Journal of the Hellenic Veterinary Medical Society

Vol. 71, 2020

A Review on Rabbit Hemorrhagic Disease with a Special Reference to Egyptian Situation

ABD EL-GHANY WAFAA A Poultry Diseases Department, Faculty of Veterinary Medicine, Cairo University, 12211 Giza, Egypt

https://doi.org/10.12681/jhvms.25065

To cite this article:

ABD EL-GHANY, W. (2020). A Review on Rabbit Hemorrhagic Disease with a Special Reference to Egyptian Situation. *Journal of the Hellenic Veterinary Medical Society, 71*(3), 2243-2250. doi:https://doi.org/10.12681/jhvms.25065

J HELLENIC VET MED SOC 2020, 71(3): 2243-2250
ΠΕΚΕ 2020, 71(3): 2243-2250

Review article
Ανασκόπηση

A Review on Rabbit Hemorrhagic Disease with a Special Reference to Egyptian Situation

Wafaa A. Abd El-Ghany

Poultry Diseases Department, Faculty of Veterinary Medicine, Cairo University, 12211 Giza, Egypt,

ABSTRACT: Rabbit hemorrhagic disease (RHD) is considered as one of the most important viral diseases that affects and threatens rabbit's flocks. This disease has affected rabbits since mid-1980. Two epidemics of RHD had been discovered; the first was in mid-1980 and known as classical RHD virus (RHDV), while the second was in 2011 and described as variant virus (RHDVb/RHDV2). Domestic and wild rabbits are susceptible to RHD. All ages can be affected, but adults are more susceptible to young kitten. RHD is presented in three forms; per-acute, acute and subacute or chronic form. Mortality rate is usually high especially in per-acute and acute stages and it is associated with disseminated intravascular coagulopathy and necrotic hepatitis. The main lesions have been observed in the liver, lungs and spleen. Diagnosis of RHD is based on the clinical picture and detection of RHDV or specific antibodies. The prevention and control strategies depend mainly on using of preventive inactivated vaccine together with adoption of hygienic measures. However, there is no specific treatment of RHDV infection. So, this review article puts a spot light on RHD regarding the epidemiology, the clinical and laboratory diagnosis as well as the prevention and control strategies with a special reference to Egyptian situation.

***Keywords*:** RHD, Epidemiology, Diagnosis, Control, Egypt

Corresponding Author:
Wafaa A. Abd El-Ghany
Poultry Diseases Department, Faculty of Veterinary Medicine, Cairo University, 12211 Giza, Egypt
E-mail address: wafaa.ghany@yahoo.com

Date of initial submission: 17-07-2020
Date of revised submission: 28-07-2020
Date of acceptance: 10-09-2020

INTRODUCTION

Egypt is considered as the fourth largest country that produce rabbit meat worldwide (FAO, 2019). Egyptians prefer rabbit's meat for its great benefits and for the characteristics of carcass traits (Alboghdady and Alashry, 2010). An improvement of rabbit industry is a goal to solve the shortage of meat after poultry industry.

Rabbits are highly susceptible to many serious diseases problems that have adverse effects on rabbits industry in Egypt (Hamed et al., 2013). One of these diseases is rabbit hemorrhagic disease (RHD) which has been considered as fetal, highly contagious, and world-wide viral disease. This disease was also previously named as rabbit viral sudden death, hemorrhagic septicemia syndrome, viral hemorrhagic pneumonia and rabbit viral hemorrhagic disease (Mitro and Krauss, 1993). RHD induces great and significant economic importance in terms of high mortality and morbidity as well as great losses in meat and fur production of Egypt (Mohamed, 2009; Fahmy et al., 2010). RHD is notifiable as authorities should be immediately reported in case of outbreaks (OIE, 2018). RHD is caused by RHD virus (RHDV) which is belonging to Calicivirus family and *Lagovirus* genus. There is only one serotype for all classical pathogenic strains of RHDV, however, two subtypes, RHDV and RHDVa, have been found (CFSPH, 2006). In 2011, other variant virus (RHDVb/RHDV2) have also been recorded. Acute RHD infection is characterized by sudden respiratory and nervous manifestations as well as by high mortality due to disseminated intravascular coagulopathy. There is no specific treatment, however, prevention of RHD is based mainly on using of vaccines as well as adoption of hygienic measures (ITAVI, 2019).

Serious studies have been conducted in Egypt to update the epidemiological situation of RHD among rabbit farms (Awad and Kotb, 2018; Magouzi et al., 2019; Erfan and Shalaby, 2020) and to spotlight on vaccine production trials (Abd El-Motelib et al. 1998; Khodeir and Daoud, 2002; Eid and Ibraheem, 2006).

Thus, this article review focuses on rabbit hemorrhagic disease regarding the epidemiology, the clinical and laboratory diagnosis as well as the prevention and control strategies with a special reference to Egyptian situation.

HISTORY AND INCIDENCE

The first clinical report of RHD was in 1980s in China where the disease killed 14 million of European Angora rabbits within 9 months (Liu et al., 1984). A year later in China, Xu (1991) recorded death of 140 million domestic rabbits due to RHD. Later, the disease showed rapid spread to Italy (Cancellotti and Renzi, 1991) and became endemic in several European, Australian, New Zealand, Asian and African countries (Berninger and House, 1995; Kovalisk, 1998; Le Gall-Recule et al., 2003; Alda et al., 2010; Abrantes et al., 2012).

The first record of RHDV in Egypt was in spring of 1991, in El-Sharkia governorate, where the virus was associated with 90% mortality rate (Ghanem and Ismail, 1992). Later, RHD was recorded in El-Kaluobia governorate (Sharawi, 1992). Salem and El-Ballal (1992) detected presence of RHDV in Upper Egypt (Assiut) governorate in winter of 1992. In 1993, RHDV was isolated from 14-16 week-old rabbits with 26.7%-100% losses in El-Minya, Assiut and Sohag governorates (El-Zanaty, 1994). Since this time, subsequent disease outbreaks have been recorded in different Egyptian governorates. During the period from 1994 to 1996, about 25 outbreaks of circulating RHDV were demonstrated in Cairo, Giza, El-Kalubia, Kafr-El-Sheikh, El-Dakahlia, El-Gharbia and Marsa-Matroh governorates (El-Mongy, 1998). Both RHDV and *Pasteurella multocida* (*P. multocida*) had been found in three cases of rabbits in Alexandria governorate (Ibrahim et al., 1999). In 2000, in Assuit governorate, clinical picture of epistaxis, incoordination of the gait, convulsion and vaginal bloody exudate were observed in rabbit flocks with RHDV (Abd El-Ghafar et al., 2000). Although presence of restricted regimen program for vaccination of Egyptian rabbit flocks against RHDV, several outbreaks of the disease were reported (Mostafa, 2001; Abd El-Lateff, 2006; Ewees, 2007; El-Sissi and Gafer, 2008). At Kafr El-Sheikh governorate, 15 outbreaks of vaccinated rabbit flocks with signs and lesions similar to that of RHDV were investigated (Metwally and Madboully, 2005). Recently, the nucleotide sequencing of viral protein (VP60) gene of RHDV was characterized from outbreaks of non-vaccinated rabbit flocks in different Egyptian governorates (Awad and Kotb, 2018; Magouzi et al., 2019; Erfan and Shalaby, 2020).

Infection and transmission

Infection by RHDV occurs mostly through oral, nasal, and conjunctival routes. The specific receptors of the virus are mainly found in the upper respira-

tory and digestive tract of susceptible animal (Ruvoen-Clouet et al., 2000). Transmission of RHDV is usually happen through faecal-oral route (Morisse et al., 1991). Close contact animals may gain the virus through aerosol (Campagnolo et al., 2003). Direct and indirect contact with the excreta of infected and dead animals are also possible routes of disease transmission (Ohlinger et al., 1993). The virus remains viable for long periods in urine, feces and respiratory secretions (Ohlinger et al., 1993) as well as fur (Mitro and Krauss, 1993). The viral RNA of RHDV may persist in the environment for 15 weeks, and the recovered animal can shed the virus for months. Contaminated bedding with urine and feces of infected animals may also be considered as a source of RHDV (Cooke, 2002). Furthermore, it has been found that RHDV can remain viable and infective on decomposed or dead carcass for 12 weeks under harsh environmental conditions (McColl et al., 2002). The lengthy persistence of infective RHDV on dead animals may help the disease spread and outbreaks in the wild (Henning et al., 2005). The virus can be excreted in the feces of predators or scavengers that fed on dead infected carcass (Merchán yet al., 2011).

Chilled and frozen rabbit carcasses can carry RHDV for several months. An outbreak of RHDV in Mixco has been reported due to imported infected carcasses from China (Belz, 2004). Due to high stability of the RHDV and resistance to environmental condition, the virus can spread via fomites and contaminated food, water, clothes, cages, and equipment (Chasey, 1997).

In addition, insects or flies may play a role in transmission of infection as the virus can persist in flies for 9 days (Asgari et al., 1998; OIE, 2008). Rodents (Broja and Larios 1990; Xu, 1991), while wild animals (Cooke, 2002) are also very important mechanical vectors of RHDV. Workers in contact with RHDV infected rabbits or with their excretions act also as mechanical vectors (CFSPH, 2006).

Susceptibility and clinical disease picture

Domestic (especially European species; *Oryctolagus Cuniculus*) and wild rabbits are susceptible to RHD (Gould et al., 1997; Muller et al., 2009; Miao et al., 2019; Urakova et al., 2019). Infection of hares with variant strain of RHDV (European brown hare syndrome virus) has been recorded. However, susceptibility of other leporid species to the virus has not been reported (Gregg et al., 1991). Pregnant and lactating does are more susceptible to RHDV infection (El-Sissi and Gafer, 2008).

The susceptibility age of rabbits to RHDV is still contradicted. The virus can cause higher mortality in adults than young kittens as deaths are not common in rabbits less than 4-weeks- old. This may be related to the presence of specific receptors in adults but not in young animals (Dalton et al., 2012). Resistance to RHD infection decreases at ages 4-12 weeks.

The incubation period of RHDV varies from16 to 48 hours and deaths appear after 2 to 3 days post infection. The disease course may last up to 30 days (CFSPH, 2006). The severity of clinical signs differs according to the breed of animal, age, immunity, geographical location, the infecting viral dose and the route of infection.

Sub-clinical infection of RHD is possible in young kittens less than 4-8 weeks old. In per-acute stage, animals in a good heath conditions, die suddenly without prior clinical signs within 12 to 36 hours of the disease onset (Belz, 2004). Severe clinical manifestations were seen in adults as well as rabbits older than 40-50 days of age (Capucci et al., 1991). Acute stage of RHD in rabbits is characterized by fever, depression, anorexia, conjunctivitis, frothy bloody nasal discharge, epistaxis, vulvar hemorrhages in pregnant does, severe respiratory distress (cough and dyspnea) and finally nervous manifestations (ataxia, convulsion, opisthotonos and paralysis) (Xu and Chen, 1989; Marcato et al., 1991; Trzeciak-Ryczek et al., 2015). Clinical picture associated with variant strains of RHDV is similar to classical strains, however the mortality rate may be comparatively lower (Le Gall-Recule et al., 2013). Severe jaundice, emaciation, lethargy, constipation or diarrhea and abdominal distension followed by death within few weeks have been observed in sub-acute and chronic stages of RHD (Capucci et al., 1991; CFSPH, 2006). Animals with sub-acute infection showed mild or minor signs with resistance to RHDV re-infection due to development of specific antibodies (Patton, 1989; Mitro and Krauss, 1993).

The morbidity rate of RHDV varies from 30-100%, and the mortality rate ranges from 40-100% within a period of 2-3 days after infection (Abrantes et al., 2012). High morbidity and mortality rates have been recorded mainly in adult animals and those kept in groups (Mitro and Krauss, 1993). Deaths occur as a result of disseminated intravascular coagulopathy

resulting in extensive hemorrhages in most organs as well as due to necrotizing hepatitis (Marcato et al., 1991; Plassiart et al., 1992).

Post-mortem lesions of RHDV infection in rabbits have been represented as generalized congestion and haemorrhages (Ueda et al., 1992; Marques et al., 2010), and acute and necrotizing hepatitis (Park et al., 1995; Alonso et al., 1998; Abrantes et al., 2012). Congestion and ulceration of nasal mucosa, haemothorax, frothy exudates in trachea, haemorrhages with multiple abscesses in lungs and pneumonia, splenomegaly, subcutaneous abscesses and congestion of the brain have also been observed (Eid and Ibraheem, 2006; Lavassa and Capucci 2008; Embury-Hyatt et al., 2012). Severe lesions of RHD appeared in liver, lungs and trachea (OIE, 2010). Animals died in sub-acute stage showed catarrhal enteritis and icterus. Hamed et al. (2013) estimated that severely affected organs as liver, lungs, spleen and kidneys in RHD outbreaks are the main causes of high mortality.

Histopathological lesions of RHD cases revealed severe congested visceral organs, dilated liver sinusoids with diffuse and focal hemorrhages and inflammatory cells infiltration, severe interstitial pneumonia and hemorrhagic alveoli, glomerulonephritis with haemorrhages, hemorrhagic tracheitis with sloughed mucosal epithelium and hemorrhagic myocarditis (Ramiro-Ibáñez et al., 1999; Ferreira et al., 2006; Soliman et al., 2016). Suppression of immune response in RHD infected animals is related to severe decrease in number of B and T lymphocytes of the liver and spleen (Marques et al., 2010).

Laboratory diagnosis

The diagnosis of RHD depends mainly on the clinical picture, histopathological lesions, detection of the virus using electron microscopy, immunostaining and molecular characterization and detection of antibodies using haemagglutination (HA) inhibition test and Enzyme-Linked Immuno-Sorbent Assay (ELISA) (Lavazza and Capucci, 1996).

The first step of laboratory diagnosis of RHDV is HA test using human type “O” (Liu et al., 1984; Pu et al., 1985; OIE, 2008) or Guinea pig and sheep erythrocytes (Sahar et al., 2011). The sensitivity as well as specificity of HA test appear to be inadequate. In Egypt, since 2007, variant strains of RHDV are circulating in rabbit’s flocks with typical signs, lesions and mortality rate similar to classical RHDV strains but these variants are non-haemagglutinating (Ewees, 2007; El-Sissi and Gafer, 2008). So, the diagnosis of RHD may not depend on the HA characters of RHDV as some variant strains showed changeable HA as negative HA strains have been turned into positive ones when passaged in susceptible rabbits (Abd El-Moaty et al., 2014).

RHD virus is ether and chloroform resistant due to lacking of the fatty envelope. The polypeptide of 60 KDa is enough to classify RHDV as Calicivirus (Clouet et al., 1995). The virus of RHD is non-enveloped, single-stranded ribonucleic acid (RNA) with icosahedral symmetry capsid and diameter 32-44 nm (Wang et al., 2013). The virus capsid is containing a protein (VP60), that encoded by RHDV genome contains specific antigenic epitope (hypervariable region E) (Capucci et al., 1998). The domain P of the virus is important for binding to host cells while P2 sub-domain is responsible for genetic variation (Wang et al., 2013). The virus also is a positive-sense RNA that contains extra structural proteins (sub-genomic RNA) of approximately 2.2 kb which is required for infection in later stages (Abrantes et al., 2012; Ismail et al., 2017). The genetic variation between viruses of RHD is mainly depends on the sequence of VP60 protein (Le Gall-Recule et al., 2003; Forrester et al. 2006’ McIntosh et al., 2007; Forrester et al. 2008; Wang et al., 2013).

Strains of RHDV belong to one serotype but the virus has a high genetic mutation rate (Gould et al., 1997). New variant strains of RHDV were detected in vaccinated rabbits for the first time in Italy and Germany in 1998 and 1999 (Capucci et al., 1998; Schirrmeier et al., 1999). The phylogenetic analysis of the strains belonging to RHDV can be classified into three groups; classical RHDV with geno-groups G1-G5, the antigenic variant RHDVa/G6 (Le Gall-Recule et al., 2003) and the new type RHDV2/RHDVb (Le Gall-Recule et al., 2013).

The liver is considered as the organ of choice for detection of RHDV where the highest virus concentration was demonstrated especially in acute or peracute disease (Abd El-Motelib, 1993; Ahmad et al., 2011). High amounts of the virus may also present in the secretions and excretions of the infected animals as well as the blood. In chronic prolonged stage of infection, the virus could be detected in spleen.

Culturing of RHDV on the tissue cultures is difficult, so detection of the viral gene or antibody are

very important for diagnosis (OIE, 2012). Reverse Transcriptase-Real Time polymerase Chain Reaction (RT-PCR) is considered as a rapid and sensitive method for characterization of specific nucleic acid of RHDV (Guittre et al., 1995; Soliman et al., 2016) as well as detection of the viral RNA in the animals's serum (Moss et al., 2002). About 98.7% homology in N-terminal part of the capsid protein which is conserved portion of RHDV has been detected by RT-PCR (Guittre et al., 1995). *In situ* hybridization or RT-Loop-Mediated Isothermal Amplification (RT-LAMP) assay also was described for detection of RHDV RNA in blood, feces and urine.

Inoculation of susceptible animals with RHDV can be used experimentally detection of the pathogenicity of the isolated strains.

Prevention and control

Management of RHD outbreaks depends on the epidemiological situation of the disease in the region and the monitoring process of the field viruses to detect any new genetic and antigenic variants (Abrantes et al., 2012).

Supplementation with hyper-immune antiserum used only for prevention of RHD and induces protection for a short time. Passively acquired immunity using hyper immune anti-serum was documented in 1993 in Egypt, where 4-months-old rabbits were inoculated intramuscularly either simultaneously with RHDV or before the virus infection. This treatment induced protection rate of 100% against RHD (Abd El Motelib, 1993). Hyper immune anti-serum may be effective only in case of absence of clinical signs of infection.

Globally, RHDV vaccines have been developed from infected animals ´tissues followed by chemical inactivation (Arguello Villares, 1991, Huang, 1991; Smid et al., 1991). They have been proved protective against variant RHDVa in domestic and wild rabbits (Capucci et al., 1998). Due to that the used vaccines have many disadvantages like variation of their efficacy according to the physiological conditions of the animal (Cabezas et al., 2006) and relatively short period of immunity which is not more than 12 months (OIE, 2010), so many modified vaccines have been developed.

The prepared RHDV vaccines may be given for animals either orally (Bertagnoli et al., 1996a; Plana-Duran et al., 1996; Martin-Alonso et al., 2003; Farnos et al., 2005) or intra-nasal (Farnos et al., 2006). Moreover, bivalent vaccines against myxomatosis (Bertagnoli et al., 1996b; Barcena et al., 2000) and pasteurellosis (Peshev and Christova, 2003) have been also used.

Initial trial for production of RHDV vaccine was conducted in Egypt by Salem and El-Ballal (1992) as inactivated formalized tissue vaccine was produced. In this trial, inactivated suspensions of liver and lung of RHDV infected animals succeeded in protection of inoculated animals 7 days post-vaccination and the immunity lasted for more than 2 months. As well, Salem and El-Zanaty (1992) tried inactivated tissue derived RHDV vaccine for prevention of animals against the infection. Other trials were conducted for preparation of formalin inactivated, aluminum hydroxide adjuvanted RHDV vaccine from the Egyptian local strain (Egypt 96) (Daoud et al., 1998a) and also preparation of rabbit pasteurellosis-RHD combined vaccines (Daoud et al., 1998b). It has been showed that cell culture (Vero cell) inactivated RHDV vaccine is more potent than tissue (liver suspension) derived vaccine (Khodeir and Daoud, 2002). A bivalent RHDV and *P. multocida* lipopolysaccharides vaccine was also developed (Khodeir and Daoud, 2002). Although, there are different effective vaccination schedules for RHDV prevention, the virus is still circulating in rabbitries of Egypt (Abd El-Motelib et al., 1998; Metwally and Madbouly, 2005; Abd El-Lateff, 2006; Ewees, 2007; El-Sissi and Gafer, 2008; El-Bagoury et al., 2014). The local commercial Egyptian vaccines (IZOVAC-MEVAX) and (SVRS-Vac) that had been used for vaccination of 1.5-months old rabbits against RHDV offered 100% protection (Eid and Ibraheem, 2006). As a result of the endemic situation of the RHD among rabbits flocks in Egypt as well as early infection of young kittens (40 days old), it has been recommended starting vaccination at 1-1.5-months of age, followed by another booster dose 15 days later and then repeating the vaccination every 4-6 months (Eid and Ibraheem, 2006).

Moreover, due to inadequate application of RHD vaccine, non-hemagglutinating RHDVa variant strains are circulating in Egyptian rabbits field and infect all ages of rabbits (Ewees, 2007; El-Sissi and Gafer, 2008; Awad and Kotb, 2018). These strains were found to cause clinical disease as well as morbidity and mortality rates similar to classical RHDV, but without HA activity of the virus. Erfan and Shalaby

(2020) concluded that there are some limitations regarding the effectiveness of currently applied RHDV vaccine strains as the vaccine formulation may not cover all the circulating strains of the virus in Egypt.

Application of genetically engineered Virus Like particle (VLP) of RHDV as a therapeutic strategy for treatment of the disease have been studied (El Mehdaoui et al., 2000; Young et al., 2006; Peacey et al., 2007, 2008; Crisci et al., 2009; Win et al., 2011). The protein of the capsid accumulates in VLP that is differ from original virions and doesn't contain the viral RNA (Nagesha et al., 1995). This treatment strategy considers VLP as immunogenic antigen that stimulate both humoral and cell mediate immune responses of infected animals (Crisci et al., 2009; Win et al., 2011).

Thorough cleaning and disinfection are a requirement for RHDV prevention and control strategies. It has been found that the virus can be inactivated using disinfectants as 1-2% formalin, 1.0–1.4% formaldehyde, 0.2–0.5% beta-propiolactone, 1% sodium hypochlorite, 10% sodium hydroxide or 10 ppm chlorine dioxide (Eleraky et al., 2002).

All mechanical sources of RHDV infection should be also taken into consideration. Field rats and flies eradication program should be applied for RHD control.

In conclusion, to overcome RHD infection in any region, constant monitoring of the epidemiological status of the disease as well as updating the development of local or autogenous vaccines are crucial issues that should be thoroughly considered.

CONFLICT OF INTEREST

The author declares that there are no conflicts of interest.

REFERENCES

Abd El-Ghafar SK, Fatma A, Aly M, Mahmoud AZ (2000) Pathological studies on the rabbit viral hemorrhagic disease. Assiut Vet Med J 43: 251-274.

Abd El-Lateff AA (2006) Clinicopathological studies on rabbit hemorrhagic disease vaccines. (Ph.D. Thesis), Fac Vet Med, Cairo Univ, Egypt.

Abd El-Moaty DAM, El-Bagoury GF, El-Zeedy SAR, Salman OGA (2014) Egyptian non-hemagglutinating isolates of rabbit hemorrhagic disease virus can change to variable HA Profile. Benha Vet Med J 26: 71-83.

Abd El-Motelib TY (1993): Role of hyperimmune serum in protection against viral haemorrhagic disease of rabbits. Assiut Vet Med J, 28 (56): 297-307.

Abd El-Motelib TY, Abd El-Gawad AM, Azzaz HH (1998) Viral haemorrhagic disease of rabbit: Comparative study between the immune response of local and imported vaccine. Assiut Vet Med J 40 (79): 150-156.

Abrantes J, van der Loo W, Le Pendu J, Esteves PJ (2012) Rabbit haemorrhagic disease (RHD) and rabbit haemorrhagic disease virus (RHDV): A review. Vet Res 43(1): 12.

Ahmad ST, El-Samadony HA, Mahgoub KM (2011) Immunological and virological studies on rabbit hemorrhagic disease virus. Global Vet 7: 545-556.

Alboghdady MA, Alashry MK (2010) The demand for meat in Egypt: An almost ideal estimation. Afr J Agric Resour Econ 4(1): 70-81.

Alda F, Gaitero T, Suarez M, Merchan T, Rocha G, Doadrio I (2010) Evolutionary history and molecular epidemiology of rabbit haemorrhagic disease virus in the Iberian Peninsula and Western Europe. BMC Evol. Biol 10(1): 347.

Alonso C, Oviedo JM, Martin-Alonso JM, Diaz E, Boga JA, Parra F (1998) Programmed cell death in the pathogenesis of rabbit hemorrhagic disease. Arch Virol 143(2): 321-332.

Arguello Villares JL (1991) Viral haemorrhagic disease of rabbits: vaccination and immune response. Rev Sci Tech 10: 471-480.

Asgari S, Hardy JR, Sinclair RG, Cooke BD (1998) Field evidence for mechanical transmission of rabbit haemorrhagic disease virus (RHDV) by flies (*Diptera: Calliphoridae*) among wild rabbits in Australia. Virus Res 54(2): 123-132.

Awad NF, Kotb GK (2018) Genetic characterization of rabbit hemorrhagic disease virus from naturally infected rabbits in Sharkia governorate, Egypt. J Virol Sci 3: 10-19.

Barcena J, Morales M, Vazquez B, Boga JA, Parra F, Lucientes J, Pages-Mante A, Sanchez-Vizcaino JM, Blasco R, Torres JM (2000) Horizontal transmissible protection against myxomatosis and rabbit hemorrhagic disease by using a recombinant myxoma virus. J Virol 74: 1114-1123.

Belz K (2004) Rabbit hemorrhagic disease. Seminars in Avian and Exotic Pet Medicine, 13(2): 100-104.

Berninger ML, House C (1995) Serologic comparison of four isolates of rabbit hemorrhagic disease virus. Vet Microbiol 47 (1–2): 157-165.

Bertagnoli S, Gelfi J, Le Gall G, Boilletot E, Vautherot JF, Rasschaert D, Laurent S, Petit F, Boucraut-Baralon C, Milon A (1996b) Protection against myxomatosis and rabbit viral hemorrhagic disease with recombinant myxoma viruses expressing rabbit hemorrhagic disease virus capsid protein. J Virol 70: 5061-5066.

Bertagnoli S, Gelfi J, Petit F, Vautherot JF, Rasschaert D, Laurent S, Le Gall G, Boilletot E, Chantal J, Boucraut-Baralon C (1996a) Protection of rabbits against rabbit viral haemorrhagic disease with a vaccinia-RHDV recombinant virus. Vaccine 14: 506-510.

Broja G, Larias OMD (1990) Determination de la viabilidad del virus de la EHV cen alfalfa, alimento balanceado commercial costales paraalimentoy ratas. (Thesis). Fac Vet Med, Unam, Mexico.

Cabezas S, Calvete C, Moreno S (2006) Vaccination success and body condition in the European wild rabbit: applications for conservation strategies. J Wild Manage 70: 1125-1131.

Campagnolo ER, Ernst MJ, Berninger ML, Gregg DA, Shumaker TJ, Boghossian AM (2003) Outbreak of rabbit hemorrhagic disease in domestic lagomorphs. J Am Vet Med Assoc 223(8): 1151-1128.

Cancellotti FM, Renzi M (1991) Epidemiology and current situation of viral haemorrhagic disease of rabbits and the European brown hare syndrome in Italy. Re Sci Tech 10: 409-422.

Capucci L, Scicluna MT, Lavazza A (1991) Diagnosis of viral haemorrhagic disease of rabbits and the European brown hare syndrome. Rev Sci Tech 10: 347-370.

Capucci L, Fallacara F, Grazioli S, Lavazza A, Pacciarimi ML, Procchi E (1998) A further step in the evolution of rabbit hemorrhagic disease virus: the appearance of the first consistent antigenic variant. Virus Research, 58, 115-126. CFSPH, Center for Food Security and Public Health, Iowa State University (2006). Rabbit Hemorrhagic Disease. Technical Factsheet. www.cfsph.iastate.edu

Chasey D (1997) Rabbit haemorrhagic disease: the new scourge of *Oryctolagus cuniculus*. Lab. Anim 31: 33-44.

Clouet RN, Blanchard D, Andre FG, Song B, Ganiere JP (1995). Detection of antibodies to RHDV: as immuno-blotting method using coated human erythrocyte membrane. J Vet Med 42 (4): 197-204.

Cooke BD (2002) Rabbit haemorrhagic disease: field epidemiology and the management of wild rabbit populations. Rev Sci Tech 21: 347-

358.
Crisci E, Almanza H, Mena I, Cordoba L, Gomez-Casado E, Caston JR, Fraile L, Barcena J, Montoya M (2009) Chimeric calicivirus-like particles elicit protective anti-viral cytotoxic responses without adjuvant. Virology 387: 303-312.
Dalton KP, Nicieza I, Balseiro A, Muguerza MA, Rosell JM, Casais R, Parra F (2012) Variant rabbit hemorrhagic disease virus in young rabbits, Spain. Emer Infec Dis 18: 2009-2012.
Daoud AM, Khodeir MH, Abbass AM, Ibrahim SI (1998a) Preparation of a specific inactivated vaccine against RHDV. In: 4th Sci Conf, Fac Vet Med, Zagazig Univ, Egypt, p. 230-234.
Daoud AM, Khodeir MH, Abbass AM, Ibrahim SI, Gergis SM (1998b): Preliminary study for preparation of rabbit pasteurellosis and rabbit hemorrhagic disease virus combined vaccine. In: 4th Sci Conf, Fac Vet Med, Zagazig Univ, Egypt, p. 191-198.
Eid AAM, Ibraheem OK (2006) Sudden death among rabbits in Sharkia Province, Egypt. Zagazig Vet J 34: 108-119.
El-Bagoury GF, Abd El-Moaty DA, El-Zeedy SA, El-Nahas EM, Youssif AA (2014) Molecular identification of RHDV Egyptian strains based on the highly variable region of VP60 gene, Benha Vet Med J 26: 84-100.
Eleraky NZ, Potgieter LN, Kennedy MA (2002) Virucidal efficacy of four new disinfectants. J Am Anim Hosp Assoc. 38(3): 231-234.
El Mehdaoui S, Touze A, Laurent S, Sizaret P-Y, Rasschaert D, Coursaget P (2000) Gene transfer using recombinant rabbit hemorrhagic disease virus capsids with genetically modified and encapsidation capacity by addition of packaging sequences from the l1 or l2 protein of human papillomavirus type 16. J Virol 74: 10332-10340.
El-Mongy FA (1998) Studies on viral hemorrhagic disease of rabbits (Ph.D. Thesis), Fac Vet Med, Zagazig Univ, Egypt.
El-Sissi FA, Gafer JA (2008) Preliminary diagnosis of non haemagglutinating strain of rabbit hemorrhagic disease virus in Egypt. Egypt J Comp Pathol Clin Pathol 21:161-175.
El-Zanaty K (1994) Some investigation on rabbit viral haemorrhagic disease in Upper Egypt. Assiut Vet Med J 30(60): 293-305.
Embury-Hyatt C, Postey R, Hisanaga T, Lynn B, Hooper-McGrevy K, McIntyre L, Millar K, Pasick J (2012) The first reported case of rabbit hemorrhagic disease in Canada. Canad Vet J 53: 998-1002.
Erfan AM, Shalaby AG (2020) Genotyping of rabbit hemorrhagic disease virus detected in diseased rabbits in Egyptian provinces by VP60 sequencing, Vet World 13(6): 1098-1107.
Ewees GAS (2007) Further studies on hemorrhagic viral disease in rabbit. (Ph.D. Thesis), Fac Vet Med, Cairo Univ, Egypt.
Fahmy HA, Arafa A, Mahmoud AH (2010) Molecular diagnosis of rabbit hemorrhagic disease virus (RHDV). Egypt J Comp Pathol Clin Pathophysiol 23: 85-101.
Farnos O, Boue O, Parra F, Martin-Alonso JM, Valdes O, Joglar M, Navea L, Naranjo P, Lleonart R (2005) High-level expression and immunogenic properties of the recombinant rabbit hemorrhagic disease virus VP60 capsid protein obtained in Pichia pastoris. J Biotechnol 117: 215-224.
Farnos O, Rodriguez M, Chiong M, Parra F, Boue O, Lorenzo N, Colas M, Lleonart R (2006) The recombinant rabbit hemorrhagic disease virus VP60 protein obtained from Pichia pastoris induces a strong humoral and cell mediated immune response following intranasal immunization in mice. Vet Microbiol 114: 187-195.FAO, Food and Agriculture Organization of the United Nations Database (2019) Available from: http://www.faostat. fao.org. Retrieved on 05-03-2019.
Ferreira PG, Costa ESA, Monteiro E, Oliveira MJ, Aguas AP (2006) Liver enzymes and ultrastructure in rabbit haemorrhagic disease (RHD). Vet Res Commun 30: 393-401.
Forrester NL, Abubakr MI, Abu Elzein EM, al Afaleq AI, Housawi FM, Moss SR, Turner SL, Gould EA (2006) Phylogenetic analysis of rabbit haemorrhagic disease virus strains from the Arabian Peninsula: did RHDV emerge simultaneously in Europe and Asia? Virology 344: 277-282.
Forrester NL, Moss SR, Turner SL, Schirrmeier H, Gould EA (2008) Recombination in rabbit haemorrhagic disease virus: possible impact on evolution and epidemiology. Virology 376: 390-396.
Ghanem IA, Ismail AN (1992) Occurrence of rabbit hemorrhagic disease in Sharkia province. Zag Vet Med J 20(4): 491-502.
Gould AR, Kattenbelt JA, Lenghaus C, Morrissy C, Chamberlain T, Collins BJ, Westbury HA (1997) The complete nucleotide sequence of rabbit haemorrhagic disease virus (Czech strain V351): use of the polymerase chain reaction to detect replication in Australian vertebrates and analysis of viral population sequence variation. Virus Res 47: 7-17.
Gregg DA, House C, Meyer R, Berninger M (1991) Viral haemorrhagic disease of rabbits in Mexico: epidemiology and viral characterization. Rev Sci Tech 10: 435-451.
Guittre CI, Baginski G, Gall LE (1995) Detection of rabbit hemorrhagic disease virus isolates and sequence comparison of the N-terminus of the capsid protein gene by the polymerase chain reaction. Res Vet Sci 58: 128-132
Hamed AM, Eid AAM, El-Bakrey RMM (2013) A review of rabbit diseases in Egypt. WARTAZOA 23 (4): 185-194.
Henning J, Meers J, Davies PR, Morris RS (2005) Survival of rabbit haemorrhagic disease virus (RHDV) in the environment. Epidemiol Infect 133: 719-730.
Huang HB (1991) Vaccination against and immune response to viral haemorrhagic disease of rabbits: a review of research in the People's Republic of China. Rev Sci Tech, 10: 481-498.
Ibrahim IS, Abass AM, ElKholy AA, Safia TK, Hussein AZ (1999) Simultaneous infection with rabbit haemorrhagic disease virus and rabbit pasteurellosis in rabbit farms in Egypt. Alex J Vet Sci 15 (3): 567-578.
Ismail MM, Mohamed MH, ElSabagh IM, Al-Hammadi MA (2017) Emergence of new virulent rabbit hemorrhagic disease virus strains in Saudi Arabia. Trop Anim Health Prod 49: 295-301.
ITAVI Institut Technique de l'Aviculture (2019) Fiches de Biosécurité en Élevage de Lapins. Available online: https://www.itavi.asso.fr/content/fiches-de-biosecurite-en-elevage-de-lapins (accessed on 11 May 2019).
Khodeir MH, Daoud AM (2002). Preparation and evaluation of an inactivated cell culture rabbit hemorrhagic disease virus vaccine. SCOVM J 1: 75-94.
Kovalisk J (1998) Monitoring the spread of rabbit hemorrhagic disease virus as a new biological agent for control of wild European rabbits in Australia. J Wildl Dis 34(8): 421-428.
Lavazza A, Capucci (1996) Viral hemorrhagic disease of rabbits. In: Manual of Standards for Diagnostic Tests and Vaccines, 3rd Ed., Reichard, rd R. Paris, France, Office International des Epizooties, Chapter, 3: 7-3.
Lavassa A, Capucci L (2008) How many caliciviruses are there in rabbits. A review on RHDV and correlated viruses. In: LagomorphBiology: Evolution, Ecology and Conservation. Springer-Verlag Berlin Heidelberg 263-278.
Le Gall-Recule G, Zwingelstein F, Laurent S, de Boisseson C, Portejoie Y, Rasschaert D (2003) Phylogenetic analysis of rabbit haemorrhagic disease virus in France between 1993 and 2000, and the characterisation of RHDV antigenic variants. Arch Virol 148(1): 65-81.
Le Gall-Recule G, Lavazza A, Marchandeau S, Bertagnoli S, Zwilgenstaein F, Cavadini P, Martinelli N, Lombardi G, Guerin JL, Lemaitre E, Décors A, Boucher S, Le Normand B, Capucci L (2013) Emergence of a new lago virus related to rabbit haemorrhagic disease virus. Vet Res 44(1): 81.
Liu SJ, Xue HP, Pu BQ, Qian NH (1984) A new viral disease in rabbits. Anim. Husbandry Vet Med 16 (6): 253- 255.
Marcato PS, Benazzi C, Vecchi G, Galeotti M., Salda LD, Sarli G, Lucidi P (1991) Clinical and pathological features of viral haemorrhagic disease of rabbits and the European brown hare syndrome. Rev Sci Tech Off Int Epizoot 10: 371-392.
Magouzi AF, Elsayed EA, Metwally AY (2019) Detection and characterization of rabbit hemorrhagic disease virus strains circulating in Egypt. Bulg J Vet Med 22(4): 409-418.
Marques RM, Costa ESA, Aguas AP, Teixeira L, Ferreira PG (2010) Early acute depletion of lymphocytes in calicivirus-infected adult rabbits. Vet Res Commun 34(8): 659-668.
Martin-Alonso JM, Castanon S, Alonso P, Parra F, Ordas R (2003) Oral immunization using tuber extracts from transgenic potato plants expressing rabbit hemorrhagic disease virus capsid protein. Transgenic Res 12: 127-130.
McColl KA, Morrissy CJ, Collins BJ, Westbury HA (2002) Persistence

of rabbit haemorrhagic disease virus in decomposing rabbit carcases. Aust Vet J 80: 298-299.
McIntosh MT, Behan SC, Mohamed FM, Lu Z, Moran KE, Burrage TG, Neilan JG, Ward GB, Botti G, Capucci L, Metwally SA (2007) A pandemic strain of calicivirus threatens rabbit industries in the Americas. Virol J 4: 96-10.
Metwally AY, Madbouly HM (2005) Study on a new isolate of rabbit heamorrhagic disease virus. Bani-suef Vet Med J 15(2): 283-243.
Merchán T, Rocha G, Alda F, Silva E, Thompson G, Hidalgo de Trucios S, Pagès-Manté A (2011) Detection of rabbit haemorrhagic disease virus (RHDV) in nonspecific vertebrate hosts sympatric to the European wild rabbit (*Oryctolagus cuniculus*). Infect Genet Evol 11: 1469-1474.
Meyers G, Wirblich C, Thiel HJ (1991) Rabbit hemorrhagic disease virus-molecular cloning and nucleotide sequencing of a calicivirus genome. Virology 184(2): 664-676.
Miao O, Qi R, Veldkamp L, Ijzer J, Kik ML, Zhu JM, Tang A, Dong D, Shi Y, Van Oers MM, Liu G, Pijlman GP (2019) Immunogenicity in rabbits of virus-like particles from a contemporary rabbit haemorrhagic disease virus Type 2 (GI.2/RHDV2/b) isolated in the Netherlands. Viruses 11(6): 553.
Mitro S, Krauss H. (1993). Rabbit hemorrhagic disease: a review with reference to its epizooitology. Europ J Epidemiol 9(1): 70-78.
Mohamed FO (2009). Clinic pathological studies on the effect of viral hemorrhagic disease (VHD) in rabbits (Master Thesis). Zagazig University, Egypt.
Morisse JP, Le Gall G, Boilletot E (1991) Hepatitis of viral origin in Leporidae: introduction and aetiological hypotheses. Rev Sci Tech 10:283-295.
Moss SR, Turner SL, Trout RC, White PJ, Hudson PJ, Desai A, Armesto M, Forrester NL, Gould EA (2002) Molecular epidemiology of rabbit haemorrhagic disease virus. J General Virol 83: 2461-2467.
Mostafa AA (2001) Characterization and pathogenicity of locally isolated virus of hemorrhagic disease in rabbits. (Ph.D. Thesis), Fac Vet Med, Beni-Suef Univ, Egypt.
Moussa A, Chasey D, Lavazza A, Capucci L, Smid B, Meyers G, Rossi C, Thiel HJ, Vlasak R, Ronsholt L, Nowotny N, McCullough K, Gavier-Widenm D (1992) Haemorrhagic disease of lagomorphs: Evidence for a calicivirus. Vet Microbiol 33(1-4): 375-381.
Muller A, Freitas J, Silva E, Le Gall-Reculé G, Zwingelstein F, Abrantes J, Esteves PJ, Alves PC, van der Loo W, Kolodziejek J, Nowotny N, Thompson G. (2009) Evolution of rabbit haemorrhagic disease virus (RHDV) in the European rabbit (*Oryctolagus cuniculus*) from the Iberian Peninsula. Vet Microbiol 135: 368-373.
Nagesha HS, Wang LF, Hyatt AD, Morrissy CJ, Lenghaus C, Westbury HA (1995) Self-assembly, antigenicity, and immunogenicity of the rabbit haemorrhagic disease virus (Czechoslovakian strain V-351) capsid protein expressed in baculovirus. Arch Virol 140: 1095-1108.
Ohlinger VF, Haas B, Thiel HJ (1993) Rabbit haemorrhagc disease (RHD): Characterization of the causative calicivirus. Vet Res 24(2): 103-116.
OIE Office of International Epizootics (2008) Terrestrial Manual. Viral hemorrhagic disease of rabbits. Chapter 2.6.2. P. 947-961.
OIE Office of International Epizootics (2010) Terrestrial Manual. Viral hemorrhagic disease of rabbits. 1-15
OIE Office of International Epizootics (2012) Terrestrial Manual. Viral hemorrhagic disease of rabbits, Rabbit Calcivirus disease. Chapter 2.6.2 pp. 1–15.
OIE Office of International Epizootics (2018) Terrestrial Animal Health Code. 2018. Available online: http://www.oie.int/en/standard-setting/terrestrial-code/access-online/ (accessed on 19 May 2019).
Patton NM (1989) Viral hemorrhagic disease. A major new disease problem of rabbits. Rabbit Res 12: 64-67.
Park JH, Lee YS, Itakura C (1995) Pathogenesis of acute necrotic hepatitis in rabbit hemorrhagic disease. Lab Anim Sci 45(4): 445-449.
Peacey M, Wilson S, Baird MA, Ward VK (2007) Versatile RHDV virus-like particles: incorporation of antigens by genetic modification and chemical conjugation. Biotechnol Bioeng 98: 968-977.
Peacey M, Wilson S, Perret R, Ronchese F, Ward VK, Young V, Young SL, Baird MA (2008) Virus-like particles from rabbit hemorrhagic disease virus can induce an anti-tumor response. Vaccine 26: 5334-5337.
Peshev R, Christova L (2003) The efficacy of a bivalent vaccine against pasteurellosis and rabbit haemorrhagic disease virus. Vet Res Commun 27: 433-444.
Plana-Duran J, Bastons M, Rodriguez MJ, Climent I, Cortes E, Vela C, Casal I (1996) Oral immunization of rabbits with VP60 particles confers protection against rabbit hemorrhagic disease. Arch Virol 141: 1423-1436.
Plassiart G, Guelfi JF, Ganiere JP, Wang B, Andre-Fontaine G, Wyers M (1992) Hematological parameters and visceral lesions relationships in rabbit viral hemorrhagic disease. J Vet Med (B) 39: 443-53.
Pu BQ, Quian NH, Cui S (1985) Micro HA and HI tests for the detection of antibody titres to so-called 'haemorrhagic pneumonia' in rabbits [in Chinese]. Chin J Vet Med 11: 16-17.
Ramiro-Ibáñez F, Martín-Alonso JM, García Palencia P, Parra F, Alonso C (1999) Macrophage tropism of rabbit hemorrhagic disease virus is associated with vascular pathology. Virus Res 60: 21-28.
Ruvoen-Clouet N, Ganiere JP, Andre-Fontaine G, Blanchard D, Le Pendu J (2000) Binding of rabbit hemorrhagic disease virus to antigens of the ABH histo-blood group family. J Virol 74: 11950-11954.
Sahar TA, Hanaa AE, Khalid MM (2011) Immunological and virological studies on rabbit hemorrhagic disease virus. Global Vet 7 (6): 545-556.
Salem B, El-Ballal SS (1992) The occurrence of rabbit hemorrhagic disease (RHVD) in Egypt. Assiut Vet Med J 27(53): 295-304.
Salem B, El-Zanaty K (1992) Tissue-derived inactivated vaccine against rabbit viral haemorrhagic disease. In: Proc 5th Sci Conf, Fac Vet Med, Assiut Univ, Egypt.
Schirrmeier H, Reimann I, Kollner B, Granzow H (1999) Pathogenic, antigenic and molecular properties of rabbit haemorrhagic disease virus (RHDV) isolated from vaccinated rabbits: detection and characterization of antigenic variants. Arch Virol 144: 719-735.
Sharawi SSA (1992) Studies on the virus causing haemorrhagic septicemia in rabbits (Master Thesis). Fac Vet Med, Zagazig Univ, Egypt.
Smid B, Valicek L, Rodak L, Stepanek J, Jurak E (1991). Rabbit haemorrhagic disease: an investigation of some properties of the virus and evaluation of an inactivated vaccine. Vet Microbiol 26: 77-85.
Soliman MA, Abdel Rahman MA, Samy MM, Mehana O, Nasef SA (2016) Molecular, clinical and pathological studies on viral rabbit hemorrhagic disease. Alex J Vet Sci 48: 20-26.
Trzeciak-Ryczek A, Tokarz-Deptuła B, Deptuła W (2015) The importance of liver lesions and changes to biochemical and coagulation factors in the pathogenesis of RHD, Acta Biochimica Polonica 62: 169-171.
Ueda K, Park JH, Ochiai K, Itakura C (1992) Disseminated intravascular coagulation (DIC) in rabbit haemorrhagic disease. Jpn J Vet Res 40(4): 133-141.
Urakova N, Hall R, Strive T, Frese M (2019) Restricted host specificity of rabbit hemorrhagic disease virus supported by challenge experiments in immune-compromised mice (*Mus musculus*). J Wildl Dis 55(1): 218-222.
Wang X, Xu F, Liu J, Gao B, Liu Y, Zhai Y, Ma J, Zhang K, Baker TS, Schulten K, Zheng D, Pang H, Sun F (2013) Atomic model of rabbit hemorrhagic disease virus by cryo-electron microscopy and crystallography. PLoS Pathogens. 9(1): e1003132.
Xu WY (1991) Viral hemorrhagic disease of rabbits in the people's republic of China: Epidemiology and virus characterization. Rev Sci Tech 10(2): 393-408.
Xu ZJ, Chen WX (1989). Viral haemorrhagic disease in rabbits: a review. Vet Res Commun 13: 205-212.
Win SJ, Ward VK, Dunbar PR, Young SL, Baird MA (2011) Cross-presentation of epitopes on virus-like particles via the MHC I receptor recycling pathway. Immunol Cell Biol 89: 681-688.
Young SL, Wilson M, Wilson S, Beagley KW, Ward V, Baird MA (2006) Transcutaneous vaccination with virus-like particles. Vaccine 24: 5406-5412.