MB-1 Mode of Action

Selected results

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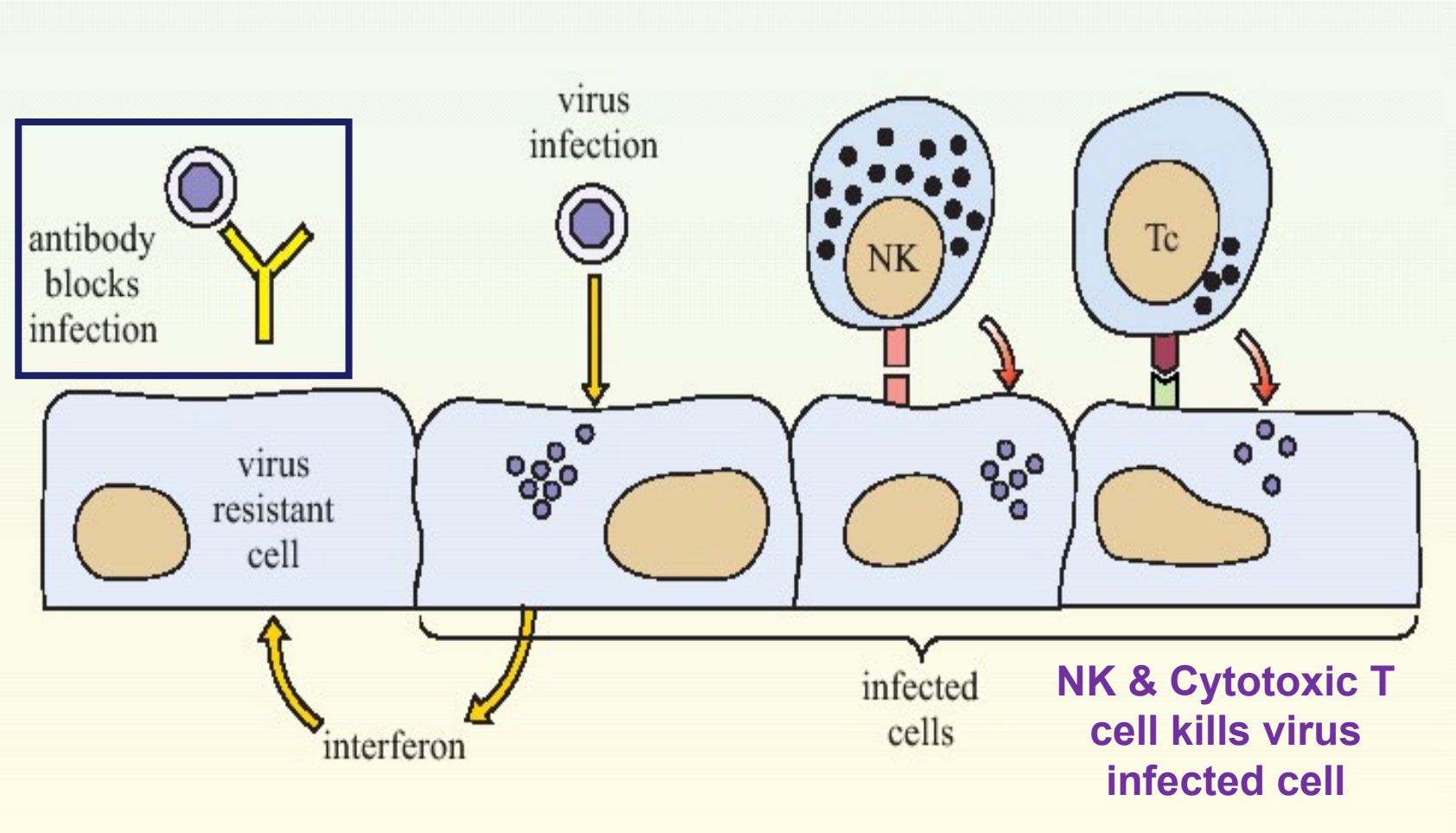
16/01/22

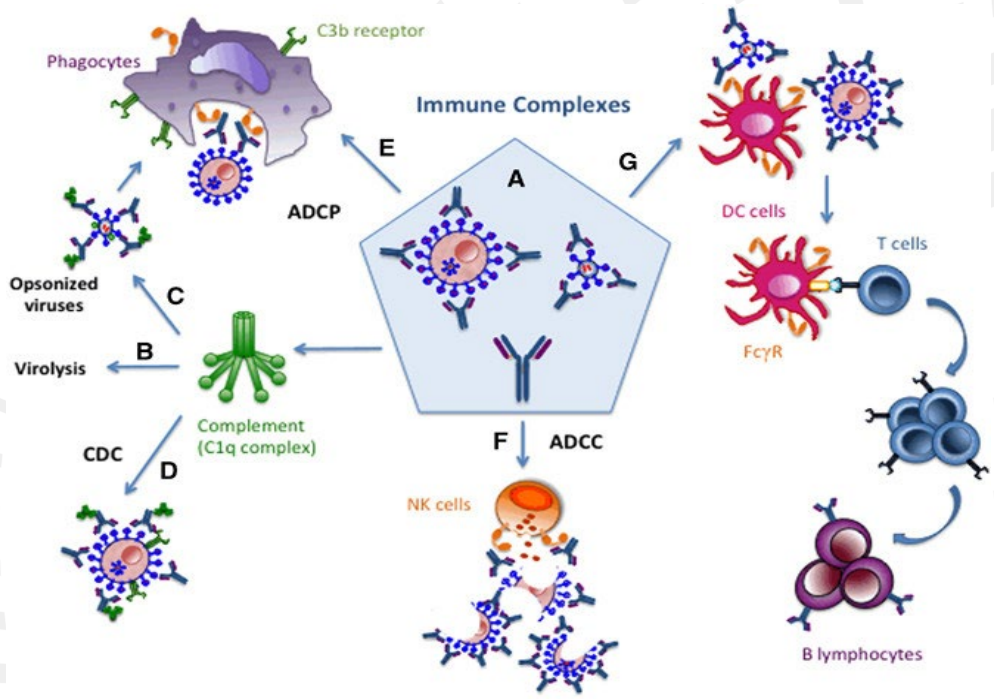
The overall anti viral immune protection:



Anti viral immune protection – Antibodies role:

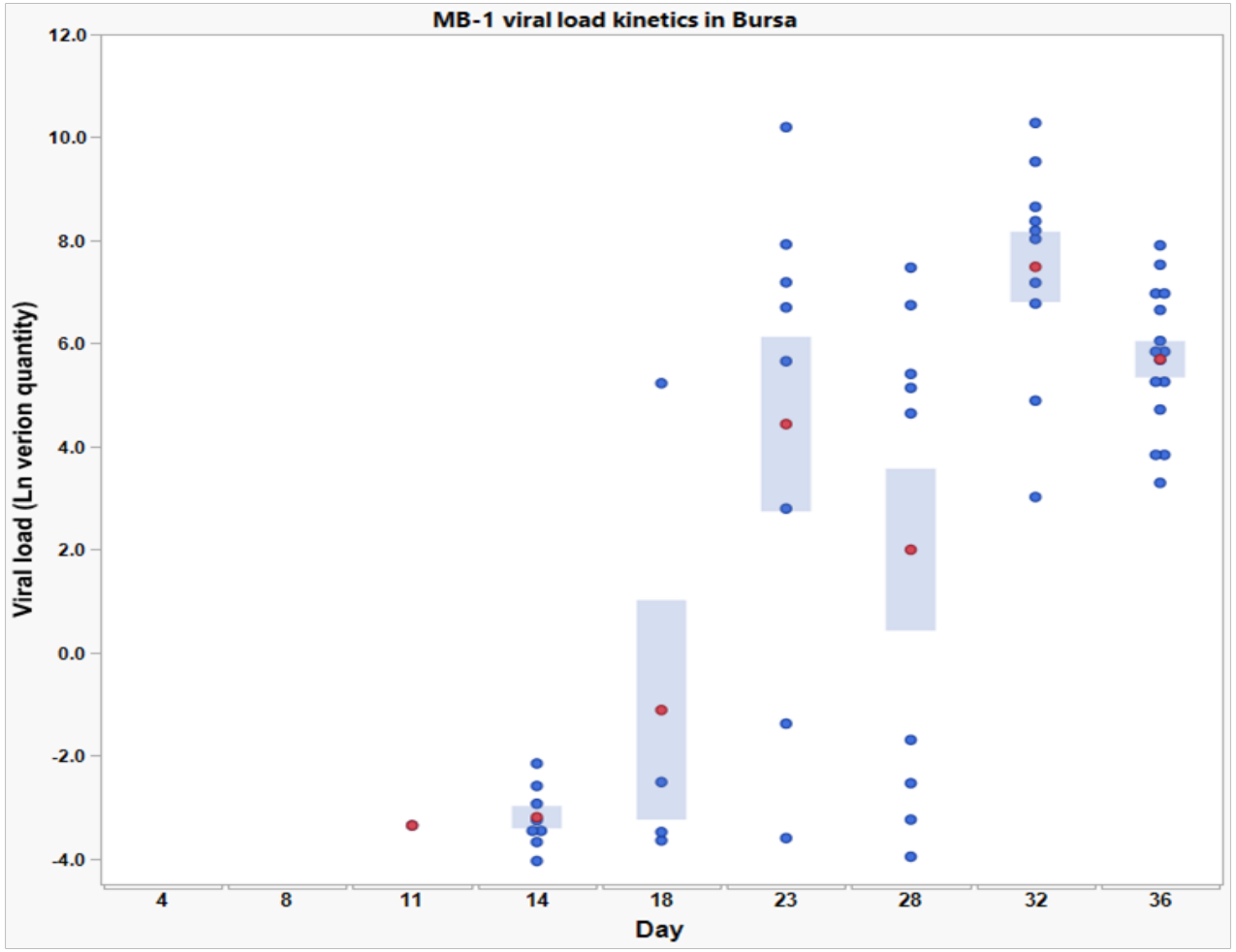
- E,C- Facilitating phagocytosis (ingesting the virus).
- B – Virus lysis.
- D – Virus neutralizing (prevents virus from attaching to healthy cells).
- F – Facilitating killing of virus infected cells by NK cells.
- G – Activating cellular and humoral immunity (T cells & antibody maturation).

- **Two important antibodies: IgY & IgA**
  - IgY prevent viremia (virus passing between cells) & neutralize virus, thus allowing faster recovery and ease of disease.
  - IgA prevent infection of healthy cells (induces mucosal immunity) thus preventing infection.



MB-1 viral load kinetics in Bursas (qPCR)

Bursa of day-1 vaccinated broilers



- Maternal antibodies protect Bursa from MB-1 vaccine strain until ~14 DOA
- High variation in viral load between days 18-28 followed by small variation between days 32-36 are due to different MDA levels

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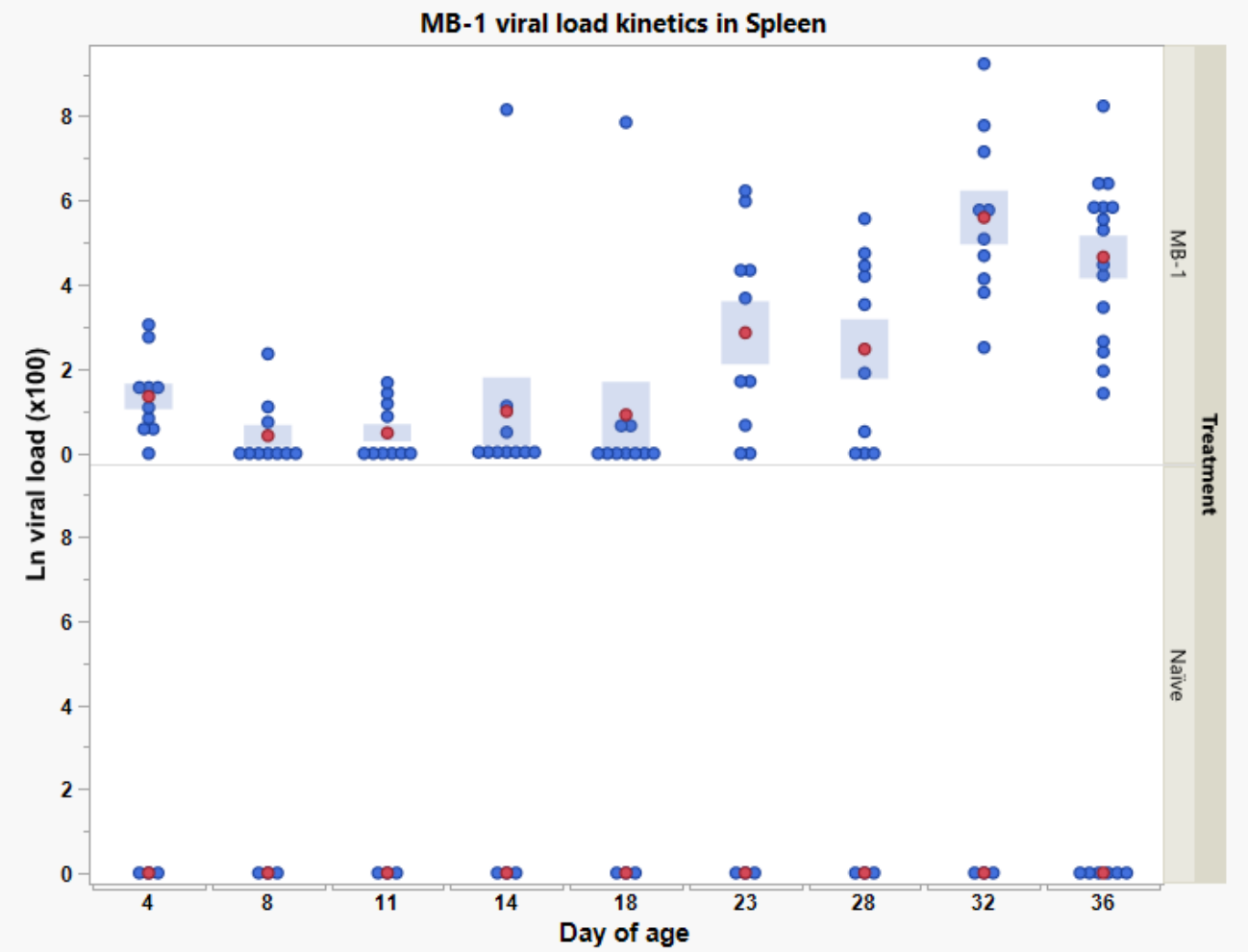
MB-1 viral load kinetics in Spleen (qPCR). Bursa of 1 day old vaccinated broilers



- No virus was found in naïve chicks (N/A)

- Virus is detectable from 4DOA and during all time points.
  - Suggesting this is the virus location!

- Viral load is low at 4 DOA, continue to decrease and starts to increase around 14-18 DOA, following minor but significant decrease at day 36:
  - Maybe due to *de-novo* immune neutralization?

- High variation in viral load (~days 14-18), following smaller variation at days 32-36;
  - Maybe due to different MDA levels?

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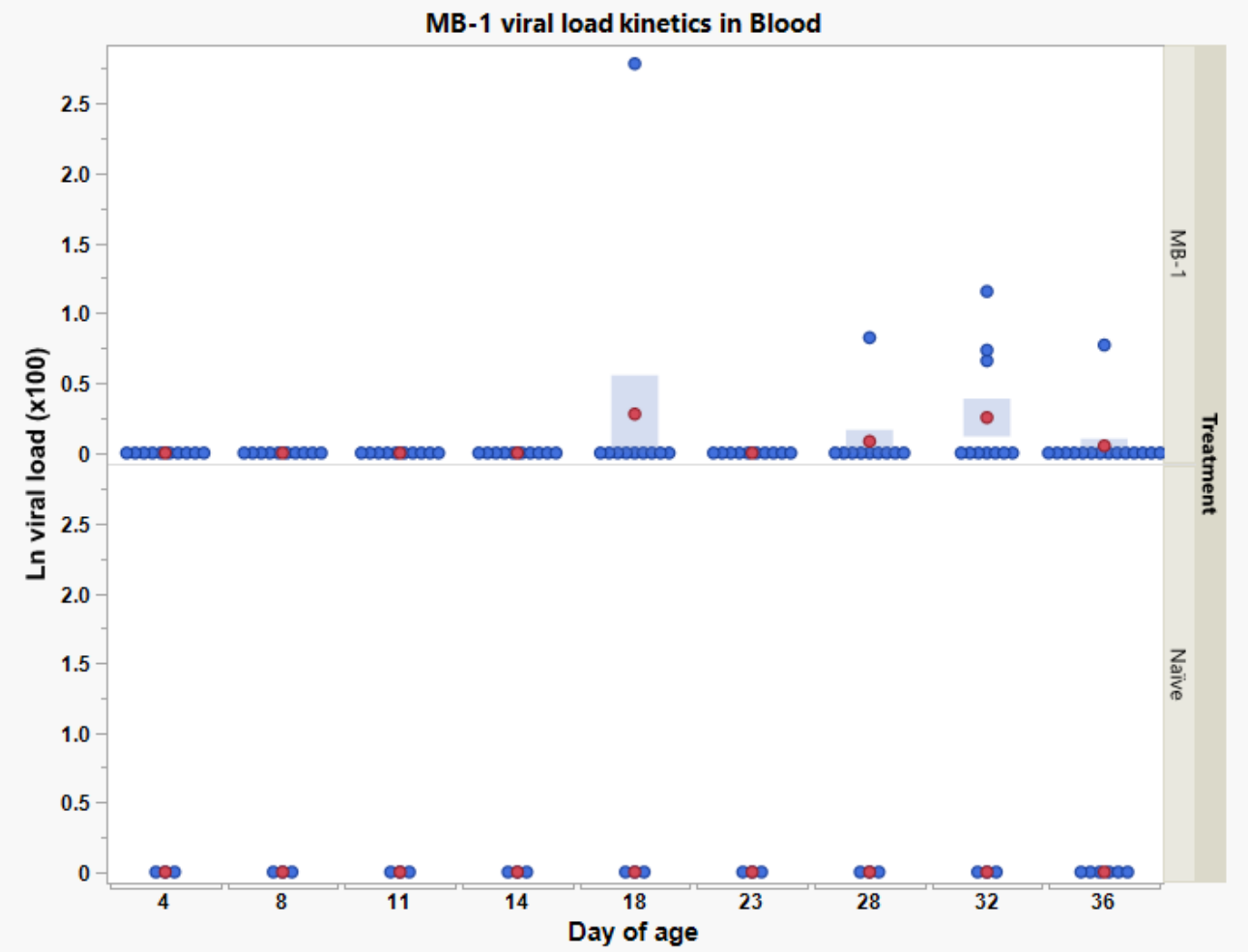
MB-1 viral load kinetics in Blood

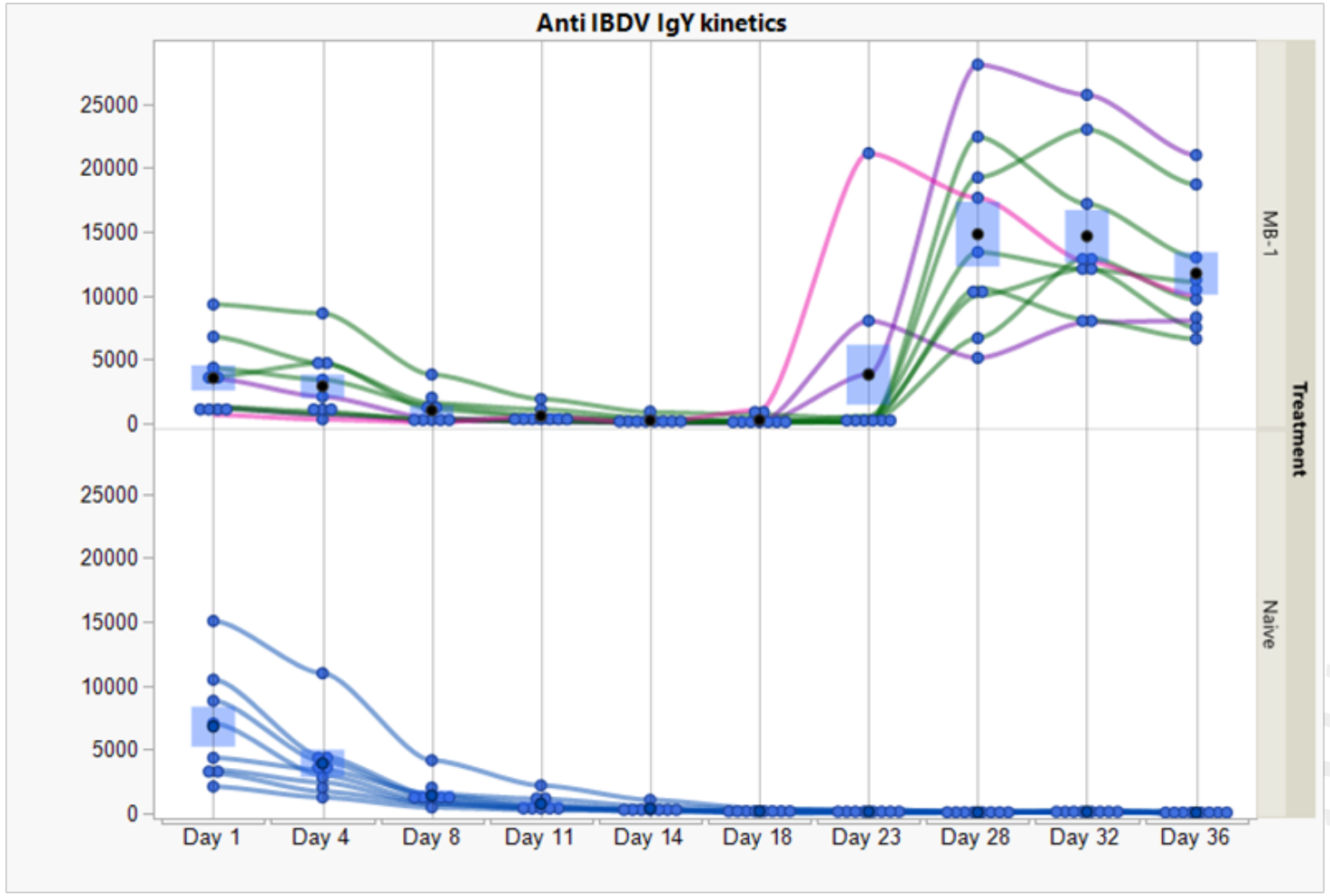


- No virus was found in naïve chicks (N/A)

- Virus (almost) not found in any time point in the blood (very low level in 32 DOA);
  - The lack of potential target cells suggest the virus location is elsewhere.

IgY (ELISA)

Kinetics of de novo seroconversion to anti IBDV IgY



- Anti *IBDV* MDA, decline during the 21 days post hatching Nadir is observed at 18 DOA.
- No seroconversion is seen in naïve chickens.
- First observations of seroconversion in MB-1 vaccinated chicks are seen in 23 DOA.
- Level of seroconversion varies between chickens and is mostly MDA dependent.

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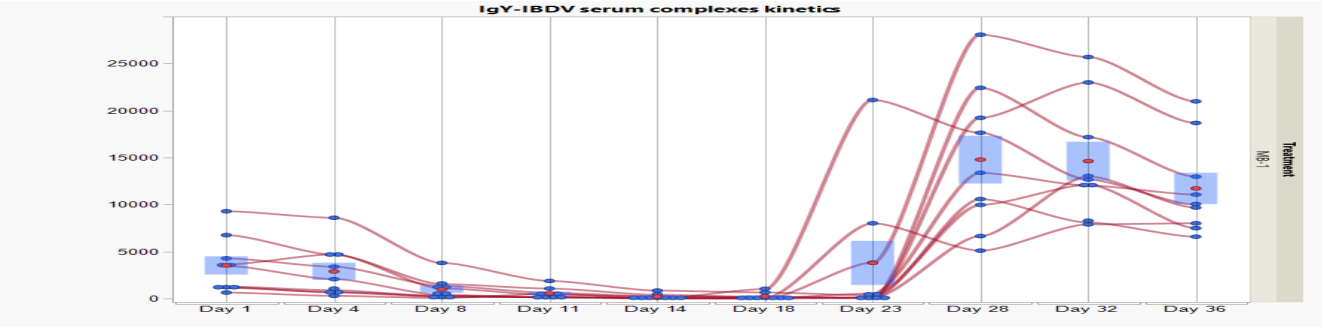
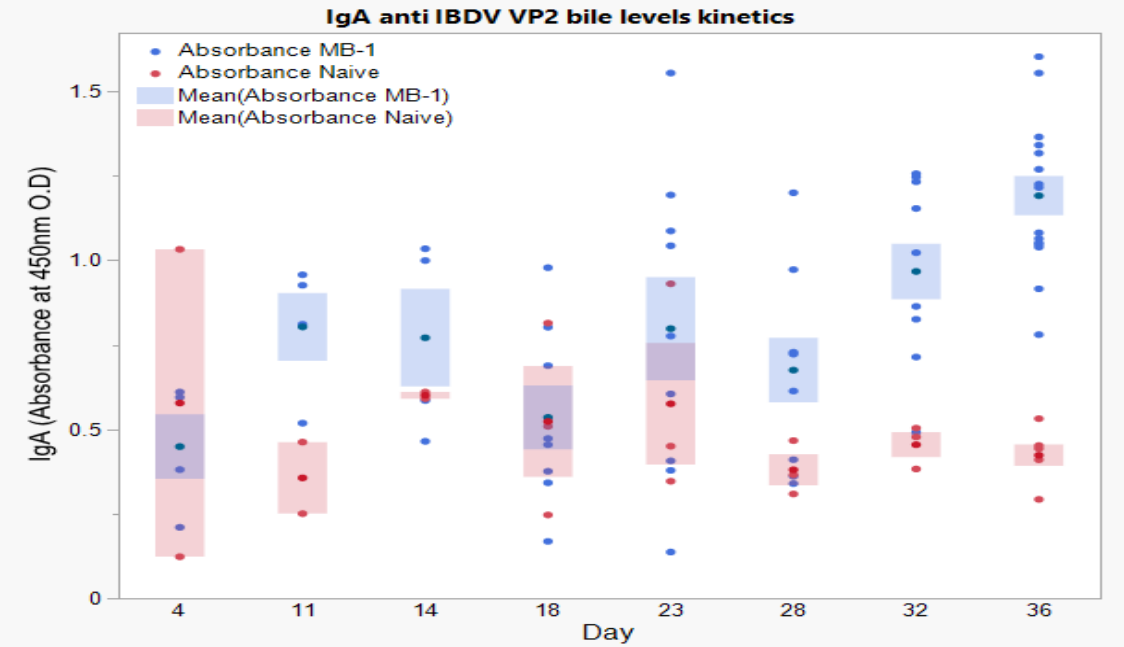
IgA (ELISA)

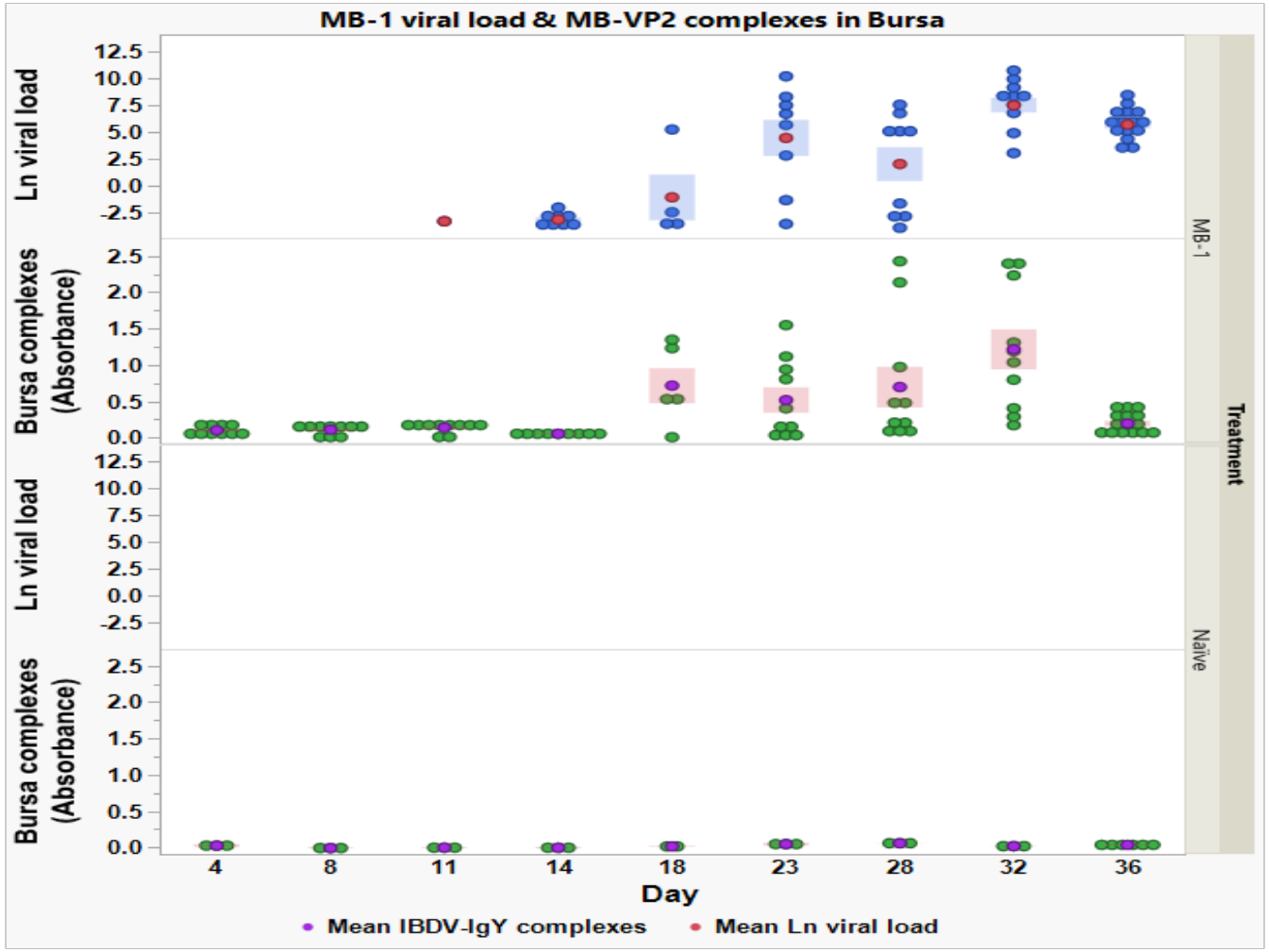
Kinetics of *de novo* seroconversion to anti *IBDV* IgA





- IgA MDA decline, reaching its nadir at ~ 18 DOA (overlapping between vaccinated and non-vaccinated chickens level).
- De-novo anti *IBDV* IgA start seroconversion at 23 DOA (similarly to serum anti *IBDV* IgY seroconversion) and increase during the rest of the time points.
- High variation in anti-IgA levels, probably due to MDA.

MB-1 IgY Immune complexes represent the evidence for Bursa maternal protection (up to 14 DOA)



- No virus and no complexes are found in naïve chicks.
- MB-1-IgY Immune complexes are observed in low level in bursa starting from 4 DOA and increasing significantly (more than spleen) from 18 DOA.
- Since viral load was not observed in bursa until 18 DOA, we assume the MB-1 found in immune complexes between 4-18 DOA originated elsewhere.
  - low level complexes represent "incoming" virus trying to invade cells.
  - high level complexes represent both "incoming" and "outgoing" virus.

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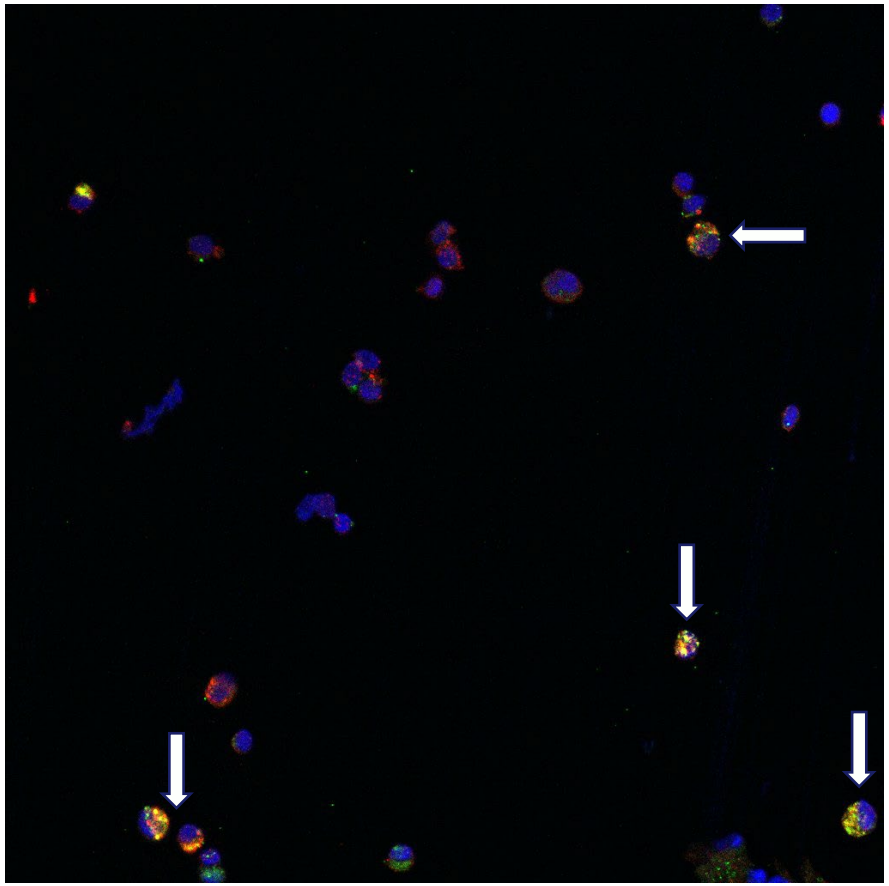
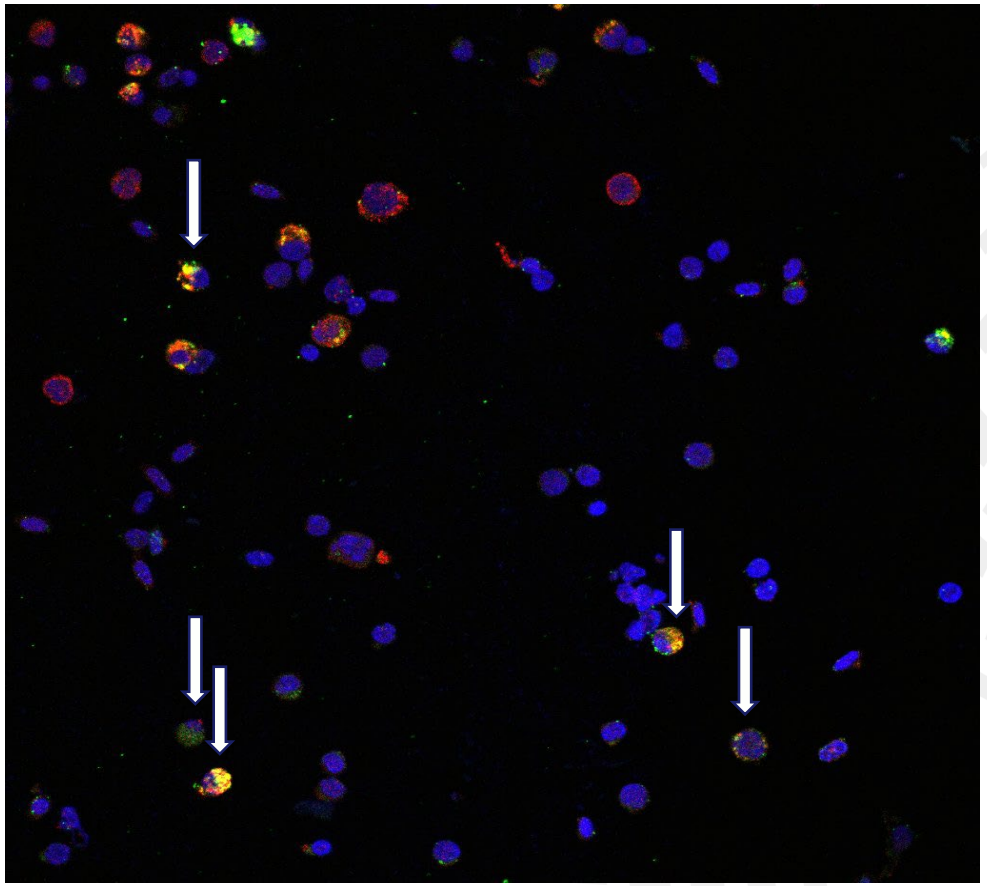
MB-1 virus resides in splenic naïve B-cells and macrophages (4 DOA)

Immature B cells



Macrophages



- **Blue** – nucleus (DAPI)
- **Red** – B cell or macrophage
- **Green** – MB1 virus

Additional Questions & Hypothesis

•Why does the virus is unable to invade the bursa until 14-18 DOA?

Due to IgY transporter saturation, only 10% of MDA deposit in yolk sac and manage to enter the blood stream (~90% are washed out in the Gastro-intestinal Tract or GIT); since bursa is anatomically connected to the GIT (differently from spleen), these antibodies reach the bursa and neutralize “incoming” virus. Serum MDA IgY levels are correlated to the GIT MDA IgY levels.

•Why does the virus reroute to the spleen?

At around the first two weeks post hatch, B naïve lymphoblasts migrate from the bursa and occupy the spleen; It means the spleen is the best second option for the virus to invade its target cells while bursa is still protected by MDA antibodies.

•Does the virus still try to invade the bursa?

Since the bursa is the organ target of the virus, the answer is yes. For this reason, we observe immune complexes in bursa fluid without elevation in viral load.