RABBIT HAEMORRHAGIC DISEASE VIRUS TYPE 2 (*R*HDV2) IN THE NETHERLANDS AND GERMANY: CLINICAL AND EPIDEMIOLOGICAL FINDINGS

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ABSTRACT

RHDV field case report. Since 1986 rabbits in farms in the Netherlands and Germany have problems suffering from RHD. In 2014 a new type RHDV2 infected 2 farms in the Netherlands. PCR or ELISA to determine RHDV were not available. Incidences occurred till outside weather temperature went down so that flying insects can't survive. In wintertime is less ventilation needed and rain makes outside air free of dust. New outbreaks were seen from spring till November each year. Virus typing was determined by State Laboratory (Friedrich-Loeffler-Institut Greifswald DE). Although does are vaccinated, weaned or suckling rabbits are not protected. Maternal antibody protection only last till 4 weeks of age. Healthy development and young age recovery of organs and body of rabbits beneath 3 weeks of age makes no clinical signs.

Key words: epidemiological, RHDV2, App, rabbit

INTRODUCTION

RHD is a fatal and infectious disease that affects wild and domestic European rabbits (OIE, 2015) with high mortality. RHDV causes disease in rabbits (*Oryctolagus cuniculus*) and hares (*Lepus europaeus*). In 2010 RHDV2 was identified in France first in wild rabbits and then in domestic farm rabbits. It was characterized by a genetic and antigenic profile clearly different from RHDV1. Data suggest that RHDV2 is spread and reported in most European countries.
In the Netherlands 1,2 million rabbits are kept as pet rabbits, 50.000 as show rabbits, 45.000 does for production meat rabbits (35 farms) (RDA report 2019). In Germany the number of pet rabbits is unknown, but 1 million rabbits are kept as show rabbits and 15.000 does (18 farms) for producing meat rabbits. Also wild rabbits and hares will bring around RHDV2 through European countries. RHDV2 was first identified in 2014 in 2 farms located south part the Netherlands and in west south part of Germany and in domestic and wild rabbits. This study describes research on RHDV2 after atypical outbreaks with sudden death, that occurred in meat rabbit farms in the Netherlands and Germany, in view of documenting the development of the epidemic and the prevention and control measures taken. The evidenced wide spread of RHDV2 in the Netherlands and Germany is cause of concern as RHDV2 outbreak is a serious problem for farmers due to the induced severe costs like feed, housing of weaned rabbits. Extra costs occur with the necessity of vaccinating weaned rabbits. Implementation of control measures is absolute necessity beside the use of specific homologues vaccines, to reduce the risk of spreading virus. The availability, cost and registration, including batch control of these vaccines, are many of the problems in the approach to control the virus outbreaks. Classical RHDV vaccines showed low cross-protection against RHDV2, revealing the need for a specific vaccine against this disease.
In the Netherlands only veterinarians are allowed to vaccinate rabbits. The high total number of rabbits in farms and the low number of veterinarians make it difficult to vaccinate all rabbits in a short time on a farm.

MATERIALS AND METHODS

RHDV isolates were collected from intensive professional rabbit farms experiencing RHDV outbreaks in the Netherlands and in Germany during 2014 - 2018.
When a few number of rabbits die suddenly with typical lying on the side body stretched and head backwards, it is the first sign for the farmer, that RHDV2 has infected the farm. Rabbits are dissected by the veterinarian in the farm. Liver, lung, spleen and trachea are collected and put in a plastic bag. Parts of these organs with typical lesions are send to a laboratory. State laboratory Friedrich-Loeffler-Institut (FLI), Insel Riems Germany tests samples using ELISA and qPCR on these organs to search for antigens of field virus.
Further determination and typing of found virus were performed using the AVID-Method VIR01-RHDV/EBHSV. In short the method describes the use of serological and microscopy tests and techniques for virus determination. The tests are described by the method: haemagglutination assay, enzyme linked immunosorbent assay (ELISA, INgenzim RHDV 17.RHD.K2 kit from Ingenasa), electron microscopy (EM), immunofluorescence microscopy, RT-PCR.
For more detailed analysis of collected RHDV infected samples and to understand the nature of RHDV epidemics in the Netherlands and Germany, VP60 gene sequences were compared among the strains. Outbreaks in pet rabbits and show rabbits were communicated by social media.

RESULTS

Outbreaks: From 2014 to 2018 34 farms in the Netherlands and 8 farms in Germany experienced RHDV outbreaks, which were confirmed as RHDV2 in cases investigated. In south of the Netherlands, RHDV2 was first detected in 2014 in 2 farms. In 2015 this virus occurred in 5 rabbit meat farms located throughout the country. In 2016 and 2017 more than twenty farms were infested by this virus. In addition many cases clinical RHDV were noticed in pet rabbits and show rabbits. In 2018 nearly all meat rabbit farms had cases with clinical signs despite well vaccination of all does and rearing does
Vaccination results: Inactivated vaccines ERAVAC (HIPRA) and FILAVAC VHD K C+V (FILAVIE) were used, evaluated and compared in an infected farm with simultaneous administration of classical RHD vaccine (CUNIPRAVAC RHD). Before registration NOVARVILAP (LABORATORIOS OVEJERO) and FILAVAC VHD (FILAVIE) were compared with simultaneous administration to different rabbits in an infected farm with a classical RHDV vaccine (ARVILAP).
In Germany inactivated vaccine CUNIVAK RHD (IDT BIOLOGIKA) was registered to defeat RHDV2. But in infected farms no immunity was build up and field RHDV2 showed clearly the same clinical signs as if no vaccination was applied. Extra vaccination with new registered vaccines ERAVAC or FILAVAC VHD K C+V was necessary to compete the invasion of RHDV2. In practice ERAVAC and FILAVAC VHD K C+V is now used, because these are registered vaccines for rabbits to defeat RHDV2 field infection. NOBIVAC MYXO-RHD PLUS (INTERVET) is not used, because of high price for rabbitfarms. Only registered vaccines in each country for species and indication can be used independent if non registered vaccines or farmspecific autovaccines give even better results in protection against RHDV field infection.
Farmers found that, wherever vaccination was carried out (with vaccines derived from RHDV and RHDV2), mortality was lower during an outbreak. On farms where no vaccination was carried out or only one vaccination in the rearing does at 10 weeks of age, up to 100% of the off spring meat rabbits died. This relation between mortality and vaccination could not be assessed statistically due to lack of data (i.e. pathogenicity of virus strains and serological response of the animals) Hypothesis raise, that RHDV-derived vaccines offer low but not negligible protection, possibly due to cross-reactivity between RHDV and RHDV2 antigens. Present outbreaks show that RHDV2 has become a wide spread in Northern Europe.
Extra biosecurity measures: Based on experience during 2014-2018 extra biosecurity measures are recommended: produce rabbits in grouping system, all in-all out system, cleaning by high pressure sprayer, disinfect with registered disinfectants, clean-dirty road, fencing around the farm against wild rabbits, spray wheels of incoming trucks with citric acid, use new dust bag in every unload of feed by the feed truck driver, hygiene lock with shower and farm clothes and footwear in unique color per stable.

DISCUSSION

In last 5 years RHDV2 in the Netherlands and Germany was reported from Southern regions starting mid-2014, but RHDV2 spread over both countries, including main islands, without any epidemiological link. In 2016 24 farms of total 45 were infestated with RHDV2 in nonvaccinated rabbits. During summer period slaughterhouses will only accept 60 % of the number rabbits produced during wintertime. Also the price per kg live rabbit varies a lot between summer and winter and is even below cost price in summer. These two items make that no continuous production in does has great impact on risk for diseases. In august production cycle starts again to supply the high demand of fresh rabbit meat at Christmas time.

Biosecurity is more important than good vaccination schemes. RHDV2 cases are first seen early spring and last the whole summer. Important vectors of introduction and spread of virus are dust and insects. Dust disappear with rainy weather, and stinging flies and mosquitoes with cold weather. Flies, mosquitoes and dust are important of transferring diseases inside a farm.

When RHDV is detected on a farm, the RHDV protocol will be put into effect. This protocol prescribes: Direct delivery of feed from feed factory to farm and back to feed factory, disinfection of all wheels of the truck with citric acid, disposable overall and boots for drivers and dustbags for each silo stay at the farm.

Direct transport of rabbits from one farm to the slaughterhouse in disinfected clean empty crates is prescribed. Driving from slaughterhouse with crates from farm to farm till the truck is filled with 5000 rabbits is a big risk in spreading diseases, especially RHDV. Only the veterinarian is allowed to go inside the farm and no other visitors. After visiting an infected farm it is forbidden to go to other rabbit farms the next two days. Removal of dead animals takes place at the edge of the farm near the main road, so that destructor trucks will not drive close to rabbit stables. Before empty bins are taken back to the farm they are disinfected at side of street. Nist boxes for bats and birds are placed around the farm so that flying insects are caught around the farm. Coolingpads at airinlet prevents insects and dust to enter the farm. Farm workers are trained to wear gloves and disinfect with alcoholgel during working with rabbits. Plastic bags inside the farm are used to remove dead rabbits, so that loose hair will not spread inside the farm. Different colors are used in each stable for work clothes and boots. Transport of infected material is reduced.

Good vaccines are important to stop and prevent endemic outbreaks in a farm. Hygienic approach, like using a new needle for each rabbit, is important. Vaccines against RHDV2 like ERAVAC, FILAVAC VHD K C+V and NOBIVAC MYXO-RHD PLUS are accepted by the national authorities for submission, because of many RVHD2 outbreaks. Vaccination failures like injection with a low protective dose and breaking the cold chain during transport vaccines from production till application is very risky. Seromonitoring is possible for RHDV in rabbits. Antibody detection will not indicate that protection is high or low for RHD infection. Humoral immunity is detected after maternal antibody intake, after fieldvirus infection, or after vaccination. A farm stays in this protocol till 12 weeks after last clinical signs are seen in new offspring rabbits. In the Netherlands there are not enough veterinarians to vaccinate all rabbits. The extended arm construction should be implemented by the law for rabbit farmers, so that they can vaccinate their own rabbits at the right time and age under supervision of the secured veterinarian .

Positive notifications of RHDV2 are placed on the webpage of FLI and University of Utrecht with location and region of origin to monitor distribution of RHDV2.A production scheme in a farm impede good planning of vaccinating and other activities. Different ages in rearing and production does and their offspring are favorable to spread infection through the whole farm. All farmers are connected *via* a group app on their mobile phones. As soon as one has clinical signs, a notification is sent in the app. All does with offspring in the farm are vaccinated and first the stable where the outbreak took place. Cleaning and disinfection stops spread of virus in the farm. When hygiene is optimal vaccination in offspring infected group and next 2 successive offspring groups needs to be done. Otherwise virus spreads easy inside the farm. Good vaccinated rabbits before infection will not be carriers of RVHD2. Vaccination during incubation period can make rabbits as virus carriers without clinical signs. These rabbits will spread virus to none immune rabbits.

Information between farmers, farm counselors and slaughterhouse is done through social media. App groups inform about solutions to disminish RHDV2 inside a farm and over the country. Manure from infected rabbits inside the farm is risky of spreading virus. Insects, hair and transport are easy distributers of virus. Good hygiene and management are key indicators for preventing spread of virus. Phylogenetic analysing highlights the diversity of RHDV strains. Results help to uncover the nature of epidemics and molecular evolution of RHDV. It is doubtful if hares are susceptible for RHDV2. In a dense area full of hares and much RHDV2, more dead hares should be found, just like how wild rabbits are living and found dead. But lifestyle of hares is different from rabbits.

CONCLUSIONS

Intensive direct or indirect contact between wild rabbits and wild hares, intensive farm rabbits, show rabbits and pet rabbits will give RHDV2 opportunities to stay alive in a big area. Prevention by displacement of this virus and biosecurity are important. Back yard rabbit farming and vaccine failures will increase the risk of a field challenge. Protocol for hygiene and vaccination scheme will prevent spreading RVHD2. Vaccines needs improvement mainly due to mode of action of the immune system of rabbits. Herd immunity is used to prevent and eliminate RHDV2 in a farm and an area.
Social media like farmers app and email and all-in all out system (ping pong) in farms will make it easier to control outbreaks. Biosecurity is the "ugly duckling" of disease control in rabbit farms. Good pre-placement disinfection is essential like footbaths, vehicle dips and sprays, staff people shower entering the farm and disinfect equipment.

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