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

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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 sub-acute 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 spotlight 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

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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 (Alboghady 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 fatal, 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-Matreh 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 Assiut 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 Madbouly, 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 (Ruvonen-Clouet et al., 2000). Transmission of RHDV is usually happen through faecal-oral route (Morrison 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 et 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 lac-

tating 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 from 16 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 health 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, opisthotonus 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; Plassart 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-Motlib, 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; Pla-

na-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 Mehdouei 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.

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