endogenous cathelicidin, 4 mM), or subjected to silencing of LL-37 (shLL-37), followed by infection with the reference strains *N. caninum* NC-1 (MOI: 0.2) and BoAHV-1 Cooper (MOI: 0.1). At 24 h post-infection, gene expression levels of key immune molecules involved in TLR mediated signaling pathways (NF-κβ, MyD88, IRF3, and IRF7) were assessed by RT-qPCR and tachyzoite replication was examined microscopically. The results indicated that *N. caninum* replication in co-infection with BoAHV-1 was significantly decreased both intracellularly and extracellularly after chloroquine pretreatment and shLL-37 compared to imiquimod and sodium butyrate treatments. This reduction was accompanied in co-infection by a significant increase, with respect to the control, in MyD88 and IRF3 expression (3.9-fold and 5.5-fold, respectively, $p<0.05$) after chloroquine treatment, and a decrease in IRF3 expression after shLL-37 (0.409-fold, $p<0.05$). While treatments with imiquimod and sodium butyrate resulted in a significant decrease, with respect to the control, in the expression of three of the four molecules analyzed (NF-κβ: 0.126 and 0.074; MyD88: 0.365 and 0.508; IRF7: 0.078 and 0.01, respectively, $p<0.05$). These findings highlight the role of TLR7 and LL-37 in co-infection, emphasizing their impact on dynamics.
TLR7 and LL-37 regulate BoAHV- 1 replication, promoting *N. caninum* replication when both pathogens are present.

CMB5- Virulence studies of *Neospora caninum* in goat trophoblast primary culture

Alvarez, B.E. [1,2]*, Rambeaud, M. [1], Dellarupe, A. [1,2], Campero, L.M. [2,3], Venturini, M.C. [1]

[1]Laboratorio de Inmunoparasitología (LAINPA), FCV, UNLP, La Plata.
[2]CONICET.
[3]Instituto de Innovación para la Producción Agropecuaria y el Desarrollo Sostenible (IPADS) (CONICET-INTA Balcarce).

*Presenting author: alvarezbetianaelizabeth@gmail.com

Neospora caninum is a well-documented cause of abortion in cattle worldwide. The relevance of the abortifacient role of this parasite in small ruminants remains under investigation, especially in areas with high seroprevalence. The objective of this study was to evaluate the virulence (tachyzoite yield [TY] and invasion rate [IR]) of a locally isolated *N. caninum* strain (*NC-Argentina LP1*) compared to a reference strain (*NC-1*) in goat trophoblast cells. Primary culture was obtained from cotyledons of a goat seronegative for *N. caninum* and *Toxoplasma gondii*, and negative by PCR, using the explant method. A total of 10^5placental cells/well (passage 4) were seeded into 24-well plates and grown to confluence (48 hours). For TY and IR studies, cultures were infected with $2x10^5$tachyzoites/well and 10^4tachyzoites/well of each strain, respectively, in 3 independent assays. At 48 h post infection, TY was quantified by qPCR, and IR was evaluated by immunolabeling of parasitophorous vacuoles (PVs) with anti-*N. caninum* hyperimmune serum and its conjugate (Alexa Fluor 488, Life Technologies) and DAPI staining. Fifty fields per replicate were counted and PV size was measured. Regarding TY, significant differences were found between *NC-Argentina LP1* and *NC-1*, with lower values for *NC-Argentina LP1* compared to *NC-1* ($p<0.01$). In addition, *NC-Argentina LP1* showed a lower IR than the reference strain ($p<0.0001$). However, the size of the PVs was larger for *NC-Argentina LP1* with respect to *NC-1* ($p<0.0001$), which could be related to its lower capacity to reinvade. These results suggest that *NC-Argentina LP1* can invade and replicate in primary cultures of goat trophoblast, although with lower virulence than the reference strain *NC-1*, and may aid in predicting *in vivo* behavior.