SciCom

SCIENTIFIC COMMITTEE
of the Belgian Federal Agency for the Safety of the Food Chain

OPINION 07-2016

Subject:

Control of lentivirus infection in sheep and goat flocks

(SciCom 2015/13)

Scientific opinion approved by the Scientific Committee on 22nd April 2016

Key terms:
lentivirus, sheep, goats, small ruminants, Maedi-Visna, Caprine Arthritis-Encephalitis

Sleutelwoorden:
lentivirus, schapen, geiten, kleine herkauwers, Maedi-Visna, caprine arthritis en encefalitis

Mots-clés:
lentivirus, moutons, caprins, petits ruminants, Maedi-Visna, arthrite-encéphalite virale caprine

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Executive summary

Control of lentivirus infection in sheep and goat flocks

Background & Terms of reference

Maedi-Visna (MV) and Caprine Arthritis-Encephalitis (CAE) are persistent lentivirus infections of respectively sheep and goats which are grouped together as the small ruminant lentiviruses (SRLVs). These lentiviruses include diverse genotypes that frequently cross the species barrier between sheep and goats and that display a great genetic variability. Most lentivirus infected sheep and goats do not exhibit clinical disease but remain persistently infected and are able to transmit the virus. Symptoms of the disease have an insidious onset and a slow progression. Small ruminant lentiviruses induce a systemic infection in sheep and goats that may affect different target organs, such as lungs, central nervous system, mammary gland and joints. The respiratory and neurologic syndromes lead to a cachectic stage and death, either by impairment of the respiratory function or by a general alteration of the nervous system. Due to the long incubation time lentiviruses can be widely spread in a flock or region before clinical cases are observed.

The control of Maedi-Visna in sheep and Caprine Arthritis-Encephalitis in goats in Belgium is based on a voluntary program mainly consisting of certification of SRLV negative flocks identified by serological testing. The true seroprevalence of lentivirus infection in sheep and goat flocks in Belgium is actually unknown.

In order to prepare a modification and a simplification of the existing legislation in regard with the control of Maedi-Visna in sheep and Caprine Arthritis-Encephalitis in goats the following questions are asked to the Scientific Committee:

1. Is it possible to use the general term ‘control of lentivirus infections in sheep and goats’ in the modified legislation instead of using separate terms for ‘the control of Maedi-Visna infection in sheep’ and ‘the control of viral Caprine Arthritis-Encephalitis infection in goats’ as is used in the actual legislation?

2. Does it make sense to distinguish between sheep and goats in the health certification for lentivirus infection? How to deal with mixed flocks?

3. Is it necessary to provide definitions for Maedi-Visna and Caprine Arthritis-Encephalitis in the new legislation?

4. The certification of flocks ‘free from MV-CAE’ is based on serological testing (first step). Is the humoral response of sheep and goats influenced by the parturition period (15 days before and after lambing)? In other words is there an increased chance of false results by applying an ELISA during that period?

5. Actually the minimum age for serological testing is 1 year. At that age positive animals are seldomly detected. What is the risk for false seronegative flock testing if the minimum age for testing is set at 2 years of age?

6. Once a flock has met the certification conditions for a ‘free from MV-CAE’ status the certificate can be prolonged twice with one year. Subsequent serological testing with 2 years interval is necessary. What is the risk for missing seropositive animals if this interval is prolonged?

7. The serological testing for prolongation of the flock's certificate 'free from MV-CAE' must be executed on 50 % of the animals in a flock with a minimum of 50 animals. What is the risk for missing seropositive animals if less than 50 % of the animals are tested?

8. What is the risk of missing seropositive animals if no serological testing is done at the start-up of new flocks with only animals from one or more flocks with a free status?

9. Is the infection transmissible between sheep and goats? Is one certificate (for one species) sufficient if both species are present on the same flock?

10. Has the Scientific Committee any remarks with regard to the diagnostic decision tree?

11. In case of a positive ELISA, is it possible to reduce the number of control tests (ELISA + immune-diffusion test + PCR) to confirm or refute the infection?

Methodology

This opinion is based on evidence from scientific literature, expert opinion and on the results of the Maedi-Visna control program in Belgium, obtained from CODA-CERVA.

Answers to the questions

Studies have confirmed that CAEV and VMV, originally established as specific pathogens in goats and sheep respectively, often cross the species barrier infecting the new host, persisting in it and spreading across the new host population. According to the Scientific Committee there is substantial evidence in scientific literature to consider that Maedi-Visna virus in sheep and Caprine Arthritis-Encephalitis virus of goats belong to the same group of small ruminant lentiviruses.

The Scientific Committee recommends using the term 'small ruminant lentivirus infections' as common name in the new legislation, instead of making a differentiation between Visna Maedi virus in sheep and Caprine Arthritis-Encephalitis virus in goats.

The Scientific Committee recommends not to make a distinction between sheep and goats in the new legislation and in the health certification as both species can be infected by the same lentiviruses. In case of mixed flocks it is recommended to test both species before declaring the flock 'lentivirus free' as prevalence, historical background and sensitivity to infection may be different between sheep and goat flocks.

Serological diagnostic methods are considered to be most convenient to detect small ruminant lentivirus infections. However, the natural immune response is slow and variable, antibody concentrations can fluctuate over time and some animals do not develop an antibody response after infection. This, together with antigenic differences between circulating strains and viral proteins used in the diagnostic tests, makes that serological tests do not always correctly determine the infection status of sheep and goats. Furthermore, the Scientific Committee is of the opinion that there is no scientific evidence related to lentivirus infection in sheep and goats showing that the result of serological testing is influenced during the peri-parturient period.

Accurate diagnosis of small ruminant lentivirus infection is of major importance in terms of the results of control programs. Most animals become infected early in life, after drinking infected colostrum or milk. The virus can also spread during close contact, probably by the respiratory route.

The incubation period for Maedi is usually more than two years; clinical signs typically develop when animals are three to four years old. The incubation period for Visna is somewhat shorter and symptoms can appear in sheep as young as two years. Seroconversion on the other hand generally occurs a few months after infection.

Actually the minimum age for serological testing is 1 year. The Scientific Committee recommends not to postpone the age of testing to 2 years because it will increase the probability of missing lentivirus spread in sheep and goat flocks.

Data from the Belgian certification program between 2010 and 2014 show that 6,4 % of the free sheep flocks and 8,3 % of the free goat flocks lose their free status after serological control at 24 months. This suggests that within a 24 month period, the disease can spread to other animals and potentially to other flocks. Prolonging the interval of testing will increase the probability that more animals and other flocks become exposed to the virus via contact or via purchase of latently infected carrier animals.

Actually the serological testing for the prolongation of the 'free from MV-CAE' flock certificate must be executed on 50 % of the animals in a flock with minimum of 50 animals. Applying decreasing sampling rates will diminish the probability of detecting seropositive animals. The Scientific Committee recommends to apply a risk-based sampling plan within the flock based on the actual sample sizes. Animals at risk are newly introduced animals, animals fed with untreated colostrum of unknown origin and animals with clinical symptoms such as unthriftiness, inappetence, lameness or chronic mastitis.

Despite the fact that animals are bought from a farm with a free status, the risk exists that SRLV infected animals are present from the start-up. The Scientific Committee is of the opinion that newly populated flocks should be able to get a similar free status of the herds of origin provided that they run the complete control program from the start. This control program is based on successive serological controls as described in the royal decree of 24th March 1993 (sheep) and in the royal decree of 27th November 1997 (goats).

Cross-species transmission of lentiviruses between sheep and goats has been shown to occur. The Scientific Committee is of the opinion that in mixed flocks (sheep and goats) the status of the flock should depend on the status of both flocks meaning that both species should be sampled to obtain or maintain an officially free status.

The certification of flocks is based on a logical sequential sampling and testing scheme described in the diagnostic decision tree. The addition of PCR analysis has an added value for determining the status of the animal. Hence it is recommended that the diagnostic decision tree is based on multiple/sequential testing including PCR.

Conclusion

Small ruminant lentiviruses are part of a very heterogeneous group of lentiviruses which can give rise to various slow-evolving syndromes depending on the species, the virulence of the viral strain and the production conditions. In order to control these infections, it is first necessary to correctly identify infected animals and herds by means of periodically executed reliable tests. The success of a voluntary control program will depend on the initial seroprevalence in the herd and in the Belgian sheep and goat population, on the strict implementation of individual flock biosecurity measures and on the cost/benefit balance of the control program for individual holders.

Samenvatting

Controle van lentivirus infecties op schapen- en geitenbeslagen

Achtergrond & referentietermen

Maedi-Visna (MV) en arthritis-encefalitis (CAE) zijn hardnekkige lentivirus infecties bij respectievelijk schapen en geiten, die samen worden gegroepeerd als de lentivirus infecties van kleine herkauwers (small ruminant lentiviruses, SRLVs). Deze lentivirussen bestaan uit diverse genotypes die frequent de species barrière overschrijden tussen schapen en geiten en die vaak een grote genetische variabiliteit vertonen. De meest schapen en geiten die geïnfecteerd zijn met lentivirussen, vertonen geen klinische ziekte maar blijven persistent geïnfecteerd en kunnen het virus overbrengen. Symptomen van de ziekte hebben een sluipend begin en een langzame progressie. Lentivirus infecties bij kleine herkauwers induceren een systemische infectie die verschillende doelorganen zoals longen, centraal zenuwstelsel, uier en gewrichten kunnen treffen. De ademhalings- en neurologische syndromen leiden tot cachexie en dood, hetzij door aantasting van de ademhalingsfunctie of van het zenuwstelsel. Door de lange incubatietijd kunnen lentivirussen op grote schaal worden verspreid in een bedrijf of in een regio voordat klinische gevallen worden vastgesteld.

De bestrijding van Maedi-Visna bij schapen en arthritis-encefalitis bij geiten is in België gebaseerd op een vrijwillig controleprogramma dat hoofdzakelijk bestaat uit certificering van SRLV negatieve bedrijven die serologisch geïdentificeerd worden. De seroprevalentie van lentivirus infectie bij schapen en geiten in België is niet gekend.

Met het oog op een wijziging en een vereenvoudiging van de bestaande wetgeving in verband met de controle van Maedi-Visna bij schapen en arthritis-encefalitis bij geiten worden de volgende vragen gesteld aan het Wetenschappelijk Comité:

1. Is het mogelijk om de algemene term 'de controle van lentivirus infecties bij schapen en geiten' te gebruiken in de nieuwe wetgeving in plaats van de afzonderlijke termen 'de controle van Maedi-Visna infectie bij schapen' en 'de controle van virale arthritis-encefalitis bij geiten" zoals in de huidige wetgeving?

2. Heeft het zin om een onderscheid te maken tussen schapen en geiten in de gezondheidscertificering voor lentivirus infecties? Hoe moet deze certificering worden toegepast op gemengde bedrijven?

3. Is het nodig om definities te voorzien in de nieuwe wetgeving voor Maedi-Visna en arthritis-encefalitis bij geiten?

4. De certificering van beslagen 'vrij van MV-CAE' is gebaseerd op serologische testen (eerste stap). Wordt de humorale reactie van schapen en geiten beïnvloed door de partusperiode (15 dagen vóór en na het lammeren)? Met andere woorden is er een verhoogde kans op valse resultaten bij toepassing van de ELISA tijdens deze periode?

5. Op dit ogenblik bedraagt de minimum leeftijd voor serologisch onderzoek 1 jaar. Op die leeftijd worden zelden positieve dieren gedetecteerd. Wat is het risico voor vals-negatieve serologische bedrijfstesten als de minimumleeftijd voor het testen wordt vastgelegd op 2 jaar?

6. Van zodra een bedrijf beantwoordt aan de voorwaarden voor een 'vrij van MV-CAE' status kan het certificaat twee maal worden verlengd met één jaar. Daaropvolgende serologische testen met

2 jaar interval zijn noodzakelijk. Wat is het risico voor het missen van seropositieve dieren als dit interval wordt verlengd?

7. De serologische testen voor verlenging van het certificaat 'vrij van MV-CAE "moet worden uitgevoerd op 50 % van de dieren in een bedrijf met een minimum van 50 dieren. Wat is het risico voor het missen van seropositieve dieren indien minder dan 50 % van de dieren wordt getest?

8. Wat is het risico van het missen van seropositieve dieren als er geen serologische test wordt uitgevoerd bij het opstarten van nieuwe beslagen met enkel dieren afkomstig uit één of meerdere beslagen met een vrije status?

9. Is de infectie overdraagbaar tussen schapen en geiten? Volstaat één gezondheidscertificaat (voor één diersoort) als de beide diersoorten zich samen op hetzelfde bedrijf bevinden?

10. Heeft het Wetenschappelijk Comité opmerkingen met betrekking tot de diagnostische beslissingsboom?

11. Is het mogelijk om bij een positieve ELISA het aantal controles te verminderen (ELISA + immunodiffusietest + PCR) om de infectie te bevestigen of te weerleggen?

Methodologie

Dit advies is gebaseerd op gegevens uit de wetenschappelijke literatuur, expert opinie en op de resultaten van het Maedi-Visna controleprogramma in België, bekomen van het CODA.

Antwoorden op de gestelde vragen

Studies hebben bevestigd dat CAEV en MV, die oorspronkelijk beschouwd werden als soortspecifieke ziekteverwekkers bij respectievelijk geiten en schapen vaak de soortbarrière doorbreken en de nieuwe gastheer infecteren, erin vermeerderen en de infectie verspreiden. Volgens het Wetenschappelijk Comité is er substantieel bewijs in de wetenschappelijke literatuur voorhanden om aan te nemen dat het Maedi-Visna virus bij schapen en het arthritis-encefalitis virus bij geiten tot dezelfde groep behoren van de lentivirussen van kleine herkauwers.

Het Wetenschappelijk Comité raadt aan om de term 'lentivirus infecties van kleine herkauwers' in de nieuwe wetgeving te gebruiken, in plaats van een onderscheid te maken tussen Visna Maedi bij schapen en arthritis-encefalitis bij geiten.

Het Wetenschappelijk Comité pleit ervoor om geen onderscheid te maken tussen schapen en geiten in de nieuwe wetgeving en in de gezondheidscertificering aangezien beide diersoorten kunnen worden geïnfecteerd door dezelfde lentivirussen. In het geval van gemengde bedrijven is het raadzaam om beide diersoorten te testen vooraleer het bedrijf "lentivirus vrij' kan beschouwd worden daar de prevalentie, de historiek en de gevoeligheid voor infecties kunnen verschillen tussen beide diersoorten in hetzelfde beslag.

Serologische diagnostische methoden worden als het meest geschikt beschouwd om lentivirus infecties bij kleine herkauwers op te sporen. Echter, de natuurlijke immuunrespons is traag en variabel, antilichaamconcentraties kunnen fluctueren in de tijd en sommige dieren ontwikkelen geen antilichaamrespons na infectie. Deze vaststellingen samen met de antigene verschillen tussen circulerende stammen en de virale eiwitten die in de diagnostische testen worden gebruikt, vormen

een verklaring waarom serologische testen niet altijd even adequaat de besmettingsstatus van schapen en geiten bepalen. Het Wetenschappelijk Comité is daarentegen van mening dat er geen wetenschappelijk bewijs is in verband met lentivirusinfecties bij schapen en geiten dat aantoont dat het resultaat van het serologisch onderzoek zou worden beïnvloed tijdens de peri-partum periode.

De accurate diagnose van lentivirus infectie bij kleine herkauwers is van groot belang in het kader van de doeltreffendheid van een bestrijdingsprogramma. De meeste dieren raken op jonge leeftijd besmet, door het drinken van besmette colostrum of melk. Het virus kan zich ook verspreiden langs de luchtwegen bij nauw contact tussen de dieren. De incubatietijd van Maedi is meestal meer dan twee jaar; klinische symptomen ontwikkelen gewoonlijk wanneer dieren drie tot vier jaar oud zijn. De incubatietijd van Visna is iets korter en symptomen kunnen optreden in schapen jonger dan twee jaar. Seroconversie gebeurt daarentegen meestal enkele maanden na infectie.

Op dit ogenblik bedraagt de minimum leeftijd voor serologisch onderzoek 1 jaar. Het Wetenschappelijk Comité beveelt aan de testleeftijd niet uit te stellen tot de leeftijd van 2 jaar omdat dit het risico zal verhogen voor het missen van de verspreiding van lentivirussen in beslagen van schapen en geiten.

Uit het Belgische certificeringsprogramma blijkt dat tussen 2010 en 2014 6,4 % van de ‘vrije’ schapenhouderijen en 8,3 % van de ‘vrije’ geitenhouderijen hun status hebben verloren na serologische controle op 24 maanden. Dit suggereert dat binnen een periode van 24 maanden, de ziekte zich kan verspreiden naar andere dieren en mogelijks naar andere bedrijven. Volgens het Wetenschappelijk Comité zal de verlenging van het interval tussen testen de kans verhogen dat er meer dieren en andere bedrijven worden blootgesteld aan het virus via contact of via de aankoop van latent besmette dieren.

Op dit ogenblik moeten de serologische tests voor de verlenging van de 'vrij van MV-CAE' status van een bedrijf worden uitgevoerd op 50 % van de dieren met een minimum van 50 dieren. Het verminderen van het bemonsteringspercentage zal de kans op het opsporen van seropositieve dieren verminderen. Het Wetenschappelijk Comité raadt aan om een risico gebaseerd bemonsteringsplan toe te passen op de beslagen gebaseerd op de huidige steekproefgrootte. Risicodieren zijn dieren die nieuw geïntroduceerd werden in het beslag, dieren die gevoederd werden met onbehandelde colostrum van onbekende oorsprong en dieren met klinische symptomen zoals lusteloosheid, afwezigheid van eetlust, manken of met chronische mastitis.

Ondanks het feit dat dieren worden aangekocht van een boerderij met een vrije status, bestaat het risico dat er SRLV besmette dieren aanwezig zijn vanaf de opstart van nieuwe beslagen. Het Wetenschappelijk Comité is van mening dat pas bevolkte beslagen in staat moeten zijn om een soortgelijke vrije status als van het beslag van oorsprong te verkrijgen, mits zij het volledige controleprogramma volgen vanaf de opstart. Dit controleprogramma is gebaseerd op de resultaten van opeenvolgende serologische controles zoals beschreven in het koninklijk besluit van 24 maart 1993 (schapen) en het koninklijk besluit van 27 november 1997 (geiten).

Het is aangetoond dat overdracht van lentivirussen tussen schapen en geiten voorkomt. Het Wetenschappelijk Comité is van mening dat in de gemengde beslagen (schapen en geiten) de status van het beslag afhankelijk moet zijn van de status van beide kuddes, hetgeen betekent dat beide diersoorten moeten worden bemonsterd om een officieel vrije status te bekomen of te behouden.

De certificering van beslagen is gebaseerd op een logische sequentiële bemonstering en testplan beschreven in de diagnostische beslissingsboom. De toevoeging van PCR-analyse betekent een toegevoegde waarde voor het bepalen van de status van het dier. Daarom is het aan te raden dat de diagnostische beslissingsboom is gebaseerd op meerdere / sequentiële testen, inclusief PCR.

Conclusie

Lentivirussen van kleine herkauwers maken deel uit van een zeer heterogene groep van lentivirussen die aanleiding kunnen geven tot verschillende traag evoluerende ziektebeelden afhankelijk van de diersoort, de virulentie van de stam en de productie omstandigheden. Om deze infecties onder controle te houden is het eerst nodig om geïnfecteerde beslagen en dieren correct te identificeren door middel van periodiek uitgevoerde betrouwbare testen. Het succes van een vrijwillig controleprogramma zal afhangen van de aanvankelijke seroprevalentie in het beslag en in de Belgische schapen en geitenpopulatie, van de strikte uitvoering van de bioveiligheidsmaatregelen in de individuele beslagen en van de kosten / batenverhouding van het controleprogramma voor individuele schapen- en geitenhouders.

Résumé

Contrôle de l'infection à lentivirus dans les troupeaux ovins et caprins

Contexte & termes de référence

Le Maedi-Visna (MV) et l'arthrite-encéphalite caprine (CAE) sont des infections à lentivirus persistantes chez, respectivement, les ovins et les caprins, et qui sont, dès lors, regroupées comme les infections de lentivirus des petits ruminants (small ruminant lentiviruses, SRLVs). Les lentivirus comprennent divers génotypes qui franchissent régulièrement la barrière des espèces entre les moutons et les chèvres et qui présentent souvent une grande variabilité génétique. La plupart des moutons et des chèvres infectés par les lentivirus ne présentent pas de maladie clinique, mais restent infectés de façon persistante et peuvent transmettre le virus. Les premiers symptômes de la maladie sont insidieux et ont une progression lente. Les infections par les lentivirus des petits ruminants provoquent une infection systémique qui peut affecter différents organes cibles tels que les poumons, le système nerveux central, les mamelles et les articulations. Les syndromes respiratoires et neurologiques conduisent à la cachexie et la mort, soit en affectant la fonction respiratoire soit le système nerveux. En raison de leur longue période d'incubation, les lentivirus peuvent largement se disséminer dans une exploitation ou une région avant que les cas cliniques ne se manifestent.

La lutte contre le Maedi-Visna chez les ovins et l'arthrite-encéphalite chez les caprins est en Belgique basée sur le contrôle volontaire, qui se compose principalement de la certification des exploitations SRLV négatives qui sont identifiées sérologiquement. La séroprévalence de l'infection à lentivirus chez les ovins et les caprins en Belgique n'est pas connue.

Afin de modifier et de simplifier la législation en vigueur concernant le contrôle du Maedi-Visna chez les ovins et l'arthrite-encéphalite chez les caprins, les questions suivantes sont posées au Comité scientifique:

1. Est-il possible d'utiliser un terme générique 'le contrôle des lentivirus chez les ovins et les caprins' dans la nouvelle législation au lieu des termes individuels "contrôle de l'infection de Maedi-Visna du mouton» et «contrôle de l'arthrite-encéphalite caprine » comme dans la loi actuellement?

2. Est-il judicieux de faire la distinction entre les moutons et les chèvres dans la certification sanitaire pour les infections à lentivirus? Comment cette certification devrait-elle être appliquée dans les exploitations mixtes?

3. Est-il nécessaire de fournir des définitions dans la nouvelle législation pour le Maedi-Visna et l'arthrite-encéphalite caprine?

4. L'attestation des troupeaux 'indemnes de MV CAE' est basé sur des tests sérologiques (première étape). Est-ce que la réponse humorale des ovins et caprins est influencée par la période d'agnelage (15 jours avant et après l'agnelage)? En d'autres termes, y-a-t'il un risque accru de résultats faux dans l'interprétation des ELISA, au cours de cette période?

5. À l'heure actuelle, l'âge minimum pour le test sérologique est d'un an. A cet âge, on détecte rarement des animaux positifs. Quel est le risque de tests sérologiques faussement négatifs si l'âge minimal pour effectuer le test est fixé à 2 ans?

6. Une fois qu'une exploitation remplit les conditions pour le statut 'indemne de MV CAE',

l'attestation peut être prolongée d'un an, à deux reprises. Des tests sérologiques ultérieurs avec des intervalles de 2 ans sont nécessaires. Quel est le risque de rater des animaux séropositifs si cet intervalle est prolongé?

7. Les tests sérologiques pour le renouvellement de l'attestation "indemne de MV CAE" doivent être effectuées sur 50 % des animaux dans une exploitation avec un minimum de 50 tests. Quel est le risque de ne pas détecter des animaux séropositifs si moins de 50 % des animaux sont testés?

8. Quel est le risque de ne pas détecter des animaux séropositifs si aucun test sérologique n'est réalisé à partir de nouveaux troupeaux constitués par des animaux d'une ou plusieurs troupeaux avec un statut indemne?

9. Est-ce que l'infection se transmet entre les moutons et les chèvres? Est-ce qu'un seul certificat (pour une espèce) suffit si les deux espèces sont ensemble dans la même exploitation?

10. Est-ce que le Comité scientifique a des commentaires sur l'arbre de décision du diagnostic?

11. Est-il possible de réduire le nombre de contrôles dans le cas d'un ELISA positif (ELISA + test d'immunodiffusion + test PCR) pour confirmer ou infirmer l'infection?

Méthodologie

Cette opinion est fondée sur les données de la littérature scientifique, des conseils d'experts et sur les résultats du programme de contrôle Maedi-Visna en Belgique, fournis par le CERVA.

Les réponses aux questions

Des études ont confirmé que la CAE et le MV, qui ont été initialement considérées comme des pathogènes spécifiques affectant, respectivement, les chèvres et les moutons, peuvent traverser la barrière d'espèce et infecter un hôte nouveau, s'y multiplier et propager l'infection. Selon le Comité scientifique, il existe des preuves substantielles dans la littérature scientifique disponible pour affirmer que le virus Maedi-Visna chez le mouton et le virus de l'arthrite-encéphalite chez la chèvre appartiennent au même groupe des lentivirus des petits ruminants.

Le Comité scientifique recommande dès lors d'utiliser le terme «infections par les lentivirus chez les petits ruminants" dans la nouvelle législation, plutôt que de faire la distinction entre Maedi-Visna chez les ovins et l'arthrite-encéphalite chez les chèvres.

De même, le Comité scientifique recommande de ne pas faire de discrimination entre les moutons et les chèvres dans la nouvelle législation et dans la certification sanitaire comme les deux espèces peuvent être infectées par les mêmes lentivirus. Dans le cas des exploitations mixtes, il est conseillé de tester les deux espèces avant que l'exploitation ne puisse être considérée comme «indemne de lentivirus » comme la prévalence, l'historique et la susceptibilité à l'infection peut différer entre les deux espèces dans le même troupeau.

Les méthodes de diagnostic sérologique sont considérées comme les plus appropriées pour déceler les infections à lentivirus chez les petits ruminants. Cependant, la réponse immunitaire naturelle est lente et variable, et les concentrations d'anticorps peuvent fluctuer au fil du temps. Certains individus ne développeront pas de réponse humorale après l'infection. Ces constatations ainsi que

les différences antigéniques existant entre les souches circulantes et les protéines virales utilisées dans des tests de diagnostic, peuvent expliquer pourquoi les tests sérologiques ne peuvent pas toujours déterminer adéquatement le statut d'infection des ovins et caprins. Par contre, le Comité scientifique estime qu'il n'y a aucune preuve scientifique en relation avec les infections par des lentivirus chez les moutons et les chèvres montrant que le résultat des tests sérologiques soit influencé pendant la période péri-partum.

Le diagnostic précis de l'infection par les lentivirus des petits ruminants est d'une grande importance pour garantir l'efficacité d'un programme de contrôle. La plupart des animaux sont infectés à un jeune âge via le colostrum ou le lait contaminé. Le virus peut aussi se propager par les voies respiratoires via le contact étroit entre les animaux. La période d'incubation de Maedi est généralement de plus de deux ans. Les symptômes cliniques se développent généralement lorsque les animaux sont âgés de trois à quatre ans. La période d'incubation de Visna est généralement plus courte et les symptômes peuvent déjà se manifester chez les moutons de moins de deux ans. Par contre, la séroconversion se produit généralement plusieurs mois après l'infection.

À l'heure actuelle, l'âge minimum pour effectuer le test sérologique est d'un an. Le Comité scientifique recommande de ne pas reporter l'âge du test sérologique à 2 ans car cela va augmenter la probabilité de manquer la propagation des lentivirus dans des troupeaux de moutons et de chèvres.

Le programme de certification belge montre qu'entre 2010 et 2014, 6,4 % des élevages de moutons «indemnes» et 8,3 % des élevages de chèvres «indemnes» ont perdu leur statut après un contrôle sérologique effectué à 24 mois. Cela suggère que, durant une période de 24 mois, la maladie peut se propager à d'autres animaux et éventuellement à d'autres exploitations. Selon le Comité scientifique l'augmentation de l'intervalle entre les tests va augmenter la probabilité que plus d'animaux et que d'autres exploitations soient exposés au virus par contact ou par l'achat d'animaux porteurs d'une infection latente.

Pour l'instant les tests sérologiques pour prolonger le statut 'indemne de MV-CAE' sont réalisées sur 50 % des animaux d'une exploitation, avec un minimum de 50 animaux. La réduction du taux d'échantillonnage diminuera la probabilité de détection des animaux séropositifs. Le Comité scientifique recommande d'appliquer un plan d'échantillonnage basé sur le risque et sur la taille d'échantillonnage actuel. Les animaux à risque sont des animaux nouvellement introduits dans le troupeau, qui ont été nourris avec du colostrum non traité et d'origine inconnue et des animaux avec des symptômes cliniques tels que la léthargie, le manque d'appétit, la boiterie ou des mammites chroniques.

Malgré le fait que les animaux soient achetés dans une ferme avec un statut indemne, le risque est qu'il y ait des animaux infectés par le SRLV dès le début des nouveaux troupeaux. Le Comité scientifique estime que les exploitations nouvellement repeuplées devraient être en mesure d'obtenir un statut 'indemne' similaire à celui du troupeau d'origine, à condition qu'ils aient suivi le programme de contrôle complet depuis le début. Ce programme de contrôle est basé sur les résultats de contrôles sérologiques successifs comme stipulé dans l'arrêté royal du 24 mars 1993 (moutons) et dans l'arrêté royal du 27 novembre 1997 (chèvres).

Il a été démontré que la transmission des lentivirus entre les moutons et les chèvres peut se produire. Le Comité scientifique est d'avis que dans les exploitations mixtes (ovins et caprins), le statut de l'exploitation devrait dépendre du statut de chacun des deux troupeaux individuels, ce qui signifie que les deux espèces devraient être échantillonnées pour obtenir un statut officiellement indemne ou pour le maintenir.

La certification des troupeaux est basée sur un plan d'échantillonnage et de testage séquentiel logique décrit dans l'arbre de décision utilisé pour le diagnostic. L'addition d'une analyse par PCR représente une valeur ajoutée pour la détermination du statut de l'animal. Par conséquent, il est recommandé que l'arbre de décision pour le diagnostic soit basé sur de multiples tests séquentiels, y compris le PCR.

Conclusion

Les lentivirus des petits ruminants font partie d'un groupe très hétérogène de lentivirus qui peut donner lieu à divers syndromes à évolution lente selon les espèces, la virulence de la souche virale et les conditions d'élevage. Afin de contrôler ces infections, il est d'abord nécessaire d'identifier correctement les animaux et les troupeaux infectés au moyen de tests fiables effectués périodiquement. Le succès d'un programme de contrôle volontaire dépendra de la séroprévalence initiale dans le troupeau et dans la population ovine et caprine belge, de la stricte application des mesures de biosécurité dans les troupeaux individuels et du rapport coût / bénéfice du programme de contrôle pour les éleveurs ovins et caprins.

1. Terms of reference

1.1. Questions

In order to prepare a modification of the existing legislation in regard with the control of Maedi-Visna (MV) in sheep and Arthritis-Encephalitis (CAE) in goats and a simplification of the procedure for certification of flocks 'free from Maedi-Visna / Caprine Arthritis-Encephalitis' the following questions are asked on behalf of the Federal Public Service of Public Health, Food Safety and Environment:

1. Is it possible to use the general term 'control of lentivirus infections in sheep and goats' in the modified legislation instead of using separate terms for 'the control of Maedi-Visna infection in sheep' and 'the control of viral Caprine Arthritis-Encephalitis infection in goats' as is used in the actual legislation?

2. Does it make sense to distinguish between sheep and goats in the health certification for lentivirus infection? How to deal with mixed flocks?

3. Is it necessary to provide definitions for Maedi-Visna and Caprine Arthritis-Encephalitis in the new legislation?

4. The certification of flocks 'free from MV-CAE' is based on serological testing (first step). Is the humoral response of sheep and goats influenced by the parturition period (15 days before and after lambing)? In other words is there an increased chance of false results by applying an ELISA during that period?

5. Actually the minimum age for serological testing is 1 year. At that age positive animals are seldomly detected. What is the risk for false seronegative flock testing if the minimum age for testing is set at 2 years of age?

6. Once a flock has met the certification conditions for a 'free from MV-CAE' status the certificate can be prolonged twice with one year. Subsequent serological testing with 2 years interval is necessary. What is the risk for missing seropositive animals if this interval is prolonged?

7. The serological testing for prolongation of the flock's certificate 'free from MV-CAE' must be executed on 50 % of the animals in a flock with a minimum of 50 animals. What is the risk for missing seropositive animals if less than 50 % of the animals are tested?

8. What is the risk of missing seropositive animals if no serological testing is done at the start-up of new flocks with only animals from one or more flocks with a free status?

9. Is the infection transmissible between sheep and goats? Is one certificate (for one species) sufficient if both species are present on the same flock?

10. Has the Scientific Committee any remarks with regard to the diagnostic decision tree?

11. In case of a positive ELISA, is it possible to reduce the number of control tests (ELISA + immune-diffusion test + PCR) to confirm or refute the infection?

1.2. Legal provisions and decision tree

Actually the control of respectively Maedi-Visna infection in sheep and Caprine Arthritis-Encephalitis in goats is based on 2 separate royal and ministerial decrees and on 2 decision trees:

- "Koninklijk besluit van 23 maart 1993 betreffende de inrichting van de zwoegerziektebestrijding bij het schaap – Arrêté royal du 23 mars 1993 organisant la lutte contre le Maedi-Visna du mouton."
- "Koninklijk besluit van 27 november 1997 betreffende de inrichting van de bestrijding van de virale caprine arthritis encephalitis. – Arrêté royal du 27 novembre 1997 organisant la lutte contre l'arthrite encéphalite virale caprine."
- "Ministerieel besluit van 11 mei 2005 houdende organisatie van de diagnose van zwoegerziekte bij schapen. – Arrêté ministériel portant organisation du diagnostic du maedi-visna du mouton."
- "Ministerieel besluit van 11 mei 2005 houdende organisatie van de diagnose van virale caprine arthritis encefalitis. – Arrêté ministériel portant organisation du diagnostic de l'arthrite encéphalite virale caprine."
- Diagnostic decision trees for certification of sheep and goat flocks 'free from VM or CAE".

1.3. Methodology

This opinion is based on evidence from scientific literature, on expert opinion and on results of the Maedi-Visna control program in Belgium, obtained from CODA-CERVA.

2. Abbreviations

MV	Maedi-Visna
MVV	Maedi-Visna virus
CAE	Caprine Arthritis-Encephalitis
CAEV	Caprine Arthritis-Encephalitis virus
SRLV	Small Ruminant Lenti Virus
CODA-CERVA	Veterinary and Agrochemical Research Center
FASFC	Federal Agency for the Safety of the Food Chain
FPSPH	Federal Public Service of Public Health, Food Safety and Environment

Considering the discussions during the work group meeting of 19th October 2015 and during the plenary sessions of the Scientific Committee on 18th March 2016 and on 22nd April 2016,

the Scientific Committee gives the following advice:

3. Introduction and aim

3.1. Context

The control of Maedi-Visna in sheep and Caprine Arthritis-Encephalitis in goats in Belgium is based on a voluntary program mainly consisting of certification of SRLV negative flocks identified by serological testing using Agar gel immunodiffusion (AGID) and ELISA. All sheep and goat breeders are free to participate at the program. In practice only studbook breeders effectively participate at the control program. The certification of SRLV negative flocks makes a distinction between sheep and goat flocks. Since 2013 new diagnostic decision trees have been used: one for sheep and one for goats.

The small ruminant sector advocates further simplification of the procedure for health certification of sheep and goat flocks and of the conditions to preserve the free status. The sector has made a number of practical suggestions to simplify the current procedures. These suggestions were reformulated by the Federal Public Service of Public Health, Food Safety and Environment into questions to the Scientific Committee in order to prepare a modification of the existing legislation.

3.2. Aim

The aim of this opinion is to give a state-of-the art on the control of lentivirus infections in sheep and goats and to provide a scientifically based answer to the different questions.

4. Risk assessment

4.1. Actual knowledge with regard to lentivirus infections in sheep and goats

4.1.1. Hazard identification & characterization

The virus

According to the OIE (2008) Maedi-Visna (MV) and Caprine Arthritis-Encephalitis (CAE) are persistent lentivirus infections of respectively sheep and goats which are often grouped together as the small ruminant lentiviruses (SRLVs). Maedi-Visna virus was at first discovered in 1960 in sheep with a chronic disease characterized by laboured breathing ('Maedi') and wasting ('Visna'). The virus of Caprine Arthritis-Encephalitis was described later in 1974. MVV and CAEV are currently referred to as small ruminant lentiviruses, due to phylogenetic proximity and natural interspecies transmission between sheep and goats (Ramirez et al, 2013; Pisoni et al, 2005).

Lentiviruses of sheep and goats belong to the *Retroviridae* family which is subdivided into 7 genuses: Alpharetrovirus, Betaretrovirus, Deltaretrovirus, Gammaretrovirus, Epsilonretrovirus, Spumaretrovirus and Lentivirus (Leroux et al., 2010). The SRLV group comprises MVV (Maedi-Visna

Virus) initially isolated from sheep and CAEV (Caprine Arthritis-Encephalitis virus) initially isolated from goats.

MVV and CAEV used to be considered as two distinct viral entities, according to the animals species (sheep versus goats) they have been isolated from. Over the last 15 years, sequence analysis and phylogenetic reconstructions based on complete and partial sequence of SRLV clearly established that they are in fact part of a viral continuum with evidence of cross-species transmission. As other RNA viruses, SRLV are rapidly evolving genomes (Leroux et al., 2010).

According to Mungujon et al. (2015) small ruminant lentiviruses include viruses with diverse genotypes that frequently cross the species barrier between sheep and goats and that display a great genetic variability. SRLV's viral genotypes have been classified into 5 groups (A, B, C, D, E) of which group A, B and E are subdivided in several subtypes (Ramirez et al. 2013). Sheep can be a source of SRLV transmission to goats, and vice versa.

The disease

Maedi-Visna is a chronic viral disease in sheep caused by lentiviruses and clinically characterized by chronic progressive pneumonia with dyspnea and wasting. Caprine Arthritis-Encephalitis is a chronic viral disease in goats caused by lentiviruses and clinically characterized by polysynovitis-arthritis, encephalomyelitis, chronic interstitial pneumonia, chronic mastitis and weight loss.

Most lentivirus infected sheep and goats do not exhibit clinical disease but remain persistently infected and are able to transmit the virus. Symptoms of the disease have an insidious onset and a slow progression. The incubation period for Maedi is usually longer than two years; clinical signs typically develop when animals are three to four years old. The incubation period for Visna is somewhat shorter and symptoms can appear in sheep as young as two years. MV can be widely spread in a flock before clinical cases are observed. CAEV encephalitis occurs primarily in goat kids aged between 2 and 6 months (OIE, 2008). The long incubation time is a complicating risk factor for viral spread during surveillance, diagnostic and control efforts.

According to Minguijon et al (2015) small ruminant lentiviruses induce a systemic infection in sheep and goats that may affect different target organs, such as lung, central nervous system, mammary gland and joints. The clinical affection appears to depend on the tropism of the virus strain, the species affected and the genetic background of each breed or animal. In general, one of the target organs is mainly affected, but it is not rare to find several of them affected in the same animal, varying in severity. In both, sheep and goats, only the respiratory and neurologic syndromes lead to a cachectic stage and death, either by impairment of the respiratory function or by a general alteration of the nervous system. The locomotive and mammary syndrome alone do not generally result in cachexia or death, although they can cause several degrees of locomotive difficulty (mostly in goats) or a decreased milk production leading to undernourished lambs or kids. Thus, animals with locomotive or mammary syndromes are prematurely culled due to suboptimal production.

Clinical and subclinical MV and CAE are associated with progressive, mononuclear cell inflammatory lesions in the lungs, joints, udder and central nervous system (OIE). Chronic mastitis is common in both species. Labored breathing associated with emaciation caused by progressive pneumonitis are the predominant features in clinically affected sheep, whereas polyarthritis is the main clinical sign in goats. However, most lentivirus-infected sheep and goats are largely asymptomatic, but remain persistent carriers of virus and are capable of transmitting infection via colostrum or milk and respiratory secretions: the existence of healthy carriers further complicates surveillance, diagnostics and control.

Diagnosis

Due to the long incubation time lentiviruses can be widely spread in a flock or region before clinical cases are observed. Usually, symptoms of the disease have an insidious onset and a slow progression. Since the main target organs of small ruminant lentiviruses are joints, lung, central nervous system and mammary glands, these predilection sites should be carefully checked during clinical examination, in order to detect any lesions.

Serological diagnostic methods, detecting specific antibodies in infected animals, are considered the most convenient to detect small ruminant lentivirus infections (de Andrés et al., 2005). Small ruminant lentiviruses usually produce persistent infections that can elicit detectable immune responses beyond the first two weeks of infection (Simard and Briscoe, 1990). However, the natural immune response is slow and variable, antibody concentrations can fluctuate over time and animals exist that do not develop an antibody response upon infection. This, together with antigenic differences between circulating strains and viral proteins used in the diagnostic tests, makes that serological tests do not always correctly determine the infection status of sheep and goats (Ramirez et al., 2013).

False positive results can also be obtained in an animal's life (Brinkhof and van Maanen, 2007), as well as diagnostic interference due to antibodies passively acquired with colostrum in lambs (Cutlip et al., 1988). Recently, vaccination against bluetongue with poorly purified inactivated vaccines has caused in several European countries false positive results by ELISA (Valas et al., 2011).

Different techniques have been used to detect antibodies against small ruminant lentiviruses, but none can be considered to be 'gold standard' (de Andrés et al., 2005). Agar gel immunodiffusion (AGID) and ELISA are the prescribed tests for international trade, whereas western blot (WB), radioimmunoassay (RIA) and radioimmunoprecipitation assay (RIPA) are more complex and have been used only as confirmatory tests (de Andrés et al., 2005; Herrmann-Hoesing, 2010). AGID is considered highly specific, but less sensitive than ELISA (Synge and Ritchie, 2010) and, therefore, nowadays AGID is almost exclusively used to confirm ELISA results. Indirect ELISA are mostly used in small ruminant lentivirus diagnosis.

Broadly reactive ELISAs have been useful in control programs of small ruminant lentiviruses. In general, ELISAs have been found to be more sensitive than PCR techniques, except in juvenile animals (Alvarez et al., 2006; Muz et al., 2012), as well as in recent infections in general. In these cases, a combination of both techniques, even though making testing more expensive, can discover all cases (including early infections) and may avoid the risk of leaving infected individuals in the flock.

Maedi-Visna may be diagnosed by nucleic acid detection techniques such as polymerase chain reaction (PCR) assays, Southern blotting and *in situ* hybridization. PCR tests are used in some laboratories for rapid diagnosis. De Regge and Cay (2013) developed a q (RT)-PCR test capable of detecting SRLV strains belonging to genotype A strains from various countries. PCR testing has the advantage that it is capable to detect infected animals directly after infection and lifelong thereafter, but its sensitivity might be hampered due to the low virus load during latent infections and due to the high genetic variability of SRLV strains which makes it impossible to detect all circulating strains with one single test.

Seroconversion generally occurs months after infection. In general, serology is of greater value in screening flocks than in diagnosing individual animals. In adult sheep and goats, a positive result indicates that the animal is persistently infected with MVV but, because most infected animals do not become symptomatic, serology does not allow to confirm that symptoms are caused by this virus.

In seropositive, symptomatic animals, histology can confirm the diagnosis in biopsy or necropsy samples. Virus isolation can also be useful; however, viral titers are variable and may fluctuate over time. These techniques are more laborious and less suitable for large-scale routine diagnostics.

4.1.2. Entry assessment

There is a growing consensus in literature that small ruminant lentivirus **between-animal transmission** occurs mainly through (in)direct horizontal routes (e.g. inhalation of respiratory secretions of faecal-oral) and that vertical lactogenic transmission (colostrum, milk), once considered the main transmission route, might be a complementary route that probably only takes real importance as a secundary mechanism, when the horizontal route is slowed down by environmental or management factors. This implies that control strategies have to take into account both vertical and mainly horizontal transmission, the latter likely being the cause of advanced situations, where infections become a clinical problem (Minguijon et al.; 2015, OIE, 2008; CFSPH, 2015).

The between-flock transmission (introduction) occurs often horizontally during transport of live animals or by introduction of new animals in a flock. Contact between uninfected flocks and untested or seropositive flocks is a risk. Mixing sheep and goats, or feeding milk or colostrums from one species to another can lead to the transfer of viruses between species (CFSPH, 2015).

4.1.3. Exposure assessment

Close contact between animals in crowded barns may enhance transmission. Genetic factors, including the breed of the sheep, influence the outcome of infection. Breed associated susceptibility for Maedi-Visna disease in sheep has been suggested (Brinkhof, 2009). Management practices can influence the prevalence of the infection and, thus, the frequency of the disease (CFSPH, 2015).

4.1.4. Consequence assessment

Close contact between sheep and goats in crowded barns is a risk factor for interspecies transmission of SRLV's. Most infections are asymptomatic, but once clinical signs appear, the disease is progressive and usually fatal. Chronic mastitis with decreased production (milk drop) of normal appearing milk appears in both syndromes. Weight gain in lambs may be decreased. Animals will show signs of pneumonia, mastitis, encephalitis and polyarthritis. These will cause suffering, slow wasting and finally death (OIE, 2008; CFSPH, 2015).

When Maedi-Visna is introduced into a new area, the mortality rate may reach 20-30%. The mortality rate is low in regions where Maedi-Visna is endemic; annual losses rarely exceed 5% in a flock, even when nearly 100% of the flock is infected. Supportive therapy may be helpful in individual animals, but it cannot stop the progression of the disease.

4.1.5. Risk estimation

The risk estimation is worked out in the answers to the different questions.

4.1.6. Uncertainties

The most important uncertainties are related to the lack of data on the actual prevalence of lentiviruses in sheep and goat flocks, the lack of knowledge on the genotype of circulating lentiviruses, lack of knowledge of transmission parameters (attack rate, R_0) and seroconversion/incubation times and the effect of immunosuppression on transmission.

4.2. Answers to the questions formulated in the terms of reference

4.2.1. Is it possible to use the general term 'control of lentivirus infections in sheep and goats' in the modified legislation instead of using separate terms for 'the control of Maedi-Visna infection in sheep' and 'the control of viral Caprine Arthritis-Encephalitis in goats' as is used in the actual legislation?

Based on limited number of complete sequences SRLVs have been initially described as two distinct genetic groups evolving independently in sheep or goats, the ovine strains being closely related to each other and distinct from the caprine ones.

However, according to Leroux et al. (2010) the description of many partial or complete sequences of caprine and ovine field isolates from various geographical regions and their phylogenetic studies clearly evidenced, over the last 2 decades, the existence of a genetic continuum with viruses that did not simply cluster according to the animal species they were isolated from. The genetic relationships are more complex than initially proposed, with caprine and ovine viruses belonging to the same genetic group.

Phylogenetic analysis comparing nucleotide sequences of Maedi-Visna virus and CAE virus has demonstrated that these are closely related lentiviruses (OIE, 2007). Phylogenetic analyses comparing nucleotide sequences of VM virus and CAE virus show clear indications of the existence and epidemiological importance of cross-species transmission between sheep and goats without demonstrating clearly that one virus has emerged from the other (Leroux et al., 1997). Results support the existence of possibly frequent cross-species transmission of SRLV in domestic and wild small ruminants (Leroux et al., 2010).

Studies have confirmed that CAEV and VMV, originally established as specific pathogens in goats and sheep respectively, often cross the species barrier infecting the new host, persisting in it and spreading across the new host population (Ramirez et al., 2013).

According to the Scientific Committee there is substantial evidence in scientific literature to consider that Maedi-Visna virus in sheep and Caprine Arthritis-Encephalitis virus of goats are both lentiviruses that are closely related and can be grouped together as small ruminant lentiviruses.

The Scientific Committee recommends using the term 'small ruminant lentivirus infections' as common name in the new legislation, instead of making a differentiation between Visna Maedi virus in sheep and Caprine Arthritis-Encephalitis virus in goats.

4.2.2. Does it makes sense to distinguish between sheep and goats in the health certification for lentivirus infection? How to deal with mixed flocks?

The Scientific Committee recommends to make no distinction between sheep and goats in the new legislation and in the health certification as both species can be infected by the same lentiviruses.

In case of mixed flocks it is recommended to apply the diagnostic protocol to both species before declaring the flock 'lentivirus free' as prevalence, incubation period, epidemiological background and sensitivity to infection may be different between sheep and goat flocks.

4.2.3. Is it necessary to provide definitions for Maedi-Visna and Caprine Arthritis-Encephalitis in the new legislation?

The Scientific Committee recommends not to include definitions of Maedi-Visna and Caprine Arthritis-Encephalitis in the new legislation provided the new insights in the classification and interspecies transmission of small ruminant lentiviruses.

It may be helpful to provide a definition of 'freedom of disease caused by lentivirus infection' in both sheep and goats by referring to the diagnostic protocol.

4.2.4. The certification of flocks 'free from MV-CAE' is based on serological testing (first step). Is the humoral response of sheep and goats influenced by the parturition period (15 days before and after lambing)? In other words is there an increased chance of false results by applying an ELISA during that period?

Theodorou et al (2007) showed that a mild general immune suppression could be observed in 2 out of 3 Greek sheep breeds during the peri-parturient period. The immune suppression was found in the 2 breeds with the highest milk production. It is unknown if immune suppression around parturition has an effect on lentivirus infection and serological status in sheep and goats. It is unknown if there is an increased chance of false results of the ELISA during the peri-parturient period.

No scientific evidence related to lentivirus infection in sheep and goats has been found showing an effect of the peri-parturient period on the results of serological testing.

In the French model no sampling is foreseen 15 days before parturition and 15 days after parturition ('Cahier des charges technique du système national d'appellation de cheptel en matière de Maedi-Visna – 2004'). No scientific basis for this practice has been reported.

4.2.5. Actually the minimum age for serological testing is 1 year. At that age positive animals are seldomly detected. What is the risk for false seronegative flock testing if the minimum age for testing is set at the age of 2 years?

Accurate diagnosis of small ruminant lentivirus infection is of major importance in terms of the results of control programs. Most animals become infected early in life, from drinking infected colostrum or milk. The virus can also spread during close contact, probably by the respiratory route. The incubation period for Maedi is usually more than two years; clinical signs typically develop when animals are three to four years old. The incubation period for Visna is somewhat shorter, and symptoms can appear in sheep as young as two years. Seroconversion generally occurs a few months after infection (and not years).

According to Alvarez et al. (2005) most animals develop detectable anti-MV antibodies 2–8 weeks after infection although the immune response fails to eliminate the virus and stop viral replication in target organs completely. The authors showed that sheep younger than 1 year do also seroconvert after horizontal transmission of lentivirus. Reina et al. (2011) showed that goat kids (< 1 year) seroconvert after experimental infection with SRLV genotype E.

The Scientific Committee recommends not to postpone the age of testing to 2 years because it will increase the probability of missing lentivirus spread in sheep and goat flocks. By postponing the testing till the age of 2 years the serological status of a complete generation of young animals will be missed. Scientific literature shows that animals below the age of 1 year seroconvert when infected. The highest probability for seroconversion occurs within the first year of life or within the first year after introduction into a positive herd.

4.2.6. Once a flock has met the certification conditions for a 'free from MV-CAE' status the certificate can be prolonged twice with one year. Subsequently serological testing with 2 years interval is necessary. What is the risk for missing seropositive animals if this interval is prolonged?

Data from CODA-CERVA (S. Roelandt, personal communication) on the Belgian certification results between 2010 and 2014 show that 6,4 % of the free sheep flocks and 8,3 % of the free goat flocks lose their free status after serological control at 24 months. This shows that within a 24 month period, the disease can be spread to other animals and potentially to other flocks via undetected infected animals or that flocks can import the disease via contact with or via purchase of latently infected animals that were seronegative at introduction. Prolonging the interval of testing will increase the probability that more animals and other flocks become exposed to the virus via contact or via purchase of latently infected carrier animals.

4.2.7. The serological testing for prolongation of the flock certificate 'free from MV-CAE' must be executed on 50 % of the animals in a flock with a minimum of 50 animals. What is the risk for missing seropositive animals if less than 50 % of the animals are tested?

The current sampling plan is historically based on Council Directive 91/68/EEC of 28 January 1991 on animal health conditions governing intra-Community trade in ovine and caprine animals. This Directive establishes the guarantees regarding animal health required for trade between the Member States. Next to minimal health conditions the Directive lays down additional controls for certain diseases including Maedi Visna, caprine viral arthritis/encephalitis, contagious agalactia or paratuberculosis. In annex A, chapter 1, heading 1 of Council Directive 91/68/EEC conditions are laid down for granting and maintenance of official Brucellosis (*Brucella melitensis*) free status. In Belgium the serological samples taken for the voluntary VM-CAE control program are also used for the control of *Brucella melitensis* (maintenance of officially free status).

The current sampling protocol corresponds with the sample sizes proposed by FAO (1988) for testing for presence of disease in small populations with 95 % confidence of detection of a seropositive animal.

According to the French 'Cahier des charges technique du système national d'appellation de cheptel en matière de Maedi-Visna – 2004' the flock sampling plan (in flocks with unknown status) should be aimed at identifying a seropositive animal with a minimal 95% chance if the flock seroprevalence is higher than 5 %.

The seroprevalence of lentivirus infection in sheep and goat flocks in Belgium is currently unknown. It makes the subject of an ongoing research project of CODA-CERVA (SRLV-BEL).

In the case of testing for prolongation of a free VM-CAE status the Scientific Committee recommends to apply a risk-based sampling plan within the flock. Animals at risk are newly introduced animals, animals fed with untreated colostrum of unknown origin and animals with clinical symptoms such as

unthriftiness, inappetence and lameness and/or other clinical signs associated with lentivirus infections (chronic mastitis). Shuaib et al. (2010) reported that large flock sizes and presence of lame aimals were positively and significantly associated with positive MVV flock status. Purchasing more than 50 new sheep in the last 5 years and unthriftiness had positive associations with MVV flock status, but did not quite achieve statistical significance.

Sample sizes for detecting seropositive animals in a flock under Belgian conditions have been calculated by S. Roelandt (CODA-CERVA, 2016) based on the following assumptions:

- most probable within flock-prevalence: 15 % (0-20 %)
- most common sheep and goat flock size in Belgium: N < 10 to ≤150 (Sanitel, 2015)
- confidence imperfect test sensitivity: 99 %
- perfect test specificity: 100 %.

In this study Roelandt concludes that the actual test protocol of sheep and goat flocks (minimum 50 animals per flock and minimum 50 % of the animals in the flock) should best be preserved as long as the true lentivirus flock prevalence and test accuracy are unknown.

The Scientific Committee recommends to preserve the actual sampling plan but proposes to review the sample size needed to test for freedom of lentivirus infection once new data become available from the SRLV-BEL CODA-CERVA research project. Applying already now sampling rates smaller than 50% in a flock will diminish the probability of detection of seropositive animals.

4.2.8. What is the risk of missing seropositive animals if no serological testing is done at the start-up of new flocks with only animals from one or more flocks with a free status?

Despite the fact that animals are bought from a flock with a free status, the risk exists that SRLV infected animals are present from the start-up of new flocks.

Data from CODA-CERVA (S. Roelandt – personal communication) show that in Belgium between 2010 and 2014 6,3 % of free VM sheep flocks and 8,3 % of “free” CAE goat flocks lost their free status after 24 months. Additionally, 3/21 (14,3 %) of new sheep herds consisting only of ‘certified MV-CAE free animals’ lost their free status at the first testing. This shows that the virus may circulate in certified ‘MV-CAE free’ flocks due to the existence of infected but seronegative animals, the applied sampling scheme, the imperfect test characteristics, introduction of SRLV infected animals, or the long incubation period of the disease.

The same argumentation holds true for introduction of animals from certified flocks in foreign countries, the more that the quality of the foreign VM/CAE control programs is unknown.

In addition, lentiviruses circulate in Belgium sheep and goat flocks especially as there is no official mandatory lentivirus eradication program.

During transport animals from free flocks may get infected due to close contact with animals from non-free flocks.

The Scientific Committee is of the opinion that newly populated flocks should be able to get a similar free status of the herds of origin provided that new flocks run the complete control program from the start. This control program is based on successive serological control as stipulated in the royal decree of 24th March 1993 (sheep) and in the royal decree of 27th November 1997 (goats). In other words it is not recommended that new flocks use historical status data from herds of origin to alleviate their own control program (maintenance of status). In this regard the Scientific Committee refers also to article 16 §3 of the royal decree of 24th March 1993 in regard with the control of

Maedi-Visna in sheep which stipulates: "… if the animal has never been tested this should be done within 6 months …".

The Scientific Committee is of the opinion that not performing serological testing of animals in new flocks, exclusively populated with animals from flocks with a certified free VM/CAE status, signifies an increased risk for introduction of SRLV infected animals in the flock.

4.2.9. Is the infection transmissible between sheep and goats? Is one certificate (for one species) sufficient if both species are present on the same flock?

Cross-species transmission of lentiviruses between sheep and goats has been shown to occur (Mungujon et al., 2015).

The Scientific Committee is of the opinion that in mixed flocks (sheep and goats) the status of the flock should depend on the status of both flocks meaning that both species should be sampled to obtain or maintain an officially free status.

4.2.10. Has the Scientific Committee any remarks with regard to the diagnostic decision tree?

The Scientific Committee is of the opinion that the certification is based on a logical sequence of tests described in the diagnostic decision tree.

In 2011, CODA-CERVA (N. De Regge – personal communication) found several flocks that were ELISA positive, immunodiffusion negative, but positive with PCR. This indicates that PCR analysis at the second sampling has an added value. In 2015, 3/33 animals were identified as PCR positive during the second sampling. Some animals were found positive in the immunodiffusion test after the first sampling, thereby confirming the obtained ELISA results.

These findings indicate that all steps and tests in the current sequential sampling and testing scheme have an added value in regard to determining the correct infection status of an animal.

4.2.11. In case of a positive ELISA, is it possible to reduce the number of control tests (ELISA + immune-diffusion test + PCR) to confirm or refute the infection?

The actual diagnostic test procedure is as follows:
- 1st sampling: an ELISA is done by DGZ/ARSIA. If positive, an immuno-diffusion (ID) test is done by CODA-CERVA. In case of a positive ID test the flock loses its free status. In case of a negative ID test the possibility exists for a 2nd sampling.
- 2nd sampling: an ELISA (by DGZ/ARSIA) is followed by a PCR by CODA-CERVA. A decision on the status of the flock is taken according to the diagnostic decision tree.

The ELISA has a high sensitivity and is therefore used as a first test. In case of a positive ELISA confirmation can be done using an immuno-diffusion test or by PCRs detecting different genotypes of the virus. A negative PCR should be carefully interpreted. It can be caused by a virus load that is below the detection limit of the PCR or because of infection of the animal with a strain that is not recognized by the implemented PCR. However, if a PCR is used that is adapted to the locally circulating strains, it is a very sensitive test (N. De Regge – personal communication).

According to Ramirez et al. (2013) a combination of serology and PCR might be optimal for detecting the infectious status of an animal).

In the ongoing CODA-CERVA research project SRLV-BEL, it is being evaluated whether the implementation of a 2nd ELISA (based on another antigen) would be helpful to determine the correct infection status in a more straightforward way compared to the use of immunodiffusion or PCR as confirmation test.

Because of the lack of data the Scientific Committee cannot adequately answer the question at this moment. However it is to be expected that no single VM/CAE test will be sensitive enough to detect all VM/CAE cases and simultaneously be specific enough to rule out all false positives. Hence it is recommended that the diagnostic decision tree is based on multiple/sequential testing including PCR.

5. Conclusions

Small ruminant lentiviruses are part of a very heterogeneous group of lentiviruses which can give rise to various slow-evolving syndromes depending on the species, the virulence of the viral strain and the production conditions. In order to control these infections, it is first necessary to correctly identify infected animals and herds by means of periodically executed reliable tests. The success of a voluntary control program will depend on the initial seroprevalence in the herd and in the Belgian sheep and goat population, on the strict implementation of individual flock biosecurity measures and on the cost/benefit balance of the control program for individual holders.

6. Recommendations

To make a lentivirus control program successful, the Scientific Committee emphasizes that next to certification of SRLV-seronegative flocks it is crucial to simultaneously apply proper biosecurity measures in all sheep and goat flocks, at trade and during transport or all activities where a direct or indirect contact with other animals is possible.

The Committee recommends that the sector as well as the authorities activate communication to sheep and goat breeders/holders to consequently apply adequate biosecurity measures. These should include avoiding introduction of animals from flocks with unknown or positive lentivirus status, avoiding using colostrum from any other flock or from other species, applying proper hygienic measures within the flock (disinfection and quarantine + testing), avoiding reintroduction of animals in the flock who participated at markets/transports, and to cull of seropositive and confirmed infected animals.

For the Scientific Committee,
Chairman,

Prof. Dr. E. Thiry (Sgd.)
Brussels, 20/05/2016

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Presentation of the Scientific Committee of the FASFC

The Scientific Committee is an advisory body of the Belgian Federal Agency for the Safety of the Food Chain (FASFC) that provides **independent scientific opinions** on risk assessment and risk management in the food chain, and this at the request of the Chief Executive Officer of the FASFC, the Minister competent for food safety or at its own initiative. The Scientific Committee is administratively and scientifically supported by the Staff direction for Risk Assessment of the Agency.

The Scientific Committee consists of 22 members who are appointed by royal decree on the basis of their scientific expertise in areas related to the safety of the food chain. When preparing an opinion, the Scientific Committee can call on external experts who are not a member of the Scientific Committee. Similar to the members of the Scientific Committee, they must be able to work independently and impartially. To ensure the independence of the opinions, potential conflicts of interest are managed transparently.

The opinions are based on a scientific assessment of the question. They express the view of the Scientific Committee which is taken in consensus on the basis of a risk assessment and the existing knowledge on the subject.

The opinions of the Scientific Committee may contain **recommendations** for food chain control policy or for the stakeholders. The follow-up of these recommendations for control policy is the responsibility of the risk managers.

Questions on an opinion can be directed to the secretariat of the Scientific Committee:
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Members of the Scientific Committee

The Scientific Committee is composed of the following members:

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Conflict of interest

Because of a conflict of interest T. van den Berg (CODA-CERVA) and N. De Regge (CODA-CERVA) participated at the activities of the workgroup as 'invited heared experts'.

Acknowledgements

The Scientific Committee acknowledges the Staff direction for Risk Assessment and the members of the workgroup for preparing the draft opinion.

Composition of the workgroup

The workgroup was composed of:

Members of the Scientific Committee:	E. Thiry (reporter), C. Saegerman, D. Berkvens
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The activities of the workgroup were attended by the following members of the administration (as observers): L. Derolez (FPS Public Health, Food Safety and Environment) and L. Vanholme (FASFC).

Legal framework

Law of 4 February 2000, on the creation of the Federal Agency for the Safety of the Food Chain, in particular article 8;
The Royal Decree of 19 May 2000, on the composition and operating procedures of the Scientific Committee, as established within the Federal Agency for the Safety of the Food Chain;
The Internal Rules as mentioned in Article 3 of the Royal Decree of 19 May 2000, on the composition and operating procedures of the Scientific Committee, as established within the Federal Agency for the Safety of the Food Chain, approved by the Minister on 9 June 2011.

Disclaimer

The Scientific Committee at all times reserves the right to modify the opinion by mutual consent, should new information and data become available after the publication of this version.