

Early colonizing bacteria in the gastrointestinal tract of day-of-hatch broilers.

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Abstract

Initial colonization of beneficial bacteria in the gastrointestinal tract in newly hatched broilers is essential for the health of the bird throughout its life. Early establishment of lactic acid bacteria (LAB) plays a vital role in stabilizing intestinal homeostasis, digestion and nutrient absorption, and nurturing mucosal conditions for immunological protection. We investigated the levels of LAB and avian pathogenic *E. coli* (APEC) in the gastrointestinal tracts of 300 day-of-hatch (DOH) birds across 5 broiler hatcheries from two US broiler companies. Breeding hens of two of these complexes were also analyzed to determine vertical transmission of microbiota to the DOH birds. Results indicate both LAB and APEC populations were highly variable in birds across flocks in the same complex and across the five complexes. The percent of birds harboring detectable levels of APEC from each complex ranged from 17% to 80%. The percent of birds harboring detectable levels of LAB from each complex ranged from 38% to 100%. Based on microbial community analysis by 16S rDNA sequencing and terminal restriction fragment length polymorphism analysis, the LAB present in the breeder hens were not always present in their respective DOH birds, suggesting that vertical transmission is limited. Microbiota consistently found in hens but missing in DOH birds included *Lactobacillus crispatus*, *L. johnsonii*, and *L. salivarius*. Interestingly, RAPD PCR fingerprint analysis of APEC isolates from breeder hens and their DOH birds provided evidence indicating vertical transmission of APEC isolates. In commercial broiler production many factors may be influencing the establishment of early colonizing bacteria such as use of antibiotics, sanitizers and egg handling methods. While these practices are credited with reducing pathogen loads and disease transmission, they may also be interfering with the colonization of native and beneficial bacteria.

Design

We investigated the levels of LAB and avian pathogenic *E. coli* (APEC) in the gastrointestinal tracts of 300 day-of-hatch (DOH) birds across 5 broiler complexes (60 birds per complex) from two US broiler companies. These bacteria were isolated and characterized to reveal their genetic diversity. Maternal breeding hens of the DOH birds from two of these complexes were also analyzed to determine vertical transmission of microbiota to the DOH birds. Terminal restriction fragment length polymorphism analysis was used to compare the LAB communities between the hen and DOH gastrointestinal tracts.

Methods

Day-of-hatch birds: Birds were harvested from the hatchery less than 24 h after emerging from the egg and shipped overnight on ice to Agro BioSciences for processing. Upon arrival the entire gastrointestinal tract from duodenal loop to ileal-cecal junction was opened, masticated, and plated with MRS agar (LAB) and Chrome agar (APEC).

Breeding Hens: Hen gastrointestinal tracts were sent to Agro BioSciences on ice overnight and were immediately processed by rinsing out the digesta then sectioning regions of the duodenum, jejunum, and ileum. Opened sections were masticated then plated with MRS agar (LAB) and Chrome agar (APEC).

Differentiation of APEC: *E. coli* isolates were screened for five virulence genes (*iron*, *ompT*, *hlyF*, *iss*, and *iutA*). Possession of at least two of these genes identifies the isolate as APEC.

Community Analysis: DNA was extracted from the mucosal content from each bird. LAB-specific primers were used to amplify the 16S rRNA gene and amplicons were digested with the Mse I restriction enzyme.

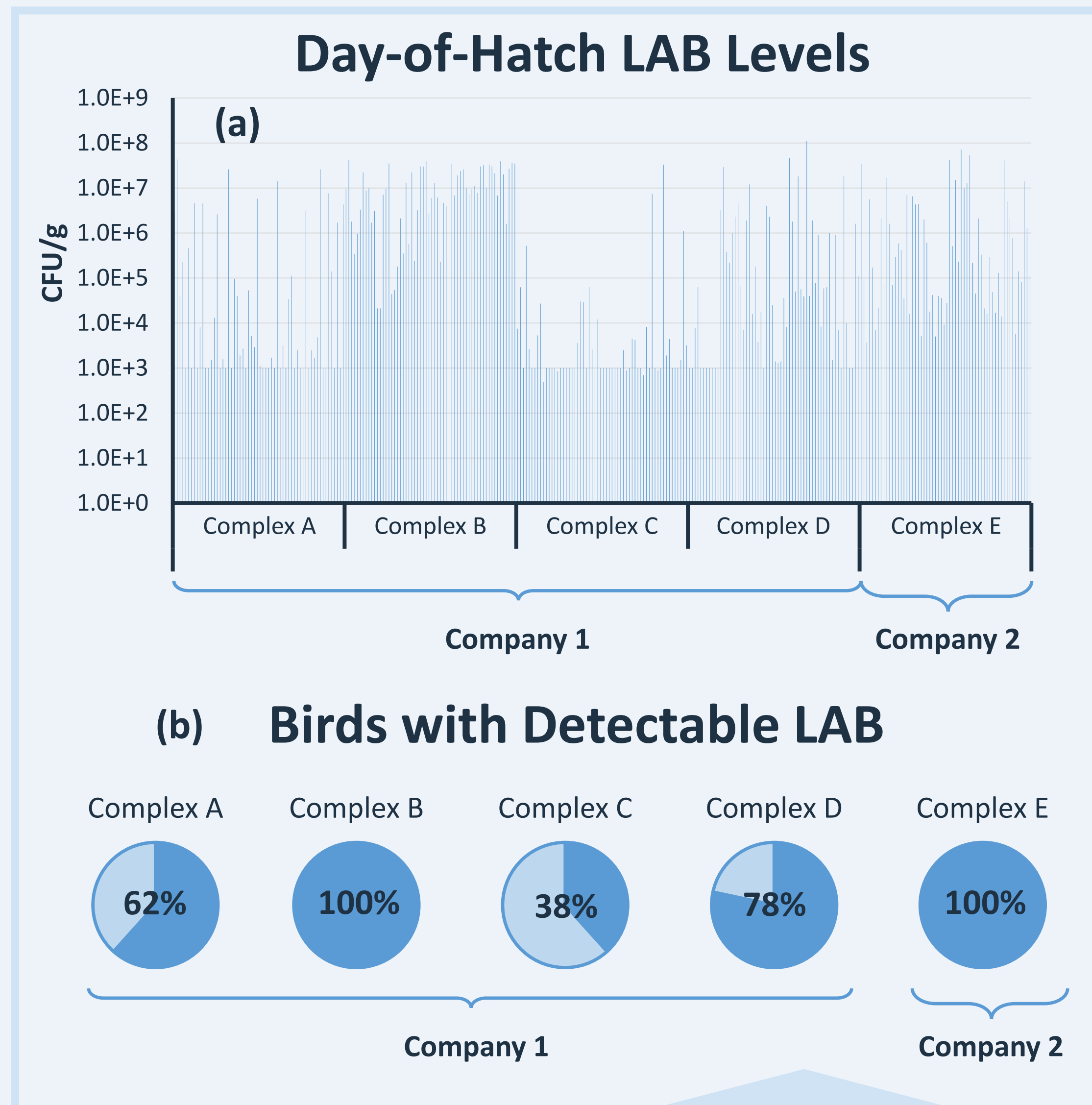


Figure 1: Levels of LAB from the GI tract of day-of-hatch birds (a) showing high variability within five complexes representing two US broiler companies. Percentage of birds that harbored detectable levels of LAB (>1000 CFU/g) (b) shows variability between complexes. 60 birds were sampled per complex.

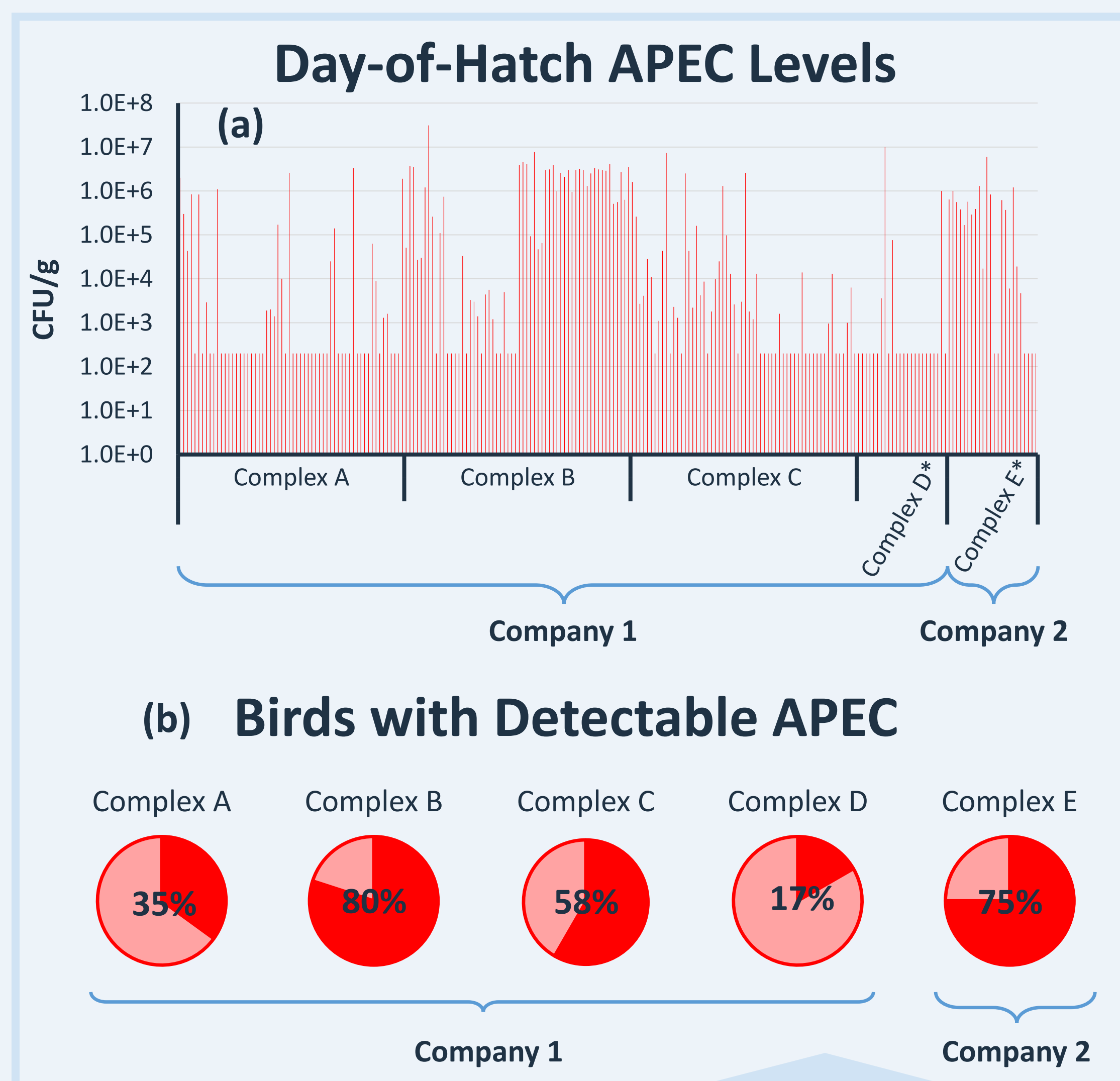


Figure 2: Levels of APEC from the GI tract of day-of-hatch birds (a) showing high variability among 5 broiler complexes. Percentage of birds that harbored detectable levels of APEC (>200 CFU/g) (b) shows variability between complexes. 60 birds were sampled per complex*.

*only 24 samples were measured for APEC levels from complexes D and E.

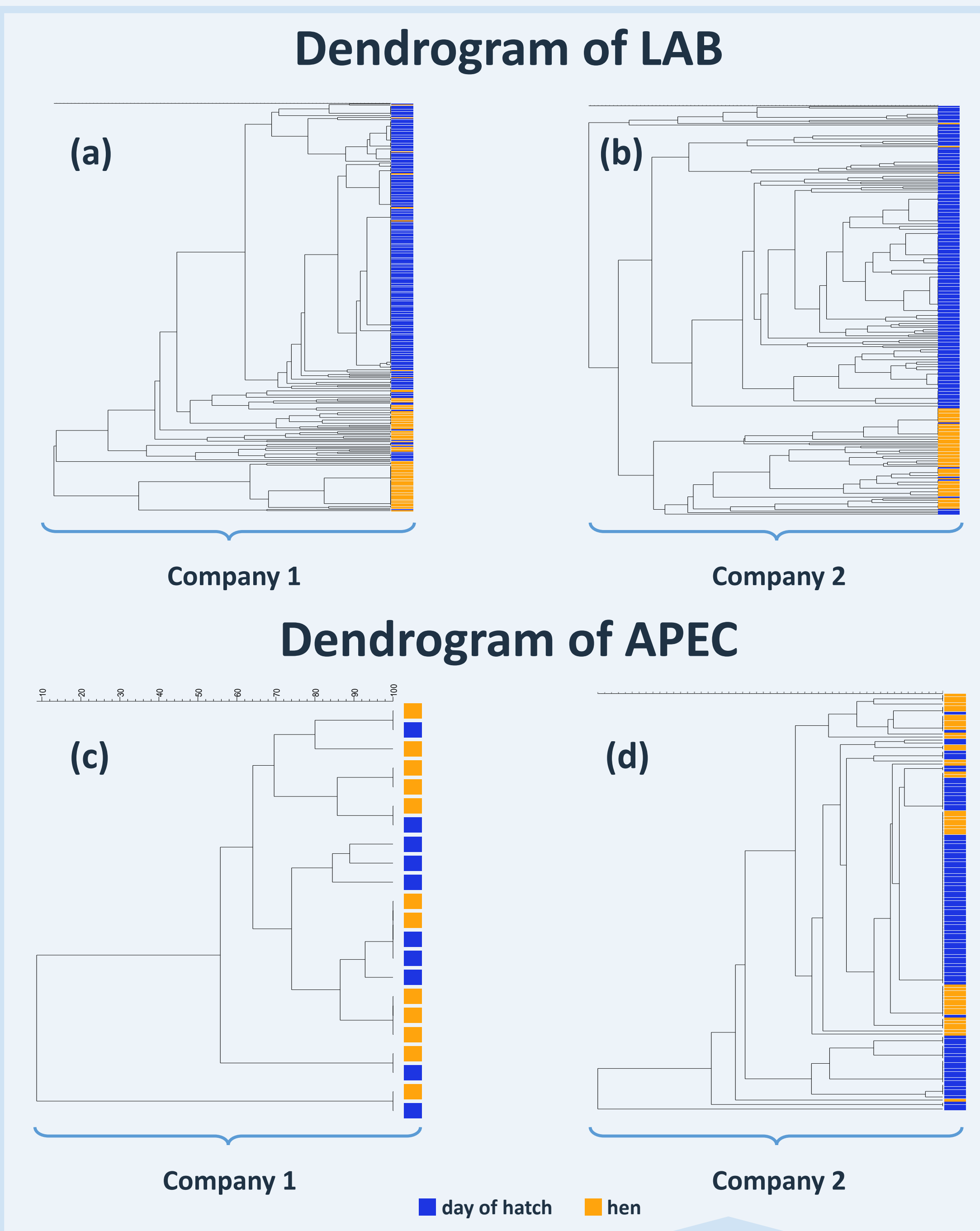


Figure 3: Dendrograms of RAPD-profiles of LAB isolates (a,b) and APEC isolates (c,d) collected from hens (orange) and DOH birds (blue). Results show genetically distinct populations between DOH and hen LAB isolates, while the same APEC populations seem to be present in both DOH and hen gastrointestinal tracts.

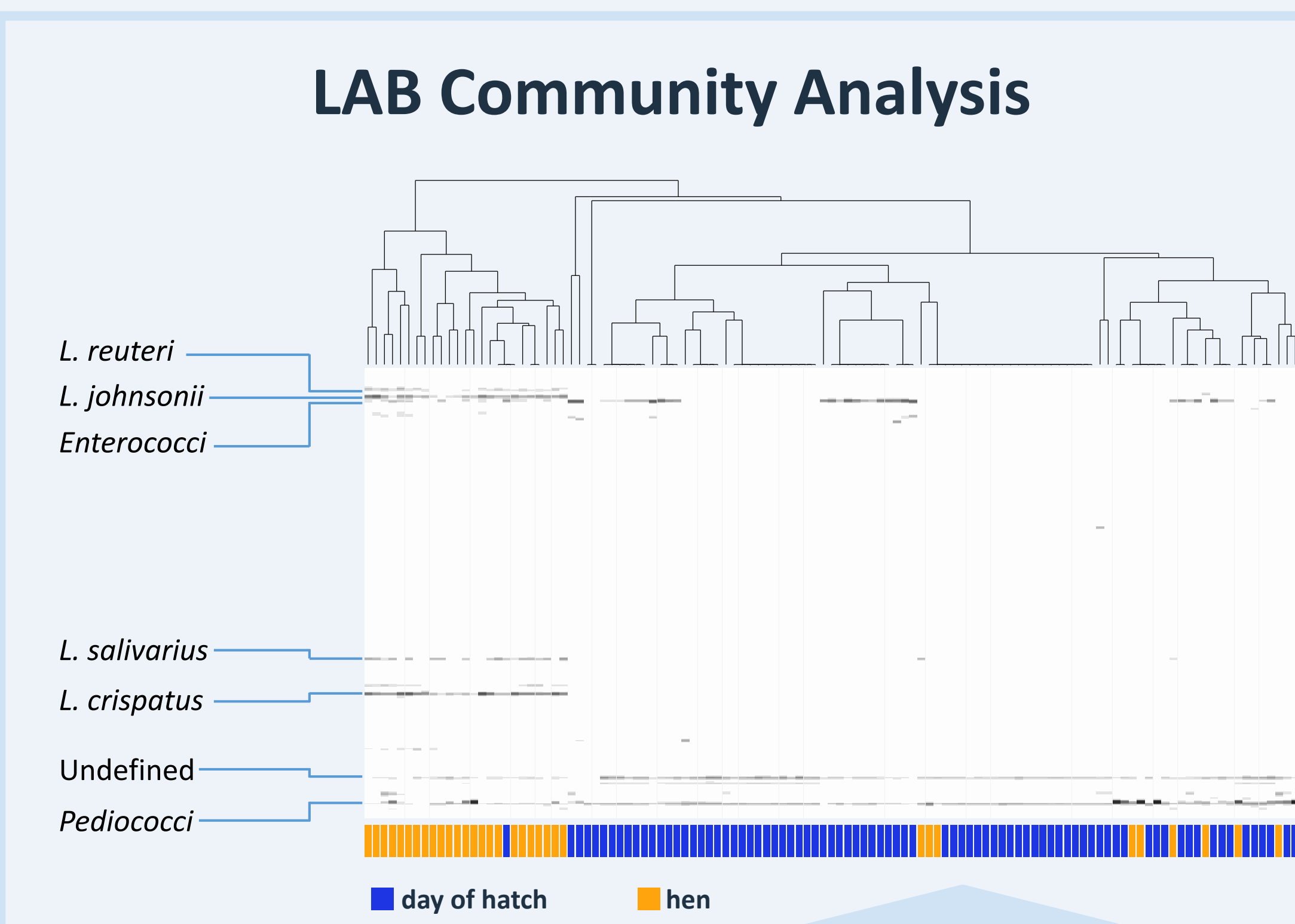


Figure 4: TRFLP dendrogram of LAB community in DOH and hen gastrointestinal tracts indicating greater LAB diversity in the hens (orange) than the DOH birds (blue). *Pediococci* and *Enterococci* are predominant in both hen and DOH birds while *Lactobacillus crispatus*, *L. johnsonii*, *L. salivarius*, and *L. reuteri* are unique to hens.

Results

Results indicate both LAB and APEC populations were highly variable in birds across flocks within the same complex and across the five complexes. The percent of birds harboring detectable levels of LAB from each complex ranged from 38% to 100% (Fig. 1b). The percent of birds harboring detectable levels of APEC from each complex ranged from 17% to 80% (Fig. 2b). RAPD-profile pairwise comparisons suggest that LAB populations of DOH and hen LAB isolates are genetically distinct (Fig. 3a,b). In contrast, DOH birds seem to harbor APEC isolates that are more genetically similar to those of hens (Fig 3c,d). These trends were consistent in both broiler companies. LAB microbial community analysis shows that the LAB present in the breeder hens were not always present in their respective DOH birds. Microbiota consistently found in hens but missing in DOH birds included *Lactobacillus crispatus*, *L. johnsonii*, *L. salivarius*, and *L. reuteri*.

Discussion

These data from two US broiler companies show that day-of-hatch (DOH) birds have highly variable levels of lactic acid bacteria (LAB) as well as avian pathogenic *E. coli* (APEC) in their gastrointestinal tracts. Comparison of the LAB isolates from maternal breeding hens and their DOH birds demonstrates that there is limited transfer of maternal LAB strains. Perhaps more importantly, the diversity among LAB in the hens is almost completely lost in the DOH birds. Moreover, microbial community analysis by 16S rDNA sequencing and terminal restriction fragment length polymorphism analysis shows that *Lactobacillus crispatus*, *L. johnsonii*, *L. salivarius*, and *L. reuteri* were consistently missing in their respective DOH birds, suggesting that vertical transmission is limited. Interestingly, APEC isolates are relatively genetically similar between breeder hens and their DOH birds which provides evidence that either APEC isolates are more easily transmitted from hen to chick, or that those APEC strains are ubiquitous in environments across production facilities.

Summary

In commercial broiler production, many factors may be influencing the establishment of early colonizing bacteria such as use of antibiotics, sanitizers and egg handling methods. While these practices are credited with reducing pathogen loads and disease transmission, they may also be interfering with the colonization of native and beneficial microbial terroir.

Citations

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