In poultry, coccidiosis is caused by species of *Eimeria*, a widespread protozoan parasite that causes gastrointestinal disease with consequences ranging from decreased growth performance during subclinical infections to high mortality in cases of infection with extremely pathogenic strains. Broilers represent the most economically important host, but *Eimeria* are pervasive across all poultry, including layers and turkeys. Estimates from 2016 data suggest that coccidiosis costs the US chicken industries $1.6 billion per year with an annual global cost of $14.4 billion (Blake et al., 2020). Inclusion of anticoccidial drugs in the feed and immunization with live oocysts are the primary means of prevention. An estimated 88% of US broilers raised on anticoccidial compounds, and though this has not been reported for turkeys, it is reasonable that, given limited vaccine options, a higher percentage are raised with anticoccidial medications (Chapman and Jeffers, 2014; Blake et al., 2020).

Rotation and shuttle programs have become popular for limiting drug resistance but despite these efforts, multi-drug resistance is prevalent within flocks (Table 1) and was documented in the early 20th century, not long after anticoccidial medications were developed, and has continued despite efforts to alternate drugs in a logical pattern (Cuckler et al., 1955; Warren et al., 1966; Hodgson et al., 1969; Martin et al., 1997; Bafundo et al., 2008; Snyder et al., 2021). Both types of program alternate medication, often based on mechanism of action, to delay the onset of resistance and extend the useful life of these products (Chapman, 2001). The concept behind these alternating medications is that if resistance develops to one compound, then it will be lost during application of the next, but this philosophy remains unproven (Chapman and Jeffers, 2014). Rotation programs involve providing a single anticoccidial medication for multiple cycles before rotating to a new medication and may include vaccination cycles. Typically, an entire complex uses the same medication for two to three months. Shuttle programs alternate anticoccidial medications more frequently with feed changes. Thus, birds are fed two or more medications within a flock when they change between starter, grower, and finisher feeds. There is no doubt that anticoccidial rotation and shuttle programs prolong the onset of resistance but given the prevalence of multi-drug resistant strains of *Eimeria* in today's poultry flocks, it could easily be argued that this strategy does not prevent resistance, and systematic testing to establish more effective rotation programs is necessary to the continued efficacy of anticoccidial compounds for the control of coccidiosis in poultry.

Extensive drug usage within intensive rearing systems is widely known to lead to resistance across all types of medications and concerns regarding multi-drug resistance in coccidia were recognized shortly after their development (reviewed by Chapman, 1997). It has also been expected that cross-resistance would occur, especially within class or mechanism of action such as monovalent ionophores. In fact, a 1975 research article reported multi-drug resistance to anticoccidials while mimicking a shuttle program for 40 passages and suggested that shuttle programs do not prevent resistance but can only delay it (McLoughlin and Chute, 1975). Rathinam and Chapman (2009) reported multi-drug resistance of turkey *Eimeria* field isolates and low incidence of sensitivity to amprolium and monensin. Thus, the pervasive existence of multi-drug resistant *Eimeria* in today's poultry industries is not unexpected, but it is approaching a critical threshold in which many producers may be left without medication options for prevention and treatment of coccidiosis.

In a review of anticoccidial compound resistance, Chapman previously suggested that sensitivity could be restored following passage of resistant lines via administration of an unrelated compound to birds (Chapman, 1997). Sensitivity to salinomycin was restored in an experiment in which broilers infected with the resistant strain were fed a diet containing diclazuril or vaccinated in various combinations across four passages, suggesting that rotation programs, especially those that include vaccination, can have some success (Chapman and Jeffers, 2015). However, this describes testing only one sensitivity profile and does not investigate other rotation programs for restoration of sensitivity. Since turkey producers have a longer growing cycle and limited medication options for control of coccidiosis, controlling resistance and restoration of sensitivity is more complicated than broiler flocks. Turning to strategies employed by chicken breeder and layer arms of the poultry industry may prove useful, but limited vaccine coverage of species prevalent in commercial operations limits effectiveness of these options.

The Poultry Enteric Health Research Laboratory (PEHRL) recently established a program with turkey and chicken veterinarians to test fecal samples containing *Eimeria* for anticoccidial sensitivity (TACS) that has highlighted the prevalence of resistance in US turkey flocks (Figure 1). Table 1 summarizes sensitivity profiles across all flocks tested in the TACS program, and an astonishing 62% are multi-drug resistant while 26% are pan-resistant. All poultry operations producing birds under raised without antibiotics standards in the US also have limited anticoccidial choices because some drugs are classified as antibiotics. This decreases the amount of “down time” for any selected medication in rotation and shuttle programs and may promote resistance. While a vaccine is currently available to the turkey industry and can help manage anticoccidial sensitivity, this is only effective against the two species provided. Thus, turkey producers need to be aware of species affecting their flocks. A recent report on species detected among 33 turkey flocks in Canada showed an average of 2.8 species per flock with *E. gallopavonis* or *E. meleagridis* present in 48% (Imai and Barta, 2018). Vaccinated flocks had fewer average species diversity, but at 2.4 species per flock, it can be assumed that vaccine containing two species does not offer full protection. This information is consistent with species detection in the TACS program at PEHRL (data not shown).

Figure 1. Representative results of test for anticoccidial sensitivity (TACS) of fecal samples from turkey integrators. PERHL has completed nearly 40 TACS samples to date.

Farm	Farm 1	Farm 2	Farm 3	Farm 4
Flock Age	36 days	36 days	14 days	5 weeks
Amprolium	SENSITIVE	RESISTANT	RESISTANT	REDUCED SENSITIVITY
Lasalocid	SENSITIVE	SENSITIVE	RESISTANT	RESISTANT
Monensin	SENSITIVE	REDUCED SENSITIVITY	RESISTANT	-REDUCED SENSITIVITY
Clopidol	SENSITIVE	SENSITIVE	SENSITIVE	SENSITIVE
Halofuginone	REDUCED SENSITIVITY	SENSITIVE	REDUCED SENSITIVITY	RESISTANT
Zoalene	RESISTANT	SENSITIVE	RESISTANT	REDUCED SENSITIVITY

Adding a period of vaccination to a rotation program has been embraced by some integrators to re-establish drug-sensitive strains on farms (Mathis and Broussard, 2006; Chapman and Jeffers, 2014). This strategy relies on re-seeding litter with drug-sensitive vaccine strains in place of drug-resistant strains as older oocysts age and die within litter during the vaccine cycle. However, recent studies have indicated that this strategy may not effectively alter drug sensitivity profiles within farms, where despite vaccinating with *Eimeria* sensitive to all medications, resistance to some was still detected after two cycles of vaccination (Snyder et al., 2021). These variable results highlight remaining

Anticoccidial sensitivity classification	% Flocks Tested
Pan-sensitive	14%
Pan-resistant	26%
Multi-drug resistant	62%
Sensitive or reduced sensitive ≥ 4	38%

Table 1. Anticoccidial sensitivity classification of farm samples tested by PERHL. Pan-sensitive = sensitive or reduced sensitivity to all drugs; Pan-resistant = sensitive to ≤ 1 drug; multi-drug resistant = resistant to ≥ 3 drugs

questions regarding how many consecutive grow-outs should be applied to overcome resistance to anticoccidials.

Anticoccidial sensitivity testing is recognized as a valuable tool by the scientific community for monitoring rotation and shuttle programs that can assist integrators in their *Eimeria* control programs (Peek and Landman, 2011). In 2020, PERHL established a TACS program for turkey integrators to screen farms and complexes to help them rotate medications likely to be effective against *Eimeria* established in their barns. The TACS service tests *Eimeria* samples against seven popular drugs for sensitivity and identifies species within samples via PCR. Identification provides information regarding the possibility that vaccination with the only commercially available option will help control coccidiosis since it contains only two of the six species known to infect turkeys. As of April 2021, nearly 150 samples have been received with 34 samples going through the entire TACS process. Nearly 100% of samples collected for testing contained *Eimeria*, which reveals the magnitude of the lack of coccidiosis control in turkeys.

Of the 34 farm samples submitted to TACS, 14% were pan-sensitive and 100% exhibited reduced sensitivity to at least one anticoccidial compound. If multi-drug resistance is classified as resistance to three or more anticoccidial compounds, 62% of samples are multi-drug resistant. Furthermore, 26% of farms exhibited either no sensitivity to all drugs or only one drug (Table 1).

In 2019, the Association of Veterinarians in Broiler Production reported to the USAHA (USAHA, 2019) that coccidiosis is the disease of highest concern in broiler production. Alongside this, the National Turkey Federation cites coccidiosis as a top concern (#8) and the Association of Veterinarians in Egg Production reported coccidiosis as the first disease of concern in cage-free pullets, second highest disease of concern in caged pullets and the fourth highest of both caged and cage-free layers. These rankings arguably make coccidiosis and its control the highest disease priority of the US poultry industries. Taken into context with multi-drug resistance reports mentioned above, it becomes apparent that coccidiosis is a ticking time bomb that can devastate a major agriculture sector within years if effective control measures are not developed. While live oocyst vaccination is a strategy that can help, it is not a solution that works on its own and control of coccidiosis continues to largely depend on routine use of anticoccidial drugs (Williams, 2002). Thus, without introduction of new drugs to control coccidia and multi-drug resistance rising, integrators are facing fewer and fewer options to keep their flocks healthy. Turkey producers need to be pro-active in their approach to management of coccidiosis through judicious use of anticoccidial compounds, resistance and species monitoring, and application of vaccines where warranted.

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