Inconspicuous post-mortem findings in rabbits from Switzerland naturally infected with Rabbit Haemorrhagic Disease Virus 2

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Unauffällige Sektionsbefunde bei Kaninchen aus der Schweiz mit einer Rabbit Haemorrhagic Disease Virus 2 Infektion

Das Rabbit Haemorrhagic Disease Virus 2 (RHDV-2) trat erstmals 2010 in Frankreich auf. In der Schweiz wurde RHDV-2 dann 2015 diagnostiziert und verdrängte das klassische Rabbit Haemorrhagic Disease Virus (RHDV). RHDV-2 verursacht wie RHDV eine virale Hepatitis mit perakutem Verlauf und erhöhter Sterblichkeitsrate in Kaninchenhaltungen. Eine RHDV-Infektion verursacht konsistente makroskopische Befunde, insbesondere in der Leber und den Atemwegen. Publikationen der makroskopischen Pathologie von RHDV-2 infiziert Kaninchen sind selten.

Die vorliegende Studie analysierte die Anamnese und Sektionsberichte von 35 Kaninchen, die im Rahmen der Routinediagnostik zwischen März 2015 und Mai 2017 untersucht wurden. Mittels einer für RHDV-2 und RHDV spezifischen Reverse-Transkriptase-Real-Time-Polymerase-Kettenreaktion (RT-qPCR) wurde bei 25 Tieren RHDV-2 und bei keinem Tier RHDV nachgewiesen. Zusätzlich wurden histologische Untersuchungen von Leber, Lunge und Niere bei 18 RHDV-2 RT-qPCR positiv getesteten Kaninchen durchgeführt.

Die Anamnese beschrieb häufiger eine erhöhte Sterblichkeitsrate bei RHDV-2-positiven Tieren (16/18, 89 %) im Vergleich zu RHDV-2 negativen Kaninchen (3/9, 33 %). Die Sektionsbefunde zeigten keine pathognomonischen Veränderungen bei RHDV-2-positiven Tieren. Histologisch wies die Leber die schwersten Veränderungen auf, gefolgt von Lunge und Nieren. RHDV-2-positive Tiere zeigten häufig Anzeichen einer Magen-Darm-Erkrankung (n = 5) und/oder einer Sepsis (n = 6), die mögliche, unspezifische Befunde einer RHDV-2-Infektion, wie eine vergrösserte Milz oder

Abstract

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Rabbit Haemorrhagic Disease Virus 2 (RHDV-2) emerged in France in 2010. In Switzerland, RHDV-2 was first identified in 2015 and apparently has almost replaced the classical Rabbit Haemorrhagic Disease Virus (RHDV) by now. Like RHDV, RHDV-2 causes a viral hepatitis with a peracute course and an increased mortality rate within the rabbitry. RHDV infection causes consistent gross pathological findings, especially in the liver and respiratory tract. Reports about gross pathology for animals naturally infected with RHDV-2 is scarce.

The present study analysed the anamnesis and necropsy reports of 35 rabbits examined during routine diagnostics between March 2015 and May 2017. A reverse transcriptase real-time polymerase chain reaction (RT-qPCR) specific for RHDV-2 and RHDV proved a total of 25 animals to be positive for RHDV-2, while none was positive for RHDV. Additionally, histological examinations were performed on liver, lung, and kidney of 18 rabbits that had tested positive by RHDV-2 RT-qPCR.

The anamnestic report more often stated an increased mortality rate in RHDV-2 positive (16/18, 89 %) compared to RHDV-2 negative rabbits (3/9, 33 %). Gross pathology did not reveal any pathognomonic changes in RHDV-2 positive animals. Histologically, the liver showed the most severe lesions followed by lung and kidney. Animals positive for RHDV-2 frequently showed signs of gastro-intestinal disease (n = 5) and/or septicaemia (n = 6) masking possible indicators of an RHDV-2 infection, such as the rather unspecific findings of an enlarged spleen or an enlarged, friable, tan-coloured liver.

The authors want to raise awareness among clinicians and pathologists that in case of sudden death in commercial or pet rabbits, RHDV-2 needs to be considered

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eine vergrösserte, brüchige, gelbbraune Leber verschleiern können.

Bei plötzlichen Todesfällen in Nutz- oder Heimkaninchenhaltungen sollte eine RHDV-2 Infektion als Differentialdiagnose in Betracht gezogen und durch eine Labordiagnose abgeklärt werden.

Schlüsselwörter: Sektionsbefunde, Histopathologie, PCR, Rabbit Haemorrhagic Disease Virus 2, Schweiz

as differential diagnosis and should be confirmed by laboratory diagnosis.

Keywords: Gross pathology, Histopathology, PCR, Rabbit Haemorrhagic Disease Virus 2, Switzerland

Introduction

Rabbit Haemorrhagic Disease Virus 2 (RHDV-2) is a ribonucleic acid (RNA) virus of the family *Caliciviridae*, genus *Lagovirus*. Genetically, RHDV-2 clusters in the group of mostly apathogenic rabbit caliciviruses (RCV) and is more closely related to the European Brown Hare Syndrome Virus (EBHSV) than to Rabbit Haemorrhagic Disease Virus (RHDV).[7]

RHDV-2 was first identified in France in 2010 and was soon detected in other European countries including Switzerland (Supplementary Table 1).[20,22] Apart from its rapid spread, RHDV-2 quickly replaced classical RHDV-strains within months after its arrival, as reported from France, Spain, Portugal, Sweden, and from wild Australian rabbits.[3,4,6,20,23,24,27]

While classical RHDV causes disease in European rabbits (*Oryctolagus cuniculus*) from five to eight weeks of age, RHDV-2 has a broader host and age spectrum and may cause disease in different leporid species of all ages, such as European rabbits (*Oryctolagus cuniculus*), Sardinian cape hares (*Lepus capensis mediterraneus*), Italian hares (*Lepus corsicanus*), European brown hares (*Lepus europaeus*) and mountain hares (*Lepus timidus*).[4,16,21,22,26,30,36,37] Despite these differences, the clinical signs of RHD are very similar no matter whether it is caused by the classical RHDV or RHDV-2. The disease is characterised by a highly contagious viral hepatitis, usually with a peracute to acute course. Therefore, rabbits often die in good body condition without showing any clinical signs prior to death.[12,30] If clinical signs are present, these are mainly foamy or bloody nasal discharge, depression, and fever.[30] Mortality in RHD caused by RHDV-2 is somewhat more variable, but overall reported to be lower (5–70% within 96 hours post infectionem), as compared to RHDV (80–90%).[29] Transmission may occur directly between animals via secretions and excretions or indirectly via fomites or contaminated insect vectors. Due to the high tenacity of the virus, recurrent infections within the same rabbitry are possible. Thus, vaccination is highly recommended.[30] Pedigree rabbits that are not vaccinated are excluded from exhibitions in Switzerland.

Post-mortem lesions of RHD are focussed on the liver and the respiratory system.[30] Lesions regularly found in rabbits infected with RHDV are acute necrotising hepatitis, pale yellow to grey colour and a marked lobular pattern, a hyperaemic tracheal mucosa and a lumen filled with frothy fluid, a congested spleen, and haemorrhages in various organs.[1,14,25] Due to the acute nature of the disease, morphological changes are often subtle and there is a lack of systematic evaluations of lesions caused by infection with RHDV-2.[30] The objectives of the present study were 1) to record the post-mortem findings of rabbits, 2) to perform a reverse transcriptase real-time polymerase chain reaction (RT-qPCR) for RHDV-2 to distinguish RHDV-2 positive and negative animals and 3) to compare the morphological findings between these two groups, aiming to identify post-mortem lesions characteristic for RHDV-2 positive rabbits.

Material and Methods

Description of samples

Samples for the present study consisted of 35 rabbits representing 29 different rabbitries located in 10 of the 26 Swiss cantons and were collected between March 2015 and May 2017. Rabbits had either been found dead by their owner (n = 30) or were euthanized in agony (n = 5). All rabbits had been submitted to the National Reference Centre for Poultry and Rabbit Diseases (NRGK) to determine cause of death or disease. If an anamnestic report was provided, mention of peracute death or increased mortality, both anamnestic findings characteristic for RHDV, was noted.[30]

Molecular diagnostics

Liver samples of all 35 individuals were collected during necropsy. Of these, 33 samples were processed using the «Total RNA Isolation Kit NucleoSpin RNA» (Macherey-Nagel, Düren, Germany) with on-column DNase treatment according to the manufacturer's protocol followed by the RHDV-2 specific quantitative RT-qPCR based on the *vp60* gene described by Duarte and others (2015).[8] Liver samples of the remaining two rabbits (15-T0085-1 and -2) had already been formalin-fixed and paraffin-embedded for histological examination and were forwarded to the Organization for Animal Health (OIE) Reference Laboratory for RHD diag-

nostics, Brescia, Italy. The RNA was extracted with a modified TRIzol protocol analyzed by a *vp60* gene based RT-PCR assay using specific primers for RHDV-2.[17,37] Additionally, 32 of 35 samples were analysed using a *vp60* based RHDV specific RT-qPCR.[15] For both RT-qPCRs, *in vitro* transcribed egfp-RNA was used as an internal amplification control.[18]

Gross pathology

Gross Pathology was performed on all 35 individuals following the standard necropsy protocol of the NRGK. The necropsy reports were analysed regarding the occurrence of typical lesions described for RHD, focusing on the presence / absence of characteristic pathological changes in liver (enlarged size, friable consistency, tan colour, reticulate pattern), spleen (enlarged, congested), and trachea (hyperaemic, presence of foam or fluid), and presence of haemorrhages.[1,14,25]

Histopathology

A total of 18 formalin fixed liver, lung and kidney samples of rabbits, which tested positive by RHDV-2 RT-qPCR, were routinely dehydrated, paraffin embedded and 3µm thin sections were haematoxylin/eosin (HE) stained. After histological evaluation, a subset of six representative fresh samples containing tissue from liver, lung and kidney was further investigated by an immune histological approach. Immune histology was performed using a pool of three monoclonal antibodies (kindly provided by Lorenzo Capucci, OIE Reference Laboratory for RHD diagnostics at Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna, Brescia, Italy) for 1 hour at room temperature detecting the lagoviruses RHDV, RHDV-2 and EBHSV.[5] Aminoethyl carbazole (Dako AEC+ High Sensitivity Substrate Chromogen, Agilent Technologies, California 95051, United States) was used as chromogen and antibody dilutions of 1:1500, 1: 4000 and 1: 8000 were tested. The samples were graded into severe, moderate, and mild positive immune histological reaction. Organ specimens of RHDV-2 negative rabbits served as negative controls.

Results

Analysis of anamnestic reports

An anamnestic report was available for 27 animals. A total of 11 reports stated both, a peracute death of the submitted animal and that other, but usually not all, rabbits of the same rabbitry had died within the last one to six days; in two rabbitries a gap of two and three weeks between cases was observed. Another 16 reports stated either a peracute death (n = 8) or an increased mortality rate (n = 8) (Figure 1).

Molecular diagnostics

A total of 25 liver samples originating from 20 different rabbitries tested positive by RHDV-2 RT-qPCR. Two of these positive samples had been taken at the beginning of the sampling period in March 2015 (Supplementary Table 2). None of 32 samples additionally tested for classical RHDV were positive.

An anamnestic report was available for 18/25 animals that tested positive by RHDV-2 PCR and for 9/10 animals that tested negative. When comparing the data given for peracute death and increased mortality rates between RHDV-2 positive and RHDV-2 negative animals, following patterns appeared: RHDV-2 positive animals showed more often an increased mortality rate (16/18, 89% vs. 3/9, 33%), while RHDV-2 negative animals showed more often a peracute death (8/9, 89% vs. 11/18, 50%) (Figure 1). However,

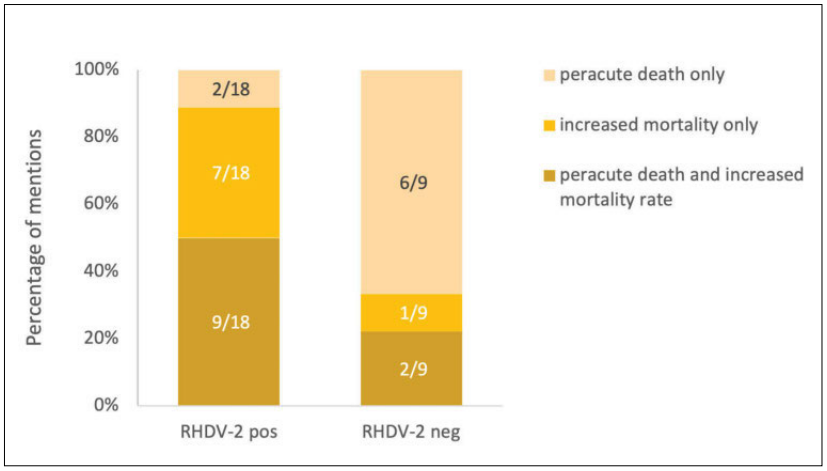

Figure 1: Comparison of the number of mentions of peracute death and increased mortality rate on the anamnestic reports of Rabbit Haemorrhagic Disease virus 2 (RHDV-2) positive and RHDV-2 negative animals. Anamnestic reports were available for 18/25 RHDV-2 positive and 9/10 RHDV-2 negative animals.

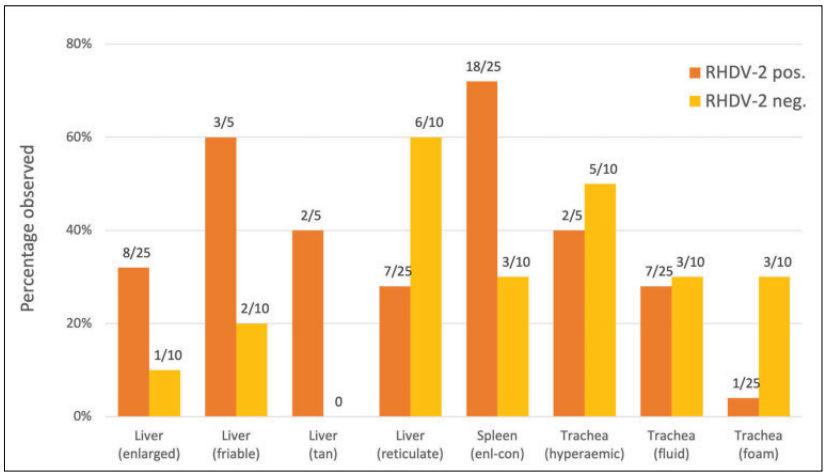

Figure 2: Percentage of observed selected gross pathological changes in liver, spleen and trachea of 10 rabbits tested negative (RHDV-2 neg.) and 25 rabbits tested positive (RHDV-2 pos.) by Rabbit Haemorrhagic Disease virus 2 specific RT-qPCR. Gross pathological changes for the liver included: enlarged size (enlarged), friable consistency (friable), tan colour (tan), reticulate pattern (reticulate); for the spleen enlarged-congested (enl-con); and for the trachea: hyperaemic, presence of fluid (fluid) and presence of foam (foam).

the chance that a peracute death was accompanied by an increased mortality rate was higher for RHDV-2 positive animals (9/11, 82%) as for RHDV-2 negative animals (2/8, 25%) (Figure 1, Supplementary Table 2)

Gross pathology

Post-mortem findings – cause of death or disease

In gross pathology 34 rabbits were of good body condition indicating an acute to peracute course of disease in almost all rabbits. One animal was cachectic. Final diagnosis was death due to infection with RHDV-2 in 25 animals, confirmed by RT-qPCR. Of these, 17 animals showed inconclusive pathological lesions, and eight animals showed lesions indicative of dysentery (n = 2/25), septicaemia (n = 3/25), or both (n = 3/25). In the ten animals that were tested negative for RHDV-2 by RT-qPCR, including the cachectic animal, the cause of death was due to dysentery (n = 5/10), coccidiosis (n = 2/10), septicaemia, pneumonia, and encephalitozoonosis.

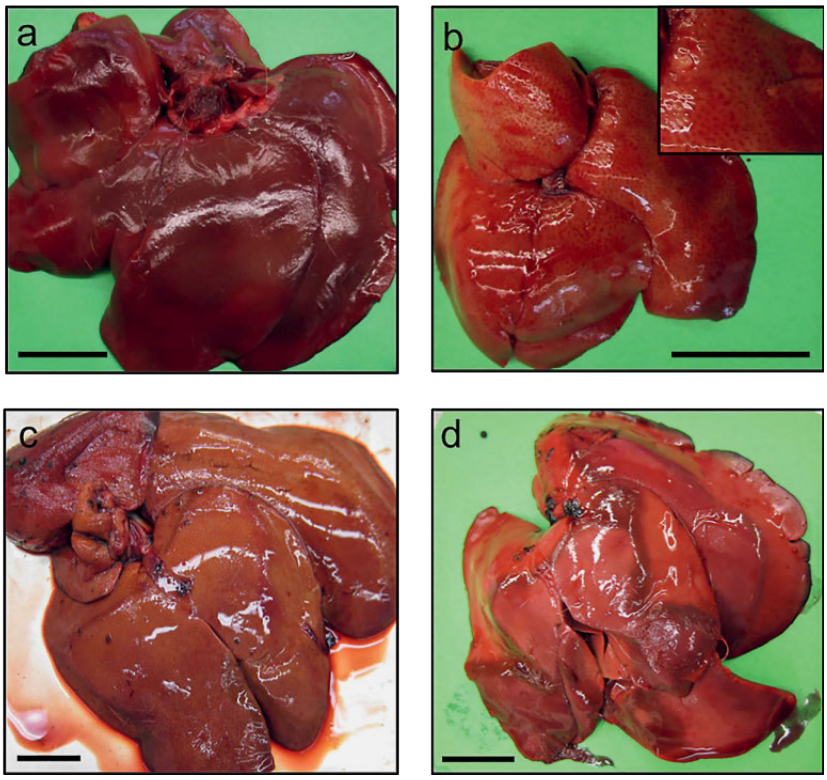

Figure 3: Gross pathological morphology of the liver of naturally infected, unvaccinated Rabbit Haemorrhagic Disease virus 2-positive rabbits. While livers could usually be assigned to more than one of the categories (enlarged size, friable consistency, tan colour, reticulate pattern), the predominant categorization is shown to demonstrate that there is no clearcut deviding line between different morphologies and that there is no morphology typical for RHDV-2 infected animals. a) enlarged (rabbit ID: 18-T0053), b) reticulate pattern (rabbit ID: 18-T0092), c) tan-coloured (rabbit ID: 16-T0421), d) friable consistency (rabbit-ID: 18-T0092); Bar = 2 cm in all pictures.

Gross pathology – in-depth analysis

An in-depth evaluation of gross pathological findings revealed an increased occurrence of a congested or an enlarged-congested spleen as the most prominent and consistent finding in RHDV-2 positive animals (18/25, 72%) compared to RHDV-2 negative animals (3/10, 30%) (Figure 2). Additionally, the occurrence of the four characteristic pathological changes in liver morphology (enlarged size, friable consistency, tan colour, reticulated pattern; Figure 3) revealed slight differences between RHDV-2 positive and negative animals. Positive animals more often showed a friable, a tan, or an enlarged liver, while negative animals more often showed a reticulate pattern (Figure 2). Also, RHDV-2 positive animals more often showed two (10/26, 38% vs. 2/10, 20%) or even three pathological changes (4/26, 15% vs. 0/10, 0%) of the liver at the same time (Supplementary Table 2). However, these findings became only evident after a detailed analysis of necropsy results and were not perceived as outstanding characteristics at the time of post-mortem examinations. The pathological examination of the trachea showed overall a lesser number of distinct pathologies in RHDV-2 positive animals compared to RHDV-2 negative animals (Figure 2). Haemorrhages were not seen in any of these animals.

Histopathology

Histologically, the infection with RHDV-2 in the selected six cases was characterised predominantly by necroses of hepatocytes, to a lesser degree of pneumocytes and even fewer necroses of renal tubular epithelia. Immune histologically, virus infected cells demonstrated a specific cytoplasmic and nuclear reaction, best at an antibody dilution of 1:8000 (Figure 4). All used lower antibody dilutions (1:1500 and 1:4000) gave a too strong, partly nonspecific reaction in immune histochemistry.

Liver:

Liver tissue of mildly affected cases showed multifocal necroses of single or small groups of hepatocytes with pyknosis, karyorrhexis, and focally mild acute haemorrhage or sinusoidal hyperaemia (Figure 2 a, b). In cases graded as moderate, partly confluent areas of necrosis with mild accumulation of cellular debris, containing single heterophils were present (Figure 2 e, f). In severe cases with larger areas of hepatic necroses, an acute, more intense infiltration of the hepatic parenchyme with heterophilic granulocytes were present (Figure 2 i, j). The borderline of acute hepatic haemorrhage and sinusoidal acute hyperaemia appeared blurred. Hence in none of the specimens investigated, evidence of haemosiderosis was observed, if haemorrhage was present, it was an acute one.

Immunohistochemistry confirmed the infection of hepatocytes with a mildly stronger positive infection of hepatocytes in hepatic zones one and three, i.e., periportal and centri-

lobular regions, compared to hepatocytes of zone two in mild and moderate cases.

Lung:
Histological specimens of the lungs were characterised by moderate acute hyperaemia and alveolar oedema with focally mild intra-alveolar acute haemorrhages and desquamation of singular alveolar lining cells, arterial constriction, and venous dilation. Comparison of HE sections with immune histological sections showed, that stronger virus infected parts of the lungs showed additionally areas of atelectasis with focal single cell necroses of pneumocytes. There was no evidence of inflammatory processes.

Kidney:
In the kidney, single immune histologically positive epithelia of collecting tubes exhibited necrosis. Individual distal lumina of tubuli demonstrated a mild protein cast formation as well as mild acute hyperaemia in the transition zone between cortex and medulla.

Discussion

Subtle indication for death by RHDV-2 from the anamnestic reports

The comparison of the mentioning of peracute death and increased mortality rate within the holding in the anamnestic reports clearly showed that a combination of peracute death and increased mortality rate was more often mentioned in RHDV-2 positive animals than in RHDV-2 negative animals, while an increased mortality rate alone was more often mentioned in RHDV-2 positive animals and peracute death alone was mentioned more often in RHDV-2 negative animals. However, it has to be kept in mind that the anamnestic reports are usually written by the owner of the submitted animal and it can be assumed that information may be omitted due to missing awareness regarding its relevance. Still, the mentioning of an increased mortality rate or a combination of increased mortality rate and peracute death in the anamnestic report may provide a stronger indication towards a possible RHDV-2 infection for the pathologist than an anamnestic report stating peracute death alone. Nonetheless, none of these findings is conclusive. In our experience, mass mortality in large rabbitries with peracute deaths of young and adults is highly suspicious, but in small pet rabbitries one dead rabbit out of three could also be RHD.

No pathognomonic post-mortem findings

A detailed analysis of the occurrence of selected pathologies of the organs liver, spleen, and trachea – all tissues previously described to exhibit typical lesions in animals suffering from RHD – revealed a distinct accumulation of findings in RHDV-2 positive and RHDV-2 negative animals. However, in spite of this detailed analysis, no typical lesions or combination of lesions could be identified for RHDV-2 positive animals. The most prevalent pathological finding in RHDV-2 positive animals was a congested or enlarged-congested spleen, which may also be caused by various other diseases such as septicaemia due to *Pasteurella multocida* (pasteurellosis) or lymphoma, but could also be an agonal change. Also, within this study, only rabbits positive for RHDV-2 showed a tan liver, which might be caused by any disease affecting the liver function including fatty liver syndrome or pregnancy toxaemia in does.[2]

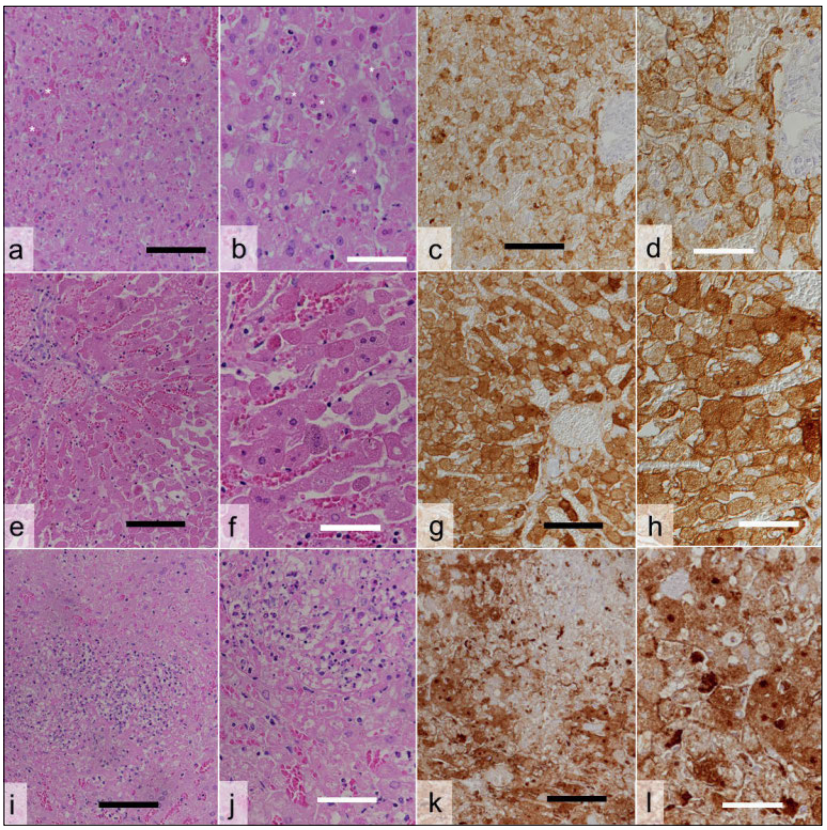

Figure 4: Histopathological images (HE and immunostaining) of the liver of naturally infected, unvaccinated Rabbit Haemorrhagic Disease virus 2-positive rabbits.

Figure 4 a–d) Mild case. **a)** Overview. Sections of the liver demonstrated multifocal diffuse hepatocellular necroses, i.e. a lack of nuclei and mild acute haemorrhage / sinusoidal hyperaemia (asterisks; 200x HE). **b)** Higher magnification. Multiple pyknotic nuclei (asterisks) within partially necrotic hepatocytes (400x, HE). **c, d)** Immune histochemistry. Scattered mild to moderately virus antigen positive hepatocytes of zones 1-3 (200/400x, anti RHDV-2 antibody).
Figure e–h) Moderate case. **e, f)** Moderately infected livers were characterised by mild haemorrhage / sinusoidal hyperaemia, dissociation and swelling of hepatocytes as well as multifocal necroses (200/400x, HE). **g, h)** Immune histochemistry. Hepatocytes of zones 1–3 demonstrated a stronger positive immune reaction (200/400x, anti RHDV-2 antibody).
Figure i–l) Severe case. **i, j)** Large, partly confluent areas of hepatocellular necrosis with accumulation of cellular debris and mild infiltrates of heterophilic granulocytes (200/400x, HE). **k, l)** Immune histochemistry. Larger areas of necrosis in which hardly any or no antigen could be demonstrated, alternating with strongly virus positive hepatocytes (200/400x, anti RHDV-2 antibody).
Black bars = 100 µm; white bars = 50 µm

This is in line with the findings of Neimanis and others (2018) in artificially infected rabbits: gross pathological lesions focused on the liver with degenerative changes (tan colour) found in all five juveniles and one of the two adults that were included in the study.[28] Additionally, both adults showed an enlarged spleen. The latter finding, an enlarged spleen without any further pathological findings in gross pathology, reflects our experience in euthanised or pretreated (e.g. fluid substitution) pet rabbits positive for RHDV-2: typical gross lesions may be absent in the liver, and an enlarged spleen (Neimanis and others 2018; own observation) may be the only macroscopic finding (own observational data, no evaluation performed).[28]

The predominant morphological alteration in tissues from liver, lung and kidney are cellular necroses of hepatocytes, pneumocytes and tubular epithelia. Most intensively affected was the liver, followed by lung and kidney. Hence no strong inflammatory reaction or evidence of haemosiderosis could be observed, the lesions were thus interpreted as acute, regardless of the intensity of the lesions. The latter most likely was related to either the infectious dose, the intensity of virus replication, or the susceptibility of the host.

RHD as differential diagnosis to gastrointestinal disease

Rabbits have a delicate intestinal tract and any condition accompanied by anorexia quickly results in gastrointestinal disease, as also evidenced in this study where 6/10 RHDV-2 negative animals died due to dysentery or coccidiosis.[11] Metabolic complications in rabbits may develop quickly either directly through changes in management/feed or indirectly in case of reduced appetite due to various causes including an infection with RHDV-2. Thus, gastrointestinal disease may be both: a differential diagnosis to RHD or a secondary condition due to RHDV-2 infection.

Unknown prevalence and distribution of RHDV-2 in Switzerland

The first detection of RHDV-2 in Switzerland was officially reported for 2016 (OIE WAHID), but our study retrospectively allowed to identify an earlier case from March 2015 from southern Switzerland (Supplementary Table 1).[34] As reported for other countries (see above), RHDV-2 seems to have largely replaced classical RHDV in Switzerland: between 2015 and 2020 there was only a single detection of classical RHDV in May 2018. However, it remains unclear, how RHDV-2 was introduced into the Swiss population of domestic rabbits. Since the wild rabbit population of Switzerland is restricted to two small colonies in the southwest of the country, the introduction via wild rabbits is highly unlikely.[19]

Prevalence or distribution of RHDV-2 in Switzerland cannot be estimated, but it is assumed that the number of actual cases is much higher than the number of submitted and confirmed cases (e.g. 20 confirmed cases in 2019, 12 confirmed cases in 2020). This assumption is based upon several factors: (i) the necropsy has to be paid by the rabbit owner, i.e. it might not be commissioned in the case that only a few animals died, or all animals of the rabbitry have died, (ii) shortly after RHDV-2 introduction, there was no suspicion of RHDV-2-infection especially in small pet rabbitries and owners did not see the need for necropsy as non-infectious causes of death (e.g. metabolic complications) were assumed, (iii) once that RHDV-2 circulation was known among rabbit owners, they might have suspected RHDV-2-infection in case of sudden increased mortality and drew their consequences without bothering to increase their financial losses by commissioning a necropsy, (iv) vaccination in pedigree rabbits has been mandatory since 2016 (Swiss rabbit breeder association).

Conclusion

RHD caused by RHDV-2 may not be diagnosed using gross pathology alone. The anamnestic report, the lack of evidence for an alternative cause of death and perhaps the presence of rather unspecific findings such as a congested spleen or a tan liver should catch the attention of the investigator to solicit further analyses.

The authors state / specify:

- In case of unclear cause of death and/or suspicious anamnestic report, the pathologist / clinician should keep in mind an infection with RHDV-2 and solicit further analysis (e.g. PCR or immunohistochemistry), even if the macroscopic picture is rather unspecific.
- Post-mortem lesions caused by RHDV-2 may be discrete, and especially in euthanized or pretreated (e.g. fluid substitution) pet rabbits typical changes in liver morphology may be missing with an enlarged spleen being the only finding.
- Rabbits are prone to develop metabolic complications in case of reduced appetite, thus in gross pathology, lesions for RHDV-2 infection may be masked by signs of dysbiosis/enteropathy caused by anorexia due to RHDV-2 infection.-

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Constatations post-mortem peu évidentes chez des lapins naturellement infectés par le virus 2 de la maladie hémorragique du lapin en Suisse

Le virus 2 de la maladie hémorragique du lapin (RHDV-2) est apparu en France en 2010. En Suisse, le RHDV-2 a été identifié pour la première fois en 2015 et semble avoir presque remplacé le virus classique de la maladie hémorragique du lapin (RHDV). Comme le RHDV, le RHDV-2 provoque une hépatite virale avec une évolution suraiguë et un taux de mortalité élevé chez les lapins. L'infection par le RHDV entraîne des constatations pathologiques bruts cohérents, notamment au niveau du foie et des voies respiratoires. Les rapports sur la pathologie macroscopique des animaux naturellement infectés par le RHDV-2 sont rares.

La présente étude a analysé les rapports d'anamnèse et de nécropsie de 35 lapins examinés lors de diagnostics de routine entre mars 2015 et mai 2017. Une réaction en chaîne par polymérase en temps réel à la transcriptase inverse (RT-qPCR) spécifique pour le RHDV-2 et le RHDV a prouvé qu'un total de 25 animaux étaient positifs pour le RHDV-2, tandis qu'aucun n'était positif pour le RHDV. De plus, des examens histologiques ont été réalisés sur le foie, les poumons et les reins de 18 lapins qui avaient été testés positifs par RT-qPCR pour le RHDV-2.

Le rapport anamnestique faisait plus souvent état d'un taux de mortalité accru chez les lapins RHDV-2 positifs (16/18, 89 %) que chez les lapins RHDV-2 négatifs (3/9, 33 %). La pathologie macroscopique n'a révélé aucun changement pathognomonique chez les animaux RHDV-2 positifs. Sur le plan histologique, le foie présentait les lésions les plus graves, suivi des poumons et des reins. Les animaux positifs pour le RHDV-2 présentaient fréquemment des signes de maladie gastro-intestinale (n = 5) et/ou de septicémie (n = 6) masquant les indicateurs possibles d'une infection par le RHDV-2, tels que les découvertes plutôt peu spécifiques d'une rate hypertrophiée ou d'un foie hypertrophié, friable et de couleur beige.

Les auteurs souhaitent sensibiliser les cliniciens et les pathologistes au fait qu'en cas de mort subite chez des lapins d'élevage ou de compagnie, le RHDV-2 doit être considéré comme un diagnostic différentiel et doit être confirmé par un diagnostic de laboratoire.

Mots clés: Pathologie macroscopique, Histopathologie, PCR, Virus de la maladie hémorragique du lapin 2, Suisse

Reperti necroscopici poco evidenti nei conigli in Svizzera naturalmente infettati dal virus 2 della malattia emorragica del coniglio

Il virus 2 della malattia emorragica del coniglio (RHDV-2) è emerso in Francia nel 2010. In Svizzera, il RHDV-2 è stato identificato per la prima volta nel 2015 e sembra aver quasi sostituito il classico Rabbit Haemorrhagic Disease Virus (RHDV). Come l'RHDV, l'RHDV-2 causa un'epatite virale con un decorso peracuto e una maggiore mortalità nell'allevamento di conigli. L'infezione da RHDV causa risultati patologici evidenti, specialmente nel fegato e nelle vie respiratorie. I resoconti sulla patologia macroscopica degli animali infettati naturalmente con RHDV-2 sono scarsi.

Il presente studio ha analizzato i resoconti anamnestici e necroscopici di 35 conigli esaminati durante la diagnostica di routine tra marzo 2015 e maggio 2017. Una reazione a catena della polimerasi in tempo reale con trascrittasi inversa (RT-qPCR) specifica al RHDV-2 e al RHDV ha dimostrato che un totale di 25 animali era positivo al RHDV-2, mentre nessuno era positivo al RHDV. Inoltre, sono stati eseguiti esami istologici su fegato, polmoni e reni di 18 conigli che erano risultati positivi alla RHDV-2 RT-qPCR.

Il resoconto anamnestico indicava più di frequente un aumento del tasso di mortalità nei conigli positivi al RHDV-2 (16/18, 89%) rispetto ai conigli negativi al RHDV-2 (3/9, 33%). I resoconti della patologia macroscopica non hanno rivelato alcun cambiamento patognomonico negli animali RHDV-2 positivi. Istologicamente, il fegato ha mostrato le lesioni più gravi seguite da polmone e rene. Gli animali positivi al RHDV-2 hanno spesso mostrato segni di malattia gastrointestinale (n = 5) e/o setticemia (n = 6), che potrebbero mascherare possibili risultati non specifici di infezione da RHDV-2, come una milza o un fegato ingrossato, friabile e di colore marrone chiaro.

Gli autori vogliono rendere attenti al fatto che in caso di morte improvvisa dei conigli commerciali o da compagnia, l'RHDV-2 deve essere considerato nella diagnosi differenziale e confermato dalla diagnosi di laboratorio.

Parole chiave: patologia macroscopica, istopatologia, PCR, virus della malattia emorragica del coniglio 2, Svizzera

Inconspicuous post-mortem findings in rabbits from Switzerland naturally infected with Rabbit Haemorrhagic Disease Virus 2

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Supplementary Table 1: Country and year of first detection of Rabbit Haemorrhagic Disease Virus 2 in Europe

year	country	reference
2010	France	20,22
2010	United Kingdom	38
2011	Italy	36
2012	Spain	6
2012/13	Portugal	1
2013	Germany	13
2013	Sweden	27,35
2014	Norway	31
2014	Azores Islands	9
2015	Denmark	32
2015*/16	Switzerland	34
2016	Finland	33
2017	the Netherlands	10

*the current study retrospectively identified two cases from 2015

Supplementary Table 2: Presence (1) and absence (0) of selected gross pathological changes in liver, spleen, and trachea of 10 rabbits tested negative (neg) and 25 rabbits tested positive (pos) by Rabbit Haemorrhagic Disease Virus 2 (RHDV-2) specific RT-qPCR, and with an anamnestic report stating (1) or not stating (0) a peracute death (peracute) or an increased mortality rate (mortality). If available, age class is given as juvenile (juv) or adult (ad).

Rabbit ID	Age class	RHDV-2	Clinical history		Liver				Spleen		Trachea		
		RT-PCR	peracute	mortality	enl	fri	tan	ret	enl	con	hyp	foa	flu
15-T0226-1	juv	neg	0	1	0	0	0	1	0	0	0	0	1
15-T0396	ad	neg	1	0	0	1	0	1	0	0	1	0	0
15-T0447	n/a	neg	1	1	0	0	0	0	0	0	1	1	0
15-T0583	juv	neg	1	0	0	0	0	1	0	0	0	0	1
15-T0621	n/a	neg	1	0	0	0	0	0	0	0	1	0	0
16-T0088	n/a	neg	1	0	0	0	0	1	0	1	1	1	0
16-T0092	ad	neg	1	0	0	0	0	0	0	0	0	0	0
16-T0123	ad	neg	1	0	0	0	0	1	0	1	0	0	1
16-T0145	n/a	neg	n/a	n/a	1	1	0	0	0	1	0	1	0
16-T0165	ad	neg	1	1	0	0	0	1	0	0	1	0	0
		total	**8/9 (89%)**	**3/9 (33%)**	**1/10 (10%)**	**2/10 (20%)**	**0/10 (0%)**	**6/10 (60%)**	**0/10 (0%)**	**3/10 (30%)**	**5/10 (50%)**	**3/10 (30%)**	**3/10 (30%)**
15-T0085-2	ad	pos	1	0	0	0	0	0	0	0	0	0	0
15-T0085-1	ad	pos	1	0	0	0	0	0	0	0	0	0	0
16-T0131	ad	pos	n/a	n/a	0	1	1	0	0	1	0	0	0
16-T0138	n/a	pos	0	1	1	1	0	0	0	1	0	0	0
16-T0143-2	ad	pos	n/a	n/a	1	1	0	0	0	0	0	0	0
16-T0143-3	ad	pos	0	1	1	1	0	0	0	0	0	0	0
16-T0151	ad	pos	1	1	0	1	1	0	0	1	0	0	0
16T0152-1	ad	pos	1	1	0	1	0	0	1	1	0	0	0
16-T0152-2	juv	pos	1	1	0	1	1	0	1	1	0	0	0
16-T0412	ad	pos	0	1	0	0	0	0	0	1	0	0	0
16-T0421-1	ad	pos	0	1	1	0	1	1	1	1	1	0	1
16-T0481	ad	pos	1	1	0	1	1	1	0	1	0	0	1
16-T0514	juv	pos	n/a	n/a	1	1	0	1	0	0	0	0	1
16-T0604	juv	pos	0	1	1	1	1	0	0	1	0	0	0
16-T0614	ad	pos	n/a	n/a	0	1	1	0	0	1	1	0	0
16-T0617	ad	pos	n/a	n/a	1	0	0	0	0	0	1	0	0
16-T0626	n/a	pos	n/a	n/a	0	1	0	0	0	0	0	0	0
16-T0656-1	ad	pos	0	1	0	0	1	0	1	1	1	0	0
16-T0656-2	ad	pos	0	1	0	0	0	0	1	1	1	0	0
16-T0662	juv	pos	1	1	0	1	1	0	1	1	1	1	0
16-T0670-1	ad	pos	1	1	0	0	0	1	1	1	1	0	1
16-T0670-2	ad	pos	n/a	n/a	1	0	0	1	1	1	1	0	1
16-T0673	ad	pos	1	1	0	0	0	1	0	1	1	0	1
16-T0116	ad	pos	1	1	0	1	1	0	0	1	0	0	0
16-T0187	n/a	pos	1	1	0	1	0	1	0	1	1	0	1
		total	**11/18 (61%)**	**16/18 (89%)**	**8/25 (32%)**	**15/25 (60%)**	**10/25 (40%)**	**7/25 (28%)**	**8/25 (32%)**	**18/25 (72%)**	**10/25 (40%)**	**1/25 (4%)**	**7/25 (28%)**

Gross pathological changes include for the liver: enl – enlarged size / fri – friable consistency / tan – tan colour / ret – reticulate pattern, for the spleen: enl – enlarged / con – congested, and for the trachea: hyp – hyperemic / foa – presence of foam / flu – presence of fluid; n/a – not available