



Intestinal avian defensin 2 and robustness of chicks

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Introduction and objectives

After hatch, chicks are susceptible to infection by entero-pathogenic bacteria such as *Salmonella* and avian pathogenic *Escherichia coli* leading to major economic losses for breeders and to a sanitary danger for humans [EFSA and ECDC Report 2017-2018]. The robustness of chicks is so far estimated by rather subjective macroscopic observations (quality score; PMID: 14717556). Among innate immunity factors, **AvBD2** is a **major antimicrobial peptide** produced by heterophils and is present in caecal tonsils of birds (PMID: 27561012). Moreover, AvBD2 **limits bacterial pathogens invasion in the intestinal mucosae** (PMID: 16702014). Therefore, **it could constitute a good candidate as a biomarker of robustness of chicks**. To test this hypothesis, caecal AvBD2 production was compared between chicks from different lines and under breeding conditions with impact on robustness.

Materials and Methods

Animals: “Standard” (S) chicks from line Ross 308, fast growing / “Label” (L) chicks from line JA657, slow growing.
 Challenging condition for chicks: T° increased and shaking during transport, delayed feeding.

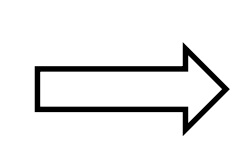


Day 0, after hatch:
 • Initial quality scoring



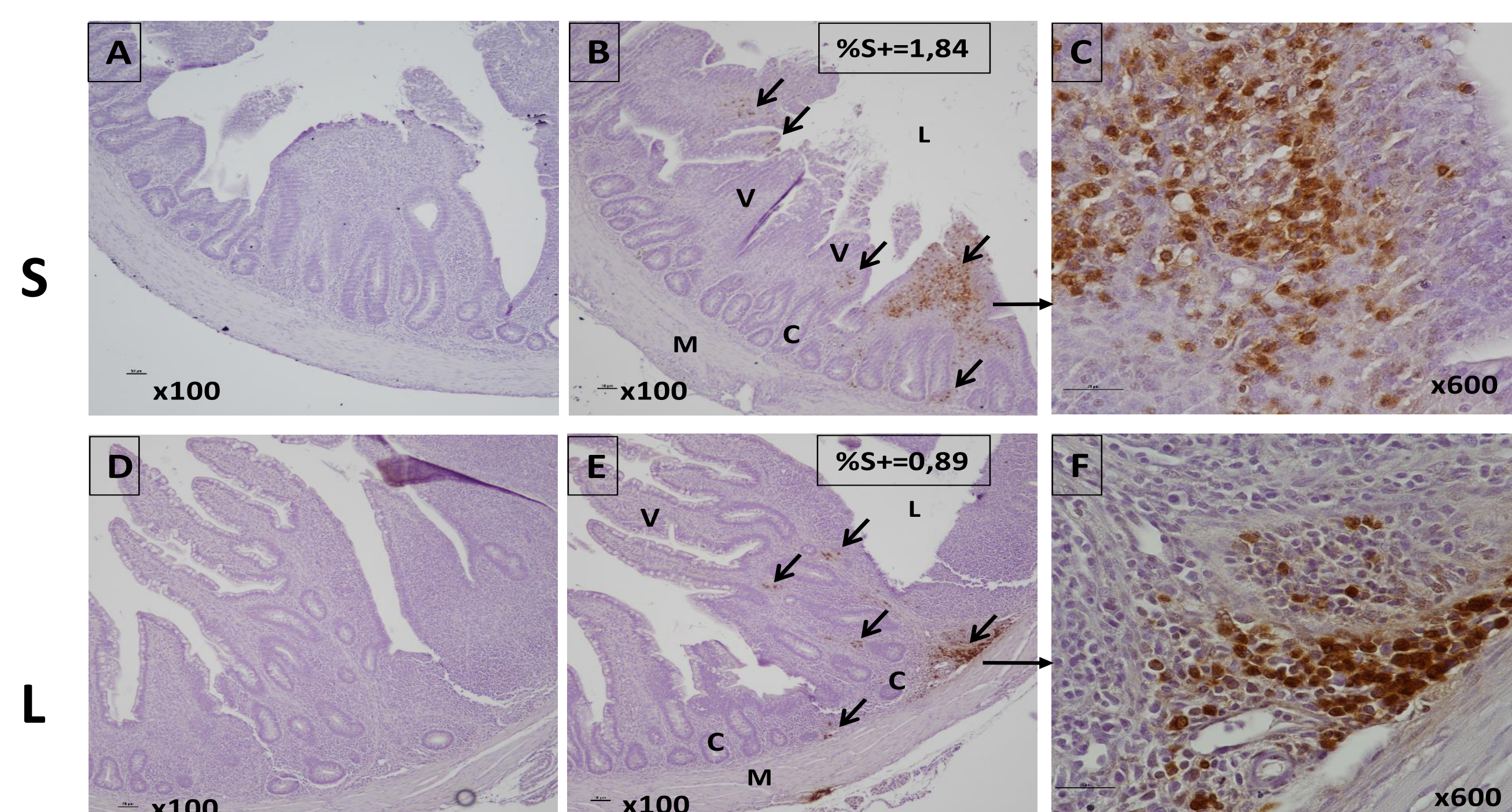
Day 1 :
 • Quality scoring after challenge

Day 7 of age:
 • Quality scoring,
 • Blood collected,
 • Caecal tonsil collected *post-mortem*.



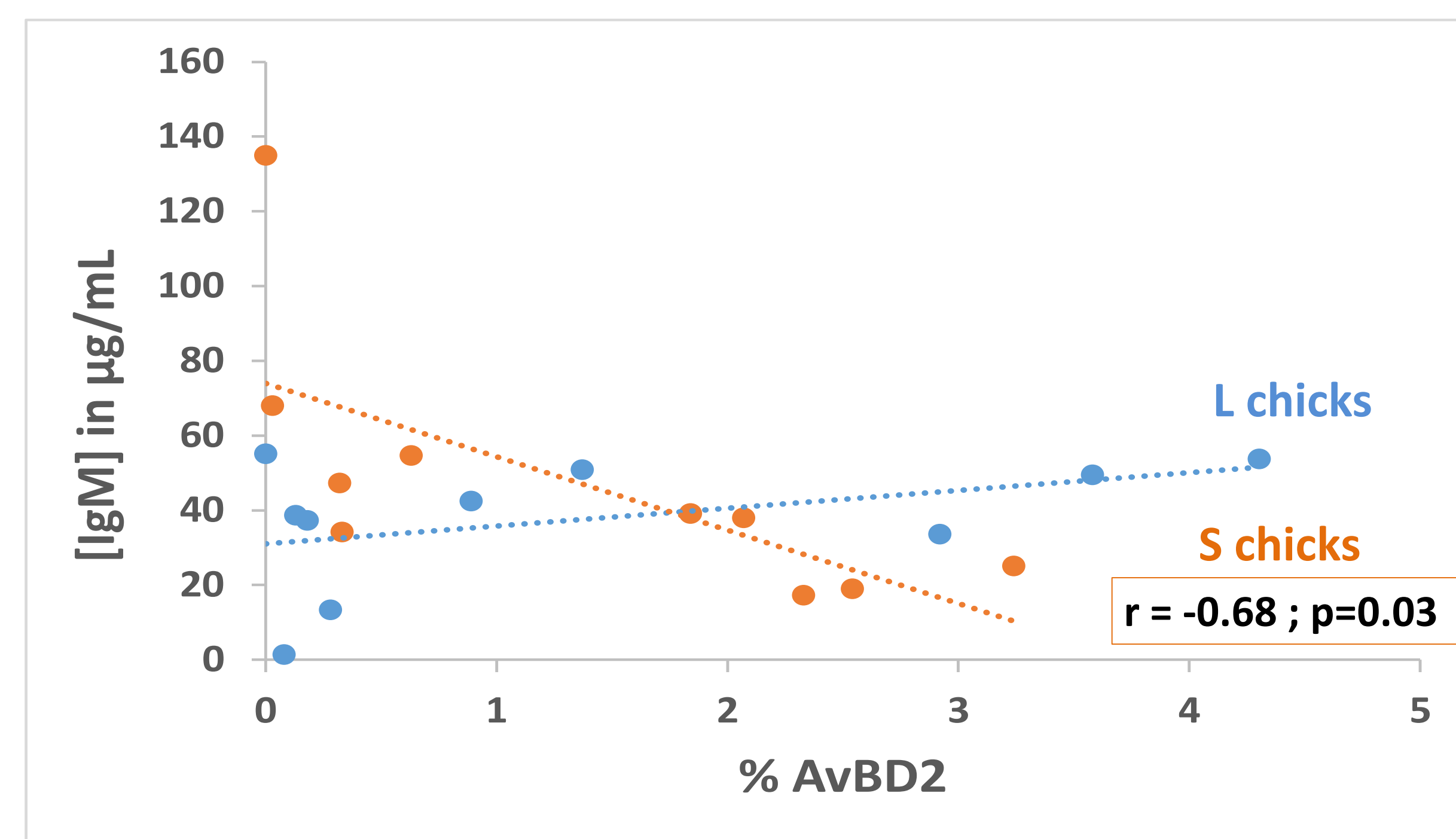
➤ IHC analysis from paraffin-embedded serial tissue sections labelled with rabbit anti-AvBD2 polyclonal antibodies,
 ➤ Ig measurement (ELISA) from serum.

Results



IHC analysis of AvBD2 in caecal tonsil from one S chick (top) and L chick (bottom). Left panels (A, D) normal rabbit Igs used as primary antibody negative control ; Right panels (B, C) and (E, F) : rabbit anti-AvBD2 polyclonal antibodies; Peroxydase conjugate goat anti-rabbit Ig was used as 2nd antibody. Images were analysed by Image J (Fiji) software to quantify percent area of the tissue section stained (%S+). V: villus; C: crypt; M: muscle; L: lumen.

- Specific labelling of AvBD2 observed in the *lamina propria* and close to crypts of caecal mucosa (Figure 1B and 1E / 1A and 1D, respectively).
- AvBD2 found close to capillaries and vessels (C and F). These mucosal locations of AvBD2 are observed whatever the line examined (not shown).



Scatterplot representation of plasma IgM concentration as a function of % AvBD2 positive area of caecal tissue. Values from L line L chicks are in blue (n=10) and from line S chicks (in orange, n=10). Linear regressions are in dotted lines for each line and their respective Pearson's correlation coefficient r is indicated with p value.

- The mean caecal level of AvBD2 is similar in both S and in L chicks, with high individual variability, similarly to quality level (not shown).
- While AvBD2 level is not correlated with the quality score, it is inversely correlated to the plasma IgM level and only for S chicks.

Discussion – Conclusion

The results could be explained by the increased immunisation of S chicks, under challenging condition, by bacteria crossing the epithelium and reaching bloodstream without being eliminated by mucosal AvBD2, that is less abundant in animals having more IgM in the peripheral blood.

The availability of anti-AvBD2 antibodies to the scientific community opens perspectives to characterize the mechanisms of mucosal regulation of this defensin.

