A Rabbit Model for Sheep-Associated Malignant Catarrhal Fever Research: from Virus Infection to Pathogenesis Studies and Vaccine Development

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Abstract

Purpose of Review Describe the implementation and use of rabbits as a laboratory model for sheep-associated malignant catarrhal fever (SA-MCF) research using cell-free ovine herpesvirus 2 (OvHV-2). Key considerations regarding the use of the model to generate consistent experimental data are presented and discussed in detail.

Recent Findings A major drawback to SA-MCF research is that OvHV-2 cannot be propagated in cell culture and experimental studies rely only on animals. An important research milestone was the finding that infectious cell-free OvHV-2 from nasal secretions of shedding sheep can be used to experimentally induce SA-MCF in various host species. Using this approach, we have demonstrated that rabbits can be infected with OvHV-2 and that infection parameters, such as dose-dependence, and clinical and pathological manifestations, resemble those in natural hosts.

Summary Rabbits are a reliable model for SA-MCF and represent an important resource for experimental research. The model is especially useful to investigate virus-host interactions and to evaluate MCF vaccine strategies.

Keywords Ovine herpesvirus 2 (OvHV-2) · Malignant catarrhal fever (MCF) · Rabbits · Animal model

Introduction

Malignant catarrhal fever (MCF) is a severe and frequently fatal lymphoproliferative syndrome of multiple species in the order *Artiodactyla*, including species in the families *Bovidae*, *Cervidae*, *Giraffidae*, and *Suidae*. MCF is caused by one of several herpesviruses in the genus *Macavirus*, subfamily

Gammaherpesvirinae [1]. These viruses exist in nature as subclinical infections in species of the subfamilies *Caprinae*, *Alcelaphinae*, and *Hippotraginae*, which serve as their carrier hosts. Domestic sheep and wildebeest are the main reservoirs for ovine herpesvirus 2 (OvHV-2) and alcelaphine herpesvirus 1 (AIHV-1), respectively, the most studied MCF viruses.

In North America, MCF is caused predominantly by OvHV-2 and represents a serious threat to American bison due to their high susceptibility to disease. Significant economic losses caused by sheep-associated MCF (SA-MCF) have prompted research into effective strategies to control the disease and mitigate virus transmission.

Sheep-associated MCF is one of the few veterinary viral diseases for which its etiological agent, OvHV-2, has not been propagated *in vitro*. The absence of a culture system to propagate OvHV-2 hinders efforts to develop tools to study the virus and to reliably reproduce the disease experimentally. However, seminal work by our group in the early 2000s demonstrated that sheep naturally infected with OvHV-2 shed infectious virus through nasal secretions [2•, 3••]. Virus from this source created new opportunities to study SA-MCF and, since then, experimental disease has been consistently induced in several clinically susceptible species, including domestic

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cattle, American bison, pigs, and rabbits [4, 5, 6•, 7]. Based on these experiments, we were able to demonstrate that susceptibility to SA-MCF varies appreciably among species. For instance, American bison are highly susceptible to the disease, whereas cattle are comparatively more resistant [5, 8].

Although natural hosts are generally the best experimental animal models for research, there are limitations on their use in SA-MCF experimental studies. Bison are difficult to handle, require special facilities, and are expensive to maintain. Routine experimental use of cattle is less problematic but because of their relative resistance to infection, reliable induction of clinical disease requires high infectious doses of OvHV-2, which is difficult to obtain. In this context, the use of a small animal laboratory model that is both easy to maintain and susceptible to SA-MCF is beneficial for experimental research. Laboratory rabbits fit these criteria. While we are not aware of MCF in free-ranging populations of rabbits, the disease can be reliably induced experimentally in laboratory rabbits using relatively low doses of OvHV-2 [6•, 9]. Importantly, initial infection, virus dissemination, disease manifestations, and host immune responses following experimental challenge with OvHV-2 in rabbits are similar to those in bison [10, 11••]. After performing OvHV-2 experimental infections in rabbits for several studies, we have now established that rabbits are a reliable and valid model for SA-MCF and in this article, we review specific aspects of their use to advance MCF research.

History of the Use of Rabbits in MCF Research

Rabbits (*Oryctolagus cuniculus*) are small mammals in the family *Leporidae* of the order *Lagomorpha*. They are commonly used as experimental models in many research fields as they are docile, easy to house, handle, and observe. Laboratory rabbits are widely available and cost-effective for research compared to larger animal models. Additionally, rabbit-specific reagents, particularly to assess immunological responses, are commercially available.

Initial reports of experimental use of rabbits in MCF research come from the 1920s and 30s [12]. From that time until the 1970s, laboratory rabbits were used for AIHV-1-transmission studies using infectious materials from cattle with wildebeest-associated MCF [13]. The majority of these studies focused on virus-host interactions, including targeted cells, lymphoid proliferation, and host immune responses [14–19]. In the early 1980s, scientists at the Moredun Research Institute in Scotland induced MCF in rabbits using tissue suspensions and leukocytes from a clinically infected red deer (*Cervus elaphus*) that had been exposed to sheep and subsequently described the clinical signs and lesions in rabbits with OvHV-2-induced MCF [20, 21]. An associated milestone from their work was the establishment of an OvHV-2-infected lymphoblastoid cell line from a rabbit with SA-MCF [22].

Although the virus does not seem to replicate in these cells and viral particles are not detected, it could induce MCF. Over the last three decades, many studies conducted in rabbits used intravenous inoculation of either OvHV-2-infected lymphoblastoid cell lines or virulent cell-associated strains of AIHV-1 (e.g., C500 strain) to investigate the pathogenesis of MCF, with particular emphasis on the nature of lymphoproliferation [23–25]. More recently, cell-free OvHV-2 obtained from sheep nasal secretions led to a reliable source of virus for in vivo studies [2•]. Experimental challenges using intranasal nebulization of sheep nasal secretions containing infectious OvHV-2 have been used extensively to infect various host species, including rabbits [3••, 4, 5, 6•, 7, 10, 11••, 26]. These experiments resulted in much of what is currently known about OvHV-2 infection and disease development in several natural hosts and validated rabbits as a suitable animal model for SA-MCF research.

Induction of SA-MCF in Rabbits Using Cell-Free OvHV-2

Rabbits can be infected with OvHV-2 following experimental inoculation of cell-associated virus derived from infected animals or lymphoblastoid cells [12, 20, 25, 27]. A disadvantage of this approach is that infection and induction of disease are inconsistent, as it is difficult to standardize inocula needed to replicate challenge studies. Cell-free infectious OvHV-2 present in nasal secretions of shedding sheep offer a more reliable source of infectious virus since these can be stored, quantified, and standardized for in vivo experiments [3••].

Below, we describe specific aspects of the cell-free OvHV-2 preparation and experimental procedures that are followed in our laboratory to study SA-MCF using the rabbit model.

Generation of Standardized Cell-Free OvHV-2 Stocks

Preparation of cell-free OvHV-2 stocks for animal experiments relies on the collection of nasal secretions from infected sheep during intense viral shedding episodes. Although OvHV-2 can be continuously detected in sheep nasal secretions, shedding of high levels of virus is unpredictable and occurs as brief episodes that usually lasts less than 24 h [2•, 28]. This makes the identification of a high-shedding sheep a laborious process. To obtain infectious virus, OvHV-2-infected adolescent sheep (6–9-month-old), which are more prone to shed the virus, must be monitored daily for intense viral shedding episodes, i.e., presence of high concentrations of viral DNA (10^5 – 10^8 OvHV-2 genome copies per microgram of total DNA) in nasal secretions [28, 29]. This involves collecting nasal secretions using a cotton-tipped swab and immediately testing them for the quantity of OvHV-2 DNA using an OvHV-2 specific quantitative PCR [30]. Once a high concentration of OvHV-2 DNA is detected in a sheep,

additional nasal secretions (4 to 8 swabs) are collected from that animal within a few hours of the first swab to obtain a larger sample. The process continues until several high-shedding sheep are identified and collected from, generating multiple nasal secretion samples containing high concentrations of OvHV-2. In our experience, about 15 sheep need to be monitored over 3 months to identify 20 to 25 high shedding episodes. Upon collection, each nasal swab is placed in cold phosphate buffer solution (PBS) containing 5% chicken egg albumin (approximately 1 ml of PBS per swab), clarified by low speed centrifugation, aliquoted, and stored in liquid nitrogen. To generate standard OvHV-2 stocks, representative aliquots from each individual sheep nasal secretions are pooled, quantified, and re-aliquoted for future use. In our laboratory, OvHV-2 stocks are stored in liquid nitrogen as aliquots containing 10^8 OvHV-2 genome copies per milliliter.

Infectivity of viral stocks is tested by inoculating groups of naïve susceptible hosts with secretions serially diluted based on OvHV-2 DNA copy numbers. Following inoculation, seroconversion, detection of viral DNA in the blood, and/or clinical signs are monitored, with the goal of identifying the minimum dose capable of establishing infection and/or disease. This approach circumvents the absence of a culture system to propagate and test OvHV-2 infectivity *in vitro* and allows standardization of inocula based on viral genome copies. In our laboratory, we have used mostly sheep to test the infectivity of new viral stock preparations and considered seroconversion and detection of viral DNA in the blood as indicators of infection. Because sheep can be infected with lower doses of virus (10^3 OvHV-2 genome copies) as compared with other animal species, their use in this type of experiment helps to conserve the stock [30, 31]. A disadvantage of this approach is that it necessitates access to OvHV-2-negative sheep, which may be challenging to find as the vast majority of adult sheep under standard husbandry conditions are infected with the virus [32, 33]. Alternatively, rabbits can be used to test OvHV-2 stocks for infectivity by using detection of viral DNA and disease development as indicators of infection. However, an inoculum containing at least 10^5 OvHV-2 genome copies is needed to reliably infect and induce disease in rabbits [9, 31].

Intranasal Nebulization Cell-free OvHV-2 obtained from sheep nasal secretions infects and induces disease in susceptible hosts when inoculated by respiratory aerosol, mimicking natural transmission. Such inocula fail to induce infection or disease when introduced intravenously or intraperitoneally [6, 7, 10, 26]. Although the reason for this is unknown, it is probably related to changes in virus components and/or cell tropism switching during initial infection and subsequent dissemination, as occurs with some other herpesviruses [34, 35].

Here, we offer a detailed protocol for inoculating rabbits with standardized cell-free OvHV-2 preparations using

intranasal nebulization. First, the virus stock is diluted in a PBS to a total volume of 2 ml containing the desired challenge dose. The inoculum is loaded into a nebulizer connected to a plastic cone covering the animal's nose and aerosolized for 6 min. During nebulization, proper handling of rabbits and accurate timing for each procedure significantly reduce variation in the inoculum dose, which is critical to ensure consistent experimental OvHV-2 infections. It is important to use the same viral stock in any series of experiments within a project. To help avoid unrelated infections, the use of specific pathogen-free rabbits and administration of prophylactic antibiotic therapy (e.g., enrofloxacin, 5 mg/kg; three intramuscular doses at 24 h interval) concurrent to OvHV-2-infected or mock nasal secretion inoculations is advised.

Defining OvHV-2 Infection Parameters in Rabbits Although SA-MCF can be consistently induced in rabbits using intranasal delivery of cell-free OvHV-2, infection parameters need to be defined in a case-to-case manner considering both the source of rabbits and the virus stock.

In our initial attempts to induce SA-MCF, outcomes differed depending on the rabbits' origin. New Zealand white rabbits from two suppliers were inoculated by intranasal nebulization with the same dose of OvHV-2 (10^7 viral genome copies) from a single pool of standardized viral stock. All rabbits had similar body weights and were inoculated at 10 weeks of age [6, 36]. In study #1, only 5 out of 8 rabbits (62%) were infected with OvHV-2 and developed SA-MCF. By contrast, all 6 rabbits (100%) in study #2 developed disease. Although susceptibility to OvHV-2 infection was not the same for each rabbit group, the time for development of disease was similar, with incubation periods averaging 30 ± 4.3 days post-inoculation (DPI) and 27 ± 1.6 DPI in studies #1 and #2, respectively (Fig. 1a). Since there was no difference in age, weight, or dose of inoculum in these two studies, the most likely explanation for the difference in susceptibility to OvHV-2 infection was the origin of the animals. Information obtained from the two rabbit suppliers revealed that distinct breeding programs were followed by each vendor. The company that supplied the rabbits for study #1 had a strictly outbred program, while the company that supplied rabbits for study #2 used a highly inbred program. Although the reason for the differences in OvHV-2 susceptibility is not completely understood, the results suggest that rabbits from different sources may show distinct infection rates, most likely reflecting genetic variations and degree of inbreeding among rabbits. Thus, pilot experiments to define the OvHV-2 inoculum dose necessary to induce SA-MCF in a given population of rabbits using a defined virus preparation are advisable to implement the model in different laboratory settings. Once these parameters are established, the model is robust and consistent among experiments.

Fig. 1 Time for development of SA-MCF in rabbits inoculated with cell-free OvHV-2 by intranasal nebulization at 10 weeks of age. **a** Studies performed with rabbits from different sources; all animals were inoculated with 10^7 OvHV-2 genome copies. **b** Rabbits inoculated with serial doses of OvHV-2 or a mock inoculum (negative control). **c** Rabbits inoculated with different doses of OvHV-2 from the same viral stock over multiple experiments in our laboratory; bars indicate mean and standard deviation

The first study to evaluate OvHV-2 infectivity in rabbits in our laboratory using various viral doses inoculated by intranasal nebulization was performed using 10-week-old New Zealand rabbits obtained from the vendor (inbred program) used in study #2 described above [9]. Four groups of rabbits ($n = 3$) were nebulized with sheep nasal secretions containing 10^7 , 10^6 , 10^5 , or 10^4 OvHV-2 genome copies. One group of rabbits received nasal secretions obtained from an OvHV-2 uninfected sheep (negative control group). As illustrated in Fig. 1b, inocula containing 10^7 and 10^6 OvHV-2 genome copies resulted in 100% infection and mortality due to MCF, while 67% mortality (2 of 3 rabbits) was observed in rabbits receiving a dose of 10^5 copies. Regardless of the inoculum

dose, all rabbits with clinical signs of MCF were euthanized between 23 and 28 DPI. The rabbit that survived challenge with a 10^5 copies inoculum was infected with OvHV-2, as demonstrated by PCR and serology, but did not develop disease until 60 DPI, when the experiment was terminated. No rabbit in the group inoculated with 10^4 copies or in the control group was infected during the 60-day post-inoculation period. The study indicated that an inoculum of at least 10^5 OvHV-2 genome copies of virus stock is necessary to infect rabbits and that reliable induction of disease requires higher doses of virus (viz. 10^6 or 10^7 copies). These results confirmed that development of MCF in rabbits is dose-dependent, as was previously demonstrated in bison, cattle, and pigs [5, 7, 8].

The results of the above study served as a guide for future experiments performed in our laboratory. Since then, additional 10-week-old, inbred New Zealand rabbits have been intranasally inoculated with 10^6 or 10^7 OvHV-2 genome copies from the same viral stock in multiple studies. Infection and development of MCF were confirmed in 46 of 48 (96%) rabbits inoculated with 10^6 OvHV-2 genome copies while all (100%, $n = 19$) animals inoculated with 10^7 OvHV-2 genome copies developed disease. Collectively, these experiments allowed evaluation of the course of infection, such as the first detection of OvHV-2 DNA in blood and the development of clinical disease, following challenge with different inoculum doses. Viral DNA in PBL was detectable in blood as soon as 14 DPI, regardless the initial dose of virus. However, rabbits inoculated with 10^7 OvHV-2 DNA copies tended to develop MCF earlier (21.4 ± 1.8 DPI) than ones receiving 10^6 (26.4 ± 3.1 DPI). Figure 1c illustrates incubation period data obtained from all the experiments combined. Although the nature of the data collected from various studies does not allow for statistical analysis, more variation and longer incubation periods were observed in animals inoculated with the lower inocula. Depending on the purpose of the experiment, and notwithstanding the absence of 100% infection rates, experiments using 10^6 OvHV-2 genome copies may be used, since it conserves viral stocks. However, this may necessitate more animals and somewhat longer studies to allow for statistical analysis.

By performing the experiments described above, we were able to define infection parameters to reliably induce MCF in rabbits using the virus stock available in our laboratory. Even though the results cannot be directly extrapolated to other research groups, they serve as a starting point and a guide for the establishment of the model in different laboratories.

SA-MCF in Rabbits

Induction of MCF in rabbits by intranasal inoculation with OvHV-2 derived from sheep nasal secretions was first reported by our group in 2008, followed by more detailed reports in

2011 and 2013 [6•, 11••, 36]. Overall, an intranasal challenge containing 10^6 – 10^7 viral DNA copies of standardized cell-free OvHV-2 causes clinical disease in rabbits. Following inoculation, rabbits should be checked at least once daily for development of clinical signs, such as fever ($>40^\circ\text{C}$), which usually occurs 2–3 weeks post-inoculation. Thereafter, they should be examined more frequently (3–4 times a day), since some animals develop severe clinical signs more quickly than others. In our laboratory, experimental animals are euthanized within 48 h of the onset of fever, or immediately if additional clinical signs, such as depression, anorexia, adipsia, diarrhea, oculonasal discharge, and neurological signs, are present. Immediately after euthanasia, necropsy is performed and selected organs collected for further analysis.

In rabbits with SA-MCF, the lesion pattern is consistent and largely mirrors that in susceptible artiodactyls. These comprise lymphoid hyperplasia, vasculitis-perivasculitis, and interstitial lymphocytic inflammation in multiple organs with the predominance of large lymphocytes [6•, 23, 36]. The most consistent gross lesions in OvHV-2-infected rabbits following aerosolization of cell-free virus are observed in the liver and to a lesser extent in the spleen, lymph nodes, and lymph node aggregates [36]. Hepatic lesions consist of disseminated pinpoint tan foci due to diffuse portal-periportal hepatitis and hepatocellular necrosis, with bridging lesions between adjacent portal areas [6•]. Cholangitis is a consistent feature, with hyperplasia and epithelial necrosis/apoptosis in biliary epithelium and with some degree of portal phlebitis-arteriolitis. Large areas of hepatocellular necrosis are associated with granulomatous inflammation, including the presence of giant cells (polykaryons). Arteriolitis-phlebitis also occurs in the bronchial circulation, concurrent with interstitial pneumonia in peribronchiolar parenchyma. Lymphoid hyperplasia involves the T cell-rich paracortex of lymph nodes and gut-associated lymphoid tissues. With the exception of hyperplastic gut-associated lymphoid aggregates in cecum, gastrointestinal lesions are mild or absent. Small, widely scattered ulcers may be present in the cecum and the tongue. Encephalitis and meningitis are absent in many terminally affected rabbits. Changes in cornea and conjunctiva can be surprisingly mild, overt clinical keratoconjunctivitis notwithstanding. Apart from above, lesions found in rabbits with SA-MCF but absent in other species are necrotizing granulomatous hepatitis and lymphadenitis [37]. In some rabbits with SA-MCF, pneumonia has also granulomatous features, specifically the presence of giant cells. The basis for this granulomatous response in rabbits is unclear. Although gross and histologic lesions in OvHV-2- and AIHV-1-induced MCF in rabbits are qualitatively similar, necrosis observed in the liver, mesenteric lymph nodes, and appendix is characteristic of rabbits infected with OvHV-2 but not present in AIHV-1-infected animals [20, 23, 37].

Consistent with these morphological changes, OvHV-2 DNA can be detected in both lymphoid and non-lymphoid tissues. The liver, mesenteric lymph nodes, and lungs tend to have higher viral loads, with an average of 10^7 – 10^8 OvHV-2 genome copies per microgram of total tissue DNA, as compared to other organs [6•, 11••]. Furthermore, OvHV-2 nucleic acids co-localized with lesions have been recently demonstrated using *in situ* hybridization [6•, 11••, 38].

In spite of advances in the last few decades regarding experimental induction of MCF using either OvHV-2 or AIHV-1 in natural and experimental hosts, the pathogenesis of the disease is still poorly understood and a matter of debate. In bison and rabbits, there is an initial round of viral replication restricted to the lungs following OvHV-2 cell-free intranasal inoculation [10, 11••]. Initial replication limited to pulmonary tissue suggests that replication in the lungs is required to establish infection and generate progeny viruses that subsequently infect lymphocytes and disseminate systemically. The first SA-MCF pathogenesis study in rabbits was reported by Buxton and collaborators in 1984. These authors proposed that disease was due to dysfunction of immunoregulatory cells, resulting in benign polyclonal T lymphocyte proliferation [21]. More recent studies have not only confirmed and expanded some of the previous finds but also, by comparing OvHV-2 and AIHV-1-infected rabbits, have identified some differences in the pathogenesis of the disease caused by these two viruses [23, 24, 36, 39, 40]. For instance, in AIHV-1-infected animals, extensive proliferation of CD8⁺ T cells expressing a cytotoxic phenotype in both lymphoid and non-lymphoid tissues has been demonstrated [23, 39]. By contrast, accumulation of cytotoxic and regulatory cells, consisting of T cells, B cells, and monocytes, is detected in SA-MCF-affected animals [23, 36, 40]. More importantly, in rabbits with AIHV-1-induced MCF, a latent infection is present in tissues, as demonstrated by the absence of detectable viral transcripts that are characteristic of productive infections [40]. The same research group provided convincing evidence for the importance of the latency-associated nuclear antigen in the development of disease, resulting in the proposal that latent infection is an essential aspect in the pathogenesis of AIHV-1-induced MCF [41]. By contrast, gene expression indicative of both latent and productive cycles has been demonstrated in cultured T cells from OvHV-2-infected rabbits [42, 43]. In addition, the concentration of ORF 25 transcripts, a marker for virus replication, was positively correlated with severity of lesions in rabbits and bison with SA-MCF [10, 11••]. Collectively, these data suggest that a mixture of latent and replicating virus, leading to immune cytotoxicity and dysregulation, may be involved in the pathogenesis of SA-MCF [10, 44]. Additional studies are necessary to clarify mechanisms leading to cell activation and immune dysregulation following OvHV-2 and AIHV-1 infection, as well as the role played by the viruses or their components in the development of MCF.

In this scenario, given the similarities of the disease in rabbits and in natural hosts, the rabbit model will continue to be an important tool to dissect the pathogenesis of the disease caused by both OvHV-2 and AIHV-1.

MCF Vaccine Development

One major goal of MCF research is the development of effective vaccines to protect clinically susceptible animals from natural disease caused by MCF viruses. It has been previously demonstrated that AIHV-1-neutralizing antibodies, induced by vaccination with an attenuated AIHV-1, can protect animals from lethal AIHV-1 challenge, confirming that achieving protection from MCF using vaccination is an accomplishable goal [45].

The absence of an in vitro system to propagate OvHV-2 not only makes an attenuated vaccine for this virus unlikely but also makes detection of neutralizing antibodies and identification of viral targets a challenging task. Therefore, alternative approaches for vaccine development and monitoring protective immune responses are necessary. The possibility of using rabbits to test new vaccine targets and to assess neutralizing antibodies proffers opportunities to advance research using a reliable animal model.

The rabbit model has been effectively used to evaluate OvHV-2 glycoproteins capable of inducing neutralizing antibody responses [46, 47]. In one of these studies, rabbits were immunized with recombinant DNA plasmids expressing OvHV-2 glycoprotein B (gB), gH, and gL delivered into the skin by a biolistic method, resulting in successful production of hyperimmune sera. The sera were then used to treat cell-free OvHV-2 prior to intranasal inoculation into rabbits. Animals infected with antibody-treated virus did not develop disease, confirming that anti-OvHV-2 gB, gH, and gL antibodies can neutralize OvHV-2 [46]. Induction of anti-OvHV-2 antibodies in rabbits has also been demonstrated using an AIHV-1 chimeric virus that had its gB-encoding gene replaced by the OvHV-2 homolog gene [47]. Testing this AIHV-1 mutant virus in challenge experiments in the rabbit model will allow for its evaluation as a potential vaccine for both AIHV-1 and OvHV-2.

Although the target animals for any SA-MCF vaccine are large ruminants, particularly bison and cattle, preliminary experiments in a laboratory animal model are helpful to assess new vaccine constructs for induction of protective immune responses and to guide more complex and expensive trials in large animals. An important feature in favor of using rabbits for these purposes is that the expression pattern of immune genes associated with both cellular and humoral responses following OvHV-2 infection in rabbits and bison is very similar [10, 11••]. Therefore, it is expected that data obtained in rabbits regarding OvHV-2 vaccine candidates, delivery

systems, adjuvants, and host responses will be critical in identifying vaccine strategies and protocols that warrant further investigation in one or more susceptible ungulate species.

Conclusion

With the lack of an in vitro system to propagate OvHV-2, SA-MCF research relies heavily on animal experiments. Since routine use of natural hosts is expensive and logistically impractical, a reliable laboratory animal model is critical for research progress. Rabbits are susceptible to OvHV-2 infection, and a disease similar to that in natural hosts can be reliably induced following initial experimental system optimization. Therefore, rabbits will continue to be used for the foreseeable future to better understand the pathogenesis of SA-MCF and for the development of MCF vaccines. By describing steps carried out in our laboratory to implement a rabbit model for SA-MCF studies using cell-free OvHV-2, this review aims to highlight important aspects to consider in order to achieve consistent and reliable experimental data when using rabbits for OvHV-2 research.

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Compliance with Ethical Standards

Conflict of Interest Cristina W. Cunha, Donal O'Toole, Naomi S. Taus, Smriti Shringi, Donald P. Knowles, and Hong Li each declare no potential conflicts of interest.

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