



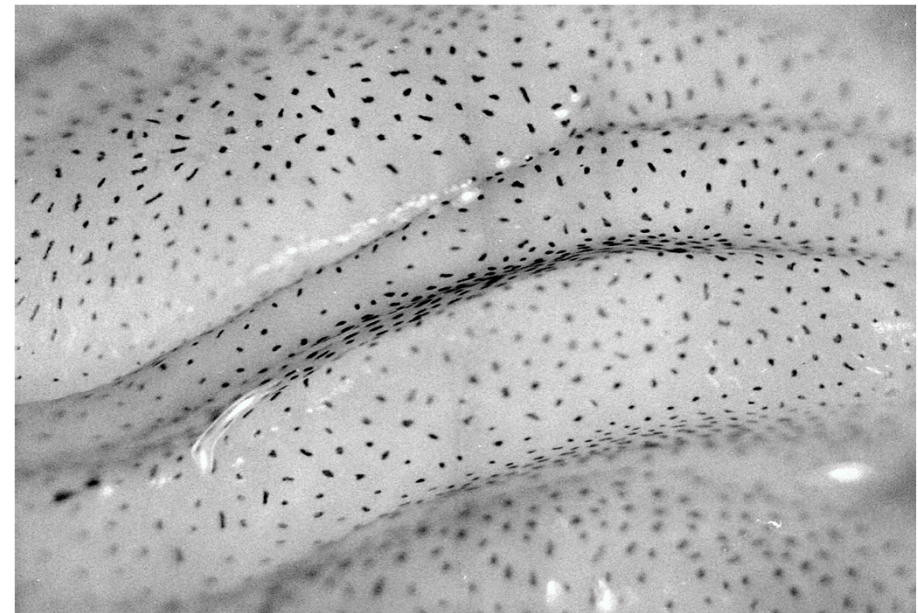
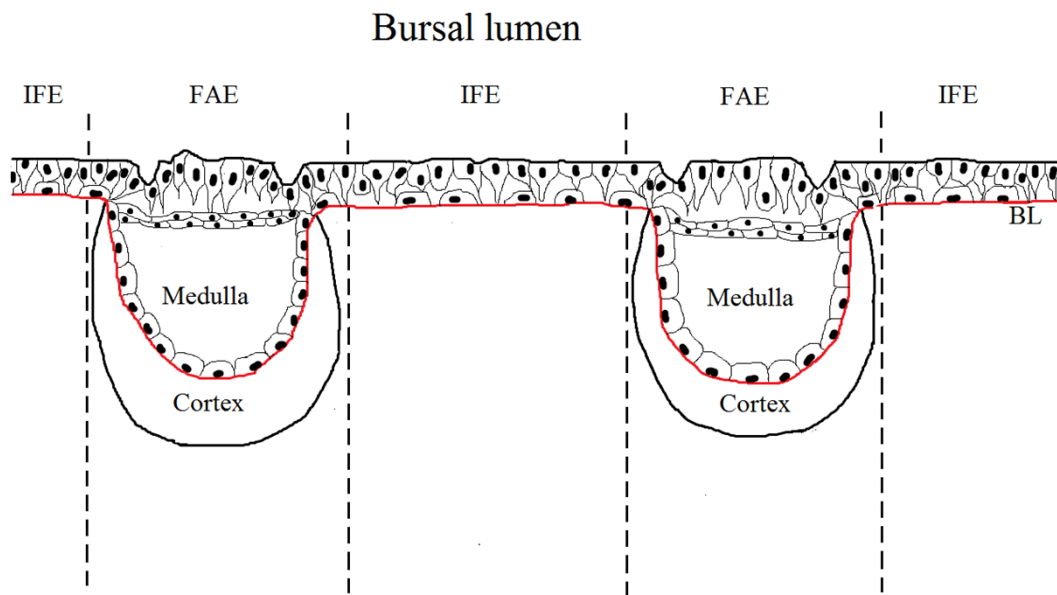
Infectious bursa disease virus IBDV influences the Caveolin-1
expression in the interfollicular epithelium of chicken's bursa of
Fabricius

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Bursa of Fabricius of chicken locates between the cloaca and the sacrum and the bursal duct opens into the cloaca. Bursa contains 12-15 plicae or folds, which are covered by interfollicular (IFE) and follicle-associated epithelium (FAE). The IFE and FAE form 90 and 10% of surface area of the bursal epithelium, respectively.



It is generally accepted, that the FAE can provide two- directional „traffic” between the follicle and bursal lumen. Terminal maturation of the bursal secretory dendritic cells results in „macrophages”, which can enter the lumen of the bursa from the follicle and from the lumen FAE can trapped and conveys antigen(s) to the follicle.

The aim of this work was to study the Caveolin-1 mediated endocytosis in the FAE using Caveolin-1 immunocytochemistry and transmission electron microscopy.

Caveolin-1 is an integral membrane protein and essential component of endocytotic caveolae. Caveolin-1 binds and carries intracytoplasmic cholesterol from Golgi apparatus to cell membrane and back to Golgi.

Experimental procedure

We have studied the Caveolin-1 expression in special pathogen free (SPF) White Leghorn birds which were infected with infectious bursa disease virus (IBDV).

Chicken

Three weeks old **control** and Winterfield 2512 **Vaccine**, Variant **Delaware** E strain and Very Virulent Field Isolate D407/02/04/TR **Turkish** virus infected birds were sacrificed at 2, 4, 7, 14, 21 and 28 days posinfection.

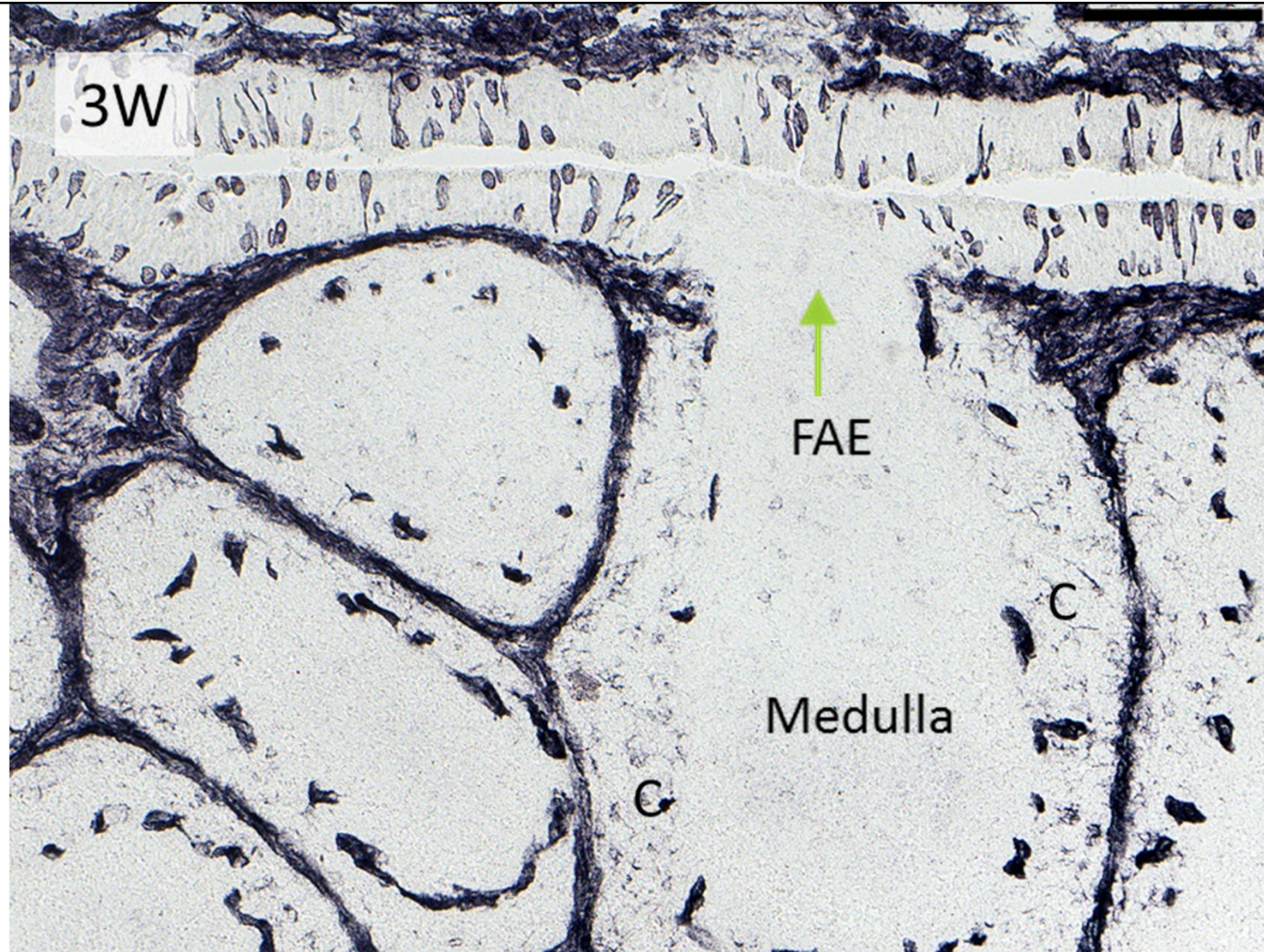
Immunocytochemistry

For immunocytochemistry the tissue samples (bursa, caecal tonsil, spleen and thymus) were embedded in liver and frozen in liquid nitrogen. The cryostate sections were fixed in cold acetone and stained with anti- Caveolin-1 (Polyclonal, rabbit anti- Caveolin-1; BD Transduction Laboratories, Biosciences, San Jose, USA) antibody 1:120 was diluted.

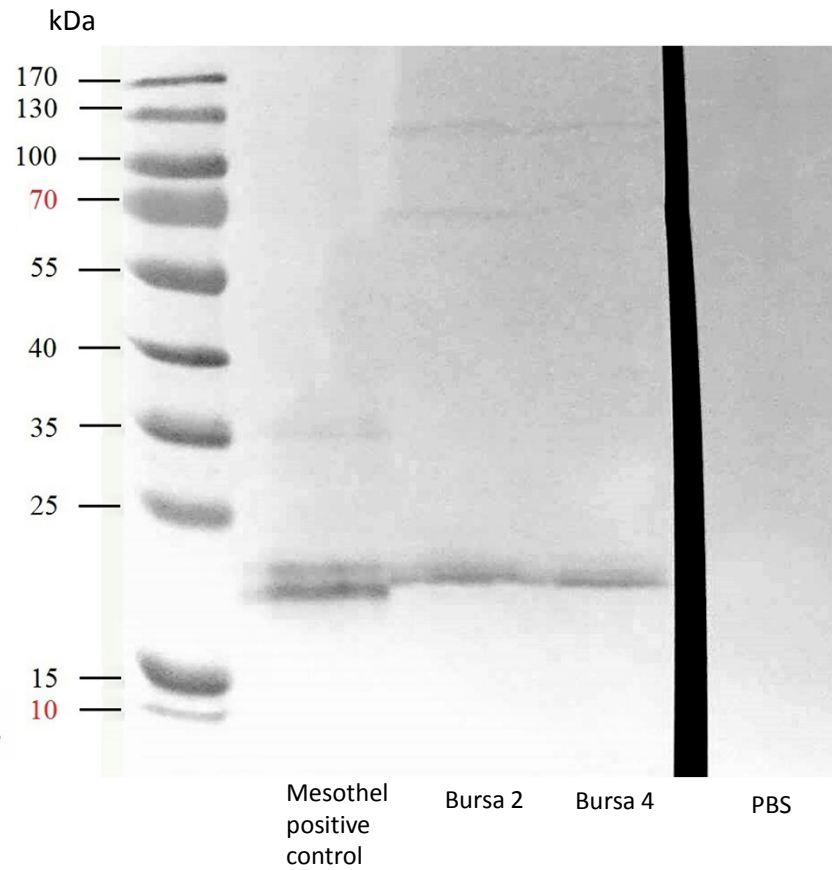
Transmission Electron Microscopy

Tissue samples for transmission electron microscopy were prepared according to general protocol.

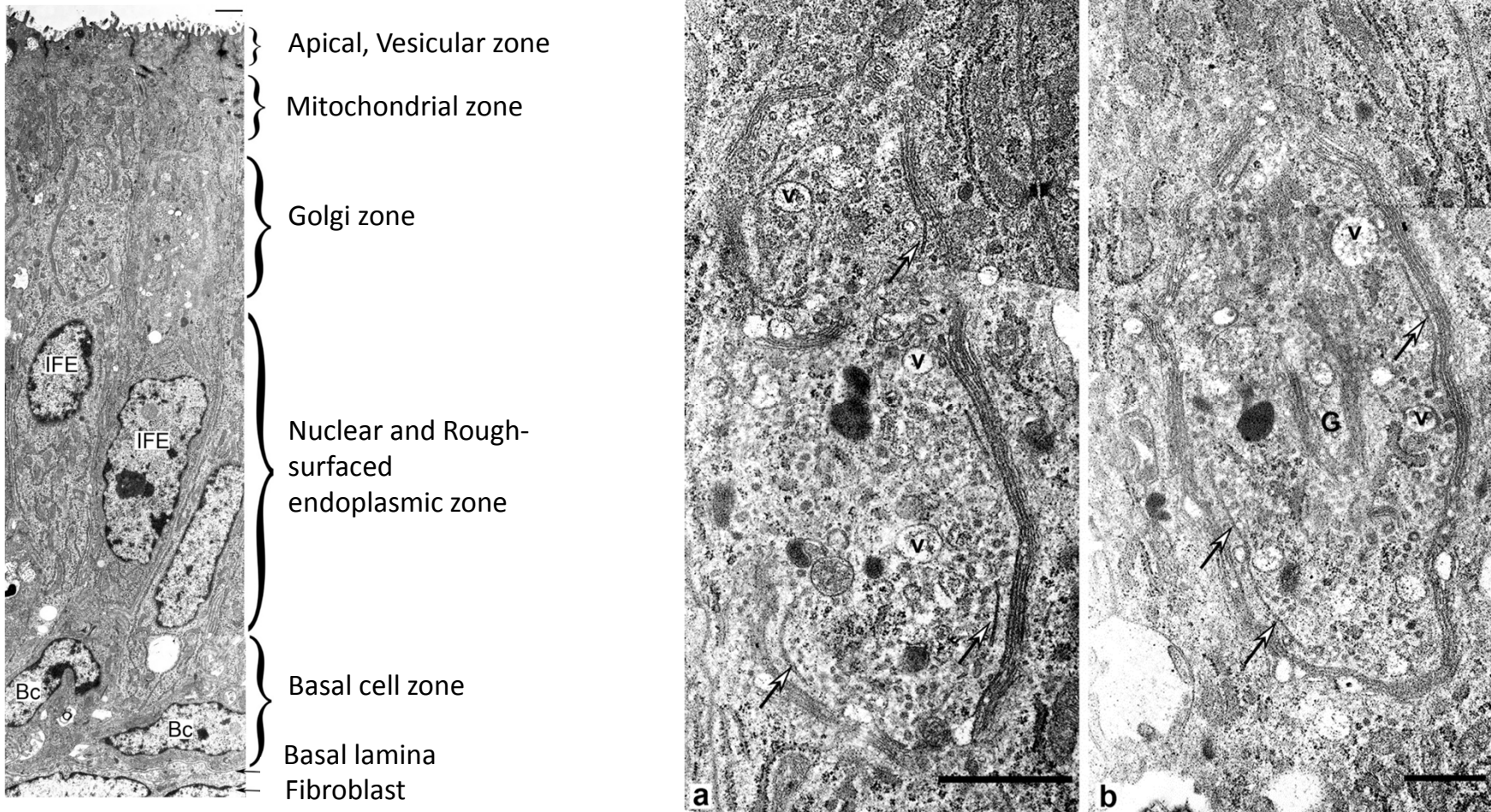
Surprisingly, the FAE and the cells of the follicle (lymphocytes, dendritic cells, epithelial cells) do not use caveolin-1 mediated endocytosis, but in the IFE Caveolin-1 is expressed in many cells.



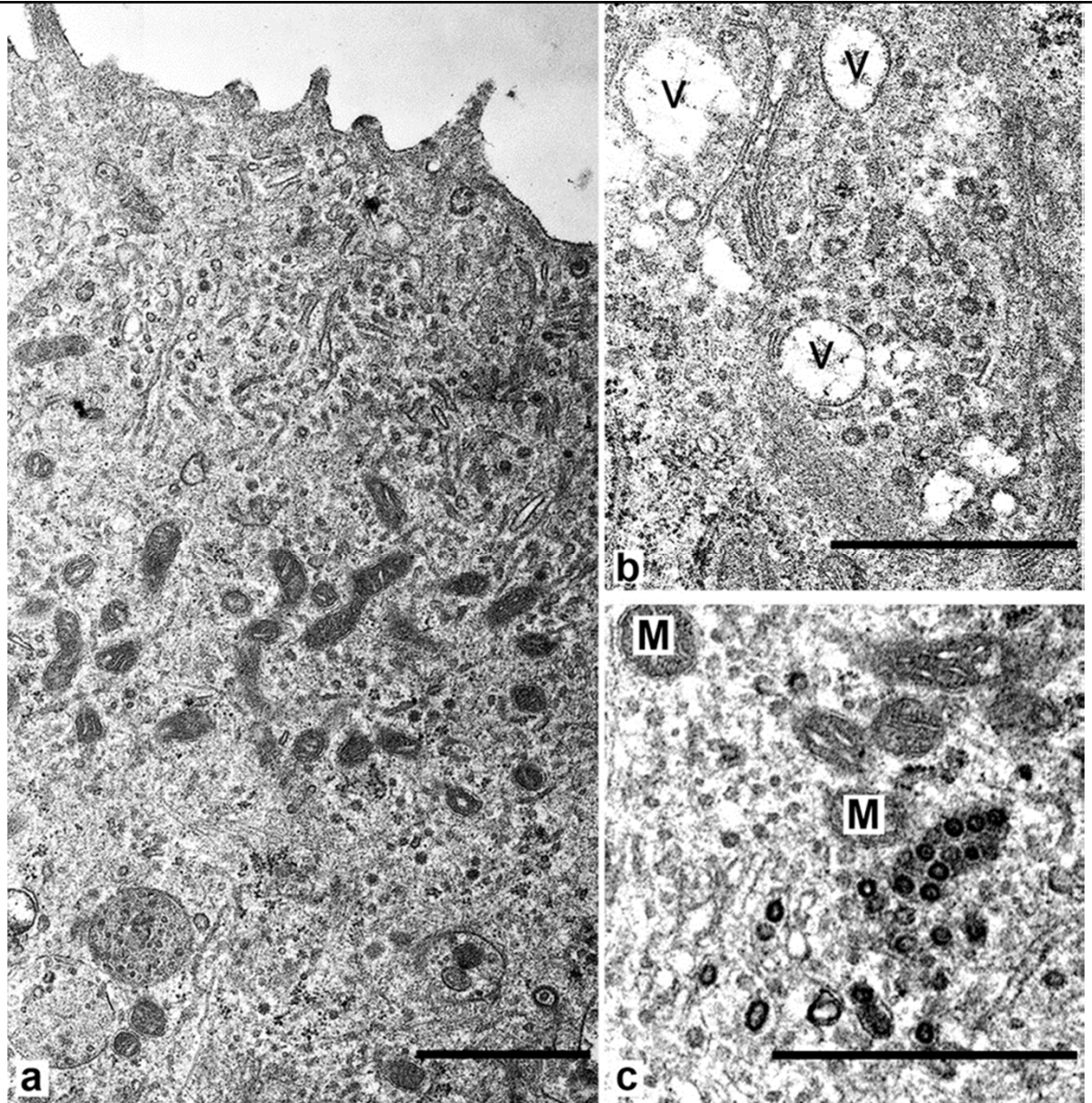
Caveolin-1 expression is found in the cell membrane and cytoplasm in granular form.
Occasionally basal cell expresses Caveolin-1.



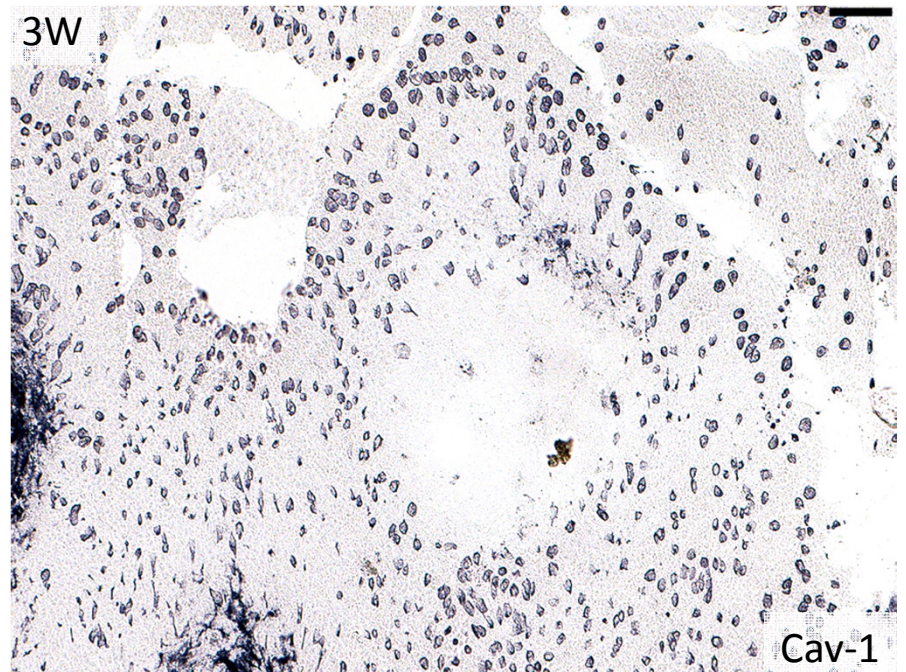
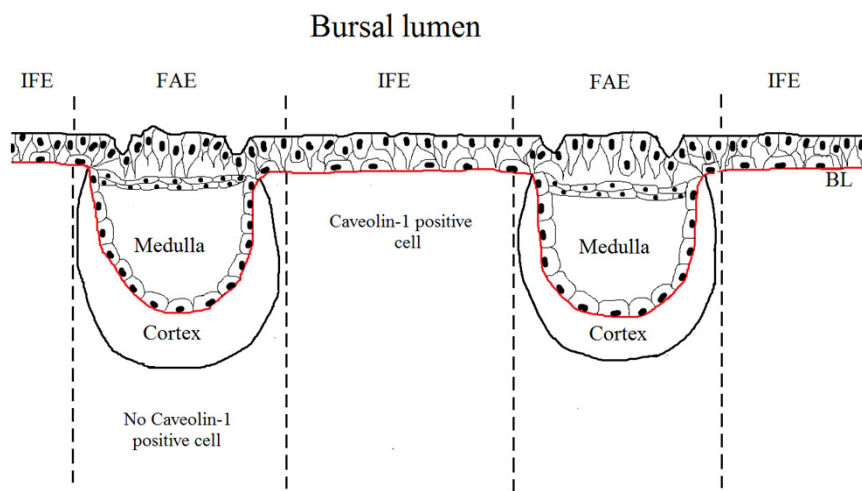
Transmission micrograph of the IFE and the basal cell layer. IFE is highly polarized. Golgi Zone extremely developed.



Appearance of caveolae (vesicles) in the cells of IFE.

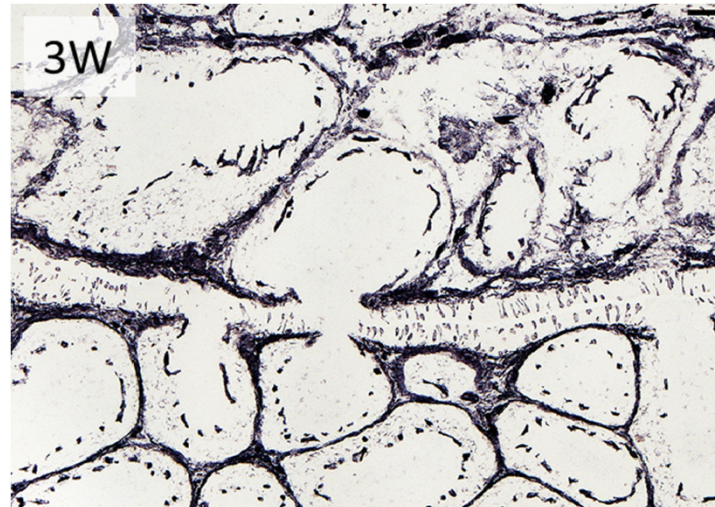
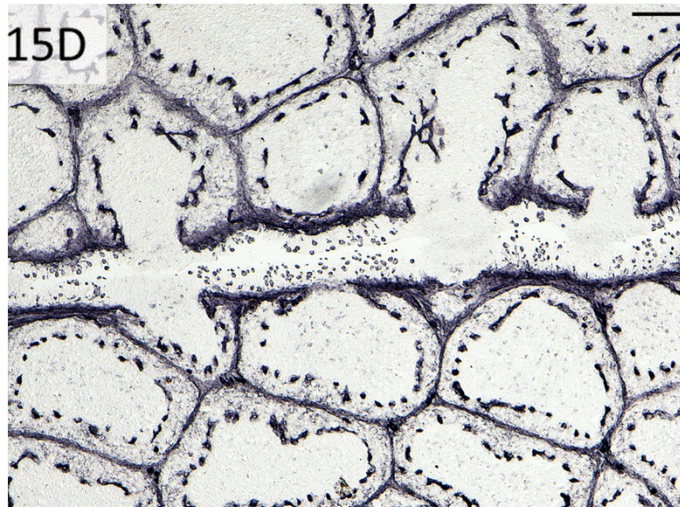
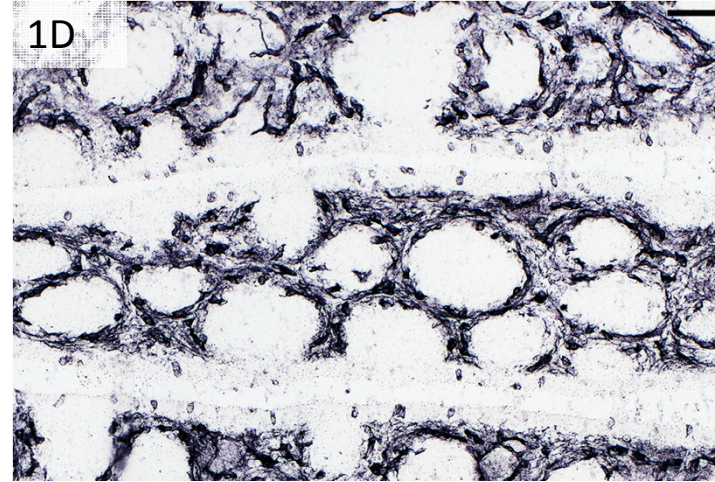
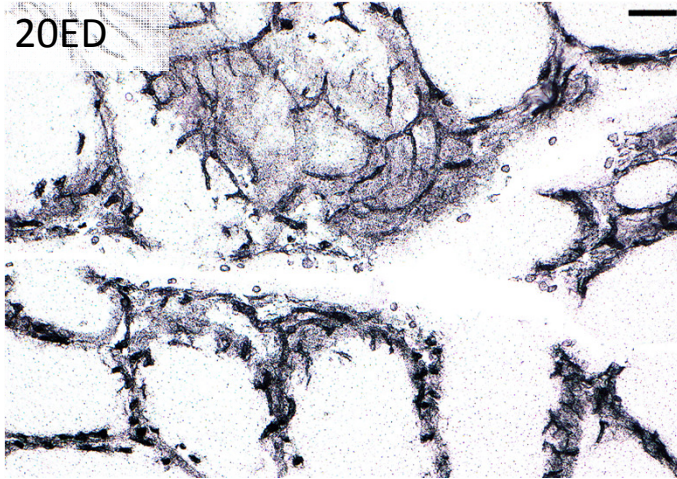


Tangentially sectioned surface epithelium of a bursal fold shows the actual density of Caveolin-1 positive cells



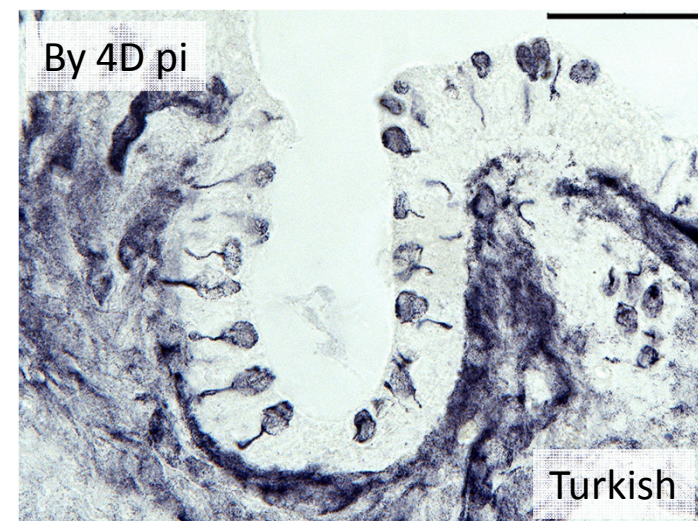
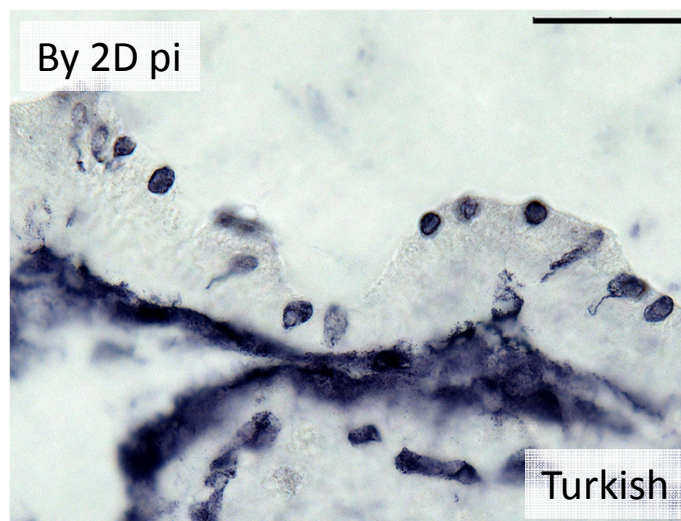
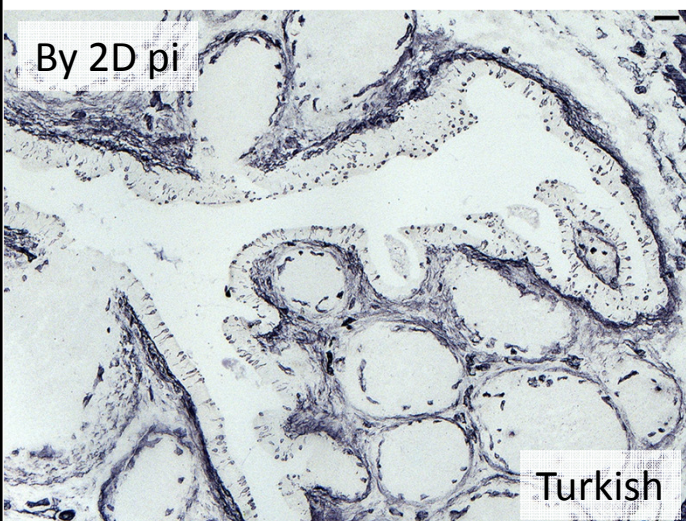
Caveolin expression by age

Changing the number of Caveolin-1 positive cells from embryonic day 20 upto 3 weeks of posthatching. The highest number of caveolin-1 positive cells seems to reach the peak by 3 weeks of age.

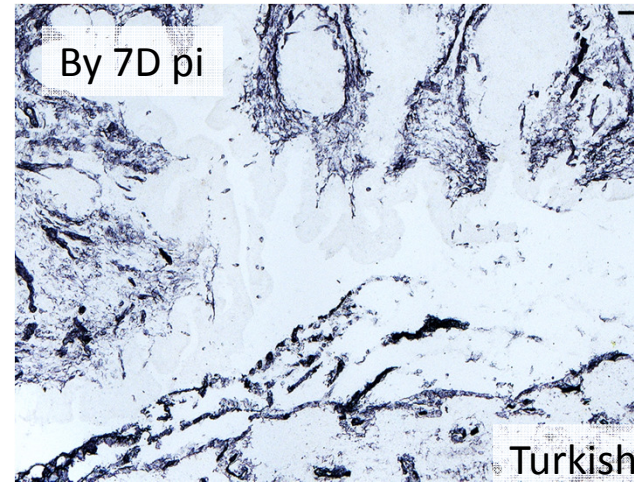
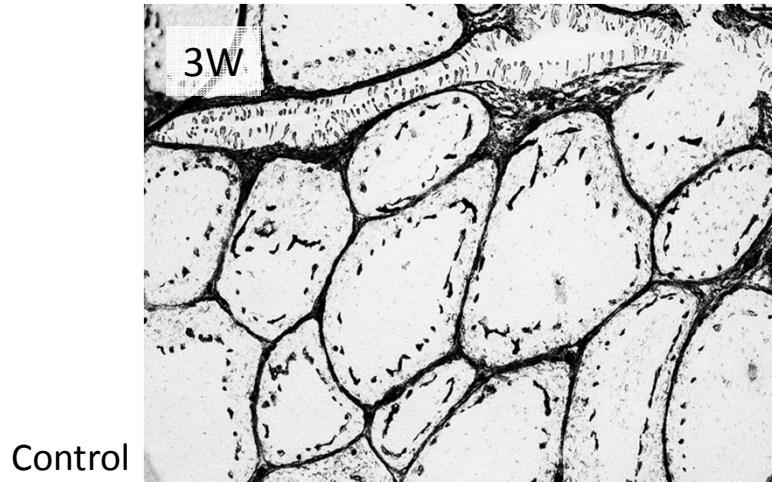


In very virulent field isolate D407/02/04/TR infected birds the number of Caveolin-1 positive cells is highly variable by day 4 pi. and many Caveolin-1 positive cells show only the „head” without „handle”.

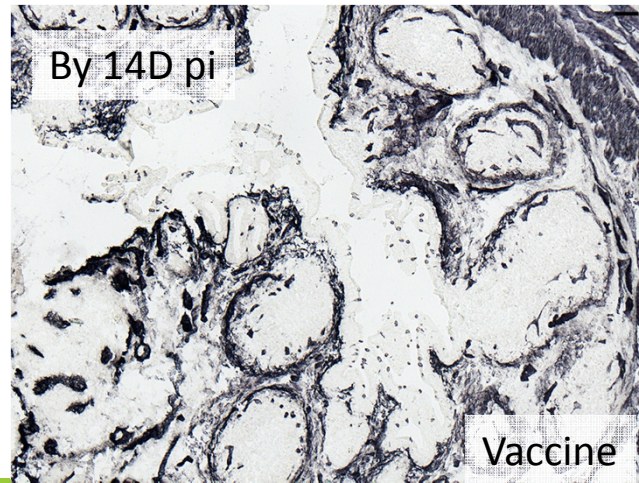
Possibly, IBDV infection inhibits basal cell differentiation being IFE. The number of Caveolin-1 positive cells decreased by day 4, which suggested that the IFE has a turnover about 4 days.



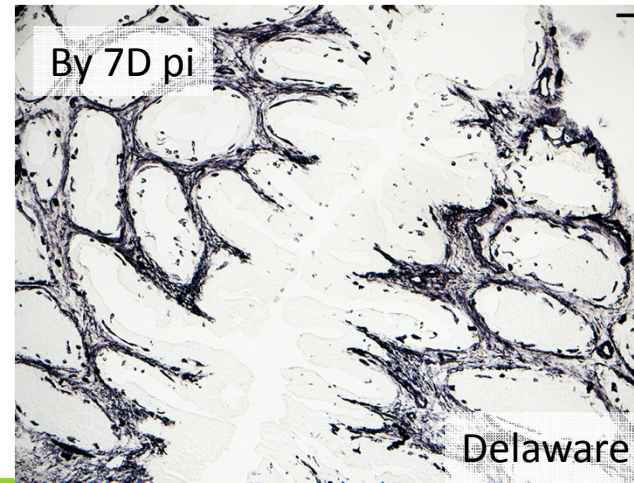
IBDV infection decreases the number of Caveolin-1 positive cells in the IFE.



Turkish by day 7 drastically decreased



Vaccine strain by day 14 decreased.

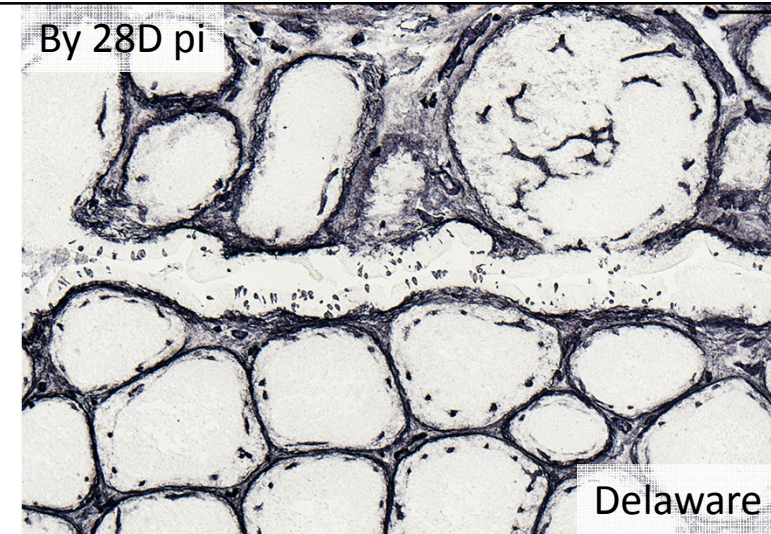
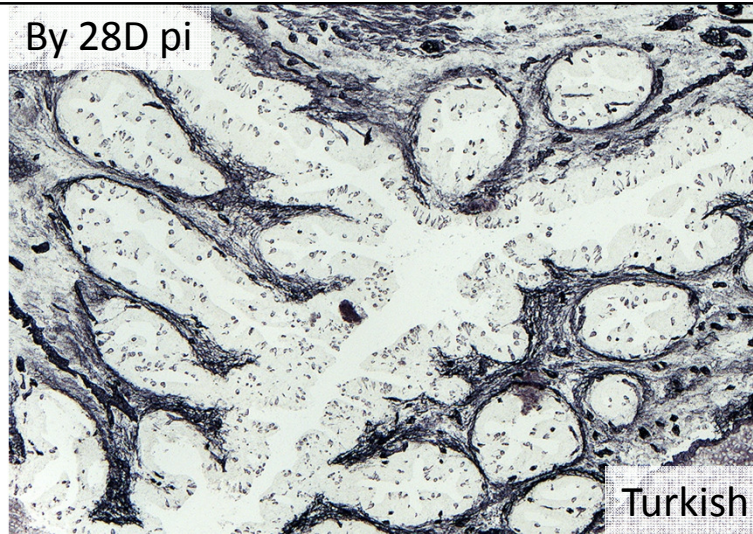


Delaware= variable in number, but decreased by day 7 pi.

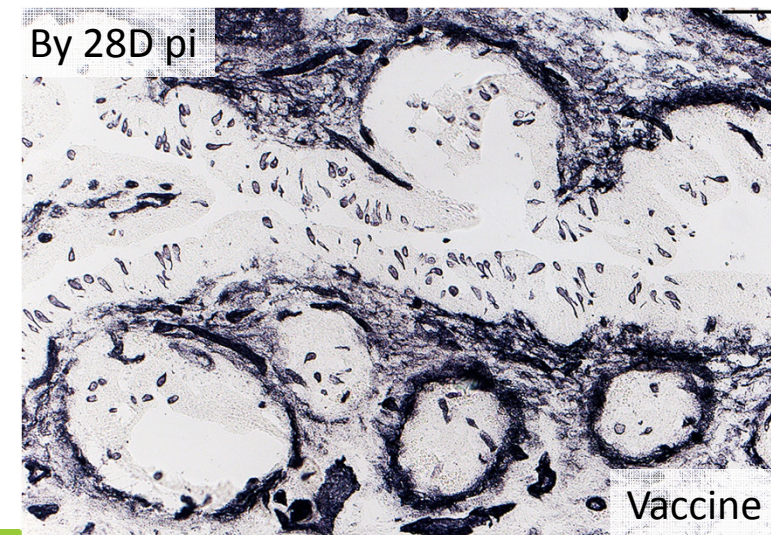
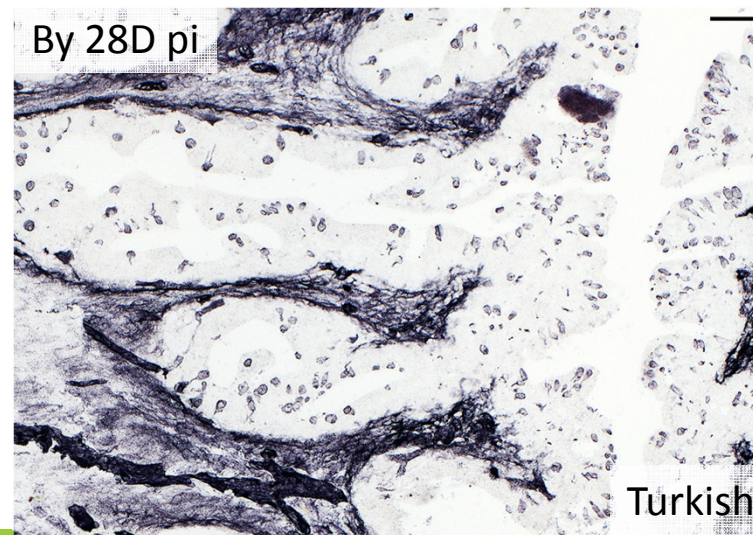
Regeneration stage

By day 28 pi.

Vaccine strain
infected birds: bursa
partially or not
regenerates

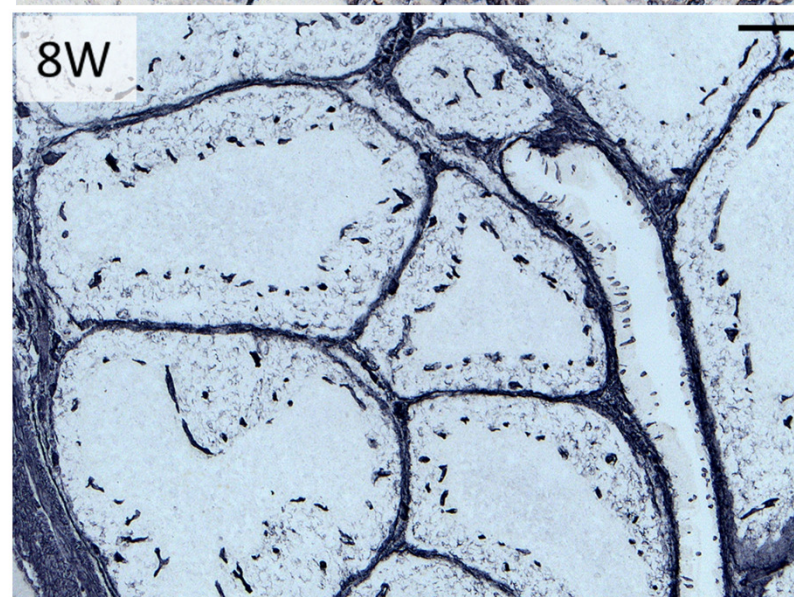
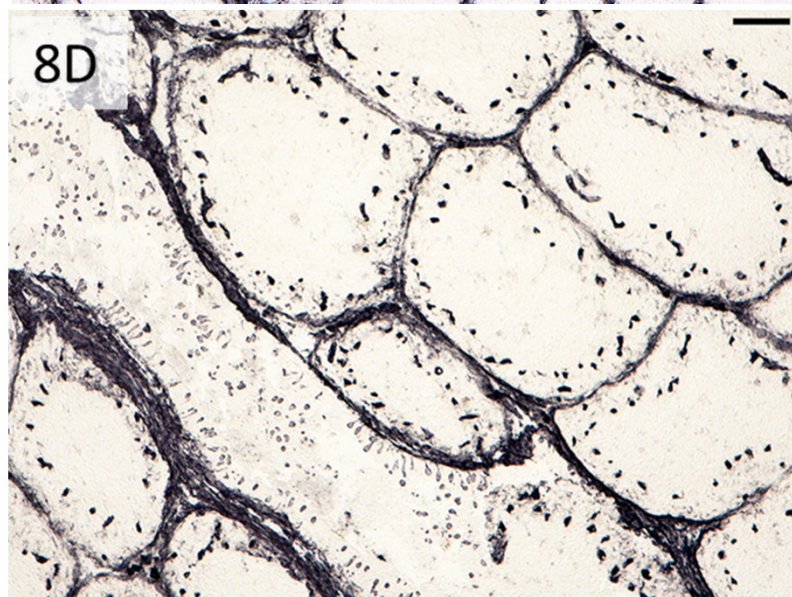
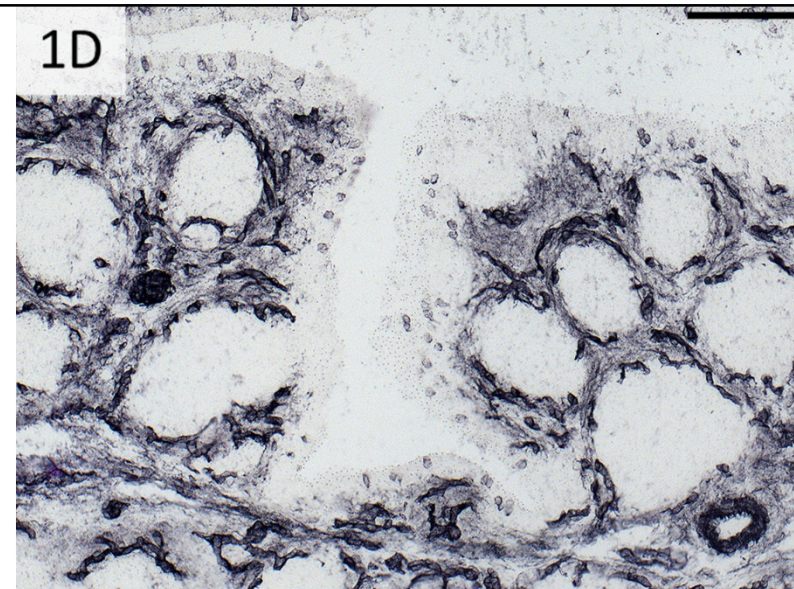
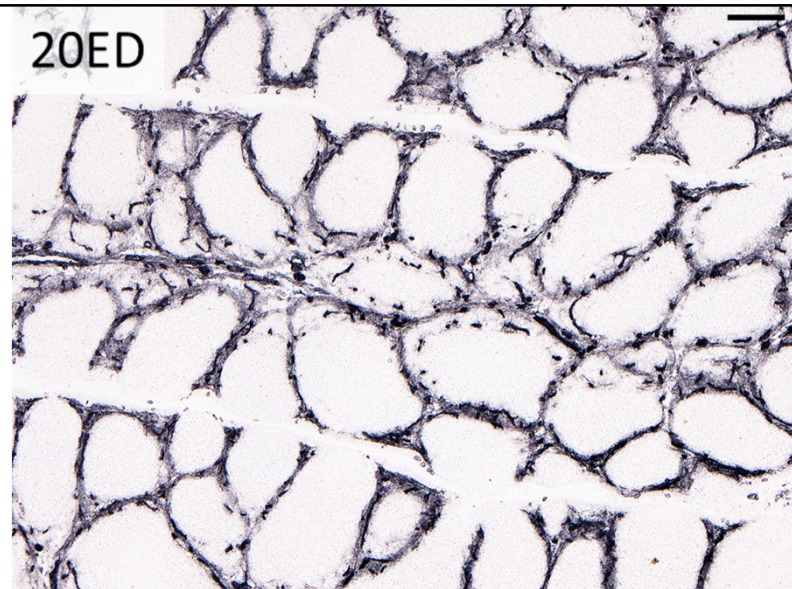


Delaware strain
infected birds:
several follicles are
completely
regenerated

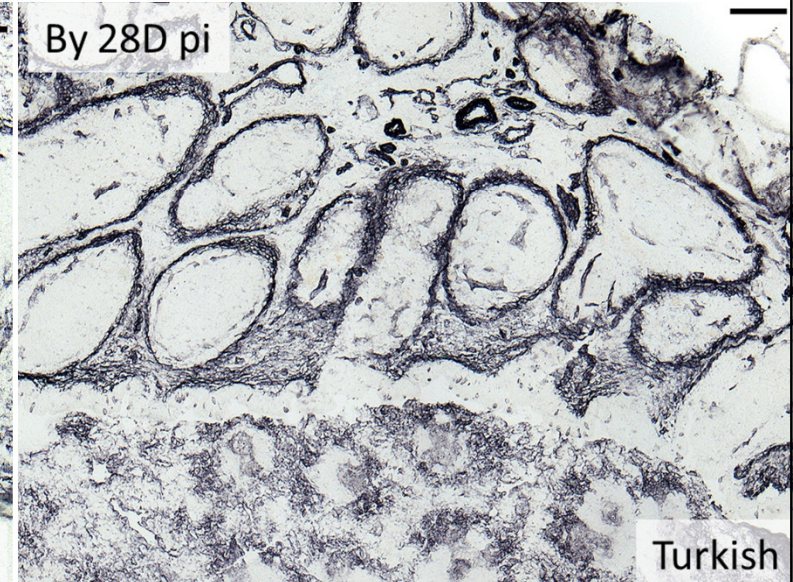
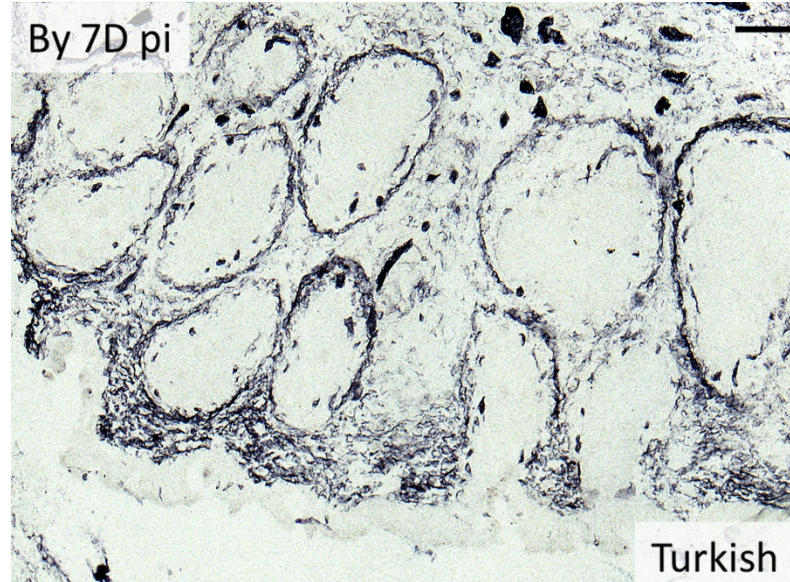
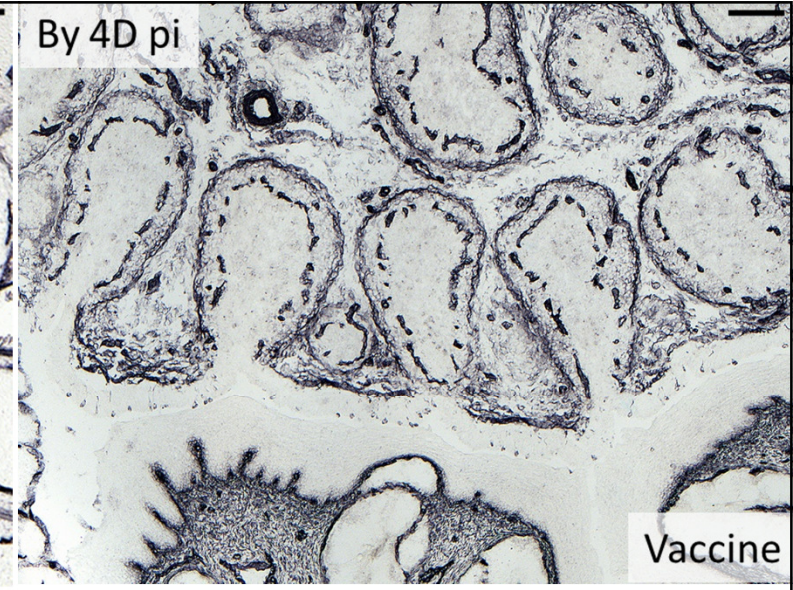
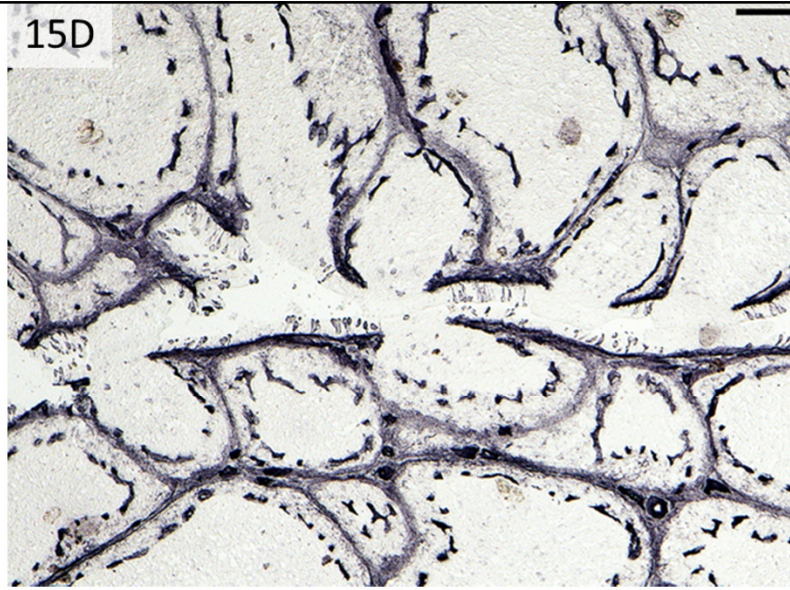


Turkish very virulent
strain infected birds:
no regeneration

Development of
capillary
network at the
cortico-
medullary
border



Capillary network and IBDV

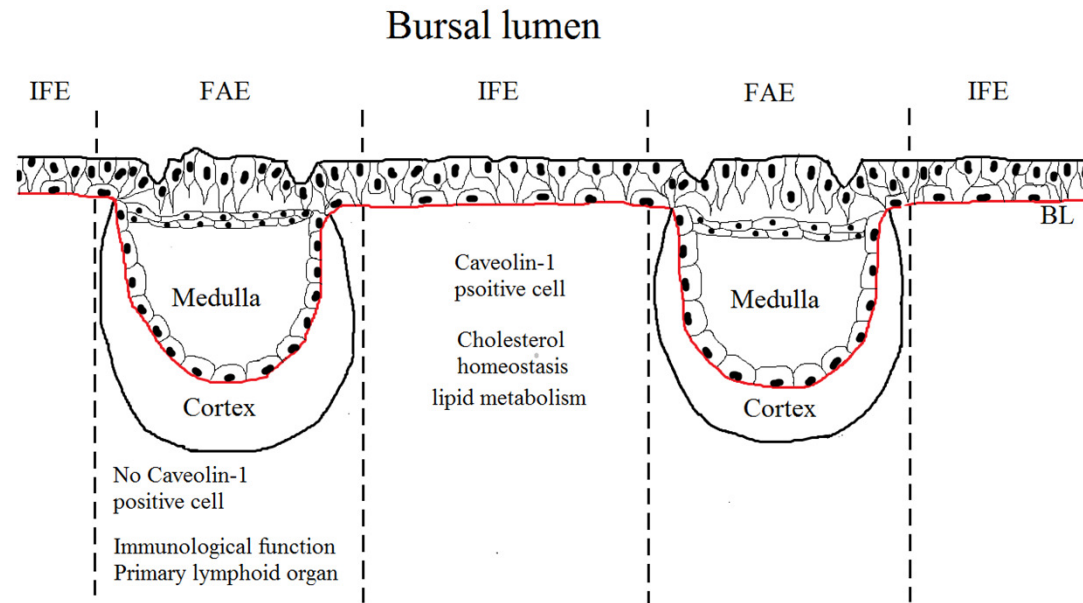


Conclusion I

- I. The FAE and cells of the medullary region of the follicles including B lymphocytes do not express Caveolin-1. No Caveolin-1 endocytosis in the FAE.
- II. Caveolin-1 is highly expressed in the IFE.
- III. The cells of the IFE differentiate from the basal cells and during differentiation Caveolin-1 is expressed.
- IV. In Delaware and Turkish strain infected birds the lowest number of Caveolin-1 positive cells occurs between day 4 and 7 pi.
In Vaccine strain infected birds the lowest number of Caveolin-1 positive cells occur around day 14.
- V. Capillary network around cortico-medullary border is destroyed: first functionally (transcytosis of endothelial cells stops) and later histologically (no regeneration of capillaries).
- VI. No follicular regeneration is in the very virulent and regeneration is variable in vaccine virus infected birds. Regeneration is more advanced in Delaware strain infected birds.

Conclusion II

- VII. Bursa of Fabricius is a complex organ; comprises two histologically and functionally different parts.
- The follicle-associated epithelium and the follicle represent **primary lymphoid organ** and serve B lymphocyte differentiation. No Caveolin-1 mediated endocytosis.
- The interfollicular epithelium contributes to the lipid metabolism; the **cholesterol homeostasis** via Caveolin-1 expression.



Contributors of this work

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Thank you for your attention!