Characterizing immune responses of MERS-CoV infected alpacas (*Vicugna pacos)*

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INTRODUCTION AND OBJECTIVES

Middle East respiratory syndrome coronavirus (MERS-CoV) emerged in 2012 in Saudi Arabia and it is a worldwide threat [1].

Dromedary camels are a major reservoir hosts for MERS-CoV and an animal source of MERS infection in humans [2].
Besides dromedaries, several animal species, including common marmosets, rhesus macaques, llamas, pigs and alpacas, are susceptible to MERS-CoV infection [3].

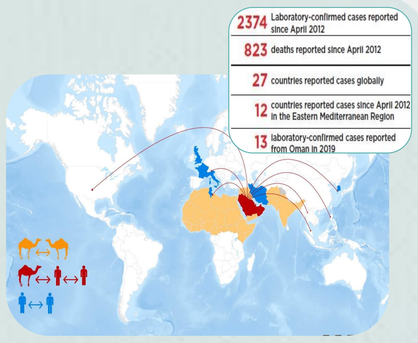


Figure 1. MERS-CoV transmission and geographic range. At the top-right corner a summary of reported cases to date is depicted [2]

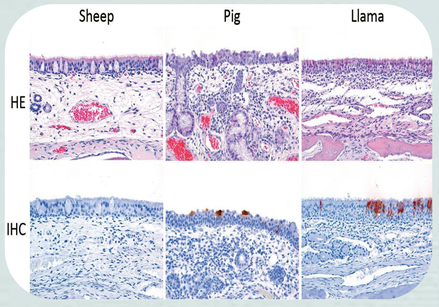


Figure 2. Histology and expression of MERS-CoV N protein by IHC at day 4 pi in the nasal epithelium of animals inoculated with MERS-CoV [6]

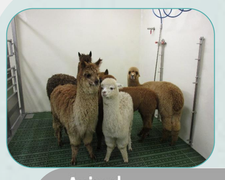
The MERS-CoV S protein mainly determinates tissue tropism and mediates viral attachment to host cells. DPP4, the MERS-CoV receptor, is presented on the surface of many cell types [4].

As it is seen in *Figure 2,* MERS-CoV preferably infects respiratory epithelial cells expressing DPP4 in llamas and pigs [5, 6].

Early IFN and proinflammatory responses were triggered in the nasal cavity of alpacas upon infection with MERS-CoV (*results not published*).

The main objective is to study the early immune responses in lungs elicited after MERS-CoV inoculation in alpacas.

MATERIAL AND METHODS



Animal identification	Group	Day of euthanasia
G1 (A13-A15)	Negative Controls	0 p.i.
G2 (A1-A3)	MERS-CoV inoculated alpacas (10^7 $TCID_{50}$)	1 p.i.
G3 (A4-A6)		2 p.i.
G4 (A7-A9)		3 p.i.
G5 (A10-A12)		4 p.i.

Figure *3.* Alpacas inside the BSL-3 animal facilities (IRTA-CReSA).
Table 1. Experimental design

Experimental design:

Fifteen alpacas were used: 3 served as negative controls (G1) and the others (n=12) were inoculated with 10^7 $TCID_{50}$ of MERS-CoV Qatar15/2015.

Sequentially necropsies were performed (days 0, 1, 2, 3, and 4 p.i., 3 alpacas/day) and respiratory tissues (nasal turbinate, trachea and lungs) were collected.

Virus detection by IHC:

Formalin-fixed tissues were embedded in paraffin and sectioned at 4 µm. To detect the presence of MERS-CoV antigen, a monoclonal antibody directed to the nucleocapsid protein (SinoBiological Inc) was used.

Cytokine quantification by RT-qPCR:

- Total RNA was extracted from methacarn-fixed slides (sectioned at 7-8µm) using the RNeasy® Mini Kit to quantify several cytokines and chemokines such as IFN-λ3 and MX1
- RT-qPCR was performed using the Luna® Universal Probe One-Step RT-qPCR Kit (New England Biolabs)
- Normalization was performed using GADPH, HPRT1 and UbC as endogenous reference gens
- Results are expressed as Fold changes of mRNA levels and the delta-delta CT method was used for data normalization with samples from negative control (G1)

RESULTS AND DISCUSSION

Virus detection:

- Viral RNA was detected on day 2 p.i. (Figure 4) mainly affecting epithelial cells and submucosa of the bronchiole. These results are in concordance with previous studies in nasal turbinate.
- No antigen-positive cells were detected afterwards, suggesting a potent innate immune response.

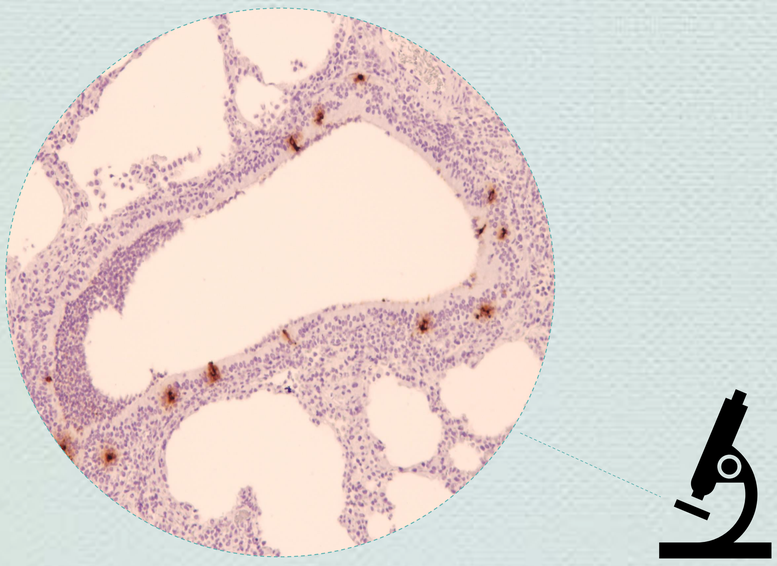


Figure 4. MERS-CoV IHC staining in lung of alpacas euthanized at day 2 p.i. Brown color indicates **nucleocapsid protein of MERS-CoV**

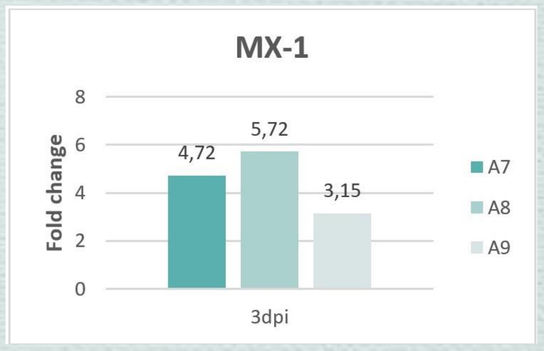


Figure 5. MX-1 expression profile in lung of MERS-CoV infected alpacas at 3 dpi

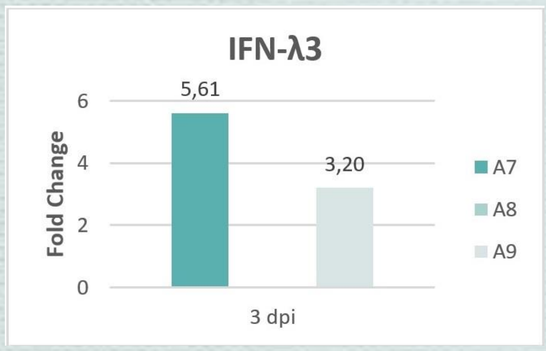


Figure 6. IFN-λ3 expression profile in lung of MERS-CoV infected alpacas at 3 dpi

Cytokine quantification:

- In lung, MERS-CoV infection resulted in a upregulation of MX-1 and IFN-λ3 on day 3 p.i. (Figure 5 and 6)
- These results are in concordance with previous studies done in the nasal turbinate of infected alpacas, although in the nasal cavities the upregulation was higher
- However, *in vitro* studies in human cells a downregulation of cytokines and chemokines was reported [7], suggesting that early immune response might have a role in controlling the MERS-CoV infection
- The lack of RNA in one sample resulted in the absence of data to calculate the IFN-λ3 quantification (Figure 6)

CONCLUSION

An upregulation of IFN-λ3 (antiviral cytokine) and MX-1 (IFN-induced protein) was detected in the lung of MERS-CoV-infected alpacas.

REFERENCES

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