# 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.**

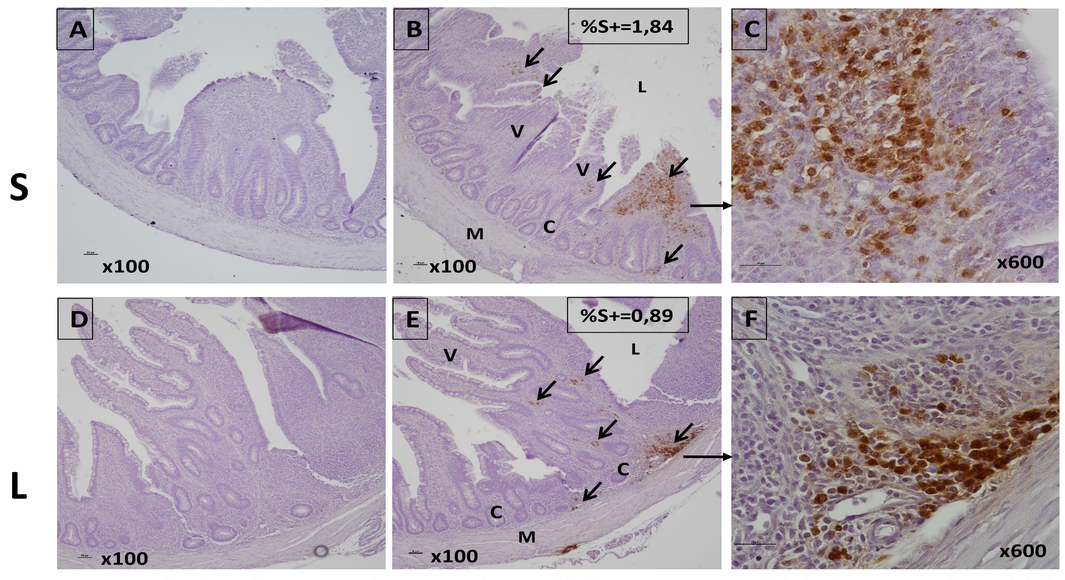
**Day 0, after hatch:**
- **Initial quality scoring**

**Day 1 :**
- **Quality scoring after challenge**

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

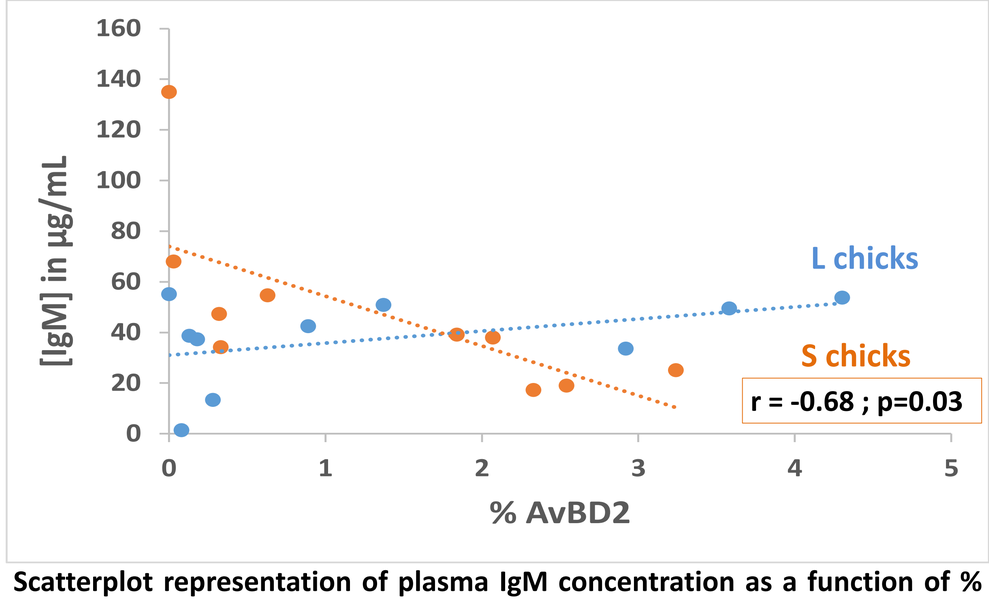
- **IHC analysis from paraffin-embedded serial tissue sections labelled with rabbit anti-AvBD2 polyclonal antibodies,**
- **Ig measurement (ELISA) from serum.**

## 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 2ry 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.**

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