Exploring Emerging PathKinex Trends

Together United Animal Health and Microbial Discovery Group are pioneering the investigation of relationships between microbial virulence genes and animal health. Our PathKinex™ platform and growing database of rectal and intestinal swab samples provide a unique resource for identifying and exploring connections between microorganisms and host or environmental factors, and we are eager to share our key findings with you!

Bacterial pathogen burden in dairy cows harboring bovine coccidia

Pathkinex™ Updates are intended to provide you with the latest research insights on emerging pathogen trends and are for your internal use.
In this update, we explore the impact of the bovine coccidia *Eimeria bovis* on gut health and opportunistic pathogen load in US dairy cows.

Eimeria bovis is a frequent cause of diarrheal disease in calves and is often carried and shed by mature dairy cows, damaging the intestinal mucosa as the oocysts mature in the gastrointestinal tract (GI) and contributing to GI disease, stress, and production loss. We examined the prevalence of an *Eimeria bovis* marker gene in PathKinex samples from US dairies and looked for associations with disease and with other opportunistic enteric pathogens including *Clostridium perfringens, Salmonella,* and virulent *E. coli.*

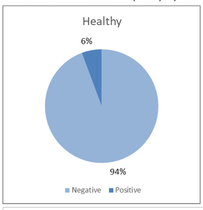
Coccidiosis in dairy cattle
Coccidia are single-celled protozoan parasites that infect the intestinal tract of many important livestock species. Among a wide variety of parasites affecting wild and domestic animals, *Eimeria* spp. are important parasites of poultry, cattle, and other ruminants, *Isospora suis* and some *Eimeria* species cause coccidiosis in swine, and *Cryptosporidium* spp. causes disease outbreaks in animals and humans.

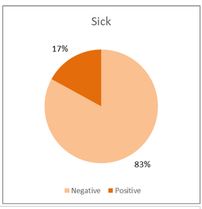
Coccidia of the genus *Eimeria* are spread via the fecal-oral route. *Eimeria* persist in manure, litter, and soil as sporulated oocysts with tough cell walls resistant to environmental damage. When oocysts are ingested and exposed to the moisture and nutrients of the GI tract, they release active sporocytes into the small intestine which invade the cells of the intestinal epithelium. Once inside the intestinal cells, the sporocytes undergo several rounds of sexual and asexual reproduction before bursting the cells and releasing thousands of immature oocysts back into the intestinal tract and feces. It is this stage of the reproductive process that damages the intestinal epithelium, causing diarrhea, weight loss, and dehydration in affected animals.

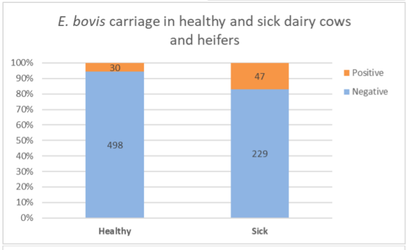
Eimeria bovis is one of three species (also including *E. zuernii* and *E. auburnensis*) capable of causing disease in dairy and beef cattle. Coccidiosis is most frequent and severe in calves, heifers, and beef cattle up to 1 year in age, but the parasite is also present in mature dairy cows which may continue to experience outbreaks of coccidiosis when exposed to new strains. Subclinical infections may lead to decreased milk production and may promote the growth of other opportunistic microorganisms in the GI tract.

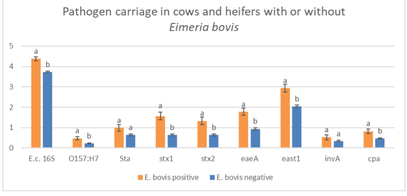
Eimeria spp. are frequently controlled with the use of coccidiostats, a class of antimicrobial feed additives not used as human antibiotics. Common coccidiostats used in the dairy industry include monensin, lasalocid, and decoquinate. Other factors including farm sanitation, cow movements, crowding, heat and cold stress, and coinfection with multiple pathogen species can also contribute to the likelihood and severity of outbreaks.

Method
Our PathKinex dataset consists of qPCR data from rectal swab DNA collected from healthy and sick cows and heifers at US dairies participating in PathKinex studies. The PathKinex™ panel includes qPCR assays for genus- or species-specific marker and virulence genes unique to bacterial, viral, and fungal pathogens of interest, including a marker gene for *Eimeria bovis,* the most prevalent species in animals over 3 months old. For this comparison, we used data from 857 cows and heifers collected between 2018 and 2021 from US commercial dairies including 88 positive for the *Eimeria bovis* marker gene. To test differences by *Eimeria bovis* marker gene presence/absence, gene data was log-transformed and analyzed via Welch's T test for unequal variance with Bonferroni correction for multiple comparisons (9 virulence genes). The Chi-square test was used to test differences in *E. bovis* frequency by health status.









Findings

- ***Eimeria bovis* is significantly more frequent in sick cows than in healthy cows**
  - In US dairy survey data, carriage of an *Eimeria bovis* marker gene is significantly higher in sick animals than in healthy animals (Chi-square test, $p < 0.001$)
- ***Eimeria bovis* carriage is associated with significantly higher levels of key pathogens as detected by virulence gene qPCR**
  - In US dairy survey data, total *E. coli* and virulence factors for enteroaggregative, enterohemorrhagic, and Shiga-toxigenic *E. coli* were significantly elevated in animals positive for an *E. bovis* marker gene ($p < 0.05$ by Welch's T test with Bonferroni correction for 9 comparisons).
    - Total *E. coli*, O157 marker gene, Shiga toxin genes *stx1* and *stx2,* intimin gene *eaeA,* and *EAST1*were significantly elevated in *Eimeria*-positive animals.
  - *C. perfringens* virulence marker *cpa* was significantly elevated in *Eimeria*-positive animals.

Action
These PathKinex™ findings illustrate the impact of *Eimeria bovis* carriage and shedding on populations of other opportunistic pathogens in the GI tract. Shedding of coccidial oocysts damages the cells of the intestinal mucosa, leading to changes in osmotic balance, nutrient availability, and impaired host defense that may promote the growth of opportunistic pathogens like virulent *E. coli* and *C. perfringens.*

Interventions like coccidiostat usage, improved sanitation, and good herd management are crucial for dealing with common, endemic parasites like *Eimeria.* Furthermore, direct-fed microbial (DFM) products may support gut integrity and provide secondary microbial control in the GI tract and the environment when gut parasites lead to polymicrobial infection with elevated levels of pathogenic bacteria. A comprehensive strategy including the use of sanitation, good herd management and DFM tools can help promote animal health and mitigate the impact of outbreaks on milk production and stress.

Discussion Question
How are parasites interacting with key bacterial pathogens in the swine, poultry, and specialty markets and where can DFMs make an impact?

Are you seeing the same trends in the field?

Respond to MDG

Is there a topic you'd like to learn more about in a future newsletter? We enjoy hearing from you! We welcome your questions, comments and suggestions on PathKinex updates. Please contact us at AnimalAg@mdgbio.com