22% among cattle that were not vaccinated (Brenner *et al.* 2009).

In addition, the study cited above comparing the SPPV RM-65 Jovivac® with the Lumpy Skin Disease Vaccine for Cattle® from OBP (Ben-Gera *et al.* 2015) shows that the incidence of the disease among the herds vaccinated with the vaccine based on JOVAC's strain, at 10 times the dose used in sheep ($10^{3.5}TCID_{50}$/ml), was 3%. Even if there was no parallel in this study involving non-vaccinated herds, this value seems much lower than the incidence observed in Israel in the study by Brenner *et al.*, which was 22%, with this vaccine at the dose used in sheep (Brenner *et al.* 2009).

Bakirköy SPPV strain - Poxvac™, Vetal Animal Health Products (Turkey) (3 to 4 times the dose used in sheep)

The experts have no information on the efficacy of this vaccine. Nevertheless, the use of this vaccine in Turkey since 2013 has not stemmed the spread of LSDV in this country (K. De Clercq, personal communication).

GTPV Caprivac® strain - Jordan Bio-Industries Center, JOVAC (Jordan)

One study compared the Caprivac® Gorgan goat pox vaccine from JOVAC and two vaccines obtained from the Ethiopian National Veterinary Institute (NVI) (a Neethling strain of LSDV and a KSGP O-180 strain). Each of the vaccines were prepared at two levels of quantification: $10^{3.5}TCID_{50}$/ml and $10^{4.5}TCID_{50}$/ml. This study focused on 35 calves divided into seven groups (5 calves per group), with six groups being vaccinated with the three vaccines at two different doses, and one non-vaccinated control group. The LSDV was administered by the IV route with a volume of 2 ml at a titre of $10^{5}TCID_{50}$/ml, 30 days after vaccination. The clinical response was measured by noting eight key clinical signs (generalised clinical signs, nodule at the inoculation site, secondary nodules at different places on the body, lymphadenopathy, ocular and nasal discharge, oedema, fever, and loss of appetite). Each criterion was rated as follows: not detected (0), moderate (1), severe (2), very severe (3). As soon as a calf had a score higher than 5, it was considered clinically ill. The animals in the two groups vaccinated with the Caprivac® GTPV were not considered clinically affected whereas for all the other groups (vaccinated or not), between 2 and 4 calves out of the 5 were considered clinically affected. This study shows that the Caprivac® GTPV seems to provide effective protection against LSD, which is not the case with the other two Ethiopian vaccines studied.

- ✓ <u>Safety</u>
  - Homologous vaccines

The study conducted in Israel and already mentioned above showed that an attenuated Neethling strain (Lumpy Skin Disease Vaccine for Cattle®) caused side effects in vaccinated cattle (Ben-

Gera *et al.* 2015). In this study, when a case was detected, an analysis of the strains present in the nodules was carried out in the laboratory. In this study, the vaccine strain was found in the lesions in nine vaccinated cattle, accounting for 0.4% of vaccinated animals (n = 2356). A single vaccinated animal had a severe case of vaccine LSD (Neethling disease), which in this study represented 0.04% of vaccinated animals. The vaccine LSD, caused by vaccination with a Neethling strain, corresponds to a generalisation of the nodules – which are much smaller than with infection by the LSDV – over the animal's body. It disappears in 4 to 10 days.

According to the report assessing the safety of the Lumpyvax® vaccine, out of the 14 cattle in the study vaccinated with the commercial doses, none presented any secondary reaction in the 4 days following vaccination.

The use of the insufficiently attenuated KSGP O-240 strain, in comparison with a Neethling strain, may cause clinical disease in vaccinated animals (Yeruham *et al.* 1994, Tuppurainen *et al.* 2014).

- Heterologous vaccines

In a study conducted in Israel, no secondary effect was observed in cattle inoculated with a vaccine based on a strain of SPPV (RM-65 Jovivac®) (n = 2338) (Ben-Gera *et al.* 2015).

According to a questionnaire survey, conducted among farmers that had vaccinated their animals, the possible side effects of vaccination, with the use of the vaccine based on the SPPV RM-65 strain (Jovivac®, dose $10^{3.5}TCID_{50}$/ml), were mainly fever, a decrease in food intake, a decrease in milk production and the appearance of skin nodules of varying sizes over the entire body (Abutarbush *et al.* 2016).

In a study conducted in Ethiopia, herds of healthy animals were used to study the side effects of three vaccines: the Caprivac® GTPV and two vaccines obtained from the NVI. 263 cattle were divided into six groups of animals with, for each vaccine, one group vaccinated by the SC route and one by the ID route. The skin reaction at the injection site was measured 48-72 hours after vaccination and then 30 days after vaccination. The study shows that the groups immunised with the Caprivac® GTPV vaccine exhibited a greater hypersensitivity reaction than the groups vaccinated with the other two vaccines. In addition, the groups vaccinated by the SC route had significantly greater hypersensitivity than the groups vaccinated by the ID route.

Moreover, the safety of these vaccines is not always guaranteed. For example, a batch of vaccines against LSD was contaminated by BTV (bluetongue virus) (Bumbarov *et al.* 2016).

In the current, fairly limited, state of knowledge on the efficacy and safety of vaccines against LSD, the choice of the Neethling strain as the vaccine strain seems to be the only option available for the moment.

The concept of efficacy is a term that is generally used, but which expresses the sum of the therapeutic indications claimed by the manufacturer. This efficacy is validated after primary vaccination until the booster, and demonstrated by laboratory and field studies. However, regarding vaccines against LSD, there are no such studies available, since no marketing authorisation application (MAA) has been submitted, either in France or to the European Medicines Agency. No experimental data on the safety and efficacy of the vaccines, as recommended by Directive 2009/9/EC, are currently available.

The degree of attenuation of the strain is an essential parameter: if it is excessively attenuated, it will be relatively ineffective; if it is insufficiently attenuated, the frequency and intensity of the adverse effects will be increased. In every case, the degree of attenuation should be the result of a compromise between safety and efficacy. Overall, in the available studies, there are very few data from which to determine the degree of attenuation constituting the best compromise.

Information available on the safety of vaccines against LSD used in the European Union, from pharmacovigilance reports, remains very fragmentary. The main adverse effects noted are in fact those of LSD, namely: a fall in milk production, fever, nodular skin lesions, abortion and death. The incidence of adverse effects is around 0.1%.

The efficacy of vaccination within the European Union is not well documented.

- **Implementation of vaccination**

✓ Vaccination in an enzootic zone

In an enzootic situation, vaccination is the only way to control the spread of the disease. Moreover, during an animal epidemic, when sanitary control measures (slaughter and restriction of movements) are no longer effective at limiting the expansion of the disease, vaccination then becomes essential for considering eradication of the disease (EFSA 2015). A vaccine presenting guarantees of safety and efficacy is then needed.

✓ Vaccination in a disease-free zone

Although vaccinating animals in zones free of LSD using a live attenuated vaccine is not recommended, because of the potential risk of spread of an attenuated viral strain (Tuppurainen and Oura 2012), there is for the moment no evidence of any dissemination of an attenuated vaccine virus. The use of these vaccines in zones where LSD is present but not enzootic, in specific situations to control the progress and to eradicate the disease, can only be considered as a short-term solution in an emergency situation (EFSA 2015).

It is not currently possible to distinguish, on a serological basis, between vaccinated and infected animals (Tuppurainen and Oura 2012). In the event of side effects following vaccination (Neethling disease), it is possible to differentiate the field strain from the vaccine strain using a PCR method (Menasherow *et al.* 2016, Menasherow *et al.* 2014).

✓ Vaccines used in Europe

Currently, no vaccine against LSD has marketing authorisation in the EU (Arsevska *et al.* 2016). However, the European Commission authorises the use of a vaccine without marketing authorisation in the event of a serious epizootic disease, which is the case with LSD[10]. Preventive vaccination has been authorised in disease-free zones on the edge of the infected regions, following EFSA's assessment of July 2016 which shows, using a model, that vaccination seems to be the most effective tool to control the situation in the Balkans (EFSA 2016b).

In Europe, the two vaccines used are homologous vaccines:

- the Lumpy Skin Disease Vaccine for Cattle® (live attenuated virus, Neethling strain, OBP);

[10]Article 8 of Directive 2001/82/EC of the European Parliament and of the Council of 6 November 2001 on the Community code relating to veterinary medicinal products

- the Lumpyvax® (live attenuated virus from MSD Animal Health, South Africa (attenuated field strain, SIS type)).

✓ Vaccination protocol

For homologous vaccines, a single injection of the vaccine, by the SC route, is recommended. Immunity develops within 10 days of vaccination and is complete after 3 to 4 weeks. The animals may be vaccinated at any age, except for calves born to vaccinated cows, which should not be vaccinated until after the age of 6 months.

The experts have no data regarding the duration of post-vaccine immunity, regardless of the type of vaccine used (homologous or heterologous).

✓ History of vaccination in Europe

In Greece, vaccination began in 2015 in the north-eastern part of the country, in Thrace, as well as in the eastern Macedonia. It was extended to the central Macedonia, first affected by LSD in April 2016. This vaccination was carried out using the two vaccines presented above. In September 2016, it was estimated that the vaccine coverage was 100% in the infected zones and partial in the buffer zones (Arsevska *et al.* 2016).

In Bulgaria, the emergency vaccination programme was accepted by the EU on 14 July 2016[11]. The vaccine used was the Lumpy Skin Disease Vaccine for Cattle®, with the choice being based on vaccine availability, as the Lumpyvax® was not available in sufficient quantities. The purpose of vaccination in this country was to obtain full vaccine coverage.

In the FYROM, a vaccination campaign, whose objective was to cover the entire country, began on 24 May 2016. Between June and July 2016, Serbia, Kosovo, Albania and Montenegro also started mass vaccination campaigns.

The countries bordering the infected zones questioned the use of vaccination as a preventive measure. The European Commission authorised Croatia to undertake preventive vaccination. To do this, it received 50,000 vaccine doses in September 2016 (information from the presentation by the European Commission during the meeting of experts on LSD that took place on 12 and 13 December 2016 in Istanbul in Turkey[12]). The two vaccines available today are interesting to use in an enzootic situation because, despite the adverse effects, EFSA considers that there is a benefit associated with the vaccination (EFSA 2016b). However, if they are used for preventive purposes, the side effects will be more visible, which could make the adoption by farmers more difficult.

✓ Use of a vaccine against LSD in France

Article L. 5141-10 of the French Public Health Code, as amended by Order No. 2010-18 of 7 January 2010 - Art. 3, provides that "*by way of derogation from the provisions of Article L. 5141-5, the French Agency for Food, Environmental and Occupational Health & Safety may authorise,*

[11] Commission Implementing Decision (EU) 2016/1183 of 14 July 2016 approving the emergency vaccination programme against lumpy skin disease of bovine animals in Bulgaria and amending the Annex to Implementing Decision (EU) 2016/645.

[12] Presentation title "Lumpy skin disease (LSD) Epidemiological situation in Europe (update since LSD2)", by D. Dilaveris, during the meeting: "Standing Group of Experts on Lumpy Skin Disease in the South East Europe region under the GF-TADs umbrella, Third meeting (SGE LSD3) 12 – 13 December 2016, Istanbul, Turkey"

when the health situation so requires and there is no suitable authorised veterinary medicinal product, the use for a limited duration:

1° - of a veterinary medicinal product that is already authorised in another Member State of the European Union or party to the Agreement on the European Economic Area;

2° - or, failing this, a veterinary medicinal product authorised in a State other than those mentioned in 1°.

In the event of an animal epidemic and in the absence of a suitable authorised veterinary medicinal product, ANSES may also authorise, for a limited duration, the use of veterinary medicinal products that have not been subject to any marketing authorisation in any State.

These temporary authorisations for use may be suspended or withdrawn at any time if the conditions laid down in this article are no longer met or if these measures are necessary to ensure the protection of human health or animal health."

In practice, ANSES - ANMV may grant a temporary authorisation for use (TAU) to a vaccine when it considers that the benefit-risk balance supports vaccination with the said vaccine.

In conclusion, while vaccination is not recommended in the disease-free zone, it is found to be the only effective means of controlling the spread of the disease in an epidemic situation, advocated by the EU (EFSA 2016b) as long as the vaccine provides adequate guarantees of safety and efficacy. For this reason, the EU has authorised vaccination in the Member States concerned. All the infected countries and some of their neighbours have established vaccine protocols.

4.1.7.1. Health control measures in Europe

- **Slaughter and zoning**

When LSD in a herd is suspected, the European regulations impose strict restriction measures. If this suspicion is confirmed, all susceptible species must be slaughtered, the carcasses and all the waste on the holding must be destroyed, the buildings must be cleaned and disinfected, and an epidemiological investigation must be conducted. In addition, after official confirmation, a protection zone with a minimum radius of 3 km and a surveillance zone with a minimum radius of 10 km around the infected holding must be put in place. Specific measures for the identification, health control, movement and maintenance of animals are established in these two zones. As LSD is a vector-borne disease, the length of time these zones are maintained is under the control of the competent authority[13]. The new European regulations impose increased surveillance and the prohibition of movements of susceptible species within a radius of 20 km around an outbreak[14].

The experience of managing the disease in Israel, retraced in EFSA's opinion of 2015, shows that the slaughter of affected animals is essential for managing the disease without the use of vaccination, but that the slaughter of healthy animals that have been in contact with infected animals is not essential (EFSA 2015).

[13] Article 4, 5, 10, 11 and 12 of Council Directive 92/119/EEC of 17 December 1992 introducing general Community measures for the control of certain animal diseases and specific measures relating to swine vesicular disease.
[14] Commission Implementing Decision (EU) 2015/1500 of 7 September 2015 concerning certain protective measures against lumpy skin disease in Greece and repealing Implementing Decision (EU) 2015/1423.

The Israeli experience also shows that zoning and the associated regulations, particularly in terms of movement of animals, are essential to avoid the spread of the disease. In 2007, the disease only spread a maximum of 12 km from the initial focus, which strongly suggests that in the absence of animal movement, the spread of the disease is fairly limited. On the other hand, the study of the 2013 outbreaks, in which the disease spread up to 100 km away from the initial focus, linked to the unauthorised movement of animals, shows that during this episode the transport of live animals or untreated animal by-products played a role in the dissemination of the virus (EFSA 2015).

Greece did however obtain certain exemptions from the European regulations in terms of movements of animals between regulated zones and the disease-free zone, as well as on the management of certain animal by-products such as meat[15]. The European Commission also authorised the movement of animals from infected zones or disease-free zones that vaccinated animals under very strict conditions[15].

Use of insecticides

EFSA's opinion of 2015 notes that there are no data on the efficacy of insecticides in the management of LSD (EFSA 2015). However, it is possible that the use of insecticides would help limit the spread of the disease. This use would need to be at two scales: that of the animal and that of the environment (for example transport trucks). Managing vectors at the scale of the environment is further complicated by the fact that the vectors of LSD in Europe are not yet well known. The European regulations nevertheless require any vehicle having been in contact with susceptible species to be cleaned and disinfected in such a manner as to inactivate the LSDV, and treated with authorised insecticides that are effective against the vectors of LSD, before leaving an infected zone[15].

Furthermore, the use of insecticides could also be extended to rendering plants. Indeed, although blood-sucking flies such as *Stomoxys* do not bite dead animals, they may be attracted by the secretions emitted by the carcasses. Moreover, a rendering plant was the most probable hypothesis behind the occurrence of an outbreak, following an epidemiological investigation in Israel (EFSA 2015).

In conclusion, the slaughter of susceptible species in an outbreak and the establishment of zones to regulate the transport of susceptible species are indispensable but, with a few exceptions, are insufficient to limit the spread of the virus. Insecticides are probably useful for controlling the disease, although the mechanical vectors are still poorly understood, especially in Europe.

[15]Commission Implementing Decision (EU) 2016/2008 of 15 November 2016 concerning animal health control measures relating to lumpy skin disease in certain Member States.

4.2. Study of the probability of a first outbreak of LSD occurring in metropolitan France

In order to respond to the first question of the formal request regarding the risk of introduction of LSD into France, and given the time available, the experts assessed "only" the probability of a first outbreak of LSD on French territory for a year, based on the epidemiological situation in January 2017, the European regulations existing on this same date, and data on trade for 2016.

They did not take into account either the dissemination from the first outbreak, or the consequences of introduction of the LSDV.

The probability of a first outbreak of LSD occurring in France results from combining the probability of the virus being introduced into France with the probability that domestic cattle or wild ruminants are then exposed to this virus on French territory.

The expert group, taking into account all the commercial and scientific data at its disposal, conducted an assessment of the risk of a first outbreak of LSD occurring in France, depending on the different virus sources and the possible ways in which they could be introduced, represented on the outbreak diagram (Figure 3). The risk assessment was carried out according to a quantitative approach for the methods of introduction regarded by the experts as most likely (movements of animals, movements of arthropod vectors). In the other cases, the approach was qualitative.

To perform this work, the experts defined the following concepts, which were then used in the risk assessment:

- **the at-risk area:** a zone from which live cattle or products can be traded and in which there is a probability that certain animals are infected, without the disease having been declared. This concerns:
    - disease-free regions of European countries recognised as infected (as of 1 January 2017: Greece, Bulgaria, FYROM, Serbia, Kosovo, Albania, Montenegro);
    - and disease-free countries bordering a country where LSD has been notified (as of 1 January 2017: Romania, Croatia, Hungary, Ukraine, Bosnia & Herzegovina).

  The countries free of LSD that vaccinate their animals are special cases, and were not differentiated in the analysis. In addition, the infected zones of infected countries were not taken into account in the risk analysis because, according to Implementing Decision (EU) 2016/2008[15], trade is possible with these zones but can only take place under the strict condition of a specific risk analysis and a bilateral agreement between the two countries concerned.

  In their analysis, the WG experts did not consider the countries of northern Europe (Finland, Estonia and Latvia) to be in the at-risk area because, although they border an infected country (Russia), the outbreaks declared in Russia are located in the south of this country (see Figure 2). Only countries belonging to the EU were taken into account for imports of live cattle. Indeed, outside the EU, only Chile, Canada and New Zealand are authorised to export, and they are not part of the at-risk area.

  This at-risk area is shown on the map below (Figure 2).

- **The adverse event** considered for the risk assessment is the occurrence of a first outbreak of LSD in France.
- **The outbreak** is defined as the presence of at least one infected native bovine in a farm in France (an imported animal that has clinical signs is not considered to be an outbreak).

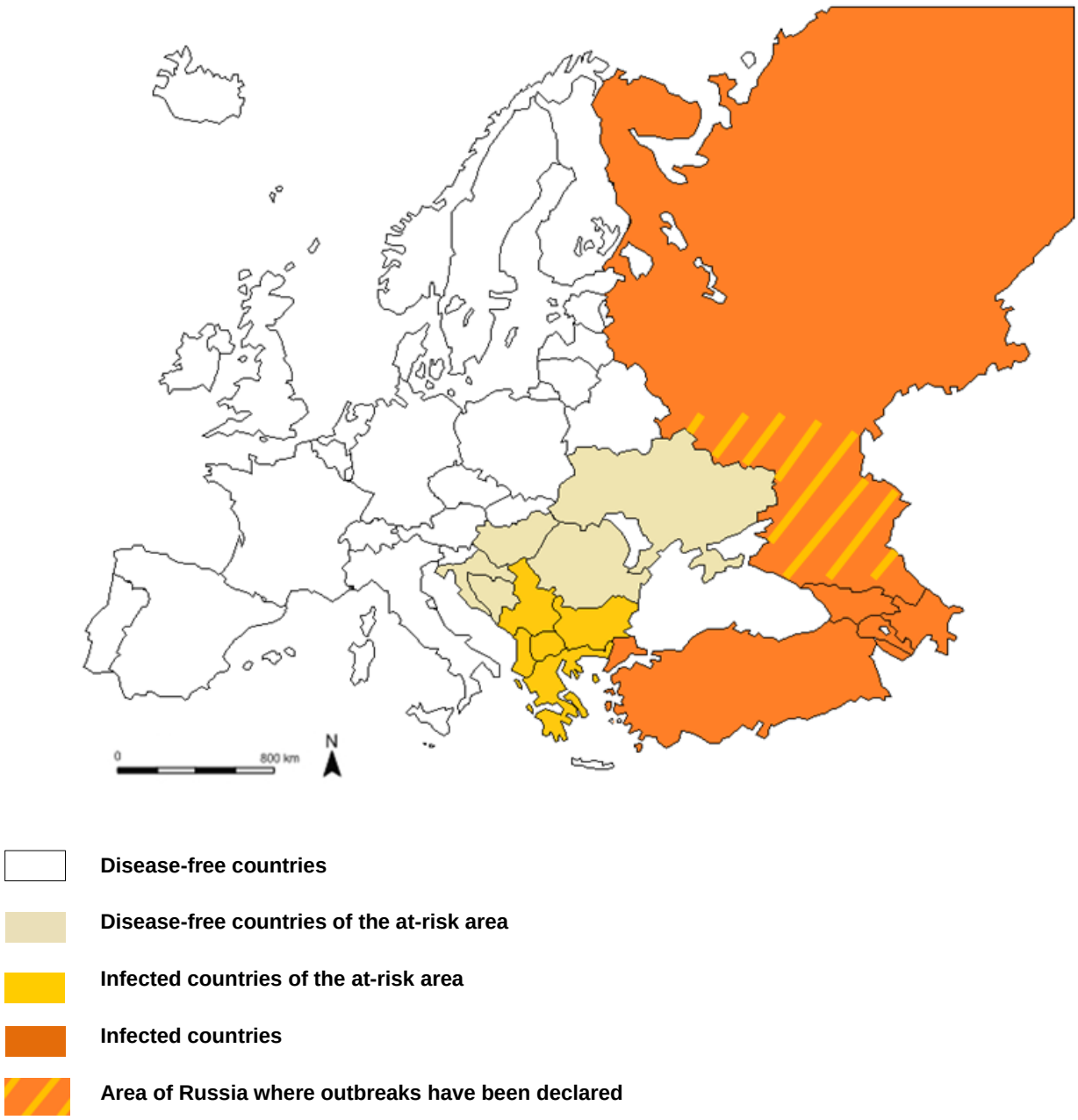

Figure 2: Map of Europe indicating the at-risk area as at 1 January 2017 (only EU countries have been taken into account for the risk of introduction of LSD via the trade in live cattle because they are the only ones in the at-risk area able to trade live cattle with France).

4.2.1. Outbreak diagram

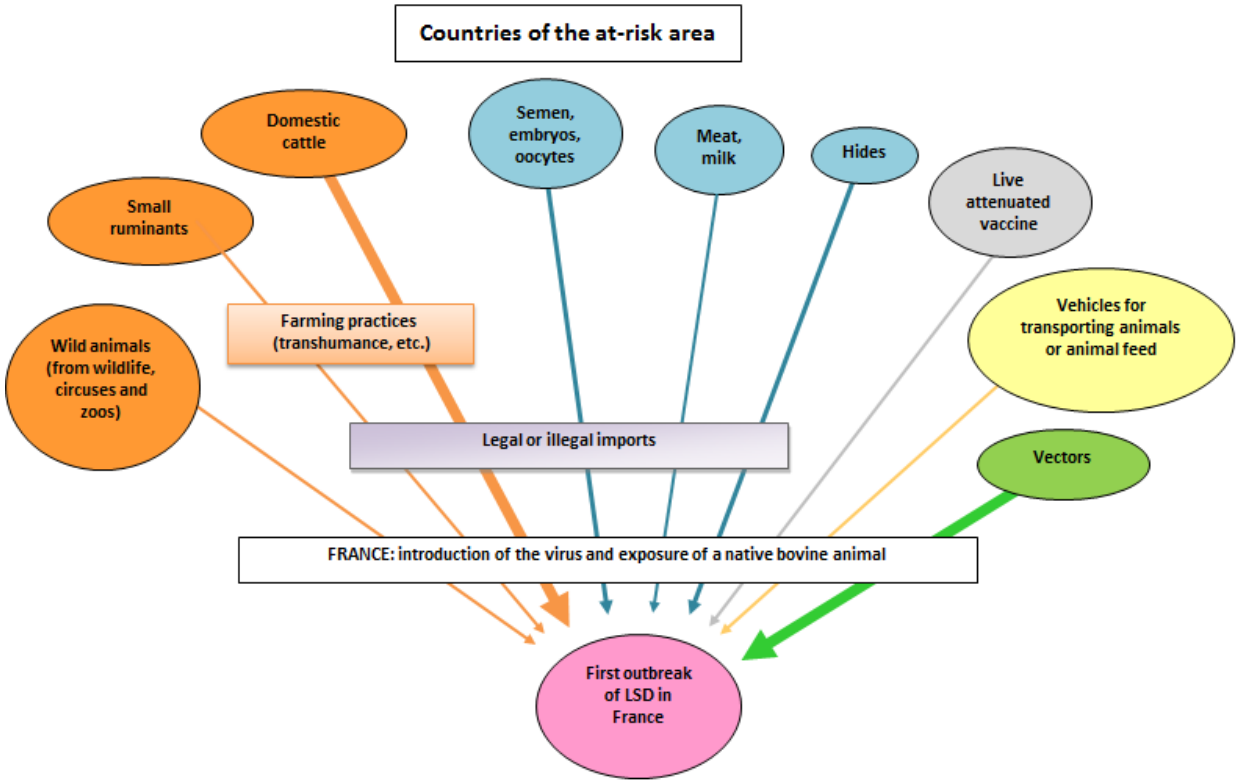

Figure 3: Outbreak diagram showing the different methods of introduction of the LSD virus into France

4.2.2. Assessment of the probability of an outbreak of LSD occurring in France

In the following sections, the probability of a first outbreak of LSD occurring is studied according to the different sources: live animals and their products (semen and embryos), vectors, inert media and other possible sources. Tables 2, 10 and 17 list the experts' arguments, and the data sources have been integrated as the text proceeds.

4.2.2.1. Arguments for the probabilities of viruses being introduced by live animals

- **Importance of the movements and introduction of live cattle**

In the analysis, only countries belonging to the EU were taken into account for imports of live cattle. In the recently affected countries, the LSD outbreaks have generally been caused by the introduction of infected live animals or vectors. For long-distance transmission, movements of infected animals, clinical or asymptomatic, seem to be the most likely cause of dissemination, along with the possible introduction of vectors transported by the wind (EFSA 2015).

In France, no introductions of live cattle from countries in Africa or the Middle East (zones where LSD is endemic) have been registered. In addition, according to the TRACES database for the period from July 2015 to July 2016, there were no movements of animals from infected Member States (Annex 6). No information was available on trade between European countries other than France (particularly between the infected countries and countries bordering France). This type of information is however of great importance for estimating the risk of importing LSD associated with movements of live animals, particularly in a changing context in which the infected zone is expanding.

■ Probability of introduction by live domestic cattle

Table 2: Summary of the elements used by the experts for the "infected live domestic cattle" method of introduction

Methods of introduction of LSD	Details	Data enabling qualification of the probability of a first outbreak of LSD occurring (Origin of the data)	Management options for reducing the probability of the first outbreak of LSD	Arguments and comments

Introduction of infected live domestic cattle	- Undeclared infected zone
	- Infected farm
	- Infected animal
	  - Not detected
	  - Contagious
	- Destination (rearing or slaughterhouse)
	- Number of animals
		Literature data
		
		TRACES data
		
		Epidemiological situation of the countries of origin (OIE data)
			Application of a screening test for the disease on entry (conditions if high risk)
			Management measures (surveillance, testing) in country of origin
				Disease spreading: neighbouring countries that may be infected without being declared
				
				Small number of introductions in 2016

As previously indicated, only animals from the EU countries belonging to the at-risk area (Greece, Bulgaria, Romania, Croatia, Hungary) were taken into account in the analysis. To estimate the probability of LSD being introduced into France via imports/trade of infected live domestic cattle, the following probabilities (Table 3) and parameters (Table 4) were taken into account.

Table 3: Probabilities taken into account in the risk of LSD being introduced into France through infected live domestic cattle

P1	Probability that the imported/traded animals come from an undeclared LSD-infected zone
P2	Probability that the imported/traded animals come from a farm infected with LSD
P3	Probability that a bovine from this farm is infected with LSD and not detected
P4	Probability that a bovine infected with LSD is contagious[16]
P5	Probability that a bovine, infected with LSD and contagious, intended for rearing, transmits the LSDV to native animals
P6	Probability that a bovine, infected with LSD and contagious, intended for slaughter, transmits the LSDV to native animals

Table 4: Initial parameters taken into account in the risk of LSD being introduced into France through infected live domestic cattle

NAR	Number of cattle introduced into a farm for rearing
NAS	Number of cattle introduced into a slaughterhouse
NCR	Number of consignments of cattle introduced for rearing
NCS	Number of consignments of cattle introduced for slaughter
NARc	Number of cattle introduced per consignment for rearing (NAR/NCR)
NAScc	Number of cattle introduced per consignment for slaughter (NAS/NCS)

According to the TRACES data for 12 months, from July 2015 to July 2016, a small number of cattle were introduced into France for rearing, from Romania and Hungary: a total of 182 animals introduced in seven consignments.

During this same period, no cattle intended for the slaughterhouse were introduced from these same countries of origin. Therefore, the experts developed a scenario using the same data for the introduction of cattle for slaughter as those used for rearing, in order to determine the potential impact of the introduction of infected cattle in this way. In the current context, this calculation has no real meaning. However if the epidemiological situation were to change with the countries present in the at-risk area and exporting cattle to France for slaughter, the weight of this mode of introduction could easily be estimated, by introducing the new data into the model.

Taking into account the probabilities (Table 3) and initial parameters (Table 4), the experts calculated the probability of imported or traded contagious cattle, from an infected farm, transmitting LSDV to native cattle, according to two methods depending on whether the cattle were intended for rearing (P7) or for slaughter (P8) (Table 5).

[16] The experts consider the term "contagious" to relate to the capacity of this animal to transmit the LSDV, either directly, by shedding it in the external environment, or by means of an arthropod vector.

Table 5: Probabilities of imported or traded cattle from infected farms transmitting the LSDV to native animals

P7 - Probability of a contagious bovine imported/traded from an infected farm transmitting the LSDV to native cattle in the destination farm	1 - (1 - P3 * P4 * P5) ^ NARc
P8 - Probability of a contagious bovine imported/traded from an infected farm and intended for the slaughterhouse transmitting the LSDV to native cattle	1 - (1 - P3 * P4 * P6) ^ NASc

Similarly, the experts calculated the probability of consignments of imported or traded cattle from the at-risk area, coming from infected farms and transmitting the LSDV to native animals, according to two methods depending on whether the cattle were intended for rearing (P9) or for slaughter (P10) (Table 6).

Table 6: Probabilities of consignments of imported or traded contagious cattle transmitting LSD to native animals

P9- Probability of consignments of imported/traded cattle intended for rearing coming from an infected farm and transmitting the LSDV to native cattle	1 - (1 - P1 * P2 * P7) ^ NCR
P10- Probability of consignments of imported/traded cattle intended for slaughter coming from an infected farm and transmitting the LSDV to native cattle	1 - (1 - P1 * P2 * P8) ^ NCS

The probabilities P1 to P6 were discussed and estimated within the LSD WG and with international specialists on LSD during the hearings and, if appropriate, were converted to probability distributions (Table 7 and Table 8).

Table 7: Arguments used for estimating the probabilities P1 to P6

Probability	Argument for the estimate
P1 - Probability that the traded cattle come from an undeclared LSD-infected zone	▪ The countries considered are the EU countries that trade live cattle with metropolitan France, either from the disease-free zones of infected countries (Greece and Bulgaria) or from countries bordering infected countries (Romania, Hungary and Croatia), i.e. cattle from the at-risk area as defined on p 47. ▪ On the basis of the experience gained during the emergence of another viral disease, bluetongue (Saegerman and Thiry 2009), whose incubation time is generally from 5 to 10 days (OIE 2013a), when estimating the probability the experts assumed that the time elapsing between the first case and its declaration, regardless of the country, was three weeks on average (minimum 7 days, maximum 60 days). This estimate was validated by the international specialists on LSD during the hearings, firstly, because the incubation period of the LSDV is 28 days and then, because in the early stage of the disease, there is a high probability of outbreaks being under-reported by farmers, because they do not notice the first symptoms, especially in zones where the disease is new. ▪ The experts calculated the incidence of the disease for a country. In 2016, of the countries in the at-risk area, six were infected and five remained free of disease (while being at risk of becoming infected). In 2016, the infected countries were on average infected after 4.5 months[17], i.e. they were considered to be at risk of being infected for 4.5 months. The countries that remained free of disease were considered to be at risk of being infected for the entire year (12 months). For the calculation, the experts therefore obtained 87 months at risk of being infected (6x4.5+5x12). The incidence is therefore 6 (countries)/87 (months at risk) = 7%. This means that a country at risk of being infected has a 7% probability of being infected during the month. ▪ **The experts estimate that this probability is between 5 and 20%, with a mode at 7%.**
P2 - Probability that the traded animals come from a farm infected with LSD	▪ The experts considered that in a recently infected country, the number of infected farms would be low (probably less than ten). ▪ This value, however, depends on farm density. ▪ In the EU, it is mainly the large farms that export. ▪ **The estimated range of probabilities is between 0.5 and 1% for the EU with a mode at 0.75%** (outside the EU this probability would likely be higher).

[17] Bulgaria and the FYROM were infected in April (3 months of being disease-free and at risk of being infected), Serbia, Kosovo and Albania in June (5 months) and Montenegro in July (6 months). Thus, on average, they were considered to be at risk of being infected for (2x3+3x5+1x6)/6 = 4.5 months.

Probability	Argument for the estimate
P3 - Probability that a bovine from this farm is infected with LSD (and not detected)	▪ Intra-herd morbidity was calculated using data from reports on declaration of infection to the OIE. The experts selected 59 farms (of more than 50 animals) declared infected between April and September 2016 in Greece and Bulgaria. The intra-herd prevalence calculated was: min: 0.3%, median: 3%, max: 25%. This value was only calculated on the number of cattle with clinical signs: Greece and Bulgaria do not perform virological tests on animals not showing clinical signs. It does not therefore relate to intra-herd prevalence but to morbidity in the herd. ▪ The estimate of intra-herd prevalence corresponds to this morbidity value multiplied by 2 because only 50% of infected animals have clinical signs (Tuppurainen and Oura 2012). ▪ However, the experts estimated that only cattle not presenting clinical signs would be exported. For this reason, the experts chose to only consider the 50% of infected cattle without clinical signs, i.e. the morbidity value calculated. ▪ **The estimated range of probabilities is therefore between 0.3 and 25%, with a mode at 3%**.
P4 - Probability that a bovine infected with LSD is contagious	▪ One animal in two expresses no clinical signs (Tuppurainen and Oura 2012). However, the asymptomatic cattle may not be contagious (Gale, Kelly, and Snary 2016) ▪ **The experts chose to consider the entire confidence interval calculated for the intra-herd prevalence (0.6% - 50%) according to a uniform distribution**.
P5 - Probability that a bovine, infected with LSD and contagious, intended for rearing, transmits the LSDV to native cattle	▪ In the farm, transmission will depend on the season. If there are many vectors, the probability of an infected bovine transmitting the LSDV to another bovine is high. In Israel, a study calculated an R0 at 16, which means that under farming conditions and in this particular region, an infected animal can transmit the virus to 16 other cattle (Magori-Cohen *et al.* 2012). Even if the environmental conditions in France may be less favourable than in this study, especially depending on the season, this value indicates that an infected animal has a 100% probability of transmitting the virus to an unaffected animal. ▪ If there are few vectors, for example in winter (P. Jacquiet, personal communication), this probability is reduced to around 30%. This value also includes other modes of transmission without vectors (for example, direct contact, semen, etc.). ▪ **The experts therefore estimated this probability to be between 30% and 95%.**

Probability	Argument for the estimate
P6 - Probability that an infected and contagious bovine, intended for slaughter, transmits the LSDV to native cattle	▪ At the slaughterhouse, there is no contact with live animals except for those that will shortly be slaughtered. ▪ There is a very low risk of vector-borne LSDV transmission from slaughtered cattle to a farm that may be located near a slaughterhouse. ▪ However, despite everything, the experts recognise that this probability is not nil. Indeed, in Greece, for certain outbreaks that occurred in the disease-free zone, the most likely assumption about the infection identified by the epidemiological investigation was the nearby slaughter of cattle originating from the infected zone (K. De Clercq, personal communication). In contrast, in Bulgaria, there have been no cases identified as linked to a slaughterhouse. ▪ **The experts estimated the values of this probability at between 0.1 and 1%.**

Table 8: Input parameters for the model analysing the risk of introduction of LSD

Input parameters		Quantitative estimate	Min.	Mode	Max.	Prob.	Probability distribution
Probability that the traded cattle come from an undeclared infected zone		5 to 20%, with a mode at 7%	0.05	0.07	0.2	**P1**	RiskPert(0.05;0.07;0.2)
Probability that the traded cattle come from a farm infected with LSD		0.5 to 1%, with a mode at 0.75%	0.005	0.0075	0.01	**P2**	RiskPert(0.005;0.0075;0.01)
Probability that a bovine from this farm is infected with LSD and not detected		0.3 to 25%, with a mode at 3%	0.003	0.03	0.25	**P3**	RiskPert(0.003;0.03;0.25)
Probability that a bovine infected with LSD is contagious		0.6 to 50%	0.006	-	0.50	**P4**	RiskUniform(0.006;0.5)
Probability that a bovine, infected with LSD and contagious, intended for rearing or slaughter, transmits the LSDV to native cattle		Rearing: 30 - 95%	0.3	-	0.95	**P5**	RiskUniform(0.3;0.95)
		Slaughterhouse: 0.1 to 1%	0.001	-	0.01	**P6**	RiskUniform(0.001;0.01)
Number of cattle introduced	Rearing	Real data	90	182	270	**NAR**	RiskPert(90;182;270)
	Slaughterhouse	Scenario	90	182	270	**NAS**	RiskPert(90;182;270)
Number of consignments of cattle introduced	Rearing	Real data	3	7	11	**NCR**	RiskPert(3;7;11)
	Slaughterhouse	Scenario	3	7	11	**NCS**	RiskPert(3;7;11)
Number of cattle introduced per consignment for rearing =						**NARc**	=NAR/NCR
Number of cattle introduced per consignment for slaughter =						**NASc**	=NAS/NCS

A Pert distribution was used when the experts were able to estimate the minimum, mode and maximum values for a distribution. This is an alternative (more plausible) distribution to the triangular distribution. A uniform distribution was used when the experts were able to estimate only the minimum and maximum values for a distribution (equiprobability that the actual value is situated between these two values).

Then, 100,000 Monte Carlo simulations were carried out using the @Risk 7.5 software, to obtain the resulting probability distributions P7 to P10. The results are summarised in Table 9 and detailed in Annex 7 (probability distributions and sensitivity analysis to show the relative influence of the different input parameters on the final result).

Table 9: Probabilities that imported or traded infective cattle or consignments of cattle transmit the LSDV to native cattle*

Probability	Parameter	Value	Qualitative expression according to the transposition grid available in Annex 8 (AFSSA 2008)
P7 - Probability of a contagious bovine imported/traded from an infected farm transmitting the LSDV to native cattle in the destination farm	2.5th percentile: Median: 97.5th percentile:	0.009 0.157 0.670	Quite high to high (7-8)
P8 - Probability of a contagious bovine imported/traded from an infected farm and intended for the slaughterhouse transmitting the LSDV to native cattle	2.5th percentile: Median: 97.5th percentile:	0.00006 0.00013 0.01	Very low to low (4-5)
P9 - Probability of consignments of imported/traded cattle intended for rearing coming from an infected farm and transmitting the LSDV to native cattle	2.5th percentile: Median: 97.5th percentile:	0.00004 0.00067 0.00326	Extremely low to low (3-5)
P10 - Probability of consignments of imported/traded cattle intended for slaughter transmitting the LSDV to native cattle	2.5th percentile: Median: 97.5th percentile:	$0.2\ 10^{-6}$ $5.7\ 10^{-6}$ $47.1\ 10^{-6}$	Nearly nil to minute (1-2)

* Probabilities calculated for one year, based on the epidemiological situation in January 2017, the European regulations existing on this same date and data on trade for 2016.

A sensitivity analysis was then carried out (Annex 7). This made it possible to view the input parameters with the greatest influence on the final result. The three most critical inputs of the quantitative risk assessment model were identified. In the case of cattle intended for rearing, it was the probabilities P3, P4 and P5: probability that a bovine from this farm is infected with LSD and not detected, probability that a bovine infected with LSD is contagious, probability that a bovine infected with LSD and contagious, intended for rearing, transmits the LSDV to native cattle. In the case of cattle intended for slaughter, it was the probabilities P3, P4 and P6 (probability that a bovine infected with LSD and contagious, intended for rearing or slaughter, transmits the LSDV to native cattle).

Probability of LSD being introduced by live domestic small ruminants

In view of the lack of literature data, it is currently difficult to decide on the role of small ruminants in the epidemiology of LSD, but it seems minor.

According to the current state of knowledge, the experts therefore estimated that the probability of LSD being introduced by the importing of small domestic ruminants into France was nil to nearly nil (0 to 1 on the AFSSA 2008 scale).

Probability of LSD being introduced by live wild ruminants, animals from zoos or circuses

There are no data on infection by the LSDV of wild ruminants present in Europe. In addition, no natural movement of the wildlife in the at-risk area to France has yet been reported. Similarly, the transport of these animals by vehicles is not documented.

The experts are unaware of any introduction into France of zoo or circus animals, during 2016, from the countries of the at-risk area. Moreover, with the current literature data, it is difficult to decide on the role of these species in the epidemiology of LSD.

Taking all these considerations into account, the experts estimated that the probability of LSD being introduced into France by live ruminants, whether wild or from zoos or circuses, was nil to nearly nil (0 to 1 on the AFSSA 2008 scale).

Probability of LSD being introduced by transhumance or other farming practices

The transhumance of potentially infected cattle currently takes place in countries without a border with France. The only transhumance movements across French borders take place with Switzerland and Spain (Annex 6).

The experts estimated that the probability of LSD being introduced into France by transhumance is nil (0 on the AFSSA 2008 scale).

In conclusion, the probability of LSD being introduced by live animals is limited to the risk of introduction by live cattle.

The probability of a first outbreak of LSD in France, following the introduction of infected live cattle intended for rearing, is estimated to be extremely low to low (probability between 0.004% and 0.32%, with a confidence interval of 95%) for a year, based on the epidemiological situation in January 2017, the European regulations existing on this same date, and data on trade for 2016.
Currently there are no cattle intended for the slaughterhouse being introduced from the at-risk area. The probability of a first outbreak of LSD in France following the introduction of infected live cattle intended for the slaughterhouse is therefore estimated to be nil.

The experts considered, however, that if there were as many cattle intended for the slaughterhouse introduced into France as the number introduced for rearing, the probability would be nearly nil to minute (probability between 0.2 10^{-6} and 47 10^{-6} per million, with a confidence interval of 95%) for a year, based on the epidemiological situation in January 2017, the European regulations existing on this same date, and data on trade for 2016.

4.2.2.2. Arguments for the probabilities of introduction by vectors

A vector can travel in three different ways: transported by birds, by vehicles, or by itself with the help of the winds. The vectors considered here are mainly the tabanids, Culicidae and *Stomoxys*.

- **Transport of LSDV vectors by birds**

The transport of vectors by birds is considered very negligible for two reasons. First of all, this type of transport is only described in ticks, and although there is little knowledge on the role of European ticks in transmission of the LSD virus (p31), the probability that a bird tick takes its blood meal on a bovine is unlikely. Secondly, France and the zone currently infected with LSD are not located on the same bird migration routes (even though certain species of birds do not follow the migration corridors and can move from east to west in Europe).

For these reasons, the probability of vectors carrying the LSDV being introduced into France by birds is estimated by the experts to be nil (0 on the AFSSA 2008 scale).

- **Transport of LSDV vectors by the winds**

This route has been considered in the spread of the LSDV in Europe and the Middle East (EFSA 2015). However, considering firstly the distance between France and the zone currently infected, and secondly the prevailing winds in Europe, the probability that vectors carrying the LSDV are transported passively by winds and transmit the LSDV to native cattle is estimated by the experts to be nil to nearly nil (0 to 1 on the AFSSA 2008 scale).

- **Transport of LSDV vectors by vehicles**

Horse-flies do not enter buildings or vehicles, so their role in long-distance transport can be regarded as nearly nil. In addition, in an enclosed environment, it has been observed that horse-flies quickly damage their wings and lose all flight ability in only a few hours (P. Jacquiet, personal observation during attempt to breed *Tabanus bromius* and *Haematopota pluvialis*).

Aedes aegypti can transmit the LSDV for up to 6 days after infection (Chihota *et al.* 2001). In France, the equivalent species *Aedes albopictus* is essentially anthropophilic, which means that it is very unlikely that it would be transported by a livestock truck.

Stomoxys can stay on smooth surfaces for long periods of time and they remain in the vicinity of their blood meal sources (horses or cattle). The risk then comes from the transport of live animals. The probability of introduction by other vehicles (food transport, cars, etc.) is estimated by the experts to be nil to nearly nil (0 to 1 on the AFSSA 2008 scale).

Just as in the previous section on the introduction of LSD by infected live domestic cattle, trucks carrying cattle may originate from an infected zone that has not yet been declared. As horses are as attractive as cattle to these vectors, account must also be taken of the transport of horses from the at-risk area to France. Indeed, contamination of a *Stomoxys* by the LSDV from cattle does not influence the possibility that the fly is then attracted by horses. One scenario would then be a mixed farm with cattle and horses, or a stud farm with a herd of infected cattle nearby. The experts therefore decided to also take into account movements of horses from the at-risk area.

In view of the distances between the current at-risk area and France, the experts estimated that a truck would travel for 2 to 3 days. Although the persistence of the virus in *Stomoxys* is unknown, a *Stomoxys* lives from 15 days to 3 weeks, i.e. generally long enough to be able to survive this

journey. Indeed, *Stomoxys*, unlike horse-flies, survive well in enclosed environments such as trucks.

In addition, *Stomoxys* always travel in association with their host (cattle, horses). Even if the truck is opened, the *Stomoxys* stay in the same place as the cattle or horses, and therefore remain in the truck. As the duration of the journey would be very short (2-3 days) compared with the lifespan of the *Stomoxys* (15 days), the model considered that 80 to 90% of *Stomoxys* survived this transport (7% mortality per day calculated if the duration of survival is 15 days).

In addition, the experts considered that every day, a relatively asymptomatic contagious bovine could generate between one and a few dozen infective *Stomoxys*. Considering that the number of *Stomoxys* is rather variable and that the proportion of infected cattle is more constant, the proportion of infective *Stomoxys* should be at least equivalent to the proportion of contagious cattle. The experts then considered that there was at least one infective *Stomoxys* per infected cattle.

Table 10: Summary of the elements used by the experts for the "vectors" method of introduction

Methods of introduction of LSD	Details	Data enabling qualification of the probability of a first outbreak of LSD occurring (origin of the data)	Management options enabling the probability of the first outbreak of LSD to be reduced	Arguments and comments
Introduction of infective vectors	- Undeclared infected zone - Infected farm - Contaminated insect - Insect eradication in vehicles - Destination of the vehicle (farm or slaughterhouse) - Number of animals	Literature data TRACE data Epidemiological situation of the countries of origin	Management measures (insect eradication in trucks) on departure of the cattle and horses	Disease spreading: neighbouring countries that may be infected without being declared Small number of introductions in 2016

As the main risk of introduction comes from *Stomoxys*, the experts estimated the probability of LSD being introduced into France via infective *Stomoxys* found in the vehicles carrying live animals (cattle or horses), taking into account the following probabilities (Table 11) and parameters (Table 12).

Table 11: Probabilities taken into account in the risk of LSD being introduced into France through *Stomoxys* found in the vehicles carrying live animals (cattle or horses)

P1	Probability that the traded cattle come from an undeclared LSD-infected zone
P2	Probability that the traded animals come from a farm infected with LSD

P3	Probability that a *Stomoxys* is infective
P4	Probability that insects are eradicated from a truck (a worst-case scenario was considered = no insect eradication)
P5	Probability that the animals are unloaded in an assembly centre (a worst-case scenario was considered = no unloading)
P6	Probability of survival of the virus in the vector
P7	Probability of survival of *Stomoxys* in the vehicle
P8	Probability that the LSDV is transmitted to native cattle by infective *Stomoxys* in the event that the cattle transport truck enters a farm
P9	Probability that the LSDV is transmitted to native cattle by infective *Stomoxys* in the event that the cattle transport truck goes to the slaughterhouse
P10	Probability that the LSDV is transmitted to native cattle by infective *Stomoxys* in the event that the cattle transport truck goes to a stud farm
P11	Probability that horses come from a mixed farm (with cattle) or that a cattle farm is located near stables
P12	Probability that horses arrive in a mixed farm (cattle/horses) or that a cattle farm is located near stables

Table 12: Initial parameters taken into account in the risk of LSD being introduced into France through *Stomoxys* found in the vehicles carrying live animals (cattle or horses)

N1	Number of *Stomoxys* entering a truck. The average number of cattle per truck is 20 (TRACES data). The number of *Stomoxys* (*S. calcitrans*) present in a dairy cattle farm affected by LSD was recently estimated to be between 20 and around 250 depending on the season (Kahana-Sutin *et al.* 2016). The experts estimated that the number of *Stomoxys* introduced into a vehicle containing cattle had to be at least equal to or higher than the number of cattle introduced, and lower than the number of *Stomoxys* present in a farm. **They therefore estimated that this number could be between 20 and 250 with a mode at 100, according to a Pert distribution.**
N1	Number of consignments of cattle transported each year to farms for rearing (data from TRACES)
N2	Number of consignments of cattle transported each year to slaughterhouses (scenario)
N3	Number of consignments of horses transported each year (data from TRACES)

For this risk analysis, the same data were used as for traded animals: statistics over 12 months (July 2015 - July 2016) for cattle intended for rearing and a scenario for the slaughterhouse using the same introduction data as for rearing.

Between September 2015 and September 2016, a low number of horses was introduced for rearing from Bulgaria, Croatia, Greece and Hungary: a total of 44 animals introduced in 44 consignments (data from TRACES, Annex 6).

Taking into account the probabilities (Table 11) and initial parameters (Table 12), it is possible to calculate the probability of vectors transported with the cattle or horses transmitting the LSDV to native cattle, according to three methods: either the cattle are intended for rearing (R2), or for slaughter (R3), or it is horses that are being transported (R4) (Table 13).

Table 13: Probabilities of LSD being introduced into France through *Stomoxys* found in the vehicles carrying live animals (cattle or horses)

Probability		Calculation
R1	Probability that an infective *Stomoxys* arrives at the destination	=1-(P1*P2*P3*(1-P4)*(1-P5)*P6*P7)^N1
R2	Probability that a native bovine is infected by *Stomoxys* that travelled with cattle intended for rearing	=1-(1-R1*P8)^n1
R3	Probability that a native bovine is infected by *Stomoxys* that travelled with cattle intended for the slaughterhouse	= 1-(1-R1*P9)^n2
R4	Probability that a native bovine is infected by *Stomoxys* that travelled with horses intended for a mixed herd (cattle/horses) or arriving in a stud farm with a herd of cattle nearby	=1-(1-R1*P10*P11*P12)^n3

The number of infective *Stomoxys* per bovine and the probabilities P1 to P11 were discussed and estimated within the LSD Working Group and shared with the international specialists on LSD during the hearings and, if appropriate, were converted to probability distributions (Table 14 and Table 15).

Table 14: Argument used for estimating the number of infective *Stomoxys* per infected cattle and the probabilities P1 to P11

Parameter	Argument for the estimate
N1 - Number of *Stomoxys* introduced into a truck	▪ At any given time, a bovine can suffer between a dozen and 50 *Stomoxys* bites (Campbell *et al.* 2001). Given that the period of activity of *Stomoxys* in a day extends from 10:00 to 18:00, the total number of *Stomoxys* bites per cattle and per day can be between a few hundred and a few thousand. Interrupted meals among *Stomoxys* are very frequent (at least 2/3 of meals are interrupted by the animals' defensive movements), which means that the same *Stomoxys* will bite several times, at several locations, on the same animal or on several different animals. In a bovine in a relatively asymptomatic phase (beginning phase or attenuated form), the probability that a *Stomoxys* bites a contaminated area (presence of the virus in or around a nodule) is low. ▪ The experts considered that, **every day**, a relatively asymptomatic contagious bovine could generate between one and a few tens of contaminated *Stomoxys*. Considering that vector-borne transmission largely predominates, this estimate is consistent with the average intra-herd prevalence (less than 20%), and the estimated R0 of 16 (Magori-Cohen *et al.* 2012). Considering that the number of *Stomoxys* is rather variable and that the proportion of infected cattle is more constant, the model used the proportion of infective *Stomoxys*, which should be **at least** equivalent to the proportion of contagious cattle. ▪ Animals affected with LSD, especially those with a fever, defend themselves less from the vectors and will therefore be bitten more than healthy animals (personal communication from the Greek official veterinarian). ▪ The average number of cattle per truck is 20 (TRACES data). The number of *Stomoxys* (*S. calcitrans*) trapped in 12 large dairy cattle farms affected by LSD was recently determined (Kahana-Sutin *et al.* 2016) by trapping, with several traps laid per farm for 48 hours. There were on average around a hundred *Stomoxys* (minimum 40, maximum 240). This corresponds to the apparent densities in these farms observed with this type of trap. The experts estimated that the number of *Stomoxys* entering a vehicle containing cattle had to be at least equal to or higher than the number of cattle introduced, and lower than the number of *Stomoxys* present in a farm (rounded to 250 units). ▪ **The experts therefore estimated that this number could be between 20 and 250 with a mode at 100, according to a Pert distribution.**
P1 - Probability that the traded animals come from an undeclared LSD-infected zone	▪ See the P1 in Table 7 ▪ **The estimated range of probabilities is between 5 and 20%, with a mode at 7%.**

Parameter	Argument for the estimate
P2 - Probability that the traded animals come from a farm infected with LSD	▪ See the P2 in Table 7 ▪ **The estimated range of probabilities is between 0.5 and 1% for the EU, with a mode at 0.75%** (outside the EU this probability would likely be higher).
P3-Probability that a *Stomoxys* is infective	▪ As indicated previously, the experts considered at least one infective vector per contagious bovine. ▪ For this reason, the experts considered that the probability of a vector being infective is the same as the probability of an infected bovine being contagious, i.e. the product of the probabilities P3 (probability that a bovine from this farm is infected with LSD) and P4 (probability that a bovine infected with LSD is contagious) in Table 7. ▪ **This calculated probability has a mode at 1%, a minimum of 0.06% and a maximum of 5.4%.**
P4- Probability that insects are eradicated from a truck	▪ The model considered the worst-case scenario, which is "there is never any insect eradication in the trucks" even though the European regulations[19] require the disinfestation of vehicles that have been in contact with susceptible animals before leaving an at-risk area. Indeed, the experts considered that despite the regulations, it is uncertain whether the quality of the insect eradication of vehicles is rigorously controlled. ▪ **This probability is therefore estimated at 0%.**
P5-Probability that the animals are unloaded in an assembly centre	▪ The model also considered the worst-case scenario: the animals are not unloaded in an assembly centre, as advocated by the regulations[18]. If the animals are taken out of the truck, the *Stomoxys* will follow them and also come out. They can then leave in another truck or remain in the assembly centre (if other animals are present). For the risk analysis, the experts considered that the animals were not unloaded between their countries of origin and their arrival in France. In view of the estimated travel time (2 to 3 days), this assumption is unrealistic, but for the model, the experts chose the worst-case scenario (no unloading of animals). ▪ **This probability is therefore estimated at 0%.**

[18] Commission Implementing Decision (EU) 2016/2008 of 15 November 2016 concerning animal health control measures relating to lumpy skin disease in certain Member States.

Parameter	Argument for the estimate
P6 - Probability of survival of the virus in the vector	▪ In a study (Chihota *et al.* 2003), the LSDV was found in *Stomoxys* (*S. calcitrans*) only on day zero after feeding on a bovine infected by the LSDV. In contrast, a positive PCR was found in several *Stomoxys* (5 out of 12 tested on day zero, and 3 out of 12 tested on day 1 after the blood meal). Subsequently and until day 20 after the blood meal, no positive PCR was found in a total of 8 to 12 *Stomoxys* tested, depending on the observation days. ▪ Following a conservative approach, the experts decided to base their estimate of survival of the virus in *Stomoxys* on all the PCR results. As it concerns a count of the number of positive *Stomoxys*, they used a binomial distribution with a view to estimating the probability of survival of the virus in *Stomoxys*, also taking into account a vehicle journey time of 2 to 3 days (see calculations in Annex 9). ▪ **Therefore, following the use of this regression model, the probability of survival of the virus within a *Stomoxys* is estimated to be between 6.5% (3-day journey) and 13% (2-day journey), according to a uniform distribution.**
P7 - Probability of survival of *Stomoxys* in the vehicle	▪ The average lifespan of a *Stomoxys* is 15 days. This corresponds to a natural *Stomoxys* mortality rate of 7% per day. ▪ Considering a journey time of 2 to 3 days and the worst-case scenario, i.e. the animals are not unloaded during their transport (see starting assumption), **this probability was estimated (rounded) to be between 80 and 90%.**
P8 - Probability that the LSD virus is transmitted to native cattle by infective *Stomoxys* in the event that the transport truck goes to a farm	▪ In the absence of relevant literature information, the experts considered that one infective vector was enough to transmit the virus to a native bovine (worst-case scenario). ▪ **This probability was estimated at 100%.**
P9 - Probability that the LSD virus is transmitted to native cattle by infective *Stomoxys* in the event that the transport truck goes to the slaughterhouse	▪ If the animals are intended for the slaughterhouse, it is most likely that the *Stomoxys* will bite cattle present in the slaughterhouse barn, and therefore the infection will not spread, even if there are native cattle nearby. ▪ In addition, as said previously, the experts considered that one infective vector is enough to transmit the virus to a native bovine. ▪ **The WG experts estimated this probability to be from 0.1 to 1%,** a value confirmed by the international specialists on LSD during the hearings.

Parameter	Argument for the estimate
P10 - Probability that the LSD virus is transmitted to native cattle by infective *Stomoxys* in the event that the transport truck goes to a stud farm	▪ The horses from the countries of the at-risk area or infected regions are primarily leisure animals; it is therefore more likely that at their destination there are no cattle nearby, and that the *Stomoxys* only bite horses found nearby. ▪ **The WG experts therefore estimated this probability to be from 0.1 to 1%,** a value confirmed by the international specialists on LSD during the hearings.
P11 - Probability that the horses come from a mixed farm (containing cattle) or that a cattle farm is located near stables	▪ Not having any information on this subject about the country of origin, the same probability as P12 was considered.
P12 - Probability that horses arrive in a mixed farm (cattle/horses) or that a cattle farm is located near stables	▪ A total of 34,500 stud farms was identified in France in 2013, of which 3420 farms have a mixed activity with cattle and horses (Interbev 2015). It was not possible to take the regional variability of these densities into account in the model. ▪ **This probability is estimated to be 10% according to a Beta distribution.**

Table 15: Input parameters for the model analysing the risk of LSD being introduced through *Stomoxys* found in vehicles carrying live animals (cattle or horses)

Input parameters	Quantitative estimate	Min.	Mode	Max.	Prob.	Distribution, value or calculation
Number of *Stomoxys* introduced into a truck	100	20	100	250	N1	RiskPert(50;100;500)
Probability that the traded animals come from an undeclared LSD-infected zone	5 to 20%, with a mode at 7%	0.05	0.07	0.2	P1	RiskPert(0.05;0.07;0.2)
Probability that the traded animals come from a farm infected with LSD	0.5 to 1%	0.005		0.01	P2	RiskUniform(0.005;0.01)
Probability that a *Stomoxys* is infective = product of probabilities P3 and P4 concerning the model relating to infected live animals	Probability that a bovine from this farm is infected with LSD, i.e. 0.3 to 25% with a mode at 3%	0.003	0.03	0.25		RiskPert(0.003;0.03;0.25)
	Probability that a bovine infected with LSD is contagious, i.e. 0.6 to 50%	0.006		0.5		RiskUniform(0.006;0.5)
Probability that a *Stomoxys* is infective	Product of the two previous probabilities	0.0006	0.01	0.054	P3	Calculation
Probability that insects are eradicated from a truck (worst-case scenario)	0%		0		P4	Worst-case scenario
Probability that the animals are unloaded in an assembly centre (worst-case scenario)	0%		0		P5	Worst-case scenario
Probability of survival of the virus in the vector	6.5 to 13%	0.065		0.13	P6	RiskUniform(0.065;0.13)
Probability of survival of *Stomoxys* in the vehicle	80 to 90% after a journey of 2 to 3 days	0.8		0.9	P7	RiskUniform(0.8;0.9)
Probability that the LSD virus is transmitted to native cattle by infective *Stomoxys* in the event that the transport truck goes to a farm	100%		1		P8	Worst-case scenario
Probability that the LSD virus is transmitted to native cattle by infective *Stomoxys* in the event that the transport truck goes to the slaughterhouse	0.1 to 1%	0.001		0.01	P9	RiskUniform(0.001;0.01)
Probability that the LSD virus is transmitted to native cattle by infective *Stomoxys* in the event that the transport truck goes to a stud farm	0.1 to 1%	0.001		0.01	P10	RiskUniform(0.001;0.01)
Probability that horses come from a mixed farm (containing cattle) or that a cattle farm is located near stables	10%	0	0.1	1	P11	RiskBeta(3421; 31081)

Input parameters	Quantitative estimate	Min.	Mode	Max.	Prob.	Distribution, value or calculation
Probability that horses arrive in a mixed farm (cattle/horses) or that a cattle farm is located near stables	10%	0	0.1	1	P12	RiskBeta(3421; 31081)
Number of consignments of cattle transported each year to farms for rearing (data from TRACES)		3	7	11	N1	RiskPert(3;7;11)
Number of consignments of cattle transported each year to slaughterhouses (scenario)		3	7	11	N2	RiskPert(3;7;11)
Number of consignments of horses transported each year (data from TRACES)		22	44	66	N3	RiskPert(22;44;66)
Outputs						
Probability that an infective *Stomoxys* arrives at the destination	Calculation				R1	=1-(P1*P2*P3*(1-P4)*(1-P5)*P6*P7)^N11
Probability that a native bovine is infected by *Stomoxys* that travelled with cattle intended for rearing	Calculation				R2	=1-(1-R1*P8)^n1
Probability that a native bovine is infected by *Stomoxys* that travelled with cattle intended for the slaughterhouse	Calculation				R3	= 1-(1-R1*P9)^n2
Probability that a native bovine is infected by *Stomoxys* that travelled with horses intended for a mixed herd (cattle/horses) or arriving in a stud farm with a herd of cattle nearby	Calculation				R4	=1-(1-R1*P10*P11*P12)^n3

A Pert distribution was used when the experts were able to estimate the minimum, mode and maximum values for a distribution. This is an alternative (more plausible) distribution to the triangular distribution. A uniform distribution was used when the experts were able to estimate only the minimum and maximum values for a distribution (equiprobability that the actual value is situated between these two values). A Beta distribution was used for the probability that horses come from or arrive in a mixed farm (containing cattle) or that a cattle farm is located near stables. Both parameters of this distribution characterise its form.

Then, 100,000 Monte Carlo simulations were carried out using the @Risk 7.5 software, to obtain the resulting probability distributions R1 to R4. The results are summarised in Table 16 and detailed in Annex 10 (probability distributions and sensitivity analysis for seeing the relative influence of the different input parameters).

Table 16: Probabilities of LSD being introduced through *Stomoxys* found in the vehicles carrying live animals (cattle or horses)*

Probability	Parameter	Value	Qualitative expression according to the transposition grid developed by AFSSA (Annex 8)
R1 - Probability that an infective *Stomoxys* arrives at the destination	2.5th percentile: Median: 97.5th percentile:	$4\ 10^{-6}$ $78\ 10^{-6}$ $612\ 10^{-6}$	Minute to very low (2-4 on a scale of 9)
R2 - Probability that a native bovine is infected by *Stomoxys* that travelled with cattle intended for rearing	2.5th percentile: Median: 97.5th percentile:	$2\ 10^{-5}$ $53\ 10^{-5}$ $440\ 10^{-5}$	Extremely low to low (3-5 on a scale of 9)
R3 - Probability that a native bovine is infected by *Stomoxys* that travelled with cattle intended for the slaughterhouse	2.5th percentile: Median: 97.5th percentile:	$0.1\ 10^{-6}$ $2.49\ 10^{-6}$ $27\ 10^{-6}$	Nearly nil to minute (1-2 on a scale of 9)
R4 - Probability that a native bovine is infected by *Stomoxys* that travelled with horses intended for a mixed herd (cattle/horses) or arriving in a stud farm with a herd of cattle nearby	2.5th percentile: Median: 97.5th percentile:	$0.01\ 10^{-6}$ $0.156\ 10^{-6}$ $1.67\ 10^{-6}$	Nearly nil (1 on a scale of 9)

* Probabilities calculated for one year, based on the epidemiological situation in January 2017, the European regulations existing on this same date and data on trade for 2016.

A sensitivity analysis was then carried out (Annex 10). This made it possible to view the input parameters with the greatest influence on the final result. The three most critical inputs of the quantitative risk assessment model were identified. They are the probability that a bovine from the

farm of origin is infected with LSD, the probability that a bovine infected with LSD is contagious, and the number of *Stomoxys* introduced into the vehicle during loading of the animals (N1). For the probability (R3) that a native bovine is infected by *Stomoxys* that travelled with cattle intended for the slaughterhouse, an additional input also needs to be considered; the probability (P9) that the LSD virus is transmitted to native cattle by infective *Stomoxys* in the event that the transport truck goes to the slaughterhouse. For the probability (R4) that a native bovine is infected by *Stomoxys* that travelled with horses intended for a mixed herd (cattle/horses) or arriving in a stud farm with a herd of cattle nearby, an additional input also needs to be considered; the probability (P10) that the LSD virus is transmitted to native cattle by infective *Stomoxys* in the event that the transport truck goes to a stud farm.

Although it is likely that more than one vector is needed to mechanically transmit the disease to a bovine, it was considered in the model that one vector was enough to ensure this transmission.

Risk due to other potential vectors (ticks, mosquitoes, horse-flies, etc.)

The experts believe that their role in the epidemiology of the disease is relatively insignificant compared to that of the *Stomoxys*. Horse-flies cannot survive in confined spaces and therefore in the trucks, because they collide with the walls, damage their wings, are no longer able to fly and die. The tick is a very unlikely vector, in a context of introduction of the disease, because it takes only one single blood meal per stage. However, they may play a role in a situation in which the disease is enzootic.

At the present time, there is no effective repellent against the bites of *Stomoxys* or horse-flies. Different commercial products based on essential oils have demonstrated partial activity that is very limited over time (a few hours at most). No synthetic pyrethroids, whether administered in pour-on or spray form, have been found to prevent the gorging of *Stomoxys* and horse flies, but cause mortality in the hours following the blood meal (Presley and Wright 1986). Cypermethrin ear tags are ineffective against *Stomoxys* and horse-flies that bite in the underparts that are not protected by these devices.

In conclusion, the risk of LSD being introduced by the long-distance road transport of vectors is limited to the risk of introduction by *Stomoxys*.

The probability of a first outbreak of LSD in France, following the introduction of infective vectors transported with cattle intended for rearing, is estimated to be extremely low to low (probability between 0.002% and 0.44%, with a confidence interval of 95%) for a year, based on the epidemiological situation in January 2017, the European regulations existing on this same date, and data on trade for 2016.

Currently there are no cattle intended for the slaughterhouse being introduced from the at-risk area. The probability of a first outbreak of LSD in France following the introduction of infective vectors transported with live cattle intended for the slaughterhouse is therefore estimated to be nil.

The experts considered, however, that if there were as many cattle intended for the slaughterhouse introduced into France as the number introduced for rearing, the probability would be nearly nil to minute (probability between 0.1 10^{-6} and 27 10^{-6}, with a confidence interval of 95%) for a year, based on the epidemiological situation in January 2017, the European regulations existing on this same date, and data on trade for 2016.

The probability of a first outbreak of LSD in France following the introduction of infective vectors transported with horses is estimated to be nearly nil (probability between 0.01 10^{-6} and 1.67 10^{-6},

with a confidence interval of 95%) for a year, based on the epidemiological situation in January 2017, the European regulations existing on this same date, and data on trade for 2016.

4.2.2.3. Arguments for the probabilities of introduction by other modes of transmission

The methods of introduction taken into account were introduction by contaminated hides, semen, oocytes and embryos, milk, meat and inert objects.

As in the sections above, the risk assessment focused only on introductions from the at-risk area as defined previously.

The key points taken into account to estimate the risk of introduction of the LSD virus are summarised in Table 17 below.

Table 17: Summary of the elements used by the experts for the "hides of infected cattle", "semen, oocytes and embryos", "meat" and "milk" and "contaminated inert objects" methods of introduction

Methods of introduction of LSD	Details	Data enabling qualification of the probability of a first outbreak of LSD occurring (Origin of the data)	Management measures for reducing the probability of the first outbreak of LSD	Arguments and comments
-Hides of infected cattle -Semen, oocytes and embryos -Milk -Meat of infected cattle -Contaminated inert media	-Establishments (slaughterhouse, insemination centre, hide collection establishment, etc.) located in an undeclared infected zone or receiving and hosting cattle or products from an undeclared infected zone -Infected farm -Proportion of infected animals -Presence of the virus in products -Treatments likely or unlikely to inactivate the virus -Introduced volumes	-Literature data -Eurostat data -Epidemiological situation of the countries of origin -Expert opinions	-Traceability -Trade restrictions -Regulatory compliance and management measures in the affected countries and the at-risk area (for example, destruction of hides from infected animals)	-Disease spreading: neighbouring countries or disease-free zone of a country recognised as infected that may be infected without being declared -Volume of introductions: nil to low in 2016 depending on the regions

For these methods of introduction, the risk assessment was carried out according to a qualitative method (AFSSA, 2008), as the experts had few data on these methods. The variables are expressed qualitatively according to a gradient from 0 (nil) to 9 (very high) (Annex 8).

Probability of introduction by fresh hides of infected cattle

✓ Characteristics of traded or imported cattle hides and associated regulations

The hides of infected cattle are regarded as potential sources of spread of the LSD virus when they are marketed.

Indeed, the skin of infected cattle is the tissue in which the virus has been isolated with the highest titres. The very highest titres (up to 8.1 - 8.3 $\log_{10}$ PFU/g between 12 and 15 days after inoculation) are found in the skin lesions (Babiuk, Bowden, Parkyn *et al.* 2008), and therefore, the amount of virus is proportional to the number of lesions present. In infected animals with no lesions, on the other hand, the amount of virus in the skin is low, making viral isolation difficult.

Traded or imported hides may fall into two categories, as designated by Commission Regulation (EU) No 142/2011[19]: untreated, when they have not undergone any treatment other than cutting, chilling or freezing; and treated, when they have undergone treatment, such as drying or salting[20]. The drying and salting aim to eliminate some of the water they contain, before they are forwarded to tanneries where they are subjected to various operations (soaking, dehairing, fleshing, deliming, curing, pickling[21]) up to the final stage of tanning.

The greatest hazard is therefore posed by fresh hides (untreated), bearing in mind that their marketing is subject to the provisions of Council Directive 92/119/EEC that excludes the marketing of fresh hides derived from cattle present in holdings subject to the restriction measures provided for in the event of suspicion and confirmation of LSD. The ban also applies (excluding exemptions) to fresh hides from cattle holdings that are not suspect or recognised as infected, located in regulated LSD zones. These hides must also meet the animal health conditions applicable to fresh meat[22] laid down in accordance with Council Directive 2002/99/EC. This provision excludes untreated hides collected from carcasses.

The trade also concerns hides treated by drying or salting in tanneries or other establishments (receiving fresh hides) approved for handling after collection. The hazard posed by these products depends on the processing conditions (temperature, whether or not inhibitor products are added), and the duration of the operation and/or their storage. Due to the virus's resistance to desiccation (it remains stable in the dried skin lesions and persists for at least 33 days at normal temperature (Weiss 1968)), drying does not contribute to eliminating the virus, unless it is for a sufficient length

[19] Commission Regulation (EU) No 142/2011 of 25 February 2011 implementing Regulation (EC) No 1069/2009 of the European Parliament and of the Council laying down health rules as regards animal by-products and derived products not intended for human consumption and implementing Council Directive 97/78/EC as regards certain samples and items exempt from veterinary checks at the border under that Directive.

[20] "Treated hides and skins" means derived products from untreated hides and skins that have been (a) dried, (b) dry-salted or wet-salted for a period of at least 14 days prior to dispatch, (c) salted for a period of at least seven days in sea salt with the addition of 2% of sodium carbonate, (d) dried for a period of at least 42 days at a temperature of at least 20°C, or (e) subject to a preservation process other than tanning.

[21] Acidification of the skin by immersion in water with H_2SO_4 to improve its preservation and prepare it for tanning.

[22] Council Directive 2002/99/EC of 16 December 2002 laying down the animal health rules governing the production, processing, distribution and introduction of products of animal origin for human consumption.

of time. Little is known about the effect of salting (usually for a fortnight at around 10°C) on survival of the virus, but the addition of certain products to the salt (for example sodium carbonate) may nevertheless accelerate its inactivation. Exemptions may thus be granted for the trade in hides, provided that these hides do not come from cattle present in an infected holding and that they have been dried for 42 days at a temperature of at least 20°C, or salted in sea salt with the addition of 2% of sodium carbonate for a period of at least seven days. These treatments, including salting for a period of at least 14 days prior to their dispatch, are also taken into consideration in Decision 2016/2008 to authorise, by way of exemption from the ban, the dispatch of leather and hides from cattle and captive wild ruminants from an infected zone.

Moreover, in the leather manufacturing process carried out in tanning, because of the duration of the operations and treatments performed (with lime during painting[23] or with acids during deliming and pickling), the experts believe that the residual viruses can be considered as inactivated.

Furthermore, to ensure their quality, the hides are examined one by one on various occasions, for example, at the washing, trimming and draining step that precedes the drying or salting phase of fresh hides, or the reshaping performed before the later steps leading to the tanning are undertaken. These visual examinations limit or rule out the possibility of failing to notice the presence of any LSD lesions that may have gone undetected during ante-mortem or post-mortem inspections at the slaughterhouse.

- ✓ Introduction of cattle hides in France

France exports more raw hides and skins (3rd largest world exporter in 2015) than it imports. The vast majority of hides introduced into France come from countries in Western Europe, mainly Switzerland, Germany and Italy. Until 2015, the share of imports from the countries of the at-risk area was very low. This share was reduced or eliminated in 2016 due to the LSD risk being taken into account by the EU regulations.

The data available (extracted from TRACES for the periods from January to December 2015 and January to June 2016) cannot be used, however, to define either the quantities of hides actually introduced from the at-risk area, or the proportion of treated and untreated hides. However, some of the countries of the at-risk area could seek to export cattle hides to France. Thus, although there are no imports of hides from these countries at the moment, the experts decided to carry out a detailed risk analysis to enable them to easily change certain variables if trading of hides were to evolve.

- ✓ Probability of the virus being introduced by fresh hides of infected cattle

This analysis focused on the risk of the LSDV being introduced into France by untreated or inadequately treated hides.

As previously indicated, the scenario considered took into account the risk of introduction of one or more infected hides from a not yet declared infected zone (LSD not yet identified or reported), therefore located in close proximity to a regulated zone, bearing in mind the propagation front of the infection. The zones to be considered may be rather large, as the slaughter of cattle can take place in any slaughterhouse in a given country, or even in a neighbouring country. The fact that few animals can be clinically affected in some herds (only 1 to 3 animals affected in 84% of

[23] Dehairing operation using a paste made of lime and sodium sulphide.

infected herds in Greece and Bulgaria in 2016) can also contribute to the late detection of the disease, especially if the animals' skin lesions are few in number and/or relatively inconspicuous.

Several parameters were taken into consideration to assess the probability of the LSD virus being introduced into France via imports/trade in hides of infected cattle.

- *Parameters taken into consideration and corresponding estimated probabilities for assessing the probability of the LSD virus being introduced into France via imports/trade in hides of infected cattle*.

The different probabilities taken into account are presented in Table 18 below.

Table 18: Table describing the probabilities taken into account to determine the probability of the LSD virus being introduced by the introduction of fresh hides from infected cattle*

Probability	Argument for the estimate	Qualitative probability estimated by the experts
P1 - Probability that imported/traded hides or consignments of hides come from cattle from an undeclared LSD-infected zone	▪ The countries considered are the countries of the at-risk area, as defined, that ship untreated or inadequately treated hides to metropolitan France. ▪ The experts assumed, when estimating the probability, that the interval between the first case and its declaration, regardless of the country, was at least three weeks (see P1 Table 7).	High (8 on a scale of 0 to 9)
P2 - Probability that the hides come from cattle belonging to an unidentified LSD-infected farm	▪ The experts considered that in a recently infected country, few farms would be infected (probably less than ten) before the disease was detected (estimate 3 weeks) (see P2 Table 7). ▪ In addition, the probability that a bovine from this farm is sent to a slaughterhouse during the period considered is estimated to be low	Low (5 on a scale of 0 to 9)

Probability	Argument for the estimate	Qualitative probability estimated by the experts
P3 - Probability that the hides come from cattle themselves infected with LSD and not detected in the farm	▪ Intra-herd morbidity was calculated using data from reports on declaration of infection to the OIE. The experts selected 59 farms (of more than 50 animals) declared infected between April and September 2016 in Greece and Bulgaria. The intra-herd prevalence calculated was: min: 0.3%, median: 3%, max: 25%. This value was only calculated on the number of cattle with clinical signs: Greece and Bulgaria do not perform virological tests on animals not showing clinical signs. It does not therefore relate to intra-herd prevalence but to morbidity in the herd. ▪ The estimate of intra-herd prevalence corresponds to this morbidity value multiplied by 2 because only 50% of infected animals have clinical signs (Tuppurainen and Oura 2012). ▪ However, the experts estimated that only the hides of cattle not presenting clinical signs would be exported. For this reason, the experts chose to only consider the 50% of infected cattle without clinical signs, i.e. the morbidity value calculated. ▪ This figure should however be lower in herds infected recently	Quite high to high (7 to 8 on a scale of 0 to 9)
P4 - Probability that the hides are themselves infected	▪ The viral titre is highest in the nodules and lowest in non-affected skin, this is even more the case in the absence of lesions. ▪ An ante-mortem examination enables animals with visible lesions to be detected.	Low (5 on a scale of 0 to 9)
P5 - Probability that the infected hide is not eliminated by visual screening	▪ The hides collected in the slaughterhouses are washed and generally examined one by one. ▪ Hides with lesions will be identified (very low probability of them not being eliminated), which is not the case with hides free of lesions (very high probability of them not being eliminated)	Very low to very high (4 to 9 on a scale of 0 to 9)
P6 - Probability of infected fresh hides being included in a consignment intended for France	▪ The countries of the at-risk area export few or no hides to France (Eurostat data, Annex 12), with this share considered very low compared to the volume produced.	Very low to low (4 to 5 on a scale of 0 to 9)
P7 - Probability that the virus present in the hides survives during transport.	▪ Assuming a transport time of one week at ambient temperature, no loss of viral titre is envisaged.	High (8 on a scale of 0 to 9)

* Probabilities calculated for one year, based on the epidemiological situation in January 2017, the European regulations existing on this same date and data on trade for 2016.

The probability of introduction is the result of successive combinations, using the combination table (Annex 11) presented in the previously mentioned AFSSA report on the qualitative risk analysis, between the various probabilities (combining P1-P2, with the result being combined with P3, etc.) (AFSSA 2008).

Ultimately, the probability of the LSDV being introduced into France in a consignment of fresh hides from infected cattle is estimated to be nearly nil to minute (1 to 2 on a scale of 0 to 9).

A qualitative analysis of the risk of introduction of LSD was carried out for Great Britain from an infected country of the EU (case of the animal outbreak in Greece), via the import of untreated hides from infected cattle that were not detected positive on the farm or the slaughterhouse in the country of origin (Gale, Kelly, and Snary 2016). Taking into account the rate of intra-herd infection, the probability that an animal is infectious in an infected herd, the probability that it is not detected (asymptomatic) on the farm or at the slaughterhouse, and the probability that the hide is exported to Great Britain and still infectious, the authors estimated this risk to be low (a rare event but one that exists), on a scale of six criteria (negligible, very low, low, medium, high and very high) (EFSA 2006, FAO 2009).

- *Probability of exposure*

Consignments of imported cattle hides are received in tanneries where the hides are examined, stored and treated up to the final stage of tanning. As indicated previously, the time needed to carry out the different stages of preparation for tanning and the treatments used contribute to eliminating any virus that may be present.

As emphasised in Table 19 below, the probability of exposure of cattle is estimated to be nil to nearly nil (0 to 1 on a scale of 0 to 9).

Table 19: Table describing the probability of exposure of native cattle to the LSD virus following the introduction of infected hides*

Probability	Argument for the estimate	Qualitative probability estimated by the experts
P8 - Probability of exposure	▪ The probability of direct contact of hides with cattle is estimated to be nil. Indirect contact, following pollution of a river by contaminated water (for example used for soaking operations) from a tannery is estimated to be nearly nil	Nil to nearly nil (0 to 1 on a scale of 0 to 9)

* Probability calculated for one year, based on the epidemiological situation in January 2017, the European regulations existing on this same date and data on trade for 2016.

In conclusion, the probability of an outbreak of LSD occurring following the introduction into France of a consignment of fresh hides shipped from an establishment located in an undeclared LSD at-risk area or one handling cattle hides from such a zone can be estimated as nil to nearly nil (0 to 1 on a scale of 0 to 9).

Semen, oocytes and embryos of infected cattle

✓ Potential role of semen, ova and embryos of infected cattle in the transmission of LSD

The semen, oocytes and embryos of infected cattle are considered as potential sources of spread of the LSD virus. Although the experts have no data validating the reality and extent of transmission of the infection by bulls infected in natural conditions, this risk must nevertheless be considered carefully. Indeed, epididymis and testis have been identified in bulls as sites of viral location and the virus is detected in the semen of clinically affected and subclinically infected subjects (Annandale *et al.* 2010). In bulls infected experimentally, the virus can be isolated by culture for up to 42 days from semen after their contamination, and its nucleic acid can be detected by PCR for up to 159 days (Irons, Tuppurainen and Venter 2005). The infection of cows inseminated with experimentally-infected semen has also been shown to be possible, and may result in the contamination of their embryo (Annandale *et al.* 2014).

Despite the limited data available (Irons 2008), the possible contamination of oocytes and embryos (at the initial development stage of a domestic bovine when it can be transferred to a breeder cow) in infected cows is also considered. In addition, the experts are unaware of any publications concerning the effectiveness of standard procedures for processing and washing embryos to eliminate the virus. The IETS (International Embryo Transfer Society) has classified the LSDV in Category 4, which means that, based on studies completed or under way, the risk of embryo transmission cannot be ruled out (insufficient number of studies) and may not be negligible even if the embryos are handled according to the recommendations of the IETS (IETS 2015).

These data are taken into account in Commission Implementing Decision (EU) 2016/2008[24], prohibiting any shipment of semen, oocytes and embryos from bovine animals and captive wild ruminants from recognised infected zones (or limiting them, with exemptions, only to shipments from vaccinated disease-free zones).

✓ Operating rules for collection centres and other approved establishments shipping semen, ova and embryos

The risk analysis presented focused on the possible introduction, into France, of semen, oocytes or embryos from semen collection centres or other approved establishments located in zones in which outbreak surveillance has not yet been able to detect a recently introduced infection.

This risk could be weighted by the regulatory requirements applicable to intra-Community trade[25], in particular in terms of surveillance and biosafety. The semen collection centres are in fact placed under the permanent supervision of a veterinarian, any bulls accommodated there for at least 30 days must not have presented any clinical manifestation of disease on the date of collection, and the semen must have been stored for a minimum period of thirty days before shipment (or, in the case of fresh semen, until the date of shipment). Evidently, the biosafety measures do not guarantee protection of the animals against insects, and clinical surveillance is ineffective for identifying donor animals that are incubating or asymptomatic during semen collection. It is however likely, if the LSDV has actually been introduced into the zone, that clinical cases will be

[24]Commission Implementing Decision (EU) 2016/2008 of 15 November 2016 concerning animal health control measures relating to lumpy skin disease in certain Member States.

[25] Council Directive 88/407/EEC as amended of 14 June 1988 laying down the animal health requirements applicable to intra-Community trade in and imports of deep-frozen semen of domestic animals of the bovine species, and Council Directive 89/556/EEC as amended of 25 September 1989 on animal health conditions governing intra-Community trade in and importation from third countries of embryos of domestic animals of the bovine species.

detected in the days or weeks following collection (at least in the 28 days corresponding to the maximum incubation period) in the centre if several animals have been infected and, failing this, it is likely that cases will appear in farms located in the same geographical area, which then becomes a restriction zone. In this case, notwithstanding the possibility of serological or PCR testing of donors and of detection of the virus by PCR in semen, the pre-storage of frozen semen for 30 days makes it possible to block consignments of potentially contaminated frozen semen. This option only applies to frozen semen, not for semen shipped when fresh.

The collection of oocytes and embryos produced *in vivo*, on the other hand, often takes place in the farms (or the slaughterhouse for oocytes). Despite strict regulations governing this activity, the experts considered that the biosafety measures and the monitoring of donor cows, especially after collection, may be less rigorous than in dedicated centres. In addition, as previously for semen, pre-storage only applies to frozen oocytes and embryos.

✓ Origin and volume of semen, oocytes and embryos introduced into France

The Eurostat data consulted for the period January 2015 to August 2016 do not report any imports of semen from the countries of the at-risk area (Annex 12). They only show probable introductions of embryos from Albania in March 2015 (i.e. more than a year before the declaration of the first outbreak on 28/06/2016), and Romania in 2015 and 2016. The data provided to the experts do not indicate any possible introductions of oocytes. Nor do they provide clarification on the volumes, or on the share of fresh or frozen products introduced.

The experts considered that, despite the limited number of countries identified as shipping these products to France, the trade could evolve. They therefore considered it useful to conduct a risk analysis, including for semen and oocytes, in the framework of this scenario.

✓ Probabilities of an outbreak of LSD occurring in France via the introduction of semen, oocytes or embryos from infected cattle

This qualitative probability arose from combining the estimated probabilities of introduction and exposure.

- *Parameters taken into consideration and corresponding estimated probabilities for assessing the probability of the LSDV being introduced*

The different probabilities taken into account are presented in Table 20 below.

Table 20: Probabilities taken into account to determine the probability of the LSDV being introduced by the introduction of semen, oocytes or embryos from infected cattle*

Probability	Argument for the estimate	Qualitative probability estimated by the experts
P1 - Probability that the semen, oocytes and embryos come from cattle from an undeclared LSD-infected zone	▪ The countries or parts of countries considered are those located in the at-risk area (as defined previously). ▪ The experts assumed, when estimating the probability, that the interval between the first case and its declaration, regardless of the country, was at least three weeks (see P1 Table 7).	High (8 on a scale of 0 to 9)
P2 - Probability that these products belong to an unidentified LSD-infected collection centre or farm	▪ The experts considered that in a recently infected country, few farms would be infected (probably less than ten) before the disease was detected (estimate 3 weeks) (see P2 Table 7). The probability that it concerns an authorised collection centre is nevertheless extremely low. ▪ The biosafety measures applied are generally not very effective against arthropod vectors.	Extremely low to low (3 to 4 on a scale of 0 to 9)
P3 - Probability that these products come from cattle themselves infected with LSD and not detected in the farm at the time of collection	▪ Intra-herd morbidity was calculated using data from reports on declaration of infection to the OIE. The experts selected 59 farms (of more than 50 animals) declared infected between April and September 2016 in Greece and Bulgaria. The intra-herd prevalence calculated was: min: 0.3%, median: 3%, max: 25%. This value was only calculated on the number of cattle with clinical signs: Greece and Bulgaria do not perform virological tests on animals not showing clinical signs. It does not therefore relate to intra-herd prevalence but to morbidity in the herd. ▪ This figure should however be lower in herds infected recently.	Quite high to high (7 to 8 on a scale of 0 to 9)
P4 - Probability that these products are themselves infected	▪ The experts considered that they are most often infected (with a very high uncertainty because of the lack of data) in the case of oocytes and embryos.	High (8 on a scale of 0 to 9)

Probability	Argument for the estimate	Qualitative probability estimated by the experts
P5a - Probability that frozen semen stored for at least 30 days before shipping is not detected as potentially infected before introduction or use in France	▪ The conditions for monitoring the animals in the approved semen collection centres and the pre-storage of frozen semen for 30 days (making it possible to verify whether or not cases occur in the centre and/or the zone) are likely to reduce to nearly nil to minute the probability of the product not being detected as potentially contaminated.	Nearly nil to minute (1 to 2 on a scale of 0 to 9)
P5b - Probability that fresh semen or oocytes and embryos frozen and pre-stored for at least 30 days before shipment are not detected as potentially infected before introduction or use in France	▪ The probability of non-detection increases if the shipping concerns fresh semen. ▪ This is also the case for frozen oocytes and embryos, even if they have been pre-stored.	Extremely low to very low (3 to 4 on a scale of 0 to 9)
P5c - Probability of non-frozen oocytes and embryos not being detected as potentially infected before introduction or use in France	▪ The probability of non-detection is greater for non-frozen oocytes and embryos.	Low to not very high (5 to 6 on a scale of 0 to 9)
P6 - Probability of infected products being included in a consignment intended for France	▪ The countries of the at-risk area export few or no products to France (Eurostat data), with this share considered very low compared to the volume produced.	Very low to low (4 to 5 on a scale of 0 to 9)
P7 - Probability that the virus present in the products survives during transport.	▪ The products are generally frozen, which increases the survival of the virus.	Very high (9 on a scale of 0 to 9)

* Probabilities calculated for one year, based on the epidemiological situation in January 2017, the European regulations existing on this same date and data on trade for 2016.

The probability of introduction is the result of successive combinations, using the combination table (Annex 11) presented in the previously mentioned AFSSA report on the qualitative risk analysis, between the various probabilities (combining P1-P2, with the result being combined with P3, etc.) (AFSSA 2008).

Ultimately, the probability of the LSDV being introduced into France in a consignment of semen, oocytes or embryos shipped from the at-risk area (in 2016, the volumes traded were very low for these products), was estimated by the experts to be nearly nil (1 on a scale of 0 to 9) for frozen semen stored for at least 30 days after collection and before shipping, and nearly nil to minute (1 to 2 on a scale of 0 to 9) for fresh semen, or frozen pre-stored oocytes or embryos, as well as for non-frozen oocytes or embryos.

- *Probability of exposure*

In the event of introduction and use of these products in France, if they are infectious, exposure will be 100%, hence the importance of ruling out any risk of introduction. The probability of exposure is therefore estimated to be very high (9 on a scale of 0 to 9).

In conclusion, the probability of an outbreak through insemination or embryo transfer after use of semen, oocytes or embryos shipped from an at-risk area, which results from combining the probabilities of introduction and exposure, can be estimated as nearly nil (1 on a scale of 0 to 9) for frozen semen stored for at least 30 days after collection and before shipping, and nearly nil to minute (1 to 2 on a scale of 0 to 9) for fresh semen, or frozen pre-stored oocytes or embryos, and nearly nil to minute (1 to 2 on a scale of 0 to 9) for non-frozen oocytes or embryos.

Meat of infected cattle

The presence of the virus in the muscles of cattle infected with LSD is contemporaneous with viraemia, which can last for up to 2 weeks (Tuppurainen, Venter and Coetzer 2005, Osuagwuh *et al.* 2007) and is probably lower in cattle that are not clinically affected. Isolation of the LSDV in the muscles is difficult, suggesting low concentrations in the meat of infected animals, which, in the absence of clinical signs or lesions detected during ante- and post-mortem inspections at the slaughterhouse, could be slaughtered for consumption. The duration of survival in meat (at the pH of maturation) is unknown, but the LSDV can persist for a long period if the carcasses are frozen.

On the assumption that cattle, reared in a recently infected geographical zone where the disease has not yet been identified or reported, were sent to a slaughterhouse during this period, the probability that they are themselves infected can be estimated as low, like the probability that their meat contains high concentrations of the virus. These elements determine the probability of introduction of infected meat, which is accordingly estimated to be low (5 on a scale of 0 to 9).

In contrast, the probability that cattle may be contaminated from potentially infected meat can be qualified as nil. Any direct or indirect contact is, in fact, entirely unlikely. The same is true for the ingestion of products derived from meat because of the ban on feeding ruminants with ruminant proteins that is currently in force in the EU.

In conclusion, in spite of the possibility of potentially infected meat being introduced into France, the probability of an outbreak of LSD occurring through this infected meat can be estimated as nil (0 on a scale of 0 to 9).

Milk and colostrum of infected cattle or buffaloes

Whether it results from possible viral shedding or contamination from skin lesions during milking, the virus can be detected in the colostrum and milk of females affected by LSD (Weiss 1968, Sharawi and Abd El-Rahim 2011). There are no precise data on the level (probably low, making viral isolation difficult) of shedding, its duration, on survival of the virus in these products, or on the

extent or even reality of this mode of transmission. The possible contamination of milk or colostrum nevertheless deserves to be taken into account when they are intended for feeding calves.

The LSDV is however sensitive to heat, so is destroyed by the suitable heat treatment of these products[26]. The experts did not, however, have any data enabling them to determine with certainty whether or not the treatments (temperatures in particular) used during the processing of the milk (often shipped as milk powder) and colostrum can destroy any virus that may be present.

The assessed risk is the one related to the introduction into France of milk or colostrum from cattle farms in the at-risk area. The Eurostat data for the period from January 2015 to August 2016 report the introduction of milk only from Romania (1,141,100 kg in 2015, only 300 kg in 2016), without specifying, however, the share dedicated to animal feed. Nor do the experts have any data on the possible introduction of colostrum from the countries of the at-risk area.

In these conditions, and considering the limited information available on the hazard represented by colostrum, the experts decided to only consider the LSD risk in light of introductions of milk from the countries of the at-risk area.

In the scenario studied, the experts considered firstly the limited number of farms possibly infected (in a recently infected country, few farms would be infected, probably less than ten) and secondly, the low proportion of infected cows (incubating or asymptomatic) in these herds (equivalent at most to the prevalence as calculated previously in infected herds, i.e. 0.3 to 25%, with a median of 3%) and lastly, the small amount of virus in the milk reported in the few publications on the subject. In these conditions, dilution of the milk produced by cows that are possibly infected in all the milk collected by the exporting dairy greatly reduces the probability that the exported consignment can represent a hazard.

Consequently, the probability of introduction via contaminated milk intended for bovine feed is estimated to be nearly nil (1 on a scale of 0 to 9). This risk is nil in the case of suitable prior heat treatment of the milk.

Even considering the risk of exposure as high (probability that should be reconsidered if the minimum infective dose by ingestion, which is currently unknown, is determined in the future), the probability of an outbreak of LSD occurring related to the introduction of dairy products from the at-risk area can be estimated as nil to nearly nil (0 to 1 on a scale of 0 to 9).

In conclusion, the probability of an outbreak of LSD occurring via milk intended for animal feed is estimated to be nil to nearly nil (0 to 1 on a scale of 0 to 9).

Contaminated inert media

The LSDV, protected in dried protein aggregates and away from the light, can survive for several months. It is nevertheless eliminated by most common disinfectants.

The risk of virus dissemination mainly concerns vehicles transporting cattle, when the animals are infected and shedding, and vehicles carrying contaminated products of animal origin, in particular

[26] In Decision 2016/2008 providing for derogations from the prohibition on the dispatch of consignments of colostrum, milk and dairy products from bovine animals and captive wild ruminants intended for animal feed from infected zones when they have undergone heat treatments (such as, among others, UHT treatment at 132°C for at least one second, or HTST at 72°C for at least 15 seconds) required to destroy the foot-and-mouth disease virus (defined in Directive 2003/85/EC).

untreated hides (in infected animals, the skin is the tissue in which the virus has been isolated with the highest titres).

The probability of the virus being introduced by vehicles used to transport cattle is at most identical to that from the introduction of infected live cattle intended for rearing, which has been estimated as extremely low to low (3 to 5 on a scale of 0 to 9). The probability of exposure takes account of the possibility of animals from potentially infected zones being transported with native animals, or native animals intended for rearing being transported in these vehicles after the unloading of traded or imported animals without the vehicles having previously been disinfected. As this risk is reduced due to regulatory obligations relating to the transport of animals and the disinfection of animal transport vehicles, the probability of exposure can be estimated as nearly nil to minute (1 to 2 on a scale of 0 to 9), placing at the same level the final probability of an outbreak of LSD occurring in response to such events.

The probability of the virus being introduced by vehicles used to transport consignments of untreated hides from infected cattle is at most identical to that from the introduction of the products in question, considered to be very low to low (4 to 5 on a scale of 0 to 9). The probability of exposure, which takes into account the place where the products are unloaded (tanneries) and the fact that the corresponding transport vehicles are not dedicated to the transport of live animals, is considered to be nil to nearly nil (0 to 1 on a scale of 0 to 9). This makes the final probability of an outbreak of LSD occurring in response to such events nil to nearly nil (0 to 1 on a scale of 0 to 9).

In conclusion, the probability of an outbreak of LSD occurring through transport vehicles that have been in contact with infected cattle is estimated to be nearly nil to minute (1 to 2 on a scale of 0 to 9) and, in contact with infected hides, this probability is estimated to be nil to nearly nil (0 to 1 on a scale of 0 to 9).

- **Risk associated with the use of a live attenuated vaccine**

Some commercial vaccines against the LSD virus are insufficiently attenuated and can cause clinical disease in vaccinated animals (Ben-Gera *et al.* 2015, Tuppurainen *et al.* 2014, Yeruham *et al.* 1994). At this stage, the experts considered the current risk to be nil because vaccination is prohibited in France and there is, for the moment, no reason to use it on French territory.

Moreover, the new OIE regulation on LSD currently being validated by the Member States provides for the possibility of introducing into a disease-free country disease-free animals that have been vaccinated against LSD, to the extent that the competent authorities can make the distinction between the vaccine antibodies and antibodies from the field strain.

4.2.2.4. Introduction by illegal imports of animals and animal products and by-products

Although, by nature, there is very little information available on possible illegal movements of live animals (cattle, small ruminants, wildlife, horses) between France and the countries of the at-risk area, the experts believe that if these movements exist, they are on a small scale. Indeed, the geographical distance between France and these countries, as well as the small number of legal transports of live animals, suggest that there are very few illegal movements. In addition, cattle, the

main source of LSDV, are difficult to introduce illegally, because of their size and especially the mandatory individual identification.

Despite the absence of information on this subject, the experts estimate, if they exist, that there are few illegal introductions of animal by-products (hides, milk, meat, semen) into France from the countries of the at-risk area, just as with the legal introductions.

In conclusion, the probability of an outbreak of LSD occurring following the illegal introduction into France of animals or animal by-products from the at-risk area can be estimated as nil to nearly nil (0 to 1 on a scale of 0 to 9).

4.3. Estimate of the size of a vaccine bank

The second question in the formal request was the following: "estimate the appropriate size for a vaccine (or antigen) bank, to manage an emergency vaccination campaign in the event that the disease were introduced". The experts considered that the purpose of this question was only to calculate the number of doses to plan for a vaccine bank. This is why this estimate does not take into account the efficacy and safety of the vaccine, or the management measures associated with this possible vaccination.

There is currently a consensus between the health authorities and scientists on the benefits of using vaccination to control LSD, as long as the vaccine provides guarantees of safety and efficacy (see the section on vaccination, page 38).

Several vaccination strategies are possible to prevent the spread of the disease, including:

- vaccination of 100% of susceptible animals before the occurrence of a first outbreak, when a territory neighbouring France is affected and the occurrence of a first outbreak in the zone is possible via vector-borne dissemination;
- ring vaccination around a first outbreak detected, assuming that restrictions on animal movement, insect eradication and disinfection of vehicles are applied immediately on all farms in the zone (and all that remains is therefore the possibility of vector-borne dissemination).

In order to estimate the appropriate size for a vaccine bank, to manage an emergency vaccination campaign, the experts considered that the disease would spread steadily from the first outbreak, and that there would be no long-distance spread. To determine the size of the zone to be considered, the following factors were taken into account:

1- The speed at which the infection travels (i.e. the distance covered per week). The value used for the calculations was the median of the dissemination speed of 7.3 km/week, from May 2015 to August 2016, with a spread from the west of Turkey to eight countries of the Balkans (Mercier *et al.* 2017).

2- The time needed to obtain good vaccination coverage of the cattle population in question:

- The time elapsing between the occurrence of the first outbreak, its detection and the establishment of control measures (estimated by the experts at 5 weeks, see page 54);
- The time needed to vaccinate the entire targeted population (estimated at 2 weeks).

3- The density of cattle in the region where the first outbreak is located. The risk of LSD being introduced into France, related to introductions of infected live cattle from the at-risk area and intended for rearing, was considered to be very low to low (Table 9). Moreover, as there are very few data on these introductions, they cannot be used to perform the simulation. The experts made the assumption that the risk of occurrence of the first outbreak was directly proportional to the number of cattle in each *département*.

Another assumption used for this estimate was that, if vaccination was established, it would be applied to all cattle in the zone concerned with a single vaccine dose per animal. The calculations

can be refined according to the age of the cattle population considered and the actual vaccination capabilities on the ground.

The product of the three values – speed of dissemination, time until detection and establishment of management measures, and cattle density – gives the size of the vaccine stock to be built up.

The calculations performed by the experts are as follows:

- Estimate of the density of cattle in each *département* (Dep.) and each region (Reg.):

$$Density\ Dep. = \frac{Number\ of\ cattle^{27}\ in\ the\ Dep.}{Area^{28}\ of\ the\ Dep.}$$

$$Density\ Reg. = \frac{Number\ of\ cattle\ in\ the\ Reg.}{Area\ of\ the\ Reg.}$$

For the calculations, tests were performed considering the total density of the region by taking into account only the density of the *départements* excluding that of the *département* concerned, but the results were identical.

For the calculations, the experts considered the *départements* as a square, with one side of:

$$Side = \sqrt{Area}$$

The experts considered that the first outbreak could appear randomly in any point of the *département*. If the radius of the vaccination zone exceeds the borders of the *département*, vaccination will apply to the corresponding zone in the neighbouring *département* within the same region (to simplify, if the vaccination zone straddled another region, the sea or a neighbouring country, the corresponding zone was not taken into account):

$$Position\ of\ first\ outbreak = \text{random number} \times Side$$

The random number was generated by the RAND() function of the Excel® software.

- Estimate of the number of animals to be vaccinated

The experts considered either the density of cattle in the *département*, or the density in the region. If the entire zone to be vaccinated was included in the *département* (the diameter was smaller than the distance between the border of the *département* and the point randomly obtained), the experts considered the cattle density of the *département* over the distance to be vaccinated (if: distance < position of first outbreak - side):

$$Number\ of\ cattle\ = Density\ Dep. \times \pi \times radius\ of\ the\ vaccination^2$$

[27] Source : Agreste - Statistique agricole annuelle semi-définitive 2014 et 2015.
[28] Source Institut National de l'Information Géographique et forestière

However, if the zone to be vaccinated exceeded the border of the *département*, the experts took into account the cattle density of the *département* but also that of the region:

$$Number\ of\ cattle\ in\ the\ region\ outside\ the\ département = Density\ Reg. \times (\varphi - \sin(\varphi)) \times radius\ of\ the\ vaccination^2/2$$

$$Number\ of\ cattle\ in\ the\ département = Density\ Dep. \times (\pi \times radius\ of\ the\ vaccination^2 - (\varphi - \sin(\varphi)) \times radius\ of\ the\ vaccination^2/2)$$

Φ corresponds to the angle formed by the two lines connecting the position of the first outbreak and the two intersections of the circle of the vaccination zone and the side (example Figure 4).

Figure 4 shows these values and the calculations performed for two zones chosen at random, the *départements* of Saône-et-Loire and Côte d'Or in the region of Bourgogne Franche Comté. The diagram illustrates two simulations (two random points) among the 20,000 carried out.

For example, in Saône-et-Loire, if the index case occurs at 42 km from the centre of the *département*, and the period between the occurrence of the first outbreak, its detection and the establishment of control measures is 7 weeks (= a radius to be vaccinated of 51.1 km), the area of the *département* to be vaccinated is 7824 km^2 with an area outside the *département* of 379 km^2.

In Côte d'Or, if the entire vaccination zone is located in the *département*, the density of cattle in the *département* is used to calculate the number of cattle, which will then be multiplied by the corresponding area.

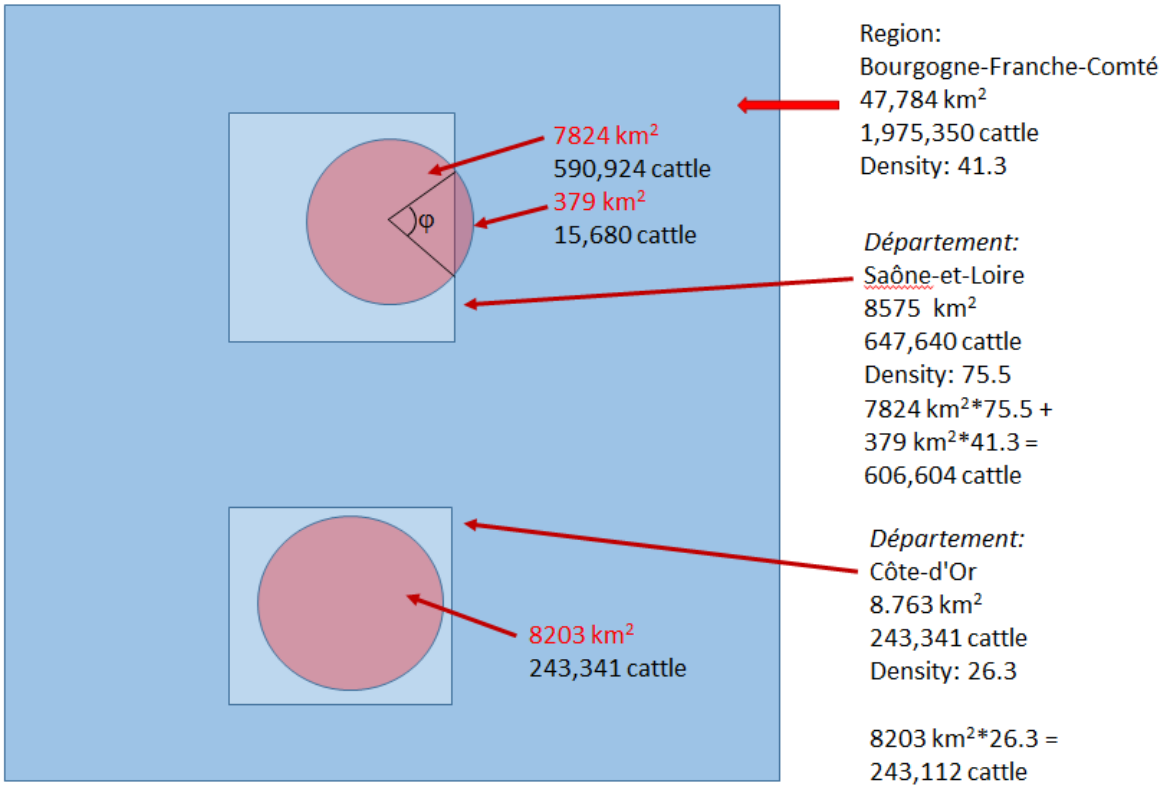

Figure 4: Representation of calculations for determining the number of cattle to be vaccinated according to the *département*, the region and the location of the first outbreak

- Results:

Table 21 shows the values obtained after 20,000 simulations using Excel® software, depending on the response time between the occurrence of the first case and completion of vaccination (values between 5 and 8 weeks). The values presented are the 50%, 75%, 90% and 95% percentiles (the values for the 90 percentile indicate the number of cattle to be vaccinated that is sufficient for 90% of simulations).

Taking into consideration a period of 7 weeks (5 to detect the disease after the occurrence of the first outbreak and 2 to vaccinate all the animals), the population in a radius of 51.1 km is around 800,000 cattle or less in 95% of simulations, which means that 800,000 vaccine doses are needed to vaccinate the entire cattle population in the zone in the event of viral spread at a steady speed of 7.3 km per week (i.e. excluding longer distance jumps). In 75% of simulations, 626,000 doses are sufficient. Table 21 gives the results for the other situations corresponding to the disease spreading over a shorter or longer period.

Table 21: Number of cattle to be vaccinated according to the dissemination time (period between the occurrence of the first outbreak and completion of vaccination of the targeted population) for 50%, 75%, 90% and 95% of the simulations performed

Weeks of dissemination	Radius (km)	50%	75%	90%	95%
5	36.5	232,532	319,492	385,627	411,434
6	43.8	334,846	460,068	555,303	591,131
7	51.1	455,763	626,204	755,829	798,128
8	58.4	595,282	809,235	987,205	1,030,416

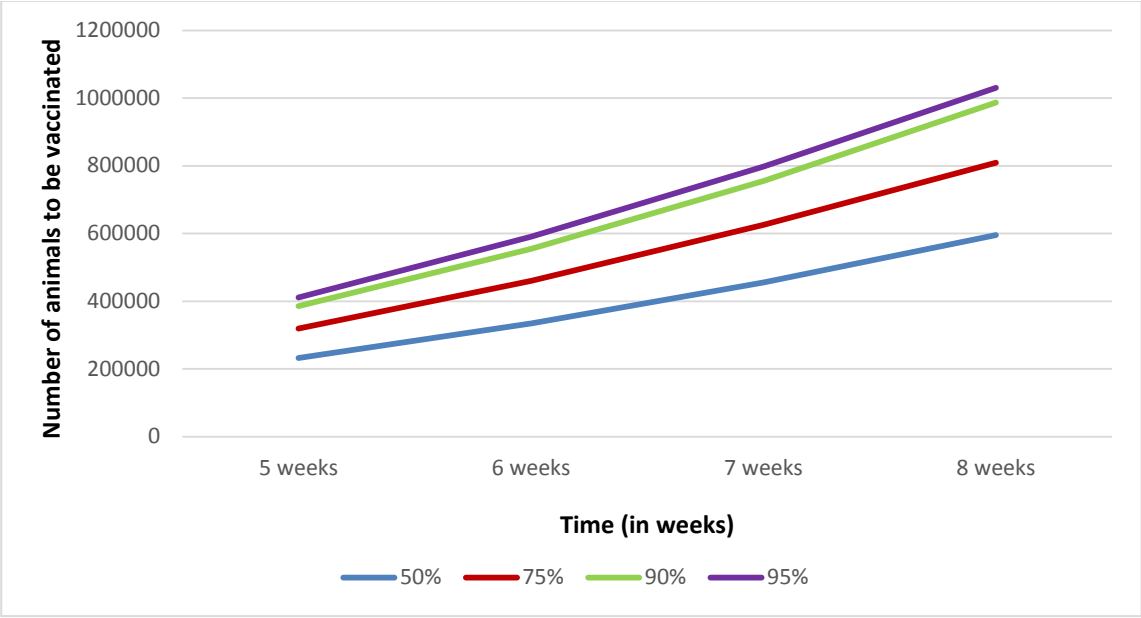

Figure 5: Number of infected cattle according to the dissemination time (period between the occurrence of the first outbreak and completion of vaccination of the targeted population)

In conclusion, for a period of 7 weeks (5 weeks before detection of the disease and 2 weeks to vaccinate the entire population) between the occurrence of the first outbreak and the end of the vaccination period, 626,204 vaccine doses would be sufficient in 75% of simulations, and 798,128 doses in 95% of simulations, to vaccinate the exposed population, taking into account the speed of viral spread following the discovery of an index outbreak (excluding long-distance spread, involving "leapfrogging"). For the French *département* with the highest density of cattle (Mayenne), the experts calculated that 945,456 doses would be necessary in 95% of simulations (Annex 13).

If the speed of dissemination of the LSDV should vary during the disease's spread in Europe, the number of vaccine doses needed may evolve. A new estimate of the number of doses required can be calculated by introducing the new assessed speed of viral spread into the model.

4.4. Uncertainties

The uncertainties related to the probability of a first outbreak of LSD in France mainly concern the limits of scientific knowledge on the LSDV, particularly regarding the epidemiological conditions of its spread in Europe. In addition, even when knowledge is available, there are often only limited surveillance and experimental data. Because the information available for some of the steps was limited, the opinion of the WG experts was sought, and the results must therefore be interpreted with the necessary caution.

The experts decided to list a few major uncertainties in Table 22 below following the reasoning behind risk assessment:

Table 22: Sources and types of uncertainties

Steps of the reasoning	Major uncertainties identified by the experts
Knowledge of the LSD virus	• Epidemiological role of small ruminants and wildlife present in Europe • Role of the direct transmission of the LSDV between cattle • Efficacy and safety of vaccines available in the EU • Minimum infectious dose in natural conditions, according to the method of transmission (bites, interaction between virus and saliva of biting arthropods, other potential routes)
Probability of introduction by live cattle	• Elapsed time between a first case of LSD in a disease-free zone and its declaration • Traceability of animal movements, in particular, transits via third countries. Lack of data on trade and transport (for example, the actual duration of the journey) • Contagiousness of cattle not expressing clinical signs • Transmission of LSD in the conditions in French farms and the French environment • Origin/traceability of imported animals in the countries neighbouring France
Probability of introduction by vectors	• Survival of the LSDV in the crop of *Stomoxys*, effectiveness of transmission by bites (number of bites needed), effective insect eradication in animal transport trucks, possibility of *Stomoxys* being transported by the wind over long distances, density of vectors in the farms (dynamic, at a given time t, etc.) • Risks associated with other European blood-sucking insects (tabanids, Culicidae, ticks, etc.) • Limitations of the available data on the persistence of the virus in the vectors: low numbers and a time period that is too long (1 day) • Lack of data on the actual duration of the transport and the number of unloads

Steps of the reasoning	Major uncertainties identified by the experts
Probability of introduction by other methods	• Effectiveness of treatments carried out on fresh hides to eliminate the LSDV; amount of virus present without visible lesions in the skin of infected animals • Lack of data on the conditions of transmission of the LSDV by semen in natural conditions • Lack of data on the survival of the LSDV in meat • Lack of data on possible transmission routes other than by arthropods
Size of the vaccine bank	• Methods of the spread of LSD in France • Location of the zones most at risk of being infected • Time between infection of a farm, detection of the disease, establishment of vaccination and the time needed to carry out the vaccination

4.5. Conclusions and recommendations

4.5.1. Conclusions

In an epidemiological context in which LSD is emerging in the EU, the DGAL formally requested ANSES to assess the risk of introduction of LSD into France and to estimate the appropriate size for a vaccine bank, to manage an emergency vaccination campaign in the event that the disease were introduced.

4.5.1.1. Probability of a first outbreak of LSD occurring in France

In order to respond to the first question of the formal request regarding the risk of introduction of LSD into France, and given the time available, the experts assessed "only" the probability of a first outbreak of LSD on French territory for a year, based on the epidemiological situation in January 2017, the European regulations existing on this same date, and data on trade for 2016. They did not take into account either the dissemination from the first outbreak, or the consequences of introduction of the LSDV.

The probability of a first outbreak of LSD occurring in France results from combining the probability of the virus being introduced into France with the probability that domestic cattle or wild ruminants are then exposed to this virus on French territory. The expert group, taking into account all the scientific and commercial data at its disposal, conducted an assessment of the risk of a first outbreak of LSD occurring in France, depending on the different sources of the virus and the possible ways in which they could be introduced (by live animals and their products: semen and embryos, by vectors, by inert media, etc.).

On the date the report was written, none of the countries bordering France had declared any infection with LSD. The experts defined an at-risk area for the purpose of the analysis: a zone from which live cattle or products can be traded and in which there is a probability that certain animals are infected, without the disease having been declared. This concerns disease-free regions of European countries recognised as infected (as of 1 January 2017: Greece, Bulgaria, FYROM, Kosovo, Serbia, Albania, Montenegro) and disease-free countries bordering a country where LSD has been notified (as of 1 January 2017: Romania, Croatia, Hungary, Ukraine, Bosnia & Herzegovina).

The risk assessment was carried out according to a quantitative approach for the methods of introduction regarded by the experts as most likely (movements of animals, movements of arthropod vectors). In the other cases, the approach was qualitative.

The values of the variables used in the model developed for the quantitative risk assessment can easily be modified later, depending on the evolution of the epidemiological situation in Europe, data relating to trade between the various Member States, and also advances in knowledge, in particular on the vectors or the methods of transmission of the LSDV. Vaccination could also be integrated in this model.

In the following sections, the probability of a first outbreak of LSD occurring is studied according to the different possible sources. The paragraphs below only detail the cases of introduction of LSD for which the probability of occurrence of a first outbreak is estimated to be equal to or greater than 3 (extremely low). The other means of introduction and their associated probabilities are shown in the summary table at the end of this section (Table 23).

- **Probabilities of an outbreak of LSD occurring following the introduction of infected live cattle**

Only animals from the EU countries belonging to the at-risk area (Greece, Bulgaria, Romania, Croatia, Hungary) were taken into account in the analysis. The probability of LSD being introduced by live animals is limited to the risk of introduction by live cattle.

The probability of a first outbreak of LSD in France, following the introduction of infected live cattle intended for rearing, is estimated to be extremely low to low (probability between 0.004% and 0.32%, with a confidence interval of 95%) for a year, based on the epidemiological situation in January 2017, the European regulations existing on this same date, and data on trade for 2016.
Currently there are no cattle intended for the slaughterhouse being introduced from the at-risk area. The probability of a first outbreak of LSD in France following the introduction of infected live cattle intended for the slaughterhouse is therefore estimated to be nil.

The experts considered, however, that if there were as many cattle intended for the slaughterhouse introduced into France as the number introduced for rearing, the probability would be nearly nil to minute (probability between 0.2 10^{-6} and 47 10^{-6}, with a confidence interval of 95%) for a year, based on the epidemiological situation in January 2017, the European regulations existing on this same date, and data on trade for 2016.

- **Probabilities of an outbreak of LSD occurring following the introduction of infective vectors**

The risk of LSD being introduced by the long-distance road transport of vectors is limited to the risk of introduction by *Stomoxys*.

The probability of a first outbreak of LSD in France, following the introduction of infective vectors transported with cattle intended for rearing, is estimated to be extremely low to low (probability between 0.002% and 0.44%, with a confidence interval of 95%) for a year, based on the epidemiological situation in January 2017, the European regulations existing on this same date, and data on trade for 2016.
Currently there are no cattle intended for the slaughterhouse being introduced from the at-risk area. The probability of a first outbreak of LSD in France following the introduction of infective vectors transported with live cattle intended for the slaughterhouse is therefore estimated to be nil.

The experts considered, however, that if there were as many cattle intended for the slaughterhouse introduced into France as the number introduced for rearing, the probability would be nearly nil to minute (probability between 0.1 10^{-6} and 27 10^{-6}, with a confidence interval of 95%) for a year, based on the epidemiological situation in January 2017, the European regulations existing on this same date, and data on trade for 2016.

The probability of a first outbreak of LSD in France following the introduction of infective vectors transported with horses is estimated to be nearly nil (probability between 0.01 10^{-6} and 1.67 10^{-6}, with a confidence interval of 95%) for a year, based on the epidemiological situation in January 2017, the European regulations existing on this same date, and data on trade for 2016.

■ Summary of the probabilities of a first outbreak of LSD occurring in France

Table 23: Summary of the probabilities of a first outbreak of LSD occurring in France

Modes of introduction of the LSDV	Assessment of the probability of a first outbreak of LSD occurring
By infected live cattle intended for rearing	**[3 to 5]** (extremely low to low) (quantitative probability between 0.004% and 0.32% with a confidence interval of 95%, for one year, based on the epidemiological situation in January 2017, the European regulations existing on the same date and data on trade for 2016)
By *Stomoxys* that travelled with the cattle intended for rearing (according to the assumptions made by the experts: no unloading and no insect eradication)	**[3 to 5]** (extremely low to low) (quantitative probability between 0.002% and 0.44% with a confidence interval of 95%, for one year, based on the epidemiological situation in January 2017, the European regulations existing on the same date and data on trade for 2016)
By infected live cattle intended for slaughter (mode not confirmed in 2016, scenario using the same introduction data as those for rearing)	**[1 to 2]** (nearly nil to minute) (quantitative probability between 0.2 10^{-6} and 47 10^{-6} with a confidence interval of 95%, for one year, based on the epidemiological situation at the beginning of 2017, the European regulations existing on the same date and data on trade for 2016)
By *Stomoxys* that travelled with the cattle intended for slaughter (mode not confirmed in 2016, scenario using the same introduction data and the same assumptions as those for rearing)	**[1 to 2]** (nearly nil to minute) (quantitative probability between 0.1 10^{-6} and 27 10^{-6} with a confidence interval of 95%, for one year, based on the epidemiological situation in January 2017, the European regulations existing on the same date, and data on trade for 2016)

Modes of introduction of the LSDV	Assessment of the probability of a first outbreak of LSD occurring
By fresh semen, or frozen pre-stored oocytes or embryos (modes not confirmed in 2016, scenario simulating a low number of introductions from the at-risk area)	**[1 to 2]** (nearly nil to minute)
By non-frozen oocytes or embryos (modes not confirmed in 2016, scenario simulating a low number of introductions from the at-risk area)	**[1 to 2]** (nearly nil to minute)
By transport vehicles that have been in contact with infected cattle	**[1 to 2]** (nearly nil to minute)
By frozen semen stored for at least 30 days after collection and before shipping	**[1]** (nearly nil)
By *Stomoxys* that have travelled with horses intended for a mixed herd (cattle/equines) or arriving in a stud farm with a herd of cattle nearby (according to the same assumptions as those for rearing)	**[1]** (nearly nil) (probability between 0.01 10^{-6} and 1.6 10^{-6} with a confidence interval of 95%, for one year, based on the epidemiological situation in January 2017, the European regulations existing on the same date, and data on trade for 2016)
By transport vehicles that have been in contact with infected hides	**[0 to 1]** (nil to nearly nil)
By live domestic small ruminants	**[0 to 1]** (nil to nearly nil)
By live wild ruminants, animals from zoos or circuses	**[0 to 1]** (nil to nearly nil)
By the milk of infected cattle or buffaloes	**[0 to 1]** (nil to nearly nil)
By illegal imports of live animals or animal by-products	**[0 to 1]** (nil to nearly nil)
By fresh hides of infected cattle	**[0 to 1]** (nil to nearly nil)
By transhumance or other animal husbandry practices	**[0]** (nil)
By the meat of infected cattle	**[0]** (nil)
By the use of a live attenuated vaccine	**[0]** (nil)

4.5.1.2. Estimate of the size of a vaccine bank

For this estimate, the experts did not take into account the efficacy and safety of the vaccine, or the management measures associated with this possible vaccination.

For a period of 7 weeks (5 weeks before detection of the disease and 2 weeks to vaccinate the entire population) between the occurrence of the first outbreak and the end of the vaccination period, 626,204 vaccine doses would be sufficient in 75% of simulations, and 798,128 doses in 95% of simulations, to vaccinate the exposed population, taking into account the speed of viral spread following the discovery of an index outbreak (excluding long-distance spread, involving "leapfrogging"). If the speed of dissemination of the LSDV should vary during the disease's spread in Europe, the number of vaccine doses needed may evolve. A new estimate of the number of doses required can be calculated by introducing the new assessed speed of viral spread into the model.

For the French *département* with the highest density of cattle (Mayenne), the experts calculated that 945,456 doses would be necessary in 95% of simulations.

4.5.2. Recommendations

The experts are able to make several recommendations following this assessment, not only regarding research, but also recommendations that focus more on preventing infection by the LSDV (the recommendations listed in the paragraphs below are not classified by order of importance).

4.5.2.1. Research recommendations

- Concerning the vectors, the experts believe it is necessary to develop knowledge on:
  - the epidemiological role of *Stomoxys* and horse-flies:
    - the infectious dose;
    - the survival time of the LSDV in the vector, with a reduction in the time unit (measurements in hours and not in days);
    - dispersion of *Stomoxys* (active and passive) and trapping methods;
  - the epidemiological role of ticks
    - the infectious dose;
    - transmission methods (mechanical versus biological) and associated issues (survival time in the vector or viral multiplication within the vector, transtadial or transovarial transmission, etc.);
  - host/pathogen interactions and in particular the effect of the vector's saliva in transmission of the LSDV;
  - *Stomoxys*, as well as on European ticks, Culicoides and Culicidae, in particular regarding vector density in farms and the methods of assessing vector densities;
  - vector control in farms: insecticide treatment with its limitations and alternative control methods to be investigated (trapping, repellents, growth regulators, Hymenoptera parasitoids of *Stomoxys*, etc.);
- Concerning vaccines against the LSDV, it is important to:
  - have access to data on the safety and clinical and virological efficacy of the available vaccines;
  - develop a DIVA vaccine conferring a higher level of protection and without any residual pathogenicity, which would enable improved control;

- Concerning the LSDV, studies are still needed in order to:
    - develop an improved experimental model of infection enabling direct infection or vector-borne transmission, and determine the minimum infectious dose;
    - better understand the epidemiological role of small ruminants, and that of native wildlife in the countries currently infected;
    - identify the determinants of natural resistance;
    - assess the actual role of artificial insemination and embryo transfer in the transmission of LSD.

4.5.2.2. Recommendations concerning prevention of the disease and its surveillance

Most of the recommendations listed below are those typically given in the context of emerging diseases.
It appears important to:

- include LSD in the list of diseases to be screened for in the framework of artificial insemination or embryo transfer from countries at risk;
- improve and validate diagnostic methods, in particular ELISA serological methods for the detection of antibodies, in view of using them when animals are introduced from the at-risk area;
- use the applicable serological and molecular diagnostic tools in the framework of a DIVA vaccination strategy (to differentiate infected from vaccinated animals);
- extend LSD surveillance, in the infected zone, to small ruminants and ruminants from circuses and zoos;
- maintain awareness among stakeholders in the sectors concerned;
- ensure the correct implementation and control of the application of insecticides and repellents in the trucks transporting livestock;
- develop a website devoted to monitoring the epidemiological situation of LSD in the EU, with a map, as is done for bluetongue[29];
- improve the traceability of live animal movements, in particular for animals from third countries;
- implement monitoring of feedback from the field regarding the situation in the Balkans and the vaccination implemented (study under way at EFSA).

Date of validation of the collective expert appraisal report by the Working Group: 18 January 2017

[29] https://ec.europa.eu/food/sites/food/files/animals/docs/ad_control-measures_bt_restrictedzones-map.jpg

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Annex 1: Formal request letter

ANSES Reçu le
18 MAI 2016

2016 -SA- 0120

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RÉPUBLIQUE FRANÇAISE

N - 0358 -D

MINISTERE DE L'AGRICULTURE, DE L'AGROALIMENTAIRE ET DE LA FORÊT

Direction Générale de l'Alimentation
Service des actions sanitaires en production primaire
Sous-direction de la santé et de la protection animales
Bureau de la santé animale
Adresse : 251, rue de Vaugirard
75 732 PARIS CEDEX 15
Dossier suivi par : Alexandre Fediaevsky
Téléphone : 01 49 55 84 61
Réf. Interne : 1605005

Le Directeur Général de l'Alimentation
au

Directeur Général de l'Agence
nationale de sécurité sanitaire de l'alimentation,
de l'environnement et du travail

Paris, le 18 MAI 2016

Objet : Demande d'expertise en urgence sur le risque d'introduction en France de la dermatose nodulaire contagieuse

Conformément aux articles L. 1313-1 et 1313-3 du Code de la santé publique, j'ai l'honneur de solliciter un avis en urgence de l'Anses pour évaluer les risques d'émergence de la dermatose nodulaire contagieuse (DNC).

Depuis quelques années la Turquie subissait une épizootie de DNC qui s'est étendue à la Grèce qui a également des difficultés à endiguer la maladie. Depuis le mois d'avril, la Bulgarie est également atteinte et des foyers ont déjà été identifiés dans plusieurs zones du pays. Une synthèse épidémiologique de la situation a été produite par la cellule de veille sanitaire internationale de la Plateforme ESA et mise en ligne (http://plateforme-esa.fr/article/extension-de-la-dermatose-nodulaire-contagieuse-%C3%A0-la-bulgarie).

Face à la situation évolutive au sein de l'Union Européenne, je souhaite avoir votre avis sur l'évaluation du risque de contamination pour la France compte tenu des différents facteurs de risque d'introduction.
Par ailleurs pourriez vous estimer la taille que devrait avoir une banque de vaccins (ou d'antigènes) pour gérer une campagne de vaccination en urgence au cas où la maladie serait introduite. Une hypothèse de détection de la maladie dans le mois suivant son introduction pourra être considérée.

L'avis de l'Agence est attendu dans un délai de six mois à compter de la réception de la saisine. Le rendu de l'avis pourra être fractionné.

Je vous remercie de bien vouloir accuser réception de la présente demande.

Le Directeur Général de l'Alimentation.
Patrick DEHAUMONT

1/1

Annex 2: Literature search profile used for the literature search

Profil de recherche bibliographique

Nom de la saisine : **Risque d'introduction de la DNC en France**

Pour la recherche bibliographique il est important de distinguer des grands ensembles qui couvriront les différentes problématiques à traiter, afin de mieux élaborer les requêtes dans les bases de données bibliographiques. (ex : ensemble1 Substance **AND** ensemble2)

Code requête	Thématique	Anglais	*Subheadings (MESH)*
#1	Populations cibles	Cattle, cow, bull, dairy cow, bovin*, calve	
#2	Types d'études	Epidemiology, emerging disease, transmission, vector, model*, risk factors, spread, diagnosis, control, surveillance, prevention, introduction, outbreak, eradication	
#3	Danger/agent	Lumpy skin disease, capripoxvirus, capripox virus, LSDV	

Termes d'exclusion	LSD	Périodicité	
Périmètre (Zone géographique)	France, Europe	Bases de données	Scopus, Pubmed
Revues spécifiques à la thématique	Revue de médecine vétérinaire des pays tropicaux		
Organismes référents sur le sujet	Cirad		

Publications déjà identifiées (en amont de la saisine ou en PJ)	Rapports identifiés	Projets identifiés (ANR, APRs Anses, ERA-NET etc.)
	Scientific Opinion on lumpy skin disease EFSA 2015 ; Etude épidémiologique de la dermatose nodulaire contagieuse bovine en Ethiopie et évaluation de son impact économique, Thèse Université de Toulouse 2011	

Espace Netvibes ☐ OUI ☒ NON

The query used in Scopus was the following:

(TITLE-ABS-KEY (cattle OR cow OR bull OR "dairy cow" OR bovin* OR calve) AND TITLE-ABS-KEY (epidemiology OR "emerging disease" OR transmission OR vector OR model* OR "risk factors" OR spread OR diagnosis OR control OR surveillance OR prevention OR introduction OR outbreak OR eradication) AND TITLE-ABS-KEY ("Lumpy skin disease" OR LSDV OR "capripox virus" OR capripoxvirus))

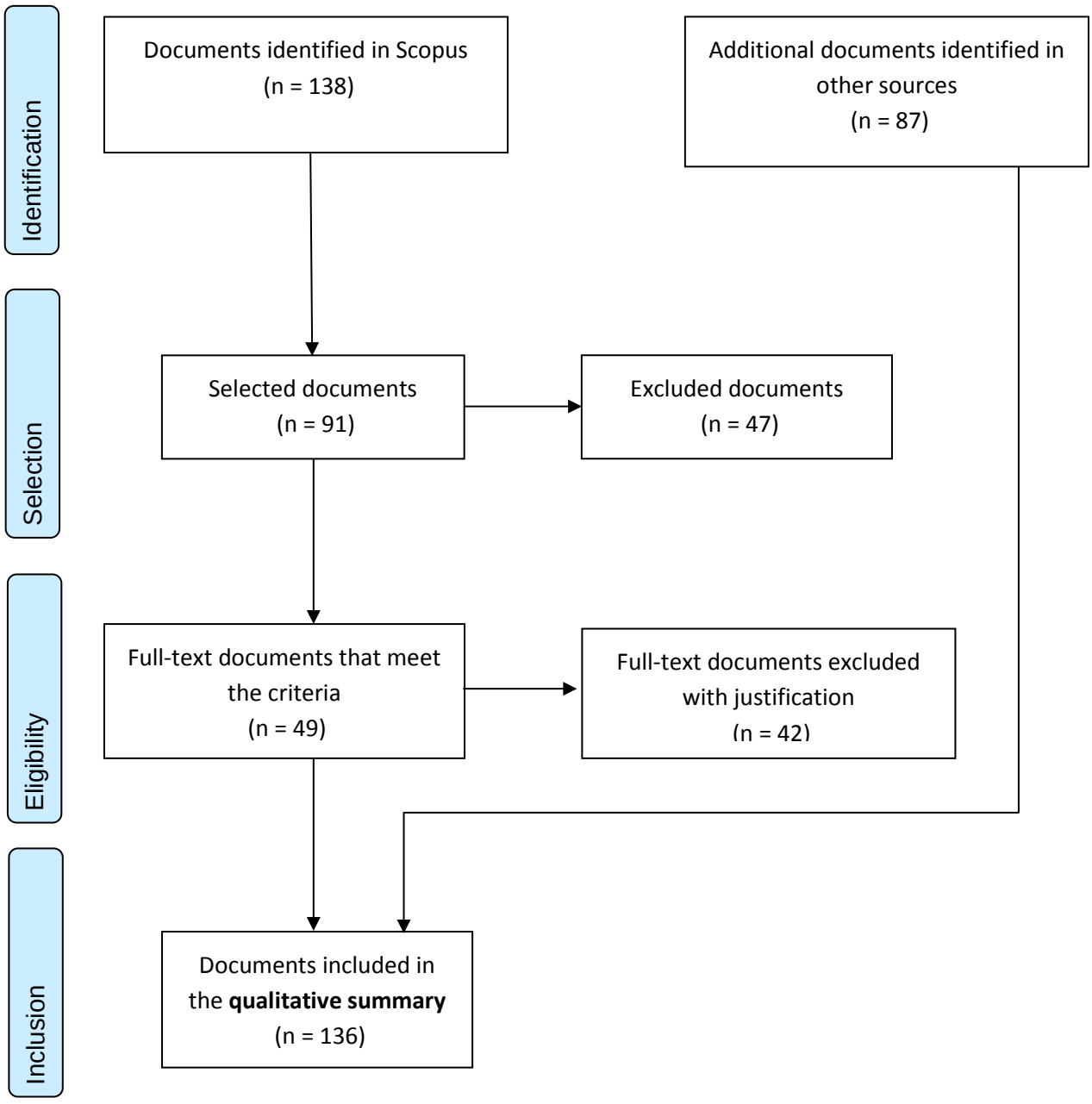
This query yielded 138 articles that the WG experts then sorted using the literature grid drawn up at the first meeting of the WG.

Annex 3: Literature grid used by the LSD WG experts to sort the 138 articles obtained following the search in Scopus.

Example with the first three articles in the list.

Title, author, year	Interest for addressing the question			Area of interest							Comments	Initials of the LSD WG expert
	No	Maybe	Yes	Methods of introduction				Epidemiological characteristics of the virus	Vaccine/ Immunology	Other		
				Live animals and by-products	Wild animals	Vectors	Other					
Abdalla and Gawad, 1992, Deutsche Tierarztliche Wochenschrift 99 (8): 347-349	X											
Abera et al, 2015, "Sero-prevalence of lumpy skin disease in selected districts of West Wollega zone, Ethiopia." BMC vet Research, 11 (1)		X						X				
Abutarbush, S. M. (2014). "Efficacy of vaccination against lumpy skin disease in Jordanian cattle." Veterinary Record 175(12).			X						X			

Annex 4: Diagram based on the PRISMA diagram[30] tracing the approach used for the literature search



[30] Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). *P*referred *R*eporting *I*tems for *S*ystematic Reviews and *M*eta-*A*nalyses: The PRISMA Statement. PLoS Med 6(7): e1000097. doi:10.1371/journal.pmed1000097

Annex 5: Clinical signs of lumpy skin disease (extract from the *Bulletin Epidémiologique* for animal health and nutrition (Arsevska *et al.* 2016))

- Incubation: 4 to 14 d, up to 1 month
- Morbidity: 5 to 45%, or even higher if the population is immunologically naive
- Mortality: less than 10%
- Lethality: 0.5 to 4.5%
- Individual resistance: only 40 to 50% of animals infected experimentally develop skin lesions

Classic Forms

Invasion phase

- Hyperthermia (4-14 d) up to 41°C and two-phase progression, listlessness, anorexia, fall in milk production
- Conjunctivitis, watery eyes, salivation, nasal discharge: initially seromucoid and then mucopurulent
- Lymph node hypertrophy (prescapular, precrural lymph nodes), 24 to 48 h after the start of the febrile phase

Rash phase

- 7 to 19 d post-inoculation, 4 to 10 d after the start of the febrile phase (first phase)
- Hard, rounded, painless nodules 0.5 to 5cm in diameter, variable in number (1-100), located on the head (periphery of the eyes and muzzle), neck, members, flanks, the udder and its teats, scrotum, perineum, as well as on the mucous membrane of the mouth, nose and eyes, and the vulvar and preputial mucosa
- Conjunctivitis and keratitis that can evolve towards blindness
- Extensive subcutaneous oedemas in the members, lumbar region, dewlap and genital organs
- Miliary form in the young: multitude of small nodules (2 to 5 mm)

Necrosis phase

- Induration of nodules and persistence (for up to several years) or necrosis and formation of a separate callus around the lesion (sequestrum)
- Nodules become dry (dry eschar) and are then shed leaving conical wounds affecting the entire thickness of the skin (evolution in 7 to 15 d)
- Healing in a few weeks (small wounds) or superinfection (large nodules) affecting the underlying tissue and then slow healing in 1 to 2 months

Serious forms

- Serious impairment of the general state, abortion
- Lameness: If pastern affected and suppurative or ulcerated lesions of the members
- Extended oedemas that can be complicated with deep ulcerated and suppurative lesions
- Skin lesions and clinical signs related to affected deep organs:
  - Painful and sonorous breathing (pharynx, larynx)
  - Pneumonia (lung parenchyma and false swallowing of necrotic tissue)
  - Cessation of rumination and bloat (oesophagus, ruminal pillars)
  - Digestive and respiratory disorders frequent in calves
- Long evolution (3 to 4 months) with sequelae: drying off, abortion, infertility, weight loss, skin impairment
- Death frequent in connection with complications:
  - Asphyxiation / bronchopneumonia, loss of the cud, severe malnutrition
  - Toxaemia and frequent septic complications (lymphangitis, abscesses, fistulas)
  - Loss of hooves/teats/tail related to the location of the nodules

Benign forms

- Mild or absent clinical signs
- Febrile reaction (2 to 5 d), lymph node hypertrophy
- Nodules heal in 3 to 6 weeks

Annex 6: TRACES data on imports of cattle and horses

Table 24: Number of consignments and cattle introduced into France from a Member State of the European Union during the period from July 2015 to July 2016 (Source: TRACES, July 2016)

Destination of cattle	Country of origin	Number of consignments	Number of cattle
Rearing			
Rearing	Germany	85	742
Rearing	Austria	4	54
Rearing	Belgium	317	8225
Rearing	Denmark	44	725
Rearing	Spain	167	3197
Rearing	France	1	9
Rearing	Hungary	1	4
Rearing	Ireland	2	50
Rearing	Italy	90	325
Rearing	Lithuania	15	1483
Rearing	Luxembourg	50	121
Rearing	The Netherlands	32	588
Rearing	Poland	15	2594
Rearing	Portugal	1	23
Rearing	Czech Republic	1	7
Rearing	Romania	6	178
Rearing	United Kingdom	44	628
Rearing	Slovenia	1	6
Rearing	Switzerland	46	202
Fattening			
Fattening	Germany	111	17966
Fattening	Andorra	6	7
Fattening	Belgium	129	1707
Fattening	Spain	182	8376
Fattening	Ireland	41	5752
Fattening	Italy	3	16
Fattening	Latvia	3	23
Fattening	The Netherlands	118	9816
Fattening	Czech Republic	64	14369
Fattening	United Kingdom	1	55
Slaughter			
Slaughter	Germany	1	18
Slaughter	Belgium	472	13536
Slaughter	Spain	747	7769

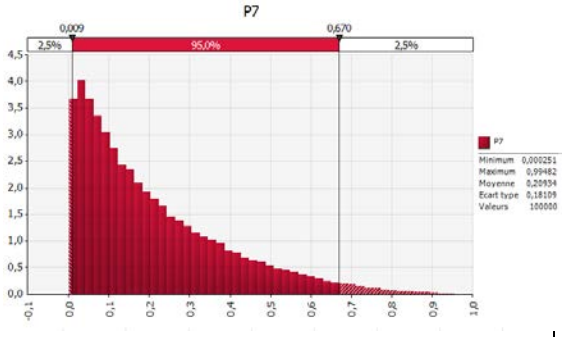
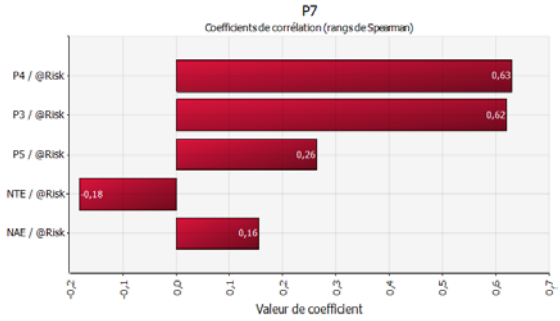
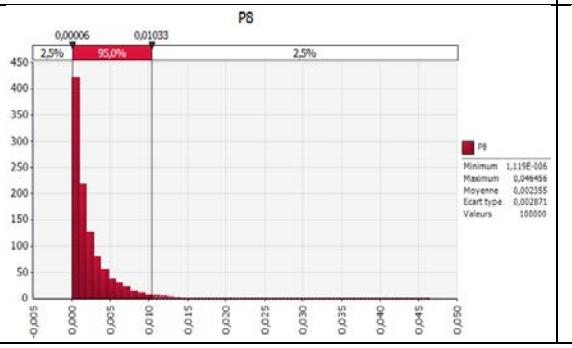
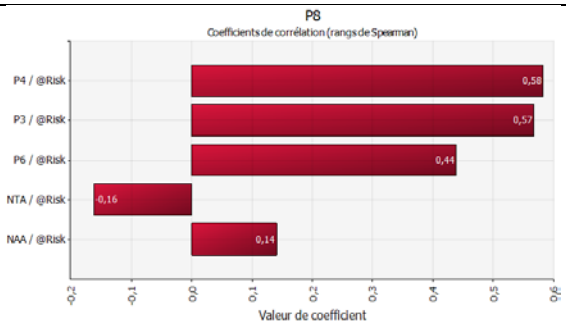
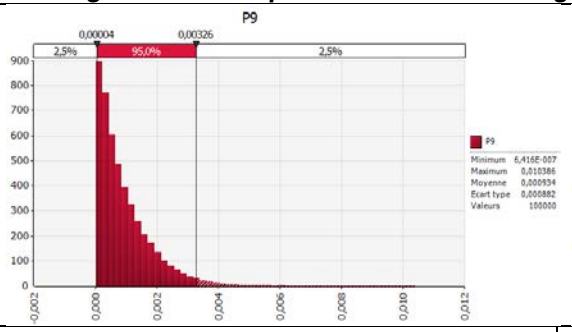
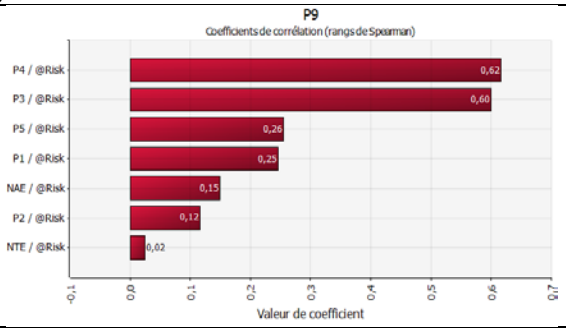
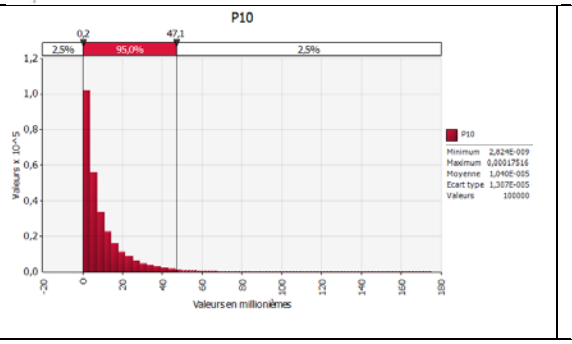
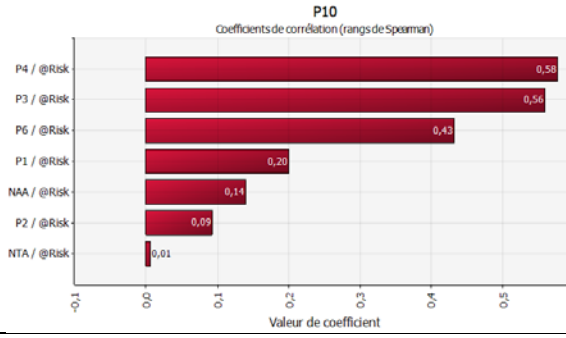
Destination of cattle	Country of origin	Number of consignments	Number of cattle
Slaughter	France	3	243
Slaughter	Ireland	6	236
Slaughter	Italy	3	8
Slaughter	The Netherlands	83	6710
Slaughter	Portugal	6	30
Transhumance			
Transhumance	Spain	4	68
Transhumance	Italy	7	314
Transhumance	Switzerland	297	6689

Table 25: Number of consignments and horses introduced into France from a Member State of the European Union during the period from September 2015 to September 2016 (Source: TRACES, September 2016)

Destination of horses	Country of origin	Number of consignments	Number of horses
Slaughterhouse			
Slaughterhouse	Belgium	1	10
Slaughterhouse	Spain	13	129
Slaughterhouse	Poland	10	173
Other			
Other	Germany	13	26
Other	Andorra	4	13
Other	Belgium	71	337
Other	Spain	335	2,168
Other	Italy	29	89
Other	The Netherlands	1	1
Other	Poland	1	2
Other	Portugal	36	185
Other	Czech Republic	1	2
Other	United Kingdom	121	243
Other	Switzerland	1	11
Rearing			
Rearing	Germany	19	30
Rearing	Belgium	10	25
Rearing	Spain	107	608
Rearing	Italy	8	34
Rearing	The Netherlands	46	312
Rearing	Poland	2	4
Rearing	United Kingdom	3	4
Rearing	Sweden	1	2
Rearing	Switzerland	3	6
Recorded in the stud book (farm)			
Recorded in the stud book	Germany	2,056	2,489

Destination of horses	Country of origin	Number of consignments	Number of horses
(farm)			
Recorded in the stud book (farm)	Austria	53	62
Recorded in the stud book (farm)	Belgium	1,504	2,908
Recorded in the stud book (farm)	Bulgaria	8	8
Recorded in the stud book (farm)	Cyprus	1	1
Recorded in the stud book (farm)	Croatia	9	9
Recorded in the stud book (farm)	Spain	1,262	2,168
Recorded in the stud book (farm)	Estonia	3	3
Recorded in the stud book (farm)	Finland	23	39
Recorded in the stud book (farm)	Greece	2	2
Recorded in the stud book (farm)	Hungary	25	25
Recorded in the stud book (farm)	Ireland	70	314
Recorded in the stud book (farm)	Italy	1,073	1,315
Recorded in the stud book (farm)	Latvia	10	10
Recorded in the stud book (farm)	Lithuania	2	2
Recorded in the stud book (farm)	Luxembourg	4	4
Recorded in the stud book (farm)	Malta	9	9
Recorded in the stud book (farm)	Norway	36	36
Recorded in the stud book (farm)	The Netherlands	1,167	1,296
Recorded in the stud book (farm)	Poland	38	81
Recorded in the stud book (farm)	Portugal	209	230
Recorded in the stud book (farm)	Czech Republic	75	78
Recorded in the stud book (farm)	United Kingdom	1,384	1,454
Recorded in the stud book (farm)	Slovakia	17	24
Recorded in the stud book (farm)	Slovenia	4	4
Recorded in the stud book (farm)	Sweden	5	5
Recorded in the stud book (farm)	Switzerland	680	1,198

Annex 7: Distribution of probabilities that imported or traded contagious cattle or consignments of cattle transmit LSD to native animals and sensitivity analysis

Probability	Probability distribution	Sensitivity analysis showing the input parameters with the greatest influence on the result
Probability that imported or traded contagious cattle transmit LSD to native animals		
P7 (for rearing)		
P8 (for slaughter)		
Probability that consignments of imported or traded contagious cattle transmit LSD to native animals		
P9 (for rearing)		
P10 (for slaughter)		

Annex 8: Probability qualifiers for the qualitative estimate of the risk

Table 26: Quantified values proposed for each probability qualifier and correspondence with the ordinal values (AFSSA 2008)

Ordinal scale	Qualitative	Lower bound	Median value	Upper bound	Order of magnitude
0	Nil	0	0	0	0
1	Nearly nil	> 0	$2.5\ 10^{-6}$	$1.3\ 10^{-5}$	10^{-6}
2	Minute	$2.5\ 10^{-6}$	$1.3\ 10^{-5}$	$6.4\ 10^{-5}$	10^{-5}
3	Extremely low	$1.3\ 10^{-5}$	$6.4\ 10^{-5}$	$3.2\ 10^{-4}$	$6.7\ 10^{-5}$
4	Very low	$6.4\ 10^{-5}$	$3.2\ 10^{-4}$	$1.6\ 10^{-3}$	$3.3\ 10^{-4}$
5	Low	$3.2\ 10^{-4}$	$1.6\ 10^{-3}$	$8\ 10^{-3}$	$2\ 10^{-3}$
6	Not very high	$1.6\ 10^{-3}$	$8\ 10^{-3}$	$4\ 10^{-2}$	10^{-2}
7	Quite high	$8\ 10^{-3}$	$4\ 10^{-2}$	$2.5\ 10^{-1}$	$4\ 10^{-2}$
8	High	$4\ 10^{-2}$	$2.5\ 10^{-1}$	1	0.2
9	Very high	0.25	1	1	1

Annex 9: Presentation of the model used to estimate the survival rate of the LSDV in the vectors

A model was developed to estimate the survival rate of the LSDV in the vectors. The probability is expressed as a binomial process:

$$L = \prod_t \binom{n_t}{k_t} p_t^{k_t} \left(1 - p_t\right)^{n_t - k_t}$$

Where n_t is the number of vectors tested on day t, k_t is the number of vectors tested as positive on day t and p_t is the probability that a vector is still infective on day t if it has had an infective meal on day t=0 (i.e. probability of survival of the vector):

$$p_t = \left(1 - r\right)^{t+1}$$

Where r is the probability of "recovery" (negativation of PCR on the vectors) on day t.

Using Bayesian analysis, the median of the *a posteriori* probability of recovery r was estimated at 0.6255 with a credible interval at 95% from 0.4969 to 0.7591. The probability distribution of survival of the virus in the vector as a function of time is shown in Figure 6.

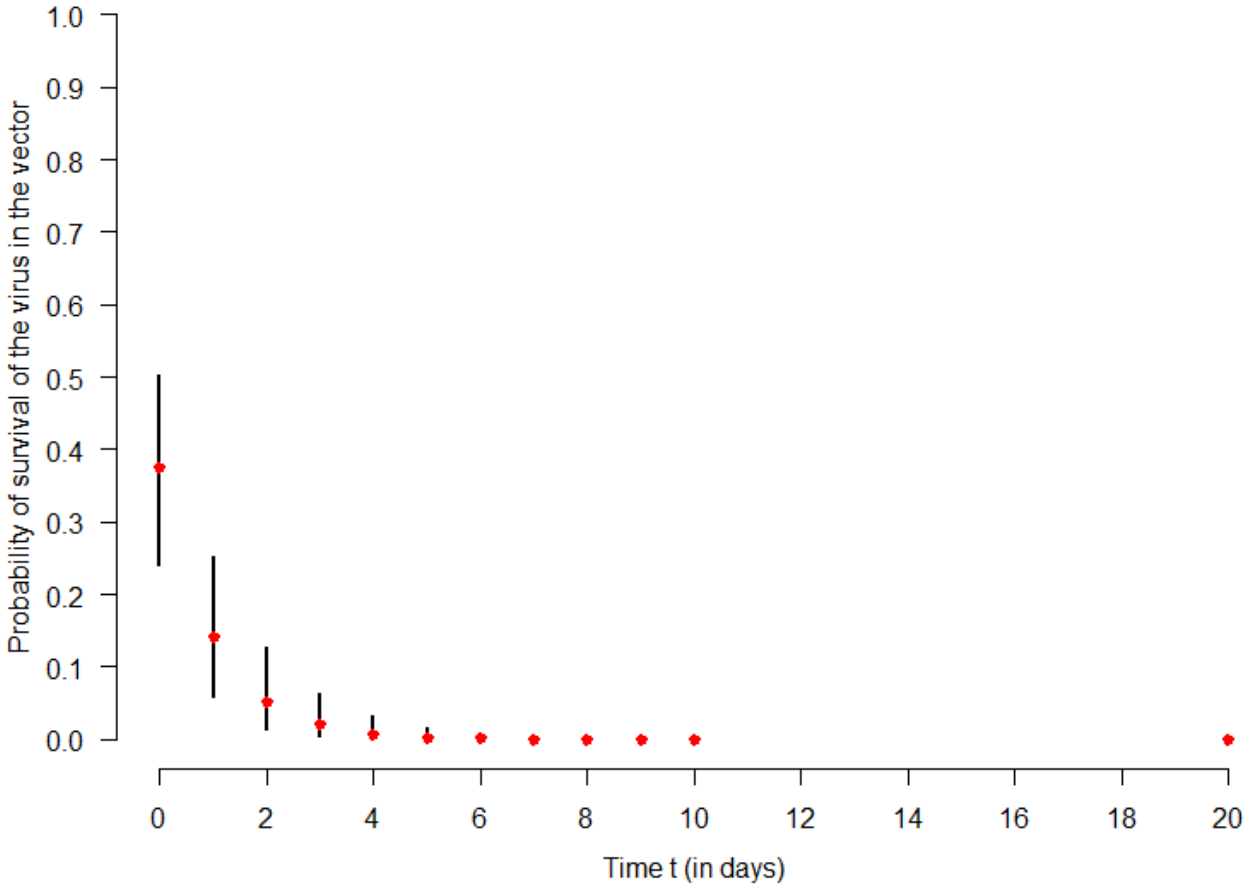


Figure 6: Distribution of the probability of survival of the virus in the vector as a function of time

When the *a posteriori* distribution is used to perform simulations that are themselves compared with experimental data (Chihota *et al.* 2003), the simulations correspond fairly well to the available experimental data (Figure 7).

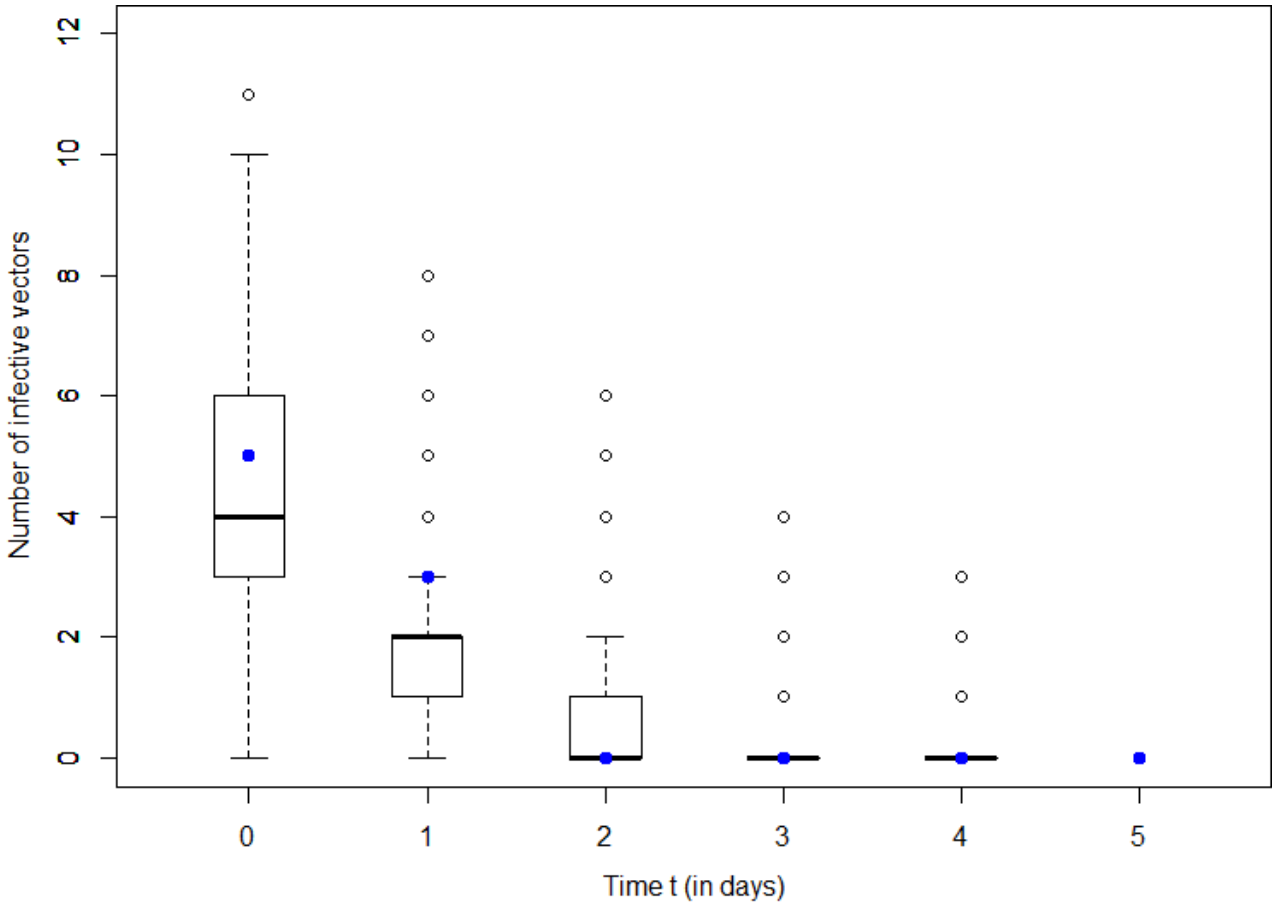
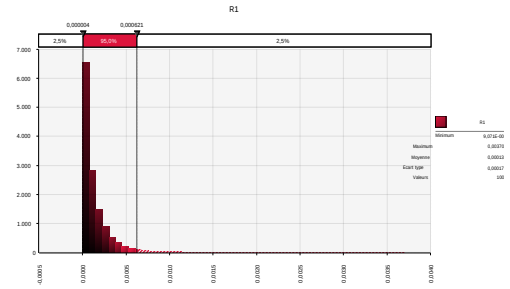
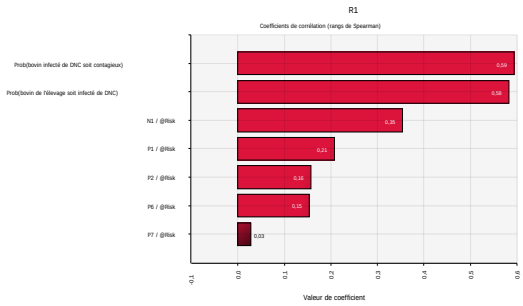
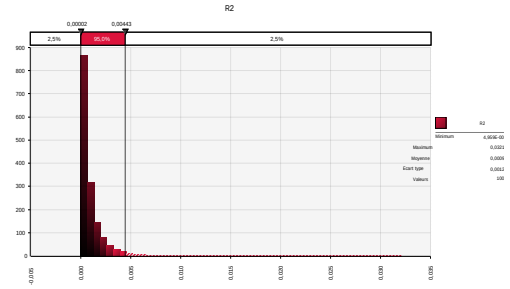
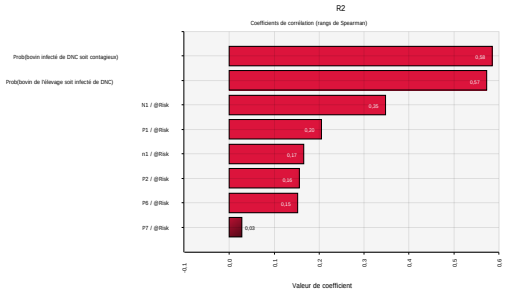
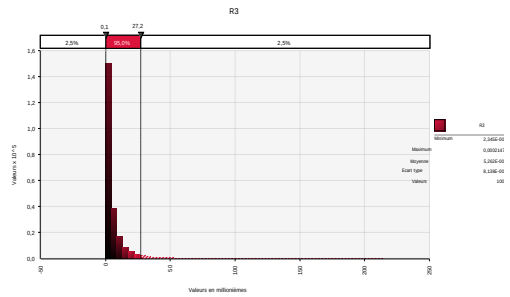
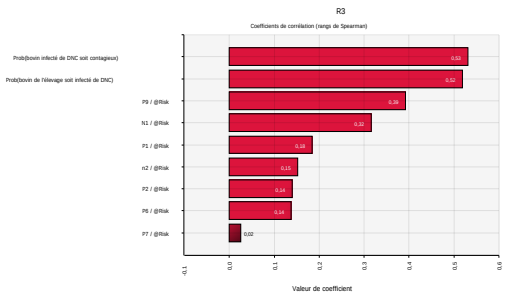
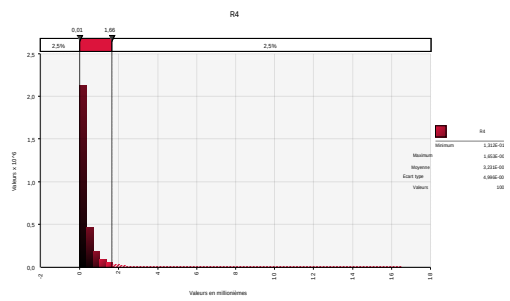
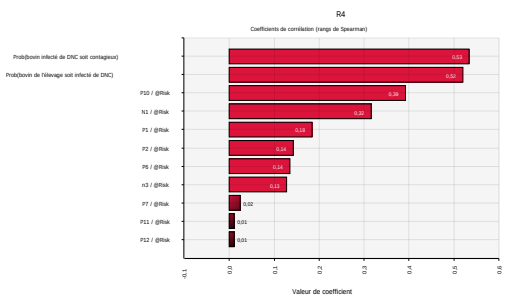


Figure 7: Distribution of the number of vectors as a function of time comparing data from simulations (box plot) and experimental values (Chihota *et al.* 2003) (blue dots)

One assumption of this model is that all vectors have had an infective blood meal at t=0, but that if certain vectors are negative at time t=0, this is because they quickly recovered. To be more precise, it would be necessary to add a second parameter, the infection parameter, which is, however, complicated to introduce here in view of the few experimental observations available to date.

Annex 10: Distribution of the probability that *Stomoxys* found in the vehicles carrying live animals (cattle or horses) then transmit LSD to native animals, and sensitivity analysis

Probability	Probability distribution	Sensitivity analysis showing the input parameters with the greatest influence on the result
R1 - Probability that an infective *Stomoxys* arrives at the destination		
R2 - Probability that a native bovine is infected by *Stomoxys* that travelled with cattle intended for rearing		
R3 - Probability that a native bovine is infected by *Stomoxys* that travelled with cattle intended for the slaughterhouse		
R4 - Probability that a native bovine is infected by *Stomoxys* that travelled with horses intended for a mixed herd (cattle/horses) or arriving in a stud farm with a herd of cattle nearby		

Annex 11: Results of the combination of probability of emission and probability of exposure

Table 27: Results of the combination of the two probabilities (according to AFSSA 2008)

			Probabilité d'émission / *Release probability*									
			N / *N*	QN / *NN*	M / *M*	EF / *EL*	TF / *VL*	F / *L*	PE / *NVH*	AE / *QH*	E / *H*	TE / *VH*
			0	1	2	3	4	5	6	7	8	9
Probabilité d'exposition / *Exposure probability*	N / *N*	0	0	0	0	0	0	0	0	0	0	0
	QN / *NN*	1	0	1	1	1	1	1	1	1	1	1
	M / *M*	2	0	1	1	1	1	2	2	2	2	2
	EF / *EL*	3	0	1	1	1	2	2	2	3	3	3
	TF / *VL*	4	0	1	1	2	2	3	3	3	4	4
	F / *L*	5	0	1	2	2	3	3	4	4	5	5
	PE / *NVH*	6	0	1	2	2	3	4	5	5	6	6
	AE / *QH*	7	0	1	2	3	3	4	5	6	7	7
	E / *H*	8	0	1	2	3	4	5	6	7	8	8
	TE / *VH*	9	0	1	2	3	4	5	6	7	8	9

N=Nul, QN=Quasi-nulle, M=Minime, EF=Extrêmement faible, TF=Très faible, F=Faible, PE=Peu élevée, AE=Assez élevée, E=Élevée, TE=Très élevée.

N=Nil, NN=Nearly Nil, M=Minute, E=Extremely Low, VL=Very Low, L=Low, NVH=Not Very High, QH=Quite High, H=High, VH=Very High.

Annex 12: Eurostat data on the introduction of hides, bull semen, bovine embryos and milk into France

Table 28: Eurostat data on the introduction of hides (quantity in tonnes) and bull semen (amount in euros)

Importing countries	Quantity of hides[1] introduced in tonnes	Amount of imports of bull semen[2] in euros
Albania	-	-
Germany	3321.0	1,235,804
Armenia	-	-
Austria	52.3	-
FYROM	-	-
Belarus	-	-
Bosnia & Herzegovina	-	-
Bulgaria	-	-
Cyprus	-	-
Croatia	-	-
Denmark	-	-
Spain	385.5	13,334
Finland	53.5	-
Great Britain	675.8	9,341,785
Greece	-	-
Hungary	-	256,441
Ireland	488.7	1,573
Israel	92.8	-
Italy	1862.4	266,920
Jordan	-	-
Kosovo	-	-
Lebanon	-	-
Lithuania	3.4	-
Latvia	-	-
Montenegro	-	-
The Netherlands	744.2	1,060,340
Poland	94.6	38,222
Portugal	63.0	-
Czech Republic	-	10,090
Romania	-	-
Russia	-	-
Serbia	-	-
Slovakia	21.1	3,996
Slovenia	67.6	-

Sweden	0	25,378
Switzerland	5078.7	-
Turkey	-	-
Ukraine	-	-

[1] EUROSTAT code 4101: Raw hides and skins of bovine (including buffalo) or equine animals (fresh or salted, dried, limed, pickled or otherwise preserved, but not tanned, parchment-dressed or further prepared), whether or not dehaired or split.
[2] EUROSTAT code 051110: bovine semen

Annex 13: Additional data used to estimate the size of the vaccine bank

Table 29: Numbers of cattle according to the French *départements*

Region	*Département*[31]	Total number of cattle[32]	Source[33]
Auvergne-Rhône-Alpes	Cantal	492300	Auvergne-Rhône-Alpes: Regional booklet (PDF: 2.2 MB) - 12/10/2016
	Allier	560300	
	Loire	316100	
	Haute-Loire	226500	
	Puy-de-Dôme	355007	
	Rhône	110200	
	Ain	184900	
	Haute-Savoie	113200	
	Isère	157500	
	Savoie	70200	
	Ardèche	53300	
	Drôme	34900	
Bourgogne-Franche-Comté	Saône et Loire	647640	Bourgogne-Franche-Comté: Regional booklet (PDF: 5.9 MB) - 04/01/2016
	Nièvre	365328	
	Doubs	249830	
	Haute Saône	201975	
	Jura	154880	
	Territoire de Belfort	18780	
	Côte-d'Or	230341	
	Yonne	106576	
Bretagne	Ille-et-Vilaine	666000	Bretagne: Regional booklet (PDF: 2.8 MB) - 21/09/2016
	Côtes d'Armor	533000	
	Finistère	455000	
	Morbihan	408000	
Centre-Val de Loire	Indre	235378	Centre - Val de Loire: Regional booklet (PDF: 2.5 MB) - 22/11/2016
	Cher	174696	
	Indre-et-Loire	84351	
	Loir-et-Cher	53370	
	Eure-et-Loir	40678	
	Loiret	41397	
Corse	Corse	66550	Corse: Regional booklet (PDF: 1.1 MB) - 15/12/2016
Grand Est	Ardennes	265800	Alsace-Champagne-Ardenne-

[31] Except for Corse, Basse-Normandie and Haute-Normandie for which the population by *département* was not available.
[32] Without distinguishing between age, sex or type of production.
[33] Agricultural booklet for each region available from the site: http://www.agreste.agriculture.gouv.fr/en-region/, accessed on 19/01/2017.

Region	*Département*[31]	Total number of cattle[32]	Source[33]
	Vosges	254200	Lorraine: Regional booklet (PDF: 1.2 MB) - 29/10/2015
	Moselle	260000	
	Meurthe et Moselle	196100	
	Meuse	229400	
	Haute Marne	206800	
	Bas-Rhin	112100	
	Haut-Rhin	59400	
	Aube	55000	
	Marne	68900	
Hauts de France	Nord	331700	Nord-Pas-de-Calais-Picardie: Regional booklet (PDF: 4.3 MB) - 06/12/2016
	Pas de Calais	379000	
	Somme	210100	
	Aisne	205000	
	Oise	114600	
Ile de France	Yvelines	7105	Ile-de-France: Regional booklet (PDF: 3.9 MB) - 12/01/2017
	Seine-et-Marne	17182	
	Val d'Oise	3295	
	Essonne	740	
Normandie	Basse Normandie	1620590	Normandie: Regional booklet (PDF: 4.4 MB) - 17/09/2015
	Haute Normandie	601378	
Nouvelle Aquitaine	Creuse	445991	Aquitaine-Limousin-Poitou-Charentes: Regional booklet (PDF: 4.1 MB) - 12/12/2016
	Haute-Vienne	366891	
	Corrèze	304967	
	Deux-Sèvres	368745	
	Pyrénées Atlantiques	275941	
	Dordogne	238539	
	Charente	147038	
	Vienne	149634	
	Lot et Garonne	73157	
	Charente-Maritime	100478	
	Landes	54054	
	Gironde	45652	
Occitanie	Aveyron	485300	Languedoc-Roussillon-Midi-Pyrénées: Regional booklet (PDF: 1.9 MB) - 17/08/2016
	Lozère	153300	
	Tarn	155300	
	Lot	136800	
	Hautes-Pyrénées	107300	
	Tarn et Garonne	63900	
	Ariège	79300	
	Haute-Garonne	99800	
	Gers	94600	

Region	*Département*[31]	Total number of cattle[32]	Source[33]
	Aude	25500	
	Pyrénées Orientales	15200	
	Gard	16000	
	Hérault	12800	
Pays de la Loire	Mayenne	627000	Pays de la Loire: Regional booklet (PDF: 1.7 MB) - 28/11/2016
	Vendée	599000	
	Maine-et-Loire	509000	
	Loire Atlantique	490000	
	Sarthe	322000	
Provence-Alpes-Côte d'Azur	Hautes Alpes	32927	Provence-Alpes-Côte d'Azur: Regional booklet (PDF: 2.4 MB) - 09/01/2017
	Bouches du Rhône	19181	
	Alpes de Hautes Provence	13942	
	Alpes Maritimes	2022	
	Vaucluse	811	
	Var	1040	

Table 30: List of the ten *départements* with the lowest and highest densities of cattle to be vaccinated (95% percentile). The values represent the number for a period of 7 weeks between the occurrence of the first outbreak and completion of vaccination.

Dep. with low density of cattle	No. doses		Dep. with high density of cattle	No. doses
Var	3,631		Allier	584,819
Vaucluse	4,649		Maine-et-Loire	591,448
Alpes Maritimes	6,245		Creuse	594,147
Essonne	7,789		Loire Atlantique	598,126
Alpes de Hautes Provence	16,751		Côtes d'Armor	633,949
Val d'Oise	21,392		Basse Normandie	642,391
Seine-et-Marne	23,357		Cantal	649,342
Yvelines	24,381		Vendée	720,312
Bouches du Rhône	28,798		Ille-et-Vilaine	781,761
Hérault	39,007		Mayenne	945,456

If the risk of introduction is considered to be directly proportional to the area of the *départements* and not to the number of cattle in the *département*, the needs in vaccine doses sufficient in 50% of simulations are close to half of those calculated for a need proportional to the number of cattle. However, the sufficient values for the population to be vaccinated in 95% of simulations are fairly similar (Table 31):

Table 31: Table showing the number of cattle to be vaccinated as a function of time of viral dissemination, regardless of the density of cattle in the *départements*

Weeks of dissemination	50%	75%	90%	95%
5	116,434	224,297	319,492	373,072
6	168,155	322,989	455,195	537,224
7	230,230	437,290	619,571	731,221
8	302,132	568,845	809,235	952,992

If the distances, i.e., the radius around the first outbreak, are taken into account, the number of cattle to be vaccinated is indicated in Table 32.

Table 32: Number of doses as a function of the radius of the zone to be vaccinated (in km)

Radius to be vaccinated (km)	50%	75%	90%	95%
30	157,086	215,832	260,510	277,944
40	265,302	364,517	439,972	469,416
50	431,037	599,534	723,638	767,660
60	623,760	862,544	1,055,934	1,103,706
70	833,509	1,162,303	1,416,349	1,460,364
80	1,084,882	1,507,620	1,836,178	1,894,530

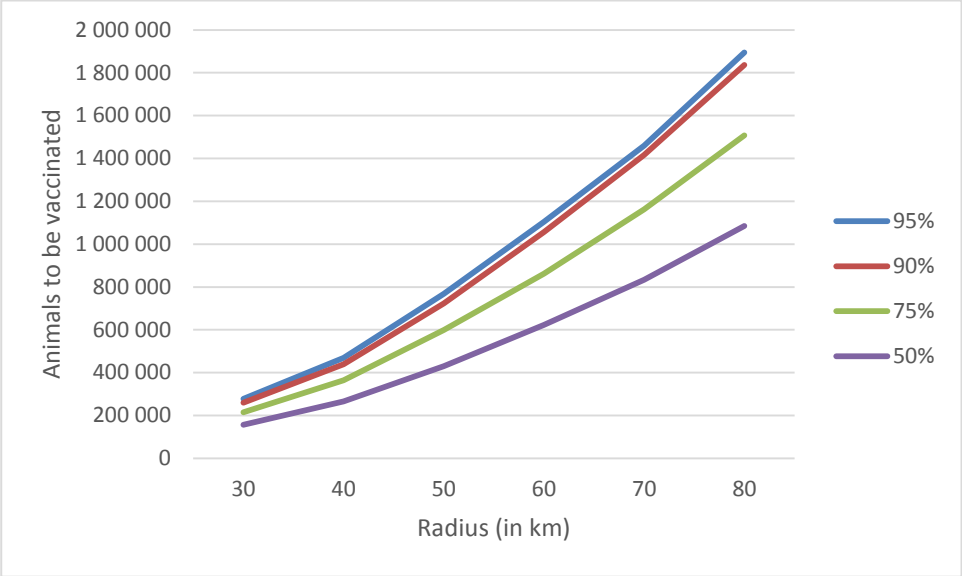


Figure 8: Number of doses as a function of the radius of the zone to be vaccinated (in km)

Notes

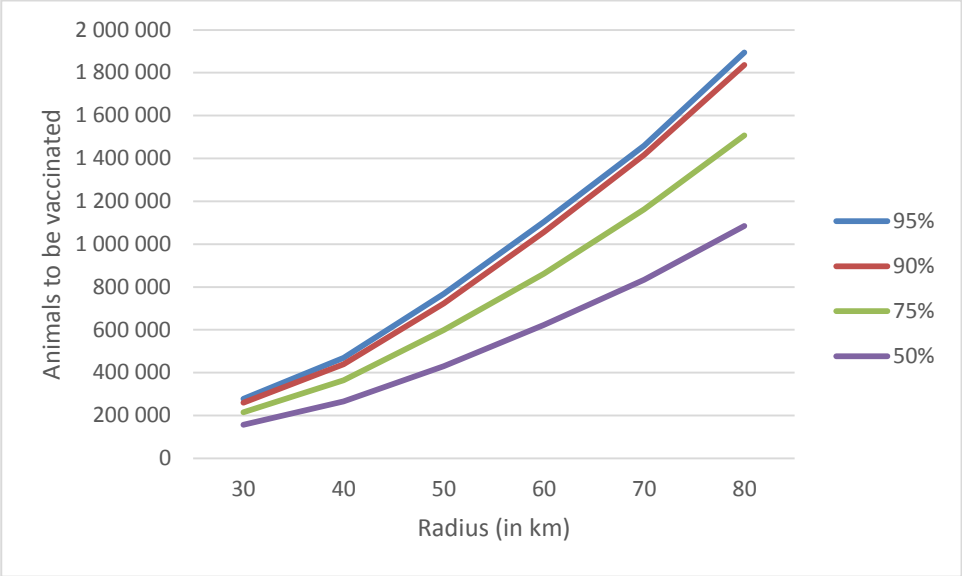


Figure 8: Number of doses as a function of the radius of the zone to be vaccinated (in km)

Notes

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