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Pestivirus Infection Does Not Affect Prevalence of *Brucella melitensis* and *Encephalitozoon cuniculi* in Small Ruminants of the State of Nuevo Leon, Mexico

Ana Fabiola ELIZONDO-RODRÍGUEZ[1], Ramiro ÁVALOS-RAMÍREZ[1], Luis Edgar RODRÍGUEZTOVAR[1], Alicia Magdalena NEVÁREZ-GARZA[1], Ricardo GOMEZ-FLORES[2] and Castillo-Velázquez U[1]*

[1]Department of Veterinary Immunology, School of Veterinary Medicine and Zootechnics, Autonomous University of Nuevo León, México

[2]Autonomous University of Nuevo León, Faculty of Biological Sciences, Department of Microbiology, Laboratory of Immunology and Virology, San Nicolás de los Garza, N.L., Mexico

Corresponding author: U Castillo-Velázquez, Department of Veterinary Immunology, School of Veterinary Medicine and Zootechnics, Autonomous University of Nuevo León, México; **E-mail:** uziel_c@hotmail.com

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Abstract

Background: Members of the Pestivirus genus cause diseases associated with immunosuppression in cattle, which are involved in important economic losses. Similarly, *Brucella melitensis* is an intracellular bacterium of goats and sheep, whose infection significantly impacts on livestock production, whereas, *Encephalitozoon cuniculi* is an intracellular parasite of small ruminants, causing a subclinical disease, which worsens if they are immunosuppressed. In the present study, susceptibility to *B. melitensis* and *E. cuniculi* in small ruminants infected with Pestivirus was investigated.

Methods: Two hundred goat serological samples were obtained in production units proportionally distributed in 4 rural development districts (RDD) in the state of Nuevo León, Mexico. For the serological identification of Pestivirus, *B. melitensis*, and *E. cuniculi*, ELISA, Rose Bengal test, and carbon immunoassay methods were respectively used.

Results: Twenty five animals selected from each RDD were positive for Pestivirus. In addition, *B. melitensis* and *E. cuniculi* seroprevalence was 96% and 97.5%, respectively, regardless of Pestivirus infection.

Conclusion: Pestivirus does not influence animals susceptibility to *B. melitensis* and *E. cuniculi* infection. In addition, *B. melitensis* and *E. cuniculi* prevalence in the state of Nuevo Leon, Mexico was demonstrated.

Keywords: *Small ruminants, Seroprevalence, Antibodies, Pestivirus, Brucella melitensis, Bovine viral diarrhea, Encephalitozoon cuniculi, Microsporidia*

Introduction

Members of the Pestivirus genus of the Flaviviridae family are the causative agents of bovine viral diarrhea, border disease, and classic swine fever [1]. These are some of the most common ailments associated with immunosuppression of cattle, which increases secondary and opportunistic infections [2]. Infected animals show marked temporary immunosuppression resulting from leukocytes viral infection [3]. In these cells, viruses replicate, leading to genetic changes that eventually cause their adaptation and interspecies interaction. Pestivirus infections persist and disseminate within domestic and wild artiodactyls, causing significant economic losses [4].

These viruses cross placenta during pregnancy, inducing embryo death and abortions, reproductive disorders such as fetal mummification, stillbirths, congenital disabilities, and malformations [5]. By colonizing the fetus during early gestational development, viruses cause persistent infection, characterized by immunological tolerance [6]. However, the disease is often subclinical and is only revealed by the presence of specific antibodies [7]. However, if the infection occurs in the first third of pregnancy before the immune system develops, the fetus will become persistently infected and produce a high viral load without developing an immune response [8]. Such animals (infected with non-cytopathic viruses) are weak and more prone to infections of the digestive and respiratory tract. If these animals are infected with another cytopathic type-strain, they develop a disease called mucosal disease with 100% mortality [9].

Pestiviruses have a worldwide distribution, whose prevalence varies among countries and regions, becoming an essential factor in virus transmission between animal species. In Mexico, there is a 33.6% prevalence of Pestivirus in cattle [10], whereas globally its prevalence ranges from 2% to 25% [11]. However, studies on goats or sheep in Mexico have not been developed to date. Some control programs against Pestiviruses are based on the application of biosecurity measures in livestock, which involve early evaluation of the herd searching for signs of infection, implementation of a vaccination

system where the disease is present, determination and elimination of persistently infected animals [12], and periodic monitoring of the herds [8]. Control and eradication models depend on the prevalence and control laws of each country and region.

In addition, *Encephalitozoon cuniculi* is an opportunistic and obligated intracellular parasite, known to be a pathogen to different species, including small ruminants [13]. Generally, the infection is subclinical, however, in immunosuppressed animals, it is common to find nephritis, non-suppurative encephalitis, and vasculitis, among other affections [14]. The transmission of this intracellular agent is by ingestion or inhalation of the spores in urine or feces.

On the other hand, *Brucella melitensis* is a Gram-negative intracellular bacterium, endemic of sheep and goats, which is highly contagious to humans. *Brucella* affects the mammary gland, uterus, and testicles, causing abortions in pregnant females, and orchitis in males [15]. This disease causes an economic impact on livestock production, especially in the countries in which ovine and caprine production is essential [16].

In the present study, prevalence of *B. melitensis* and *E. cuniculi* in goats and sheep of Northeast Mexico, infected with Pestivirus was investigated.

Methods

Study Area and Animals Source

This study was developed with serum samples from 200 small ruminants (goats) proportionally distributed in the rural development districts (RDD) Anahuac, Apodaca, Montemorelos, and Galeana of the state of Nuevo Leon, Mexico. Animal serum samples were evaluated for the presence of *B. melitensis* and *E. cuniculi* antibodies by the Rose Bengal test and the carbon immunoassay (CIA) method respectively.

Detection of Antibodies against Pestivirus

A competitive ELISA (INgezim BVD Compac, Ingenasa, Madrid, Spain) was used to detect Pestivirus specific antibodies against NS2-3 antigen (p80/p125 inactivated viral antigens) in goats and sheep. Sensitivity and specificity of the test are 95% and 92.0%, respectively, according to the manufacturer.

Detection of Antibodies to *B. melitensis*

The hemagglutination Rose Bengal test was used to detect *B. melitensis* antibodies in serum [17]. Antigen stained with Rose Bengal was used and buffered at a low pH; 25 µl of serum samples were placed in a plate, along with 25 µl of antigen. Mixture was then stirred for approximately 4 minutes until hemagglutination was observed; if there was no evidence of agglutination, it was considered as unfavorable.

Detection of antibodies to *E. cuniculi*

CIA method was performed according to manufacturer's instructions (Medicago, Uppsala, Sweden). It consists of using *E. cuniculi* complete spores (killed by heat) suspended with carbon contained in the kit, and exposing them to serum samples; presence of antibodies will be visualized as agglutination under optical microscopy at 40X [18].

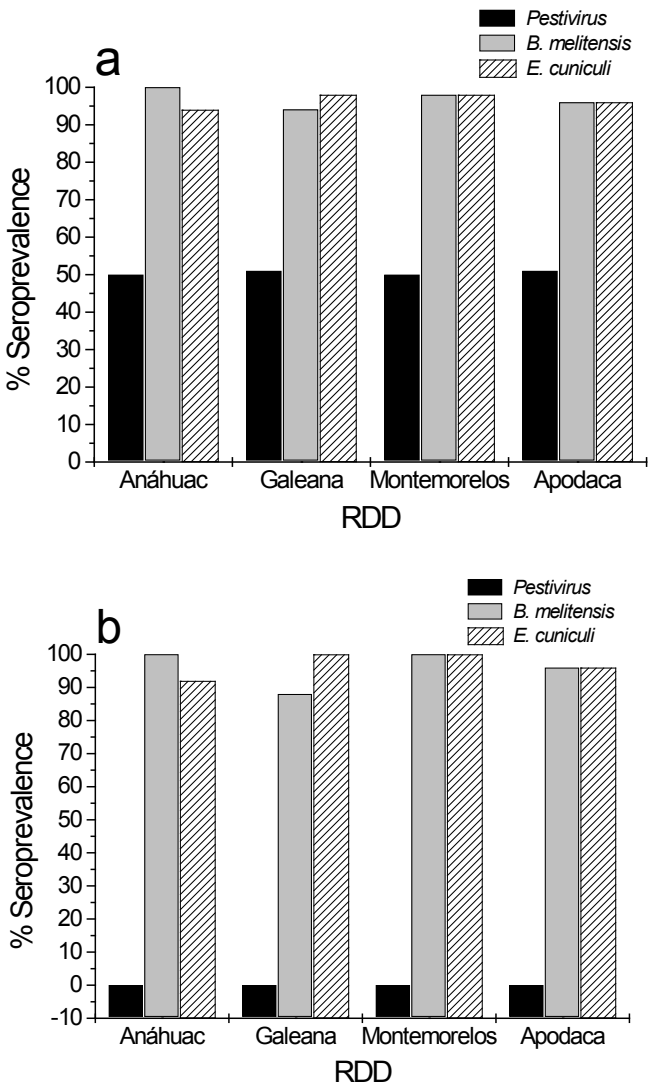

Figure 1: Seroprevalence of Pestivirus, *B. melitensis,* and *E. cuniculi* in RDD goats positive (a) and negative (b) for Pestivirus infection.

Results

As shown in Figure 1a, 50.5% ± 0.6% of animals from RDDs were positive for Pestivirus, whereas seroprevalence for *B. melitensis* and *E. cuniculi* was 97% ± 2.5% and 96.5% ± 1.9%, respectively. In Pestivirus-free animals, *B. melitensis* and *E. cuniculi* seroprevalence was 96% ± 5.65% and 97% ± 3.8% respectively (Figure 1b).

Discussion

Pestivirus infection causes immunosuppression, affecting IFN-γ and IL-2 production, phagocytosis and elimination of microorganisms, lymphocytes, and MHC regulation, which may reactivate some microorganisms already present or allow other opportunists to impair the infection [8,9,20].

The aim of the present study was to evaluate the role of Pestivirus infection on *B. melitensis* and *E. cuniculi* prevalence, potentially impairing the development of disease in small ruminants. We expected that those individuals infected with Pestivirus would be more

susceptible to infection by *Brucella* and *Encephalitozoon*. However, there was no difference on the prevalence of these microorganisms in control animals not infected with Pestivirus. This can be explained by not considering additional environmental or management factors such as malnutrition and hygiene conditions in pens.

B. melitensis and *E. cuniculi* co-infection was present in goats of the state of Nuevo Leon, Mexico, with a combined seroprevalence of animals infected and not infected with Pestivirus of about 97%, which is very high, compared with that of other states such as Veracruz (18.18% seroprevalence) [21], and with results from other countries, including Nigeria (9.6% seroprevalence) [22] and Colombia (1.2 % seroprevalence) [23]. The high prevalence of *E. cuniculi* should be taken into consideration, since this disease is not endemic of Nuevo Leon or even Mexico. There are no studies reporting seroprevalence or cases of this microorganism in small ruminants. However, presence of microsporidia in ruminants has been reported [24]; Juránková et al. [24] showed no significant difference between *Enterocytozoon bieneusi* prevalence of 17.5% and 13.33% in bovine herds positive and negative for Pestivirus respectively. Despite there are no reports to date about co-infections with *E. cuniculi*, these data indicated the potential of microsporidia to use ruminants as hosts [25].

The first report of specific antibodies against *E. cuniculi* in ruminants showed that were 43.6% seropositivity of Slovakia cows [26], in which immunohistochemical tests showed the presence of spores, suggestive of *E. cuniculi* in the placenta, brain, liver, myocardium, kidneys, and lungs of aborted fetuses. These findings are compatible with those observed in fetuses aborted by microorganisms of the *Brucella* genus, although they corresponded to *E. cuniculi*. This is one of the main reasons why the family *Brucella* was considered within the organisms to study in this investigation [26,27].

Pestivirus infection can maintain an immunosuppression status in animals because of the decrease in CD4 + and CD8 + lymphocytes in peripheral blood, as observed in HIV infections [20]. Other reports of Pestivirus-induced immunosuppression, resulted in the presence of opportunistic pathogens such as *Neospora caninum, Mycoplasma bovis*, and *Salmonella typhimurium* in ruminants [28,29].

Conclusion

We have demonstrated that Pestivirus infection is not a predisposing factor to acquire other opportunist intracellular microorganisms, since *B. melitensis* and *E. cuniculi* were prevalent in small ruminants in the absence of the virus. In addition, a significant prevalence of 97% for *B. melitensis* was observed, whereas *E. cuniculi* seroprevalence was 96.5% in goats of the state of Nuevo Leon, Mexico, that were infected with Pestivirus. Similarly, in Pestivirus-free animals, prevalence of *B. melitensis* and *E. cuniculi* was 96% and 97% respectively. Furthermore, we believe this is the first report on *E. cuniculi* seroprevalence in small ruminants in Mexico.

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