Is *Enterococcus faecalis* a Trojan Horse for Poultry Hatcheries?

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The commercial poultry industry is renowned for its innovations in production efficiency. Over the past several decades advances in genetic selection, nutritional improvements, disease control measures and management practices have made commercial poultry production one of the most efficient and productive animal farmed species.(1) Modern advances in incubators and hatchers have also improved incubation methods and sensors which have gained efficiencies in monitoring and lowering incubation costs. However, it has been estimated that 20% of breeding eggs are lost as hatchery waste.(2) These losses are primarily due to low hatchability and infertility. Management guides for egg layer breeder companies report expected hatchability to be between 78% and 83%.(3-5) The conditions by which these management guides derive their data are typically favorable and/or ideal. Real-world conditions may affect the hatchability so as not to achieve the results reported in the management guides. In recent communications with laying hen industry personnel that operate a state-of-the-art, modern hatchery, with a high level of hygiene and biosecurity, they achieve only 78% hatchability. The current situation with turkey hatcheries, based on personal communications with the turkey industry, is similar and often only the lower range of hatchability (78%) is realized. This aligns with a twenty-year survey of turkey egg hatchability that reported the range of hatchability over the twenty-year period to be from 76% - 80%. It is also worth noting that during this time advancements in nutrition, genetic selection, and management did not result in an increase in hatchability(6). Additionally, the decreased fertility / hatchability of turkey eggs has been reported to decrease with time within a production cycle.(7) According to USDA hatchery reports, the hatchability of broiler chickens has steadily decreased over the past decade from 85% to less than 80%.(8) The reason(s) for this decrease has yet to be determined.

It is surprising to note that other performance parameters have increased production efficiency over time, but hatchability rates have stayed nearly the same for decades. The seemingly inability to increase hatchability and fertility has stagnated production efficiency. If even modest to small increases in hatchability and fertility could be achieved, it would have significant economic benefits for the poultry industry. For example, a laying breeder hen typically produces 95 saleable hen chicks in her productive life (i.e., at 65 weeks of age). If the hatchability were increased by 2% then this would equate to an additional 2.4 chicks being produced or an increase of 2.5% in saleable hen chicks. The number of broiler chickens produced in the U.S. is approximately 9.2 billion. USDA places the value of the US broiler

industry at over thirty-one billion dollars and the US turkey industry at nearly 6 billion dollars. The value of eggs is estimated at 8.7 billion dollars.(9) A quick arithmetic calculation reveals that a 1% decrease, or increase, in broiler hatchability equates to a loss, or gain, of ninety-two million broiler chickens annually.

There are many factors that can affect egg hatchability including incubation conditions such as temperature, humidity, ventilation and turning. Egg collection, handling and storage are other factors. Typically, these factors are intensely scrutinized and controlled. Breeder flock age, health, nutrition, and breed can also affect hatchability. However, these factors are also carefully monitored and evaluated. As mentioned above, these factors, as well as other advances in nutrition, genetic selection, and management, do not account for either decreases or the lack of increases in hatchability.(6) It is recognized that bacterial pathogens also play a role in hatchability, although comprehensive work has not been done in this area. Recently, we have found that some virulent strains of the bacteria *Enterococcus faecalis* (*E. faecalis*) are present in embryos at hatch, can cause embryo mortality, and prevent fertile eggs from developing. However, other *E. faecalis* strains found at hatch are avirulent and their effects on embryos seem to be minimal. We have found that small numbers of virulent *E. faecalis* were lethal to chick embryos at all stages of embryonic development. To our surprise, we discovered that when fertile chicken eggs were inoculated with *E. faecalis* and then placed into the incubator they failed to develop and appeared as infertile (i.e., clear) eggs. **We hypothesize that virulent strains of *E. faecalis* account for some of the decreased hatchability that is attributed to infertility and unhatched eggs / embryos.** Because of this, we feel that *E. faecalis* may be a type of "Trojan Horse." That is, it is often found in the hatchery environment and unhatched embryos but not recognized as a pathogen. Therefore, we believe that some virulent strains of *E. faecalis* may be significantly contributing to decreased hatchability and weak hatchlings.

Enterococcus faecalis was originally classified as streptococci of Lancefield group D and has long been considered part of the normal bacterial intestinal flora of humans and poultry (10-12). *Enterococcus faecalis* has been found in day-old chicks but infrequently in 3-5 week-old broiler chickens.(10) Kuntz, et al., reported isolating *Enterococcus faecalis* from feces of broilers but rarely from broiler litter and concluded that broiler litter selects against the organism and is an unlikely environmental source (11). In humans, *Enterococcus* spp. has been considered an opportunistic pathogen and has often been associated with nosocomial infections (12, 13). Early reports in poultry associated a streptococcus microorganism with a malabsorption enteric disease of chicks (14). This report concluded that a filterable agent was involved; however, antibiotics improved, but did not eliminate, the condition. *Enterococcus faecalis* has been implicated as causing pulmonary hypertension in broilers (15). However, it should be noted that high doses ($3X10^6$, $1.5X10^7$ and $2X10^7$) of the bacterial inoculum were used in this study and the inoculum was administered by either the intra-abdominal or intravenous route (15). Landman, et al, reported on a syndrome occurring in laying chickens where the birds displayed growth depression and amyloid arthropathy (16). *Streptococcus faecalis* was isolated from two of six flocks reported with this condition. A ten-year study involving 3,100 broiler breeder chickens was conducted to investigate correlations between multilocus sequencing types of *Enterococcus faecalis* and lesion types (17). Of the 3,100 birds tested, *Enterococcus faecalis* was isolated from 167 birds (5.4%). Sixty-nine isolates originated from eight diseased flocks and twenty isolates from two healthy flocks. The study concluded that no correlation or relationship could be established between multilocus sequence types of *Enterococcus faecalis* and healthy birds or

diseased birds (17). Olsen, et al, explored first-week mortality in fifty layer flocks (18). It was reported that fifty percent of the mortality was due to an infectious cause. Postmortem results from 983 chicks revealed that approximately 50% had died from infectious causes and *Escherichia coli* and *Enterococcus faecalis* were found to be the most significant pathogens associated with first-week mortality. The first report of *Enterococcus faecalis* potentially being egg transmitted was in 1992 whereby, Hassan, et al, reported various microorganisms being isolated from 100 dead in-shell embryos sourced from four different hatcheries (19). Thirty-six isolates representing eight different bacterial genera and one fungal genus were identified. Sixty-five percent of the isolates occurred as mixed cultures. *Streptococcus* spp. was identified from one culture but was mixed with *E. coli.*(19). It is unclear if this was in fact *Enterococcus faecalis* or another organism. Fertner, et al, presented convincing evidence of *Enterococcus faecalis* being egg transmitted in a study whereby eggs were fumigated prior to hatch to ensure no bacterial contamination was present at hatch either on the eggs' surface or within the hatcher environment. The hatched chicks were then sampled for *Enterococcus faecalis* (cloacal swabs) at 0 and 24 hours following hatch (20). Two lines of layer chickens were studied: Lohman Brown and Lohman White. It was found that 14% of the Brown line chicks were positive for *Enterococcus faecalis* at hatch (0 hour) and 97% were positive at 24 hours post hatch. Whereas 0.5% of the White line chicks were positive at hatch and 23% were positive at 24 hours post hatch. It was concluded that *Enterococcus faecalis* could be egg transmitted and "the present findings demonstrated a high potential of a few contaminated eggs or embryos to rapidly facilitate the spread of *E. faecalis* to almost all chickens during hatch."(20). More recently, a case report by Reynolds and Loy presented clinical evidence for egg transmission of *Enterococcus faecalis* and that *Enterococcus faecalis*, as the sole pathogen without involvement of other culturable microorganisms, was responsible for embryo death resulting in decreased hatchability of ring-neck pheasant eggs where the hatchability was reported to be 14 – 15% (normal hatchability is about 75%).(21)

Using the *E. faecalis* isolated from pheasant eggs / embryos (as indicated above) we initiated preliminary studies where we propagated this organism and inoculated chicken embryos with *E. faecalis* at various stages of incubation. We found that small numbers (less than 100 CFUs) of this *E. faecalis* isolate were lethal to the embryos at all stages of embryonic development. We established a chicken embryo lethality assay (cELA) whereby, 10-day-old specific pathogen free (SPF) chicken embryos are inoculated with *E. faecalis* by the chorioallantoic route and assessed for viability / mortality 4 days post-inoculation. Using this cELA, we demonstrated that not all *E. faecalis* isolates are embryo lethal. From our repository in the Nebraska Veterinary Diagnostic Center (NVDC) we recovered *E. faecalis* isolates originating from a turkey flock, a broiler flock and a layer flock and determined their virulence using the cELA. The turkey isolate was as virulent as the pheasant isolate causing 100% embryo mortality. The *E. faecalis* isolates from broiler and layer chickens caused no embryo mortality and were deemed avirulent. This demonstrates that strain-level *E. faecalis* virulence capability differs and should be further studied. To emulate transovarial transmission, we inoculated the yolks of fertile chicken eggs with *E. faecalis* and then placed them into an incubator where they failed to develop, and they appeared as infertile eggs. These results were reported at the recent 74th North Central Avian Disease Conference.(22)

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