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Distribution of bovine alpha-herpesviruses and expression of toll-like receptors in the respiratory system of experimentally infected calves

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ABSTRACT

This study provides an initial analysis of the toll-like receptors (TLRs) that might be implicated in alpha-herpesvirus infection of the bovine respiratory system. A significant variation in the expression of TLR3 and TLRs 7–9 during bovine herpesvirus type 1 (BoHV-1) and 5 (BoHV-5) acute infections and particularly an up-regulation during viral reactivation in respiratory tissues has been demonstrated. Furthermore, viral distribution in the respiratory tract of BoHV-1- and BoHV-5-infected calves at different stages of the infectious cycle was analysed. The wide distribution of BoHV DNA in the respiratory tract during acute infection was restricted during latent infection and the subsequent reactivation of BoHV-1 and BoHV-5. Overall, the findings presented here contribute to the knowledge on the replication and dissemination of bovine alpha-herpesviruses. Furthermore, some of the immune factors triggered in the host that determine the different outcomes of infection by two closely related pathogens of cattle have been elucidated.

Toll-like receptors (TLRs) are a broad family of innate immune receptors that recognise pathogen-associated molecular patterns. TLRs 3, 7, 8 and 9 recognise microbial nucleic acids, particularly of viral origin, and constitute a powerful sensor system that detects viral invasion. Activation of TLRs induces the expression of pro-inflammatory mediators and anti-microbial effector molecules, which are required for the host's defence against invading pathogens and for tissue repair (Mogensen, 2009).

Bovine herpesvirus types 1 (BoHV-1) and 5 (BoHV-5) are two closely related alpha-herpesviruses that infect cattle. BoHV-1 is a major virus associated with respiratory disease (Muylkens et al., 2007). BoHV-5 is the primary etiological agent of non-suppurative necrotising meningoencephalitis in calves (Pérez et al., 2002) and it has occasionally been isolated from the genital and respiratory tracts of cattle (Schudel et al., 1986; Kirkland et al., 2009).

Little is known about herpesvirus replication and host invasion at primary replication sites, such as the bovine respiratory tract. Furthermore, the innate immune mechanisms underlying the clinical manifestations after BoHV infection have not been well-defined. The aim of this study was to determine the distribution of bovine alpha-herpesviruses and the variations in TLRs expression at different stages of the infectious cycle in the respiratory tract of their natural host.

Fourteen crossbred calves were randomly assigned for experimental challenges with the reference strain Cooper (BoHV-1.1) or the Argentinean BoHV-5 field strain (97/613). Samples from the respiratory system of the infected animals upon primary acute infection, latency or viral reactivation and from mock-infected animals were collected after euthanasia for virus isolation, DNA detection and TLR expression studies by real-time PCR (Marin et al., 2014, 2016), according to the details of the supplementary file.

Nasal secretions were present during acute virus infection and intermittently detected during latency and reactivation. Ocular secretions were detected during primary infection and reactivation. Respiratory or neurological signs were not observed. Rectal temperatures differed ($P < 0.05$) with respect to mock-infected calves only in BoHV-1-latently infected calves at 22 and 23 dpi.

In acutely infected calves, BoHV-1 was isolated from the epithelium of nasal mucosa and trachea and BoHV-5 from the trachea epithelium and retropharyngeal lymph nodes of one calf. During reactivation, only BoHV-1 was isolated from the epithelium of nasal mucosa of one calf. Virus isolation from tissue samples of latently-infected calves or uninfected animals was negative.

Virus DNA was mainly present in the epithelium of nasal mucosa, trachea and retropharyngeal lymph nodes. During primary acute infection BoHV DNA was consistently detected in tracheal epithelium (4/4), retropharyngeal lymph nodes, epithelium of the nasal mucosa and

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bronchi (3/4), apical, middle and diaphragmatic lobes of the lungs (1/2 BoHV-1-infected-calves), bronchial lymph nodes of both BoHV-1-infected calves and in mediastinal lymph nodes of a BoHV-5-infected calf. During latency, virus DNA was detected in the bronchial lymph nodes from a BoHV-1- and a BoHV-5-infected calf, in the retropharyngeal lymph nodes from a BoHV-5-infected calf and in tracheal epithelium from a BoHV-1-infected calf. Upon BoHV-1 reactivation, virus DNA was detected in the epithelium of the nasal mucosa, tracheal epithelium and lung diaphragmatic lobe. During BoHV-5 reactivation, virus DNA was only present in the retropharyngeal lymph nodes of one animal. Viral DNA was not detected in samples from uninfected calves (Table 1).

During acute infection and latency, a significant increase of TLR3 mRNA in the different respiratory areas was not observed ($P > 0.05$). TLR3 was only downregulated in the tracheal epithelium of BoHV-5-acutely infected calves (0.14-fold decrease) ($P < 0.05$). During reactivation, TLR3 expression was strongly upregulated. For BoHV-1, mRNA levels were 346.09- and 25.99-fold higher in the epithelium of the nasal mucosa and trachea, respectively. For BoHV-5, TLR3 mRNA levels were 260.28-fold higher in the epithelium of the trachea. Variations in TLR3 levels were not detected in any other area (Fig. 1A).

TLR7 (Fig. 1B) mRNA increased 3.5-fold during acute BoHV-1 infection, particularly in nasal mucosa and lungs ($P < 0.05$) and there was a significant decrease ($P < 0.05$) (0.29-fold) in the tracheal epithelium. TLR7 expression was not affected ($P > 0.05$) during acute BoHV-5 infection or BoHV-1 or BoHV-5 latency. During BoHV-1 and BoHV-5 reactivation, TLR7 expression was upregulated in the diaphragmatic lobe of the lungs (16.79- and 9.88-fold, respectively). TLR7 mRNA also increased ($P < 0.05$) in the tracheal epithelium (1.26-fold) during BoHV-1 reactivation.

Upregulation of TLR8 was detected in the epithelium of nasal mucosa (7.44-fold increase) of BoHV-1-acutely-infected animals. For BoHV-5, at this stage, the relative levels of TLR8 mRNA did not change significantly ($P > 0.05$). Similarly, during BoHV-1 and BoHV-5 latency, significant levels of TLR8 expression in the respiratory tissues were not detected ($P > 0.05$). However, TLR8 expression increased in the trachea and lungs during reactivation. TLR8 was strongly upregulated in the tracheal epithelium and diaphragmatic lobes of the lungs during BoHV-1 reactivation (46.37- and 10.55-fold increase, respectively) ($P < 0.05$). In BoHV-5 reactivation, TLR8 levels in the tracheal epithelium and lungs were 19.36- and 6.43-fold higher, respectively, but differences were not observed ($P > 0.05$) in the lungs (Fig. 1C).

Relative TLR9 mRNA levels in the tracheal epithelium of BoHV-1- and BoHV-5-acutely-infected animals were significantly lower ($P < 0.05$) than in mock-infected animals (0.13- and 0.22-fold decrease, respectively) (Fig. 1D). Variations in TLR9 levels in other areas of respiratory tissue at this stage of infection, or during latency, were not observed ($P > 0.05$). During reactivation, TLR9 was strongly upregulated in the tracheal epithelium and diaphragmatic lobes of the lungs during BoHV-1 reactivation (32.11- and 5.9-fold increase, respectively) ($P < 0.05$). For BoHV-5 reactivation, TLR9 levels in tracheal epithelium and lungs were 34.78- and 4.1-fold higher, respectively, but differences in the lungs were not significant ($P > 0.05$).

BoHV-1 and BoHV-5 can cause distinct syndromes in cattle. However, they can also be isolated from very similar clinical conditions (Silva et al., 2007). Therefore, it is important to study both viruses together. The immune mechanisms triggered after invasion of the primary replication sites, such as the respiratory tract, and the role of TLRs during respiratory BoHV infection have not been elucidated. In this study, the findings on viral DNA detection in different areas of the respiratory tract also contribute to the understanding of bovine alphaherpesviruses dissemination within the host. BoHV-1 DNA was widely distributed in the bovine respiratory system of acutely infected calves. With the only exception of the mediastinal lymph nodes, the virus genome was detected in all other samples. As expected, BoHV-5 distribution in the respiratory tract during primary acute infection was more restricted. The virus only reached the upper respiratory tract, and BoHV-5 DNA was not detected in bronchial lymph nodes and lungs. This wide distribution of virus genome a few days after infection was not evident during latency and reactivation. Virus detection in the bronchial lymph nodes of latently-infected calves suggests that these tissues might play a role as non-neural sites of latency, which requires further research. Overall, these findings confirmed that after primary infection, latency and subsequent reactivation, BoHV-1 and BoHV-5 displayed similar biological features and consequently need to be considered together for the control of BoHV infection.

In this study, the detection of viral DNA by real-time PCR with HRM analysis allowed a better description of herpesvirus dissemination in the respiratory tract and confirmed the importance of applying molecular techniques to improve the sensitivity of the tests used for pathogens identification. HRM was successfully used as a novel procedure for the analysis and differentiation of BoHV types in tissue samples.

The most relevant finding of this study was the significant variation in the expression of TLR3 and TLR7-9 in respiratory tissues from BoHV-5- and BoHV-1-infected calves during acute infection and reactivation. Knowing how BoHV infection compromises host defences in the respiratory tract is essential for the rational design of control and/or therapeutic measures for viral respiratory diseases in cattle. The broad distribution of BoHV-1 DNA during acute infection when compared with BoHV-5 is in agreement with the higher stimulation of the receptors involved in the innate immune response, particularly TLR7 and TLR8, in the epithelium of the nasal mucosa and lung only during BoHV-1 acute infection. A previous study by Marin et al. (2014) also

Table 1
Detection of viral DNA by real-time PCR with high resolution melting (HRM) analysis in respiratory sections of calves inoculated with BoHV-1 or BoHV-5.

Groups	BoHV type	Calf no.	Epithelium			Lung lobe			Lymph nodes		
			Nasal mucosa	Trachea	Bronchi	Apical	Middle	Diaphragmatic	Retropharyngeal	Bronchial	Mediastinal
Group 1	BoHV-1	1	+	+	+	+	−	−	+	+	−
Acute infection		2	+	+	+	−	+	+	−	+	−
	BoHV-5	3	−	+	−	−	−	−	+	−	+
		4	+	+	+	−	−	−	+	−	−
Group 2	BoHV-1	5	−	−	−	−	−	−	−	+	−
Latency		6	−	+	−	−	−	−	−	−	−
	BoHV-5	7	−	−	−	−	−	−	−	+	−
		8	−	−	−	−	−	−	+	−	−
Group 3	BoHV-1	9	+	+	−	−	−	+	−	−	−
Reactivation		10	+	−	−	−	−	−	−	−	−
	BoHV-5	11	−	−	−	−	−	−	−	−	−
		12	−	−	−	−	−	−	+	−	−
Group 4		13	−	−	−	−	−	−	−	−	−
Controls		14	−	−	−	−	−	−	−	−	−

+: Viral DNA was detected in the respiratory sample analysed; −: Viral DNA was not detected in the respiratory sample analysed.

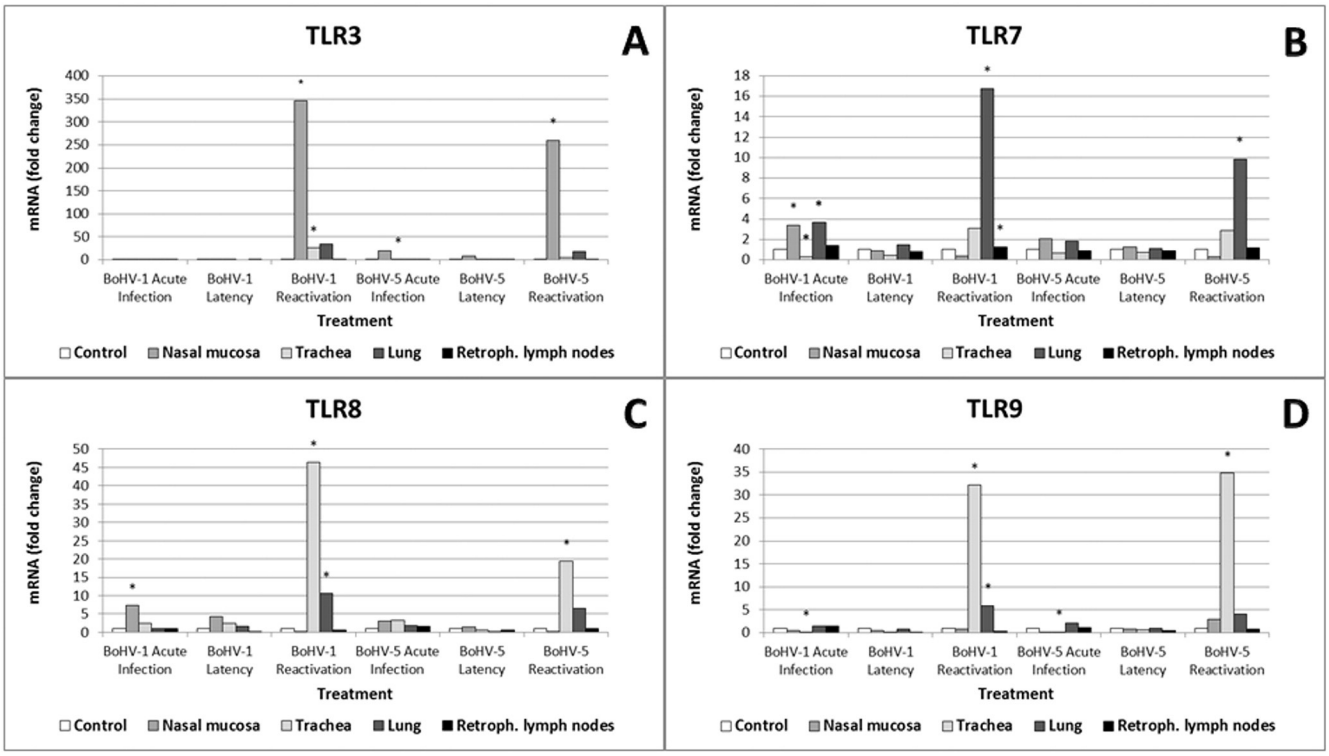

Fig. 1. Relative expression of TLR3 (A) and TLR7-9 (B, C and D) in the respiratory tracts of BoHV-1- and BoHV-5-infected calves. The results represent the mean fold change of TLR transcription levels in specific areas of the respiratory tract of infected calves over levels detected in tissue sections of uninfected calves, which served as the control group.; *: statistically significant differences ($P < 0.05$) with respect to the uninfected control.

demonstrated that Imiquimod, an agonist of TLR7/TLR8, impairs BoHV replication in MDBK cells.

During reactivation, TLR3 mRNA increased in the epithelium of nasal mucosa from BoHV-1- and BoHV-5-infected calves and in the tracheal epithelium of BoHV-1-infected calves. TLR7 levels increased in the lung during reactivation of both viruses. This was the only receptor that was significantly affected by infection in the retropharyngeal lymph nodes during BoHV-1 reactivation, suggesting that it might play a role upon viral reactivation in lymphoid tissues. At this stage of infection, upregulation of TLR8 and TLR9 was detected in the tracheal epithelium of BoHV-1- and BoHV-5-infected calves and lungs of BoHV-1-infected animals. The fact that all TLRs evaluated are particularly upregulated during virus reactivation must be highlighted. TLR activation might be important to halt virus dissemination as evidenced by the limited distribution of viral genome during latency and reactivation. Therefore, understanding the mechanisms involved in TLR activation during a viral disease will provide evidence for the future use of TLR agonists in the control of infection. This study contributes to the knowledge on the tissue dissemination of bovine alpha-herpesviruses and on the immune factors that might determine the different outcomes of BoHV infection.

Conflict of interest statement

The authors declare no potential conflicts of interest with respect to the research, authorship, publication of this article and/or financial and personal relationships that could inappropriately influence this work.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.rvsc.2016.01.011.

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