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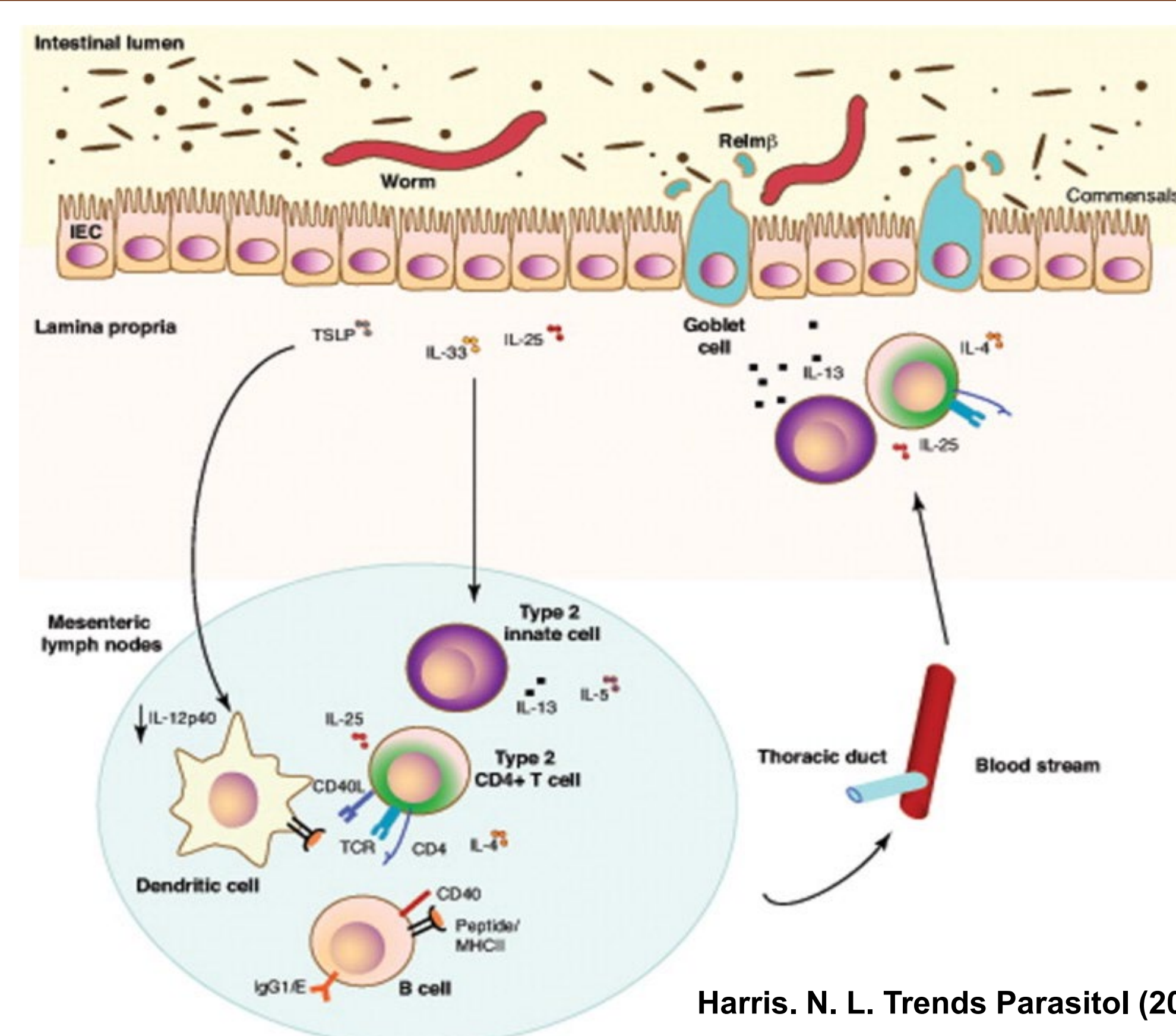
## Introduction

- *Heligmosomoides polygyrus bakeri* (Hpb) is a natural nematode parasite with mice as its definitive host
- Hpb elicits a strong polarized T helper 2 (Th2) immune response in gastrointestinal tract of mice
- Depending on the strain of mice infected, Hpb can survive for many weeks during primary infection and secondary infection provokes a strong sterile immunity resulting in the expulsion of worm by 28<sup>th</sup> days post infection (DPI)
- Hpb derived excretory secretory products contains various molecules which are potent immunomodulatory substances
- The exact mechanism involved in the survival of the parasite during primary infection is unknown

## Objective

- To isolate parasite derived exosomes which play an important role in modulating Th2 immune response elicited during the primary infection

## Immune response to worm



## Methodology

- Infected the mice orally with 200 infective stage L3 larvae of Hpb, per mice
- Adult parasites were isolated from infected mice on 14 dpi
- The adult parasites were maintained in specific culture medium for 14 days
- Spent medium were replaced every 3<sup>rd</sup> day of culture
- The spent medium were used to purify and concentrate parasite derived excretory secretory products (HES) using Amicon® Ultra Centrifugal Filters at 3kDa (MWCO)
- HES is also subjected to ultracentrifugation at 100,000g to separate exosomes from HES.
- Purified and concentrated HES is analyzed by silver staining
- Purified exosomes were analyzed via scanning electron microscopy (SEM) and by uptake assay with murine intestinal epithelial cell line (MODE-K)

## Results

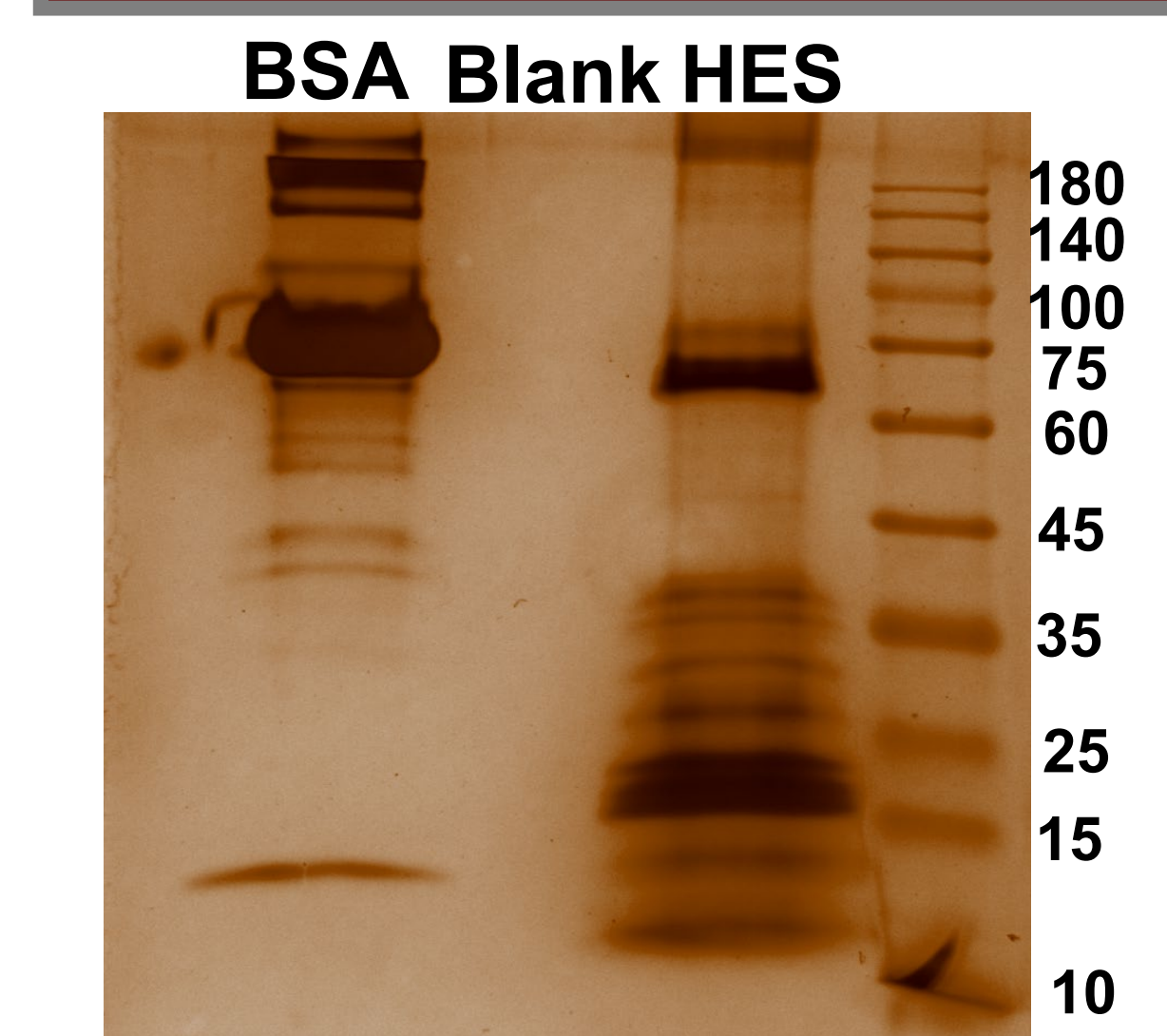
### L3 Larva of Hpb



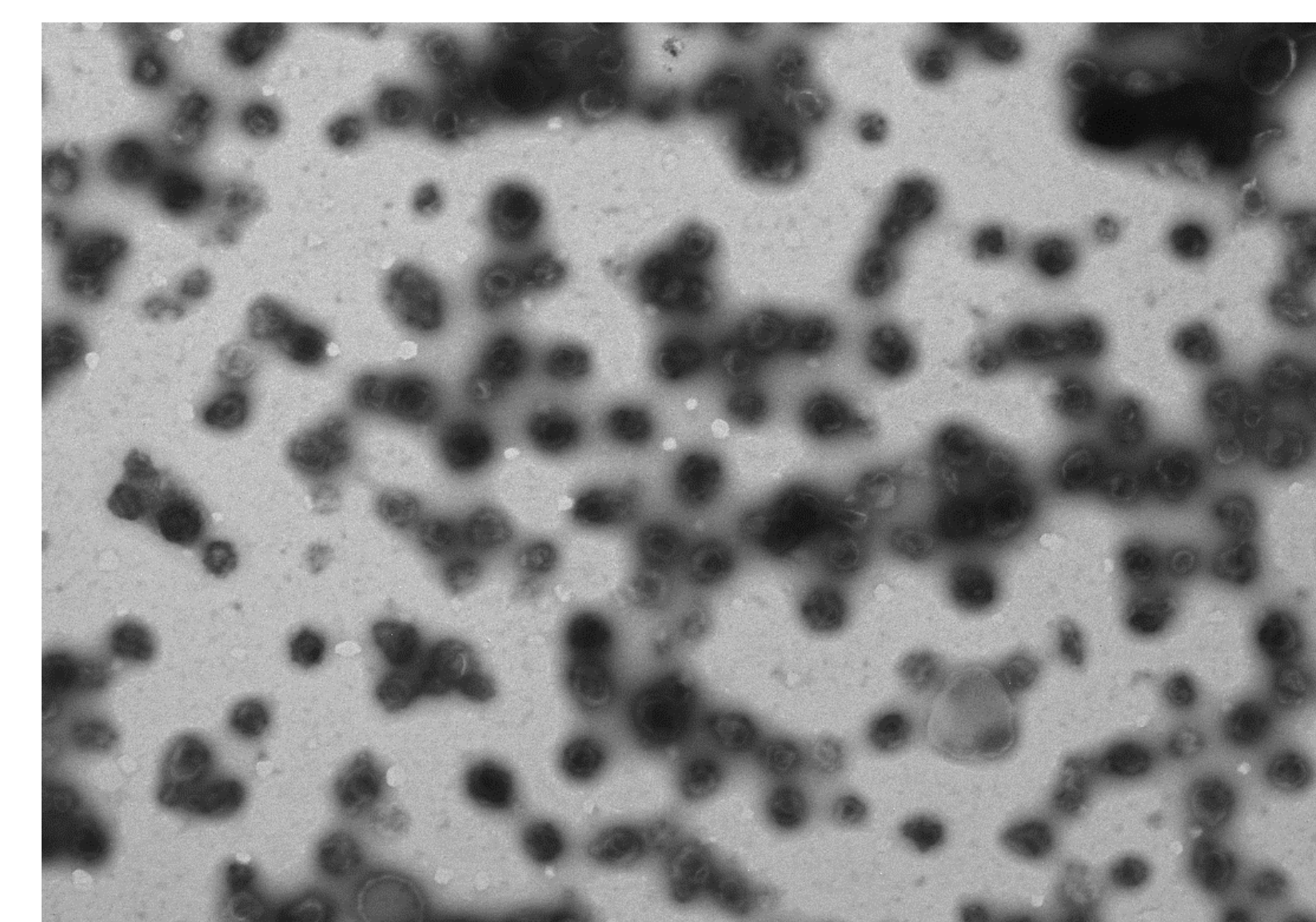
### Adult Hpb



### Silver staining of HES

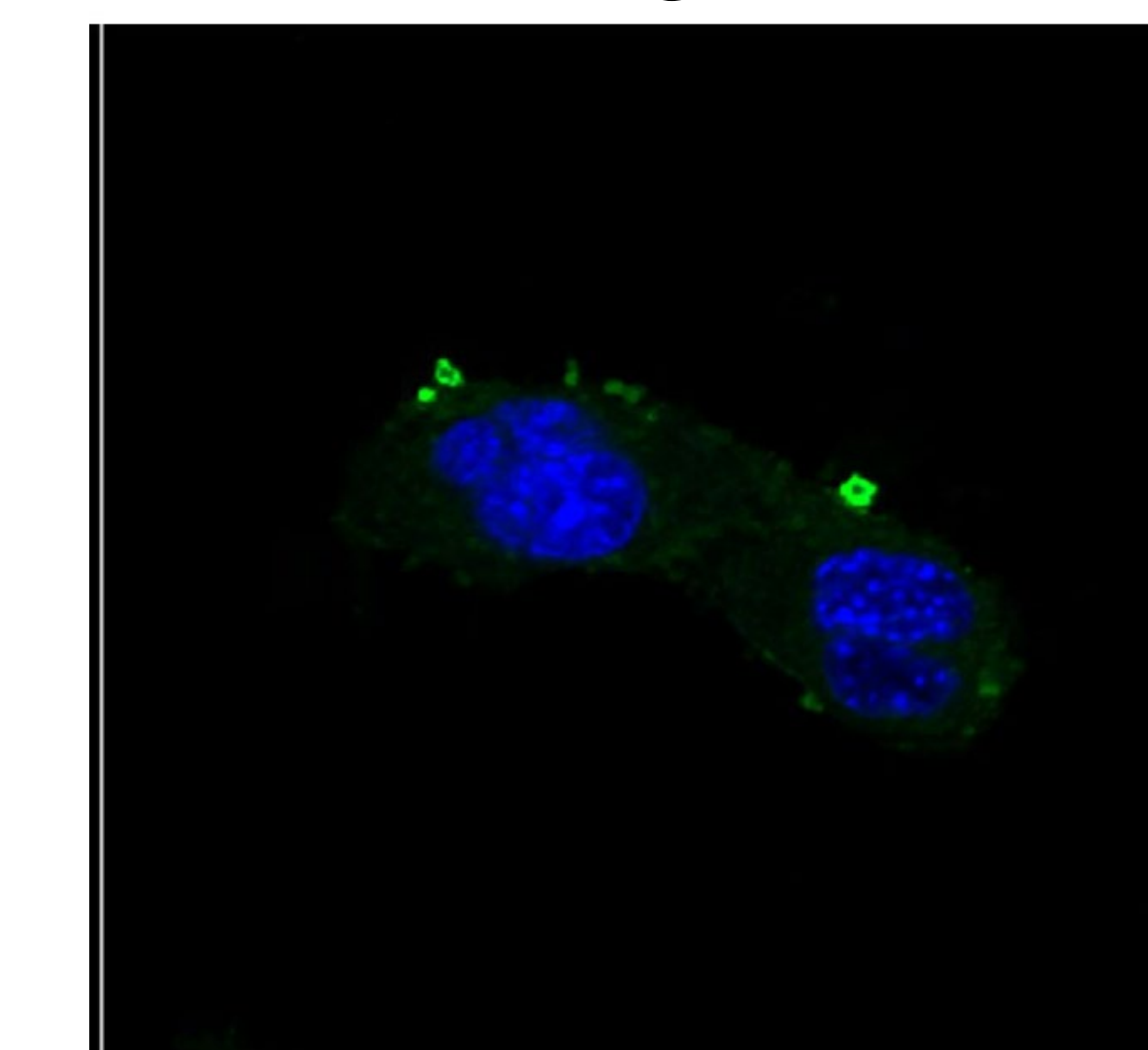


### SEM image of Hpb derived exosomes

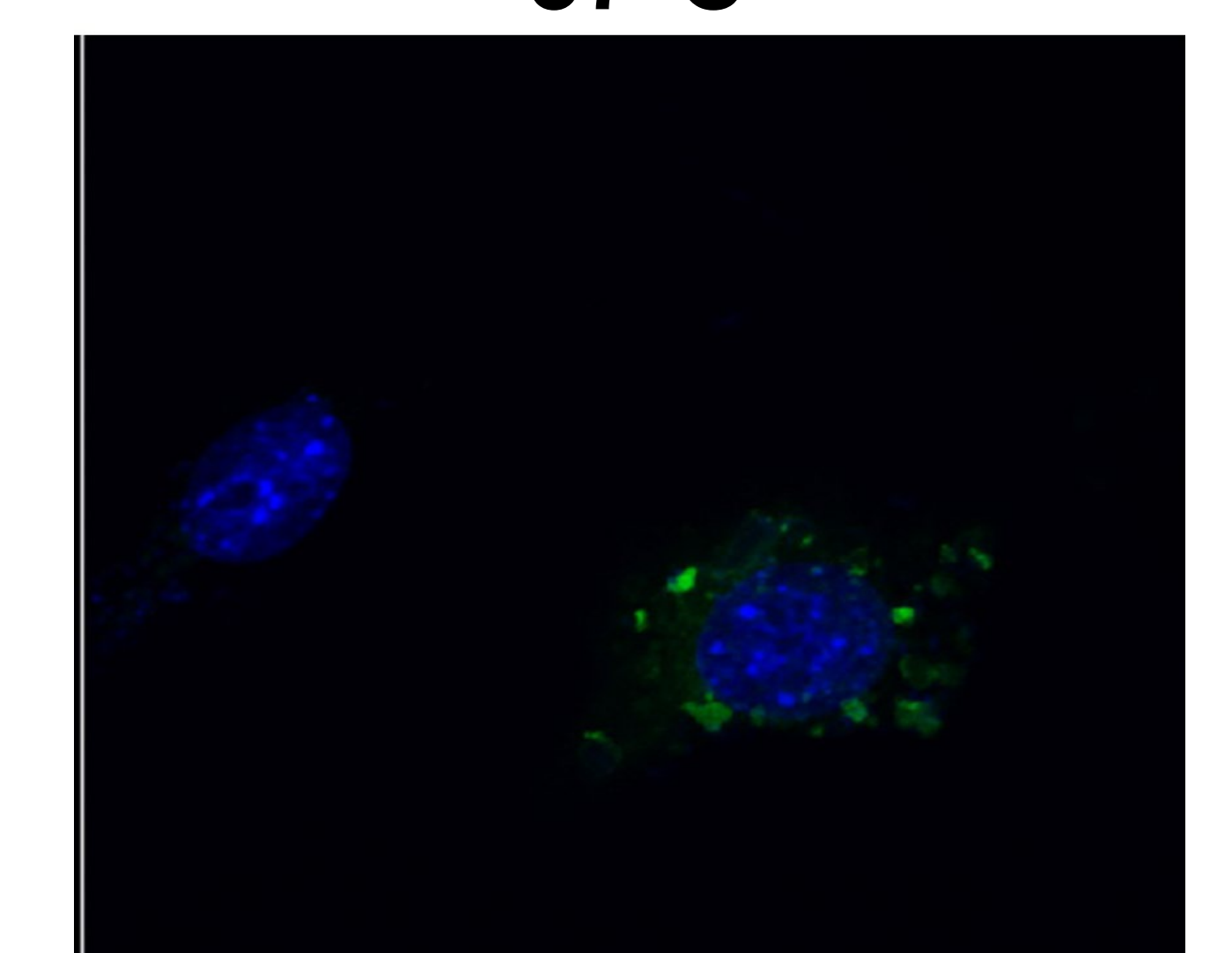


### MODE-K Cells up taking the PKH-67 stained exosomes

4°C



37°C



## Conclusion

- Successfully isolated and purified the parasite derived exosomes from *Heligmosomoides polygyrus bakeri*

## Reference

- Buck, A.H., Coakley, G., Simbari, F., McSorley, H.J., Quintana, J.F., Le Bihan, T., Kumar, S., Abreu-Goodger, C., Lear, M., Harcus, Y., Ceroni, A., Blaxter, M., Ivens, A., Maizels, R.M., 2014. Exosomes secreted by nematode parasites transfer small RNAs to mammalian cells and modulate innate immunity. Nat. Commun. 5, 5488. <https://doi.org/10.1038/ncomms6488>
- Camberis, M., Le Gros, G., Urban, J., 2003. Animal Model of *Nippostrongylus brasiliensis* and *Heligmosomoides polygyrus*, in: Coligan, J.E., Bierer, B.E., Margulies, D.H., Shevach, E.M., Strober, W. (Eds.), Current Protocols in Immunology. John Wiley & Sons, Inc., Hoboken, NJ, USA.