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30 June – 4 July 2008

XX
III

Selected presentations
from the

World's Poultry Congress



The XXIII World's Poultry Congress in Brisbane, Australia, brings together scientists from all over the globe to present new ideas for improving flock health, nutrition, welfare, environment and many other factors vital to the success of the poultry industry.

Intervet/Schering-Plough Animal Health is proud to be a Ruby Sponsor — the highest level of support provided by allied industry to this important educational congress.

Our scientists and cooperators were also invited to present 12 papers on topics related to intestinal health, Newcastle disease, laryngothacheitis and Marek's disease.

With the support of the congress' organizers, we are pleased to publish this booklet, which contains detailed summaries of those presentations. For more information, please contact your Intervet/Schering-Plough Animal Health representative.

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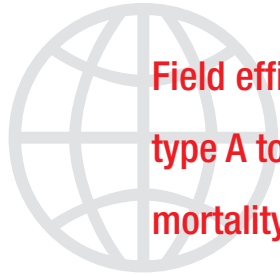
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Field efficacy of Netvax, a *Clostridium perfringens* type A toxoid breeder vaccine, in preventing mortality due to necrotic enteritis in broilers

Summary

KEY POINTS



The efficacy of Netvax, a *Clostridium perfringens* type A toxoid, was assessed at an antibiotic-free broiler company with a history of sporadic necrotic enteritis (NE), especially during winter.

- **Mortality was lower in chicks from hens vaccinated with Netvax than in standard broilers from hens that did not receive the toxoid, particularly during the 22- to 28-day time period and during winter months.**
- **Vaccinating breeder hens with Netvax, a *C. perfringens* type A toxoid, can significantly reduce mortality related to NE in progeny chicks under field conditions.**

An antibiotic-free broiler company with a history of sporadic necrotic enteritis (NE), especially during the colder months, was chosen to evaluate the efficacy of Netvax, a *Clostridium perfringens* type A toxoid breeder vaccine. The toxoid is administered to hens, which in turn convey immunity to their progeny. Coccidiosis was controlled with a non-attenuated coccidial vaccine.

Mortality in the progeny of toxoid-vaccinated hens was compared to a standard group of broilers from hens that did not receive the toxoid. Birds in both groups were evaluated under commercial conditions that provided separate but similar housing and similar management conditions.

Mortality data from weekly settlement records for a period of 17 weeks were analyzed from August 2005 to January 2006. The weekly number of chicks enrolled ranged from 12,900 to 138,000 in the trial group and from 200,600 to 332,932 in the standard group.

In the trial group, average weekly mortality by placement week ranged from 3.9 to 8.9%, compared to 5.3 to 13.9%

in the standard group. The odds for mortality in the standard group were 32% higher compared to the trial group (7.08% versus 5.47%) over all ages, and during the time period of 22 to 28 days, there were 47% higher odds of mortality for the standard group compared to the trial group (1.45% versus 0.98%).

Additionally, a seasonal effect was observed for mortality in the 22- to 28-day group, with a 73% higher odds ratio for mortality in the standard birds compared to the trial birds the same age.

These results demonstrate that vaccinating breeder hens with Netvax can significantly reduce mortality related to NE in progeny chicks under field conditions.

Introduction

Necrotic enteritis (NE) is an enteric disease of poultry typically reported in 2- to 5-week-old broiler chickens raised on litter.¹ NE was first recognized as a problem in poultry in 1961.²

Enterotoxins of *C. perfringens* are believed to be responsible for the fibrinonecrotic lesions observed with this disease. The nature of the ration has been a factor shown to exacerbate or even predispose



chickens to NE,³ along with intestinal mucosa damage caused by coccidiosis.⁴

In addition to the classic form most often described in the literature, a milder sub-clinical form of NE affects performance and has been estimated to have an economic cost of up to USD \$0.05 per bird.⁵

The standard approach for protecting against NE in the poultry industry has been subtherapeutic, in-feed antibiotics and/or antibiotic ionophores. With the recent movement in the poultry industry to remove or minimize the use of in-feed subtherapeutic antibiotics, the incidence of NE has increased. Additionally, NE is a major concern for companies that produce antibiotic-free birds.

This paper will describe the prevention of mortality due to NE in broiler flocks via passive immunity originating from Netvax, a *C. perfringens* type A toxoid administered to hens. Broilers from toxoid-vaccinated hens were compared to progeny from unvaccinated hens in an antibiotic-free commercial production system.

Methods

A total of 79,833 female chickens were vaccinated at 10 and 18 weeks of age with a full dose (0.5 mL) of the *C. perfringens* type A toxoid vaccine administered intramuscularly. Eggs from toxoid-vaccinated hens were collected and hatched separately.

Chicks that hatched from vaccinated hens were designated as trial flocks, while chicks from unvaccinated hens were designated as standard flocks. All chicks in the trial group were from toxoid-

vaccinated hens, while the standard group was determined to be any group with less than 100% progeny from toxoid-vaccinated hens.

Flocks in each group were housed separately, but under similar management conditions with a diet consisting of an *ad libitum* corn/soy ration that complied with the minimum nutritional requirements for chickens at this site.

Weekly trial flock placements were initiated on 29 August 05 and terminated on 30 January 06. During the 17-week

Enterotoxins of *C. perfringens* are believed to be responsible for the fibrinonecrotic lesions observed with this disease.

settlement period, there were 1,309,600 chickens settled with a weekly range of 12,900 to 138,000 chickens in the trial group and 4,628,547 settled in the standard group with a range of 200,600 to 332,932.

Percent mortality was calculated for all flocks placed during each week of the study and was used as an indicator of vaccine efficacy. The time periods examined — 8 to 14, 15 to 21 and 22 to 28 days — corresponded to the typical NE-associated mortality observed in this company. Additionally, total mortality was calculated, summarized and statistically

analyzed. Flocks experiencing mortality greater than 160 birds per day were treated with sulfamethoxazine for 3 days to reduce mortality.

Differences in mortality were analyzed over the 22- to 28-day period (the high risk period) and over the entire grow-out period. A second analysis was performed to assess the seasonal impact of the toxoid vaccine, which coincided with higher NE-associated mortality.

Differences in total mortality were analyzed in a mixed logistic regression model (SAS GLIMMIX), with the group as fixed effect and the week as a random effect. The probability of statistical differences in the mortality model estimates were determined by least square means.

Results

Mortality during the 22- to 28-day range for the period measured was 0.98% for the trial group compared to 1.45% for the standard group (Figure 1). The resultant odds for mortality during this time period were 47% higher for the standard group ($P=0.0078$).

Average total mortality by placement week was 3.9% to 8.9% in the trial group compared to 5.3% to 13.9% in the standard group (Figure 2). There was a 32% higher odds of mortality over the life of the flock for the standard group (7.08%) compared to the trial group (5.47%, $P=0.0005$).

A seasonal effect on disease occurrence was also observed. Local temperature data

Field efficacy of Netvax, a *Clostridium perfringens* type A toxoid breeder vaccine, in preventing mortality due to necrotic enteritis in broilers

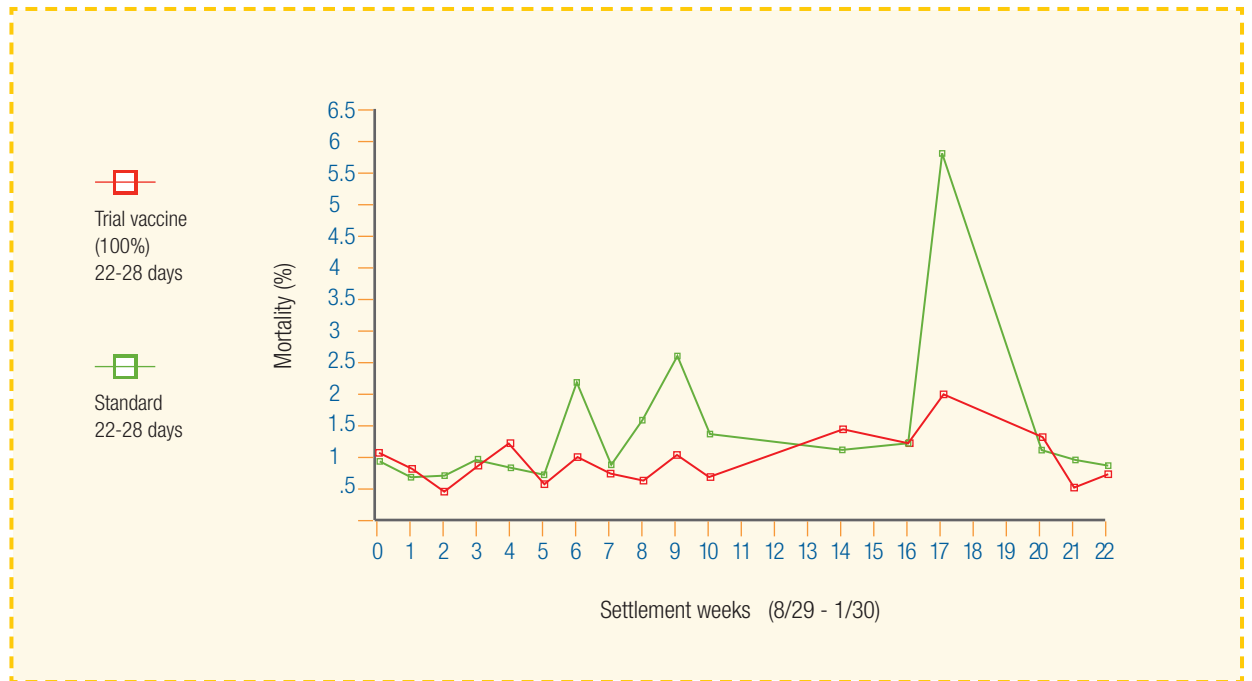


Figure 1. Weekly mortality at 22 to 28 days was lower in the trial group of broilers from toxoid-vaccinated hens than in standard broilers from unvaccinated hens.

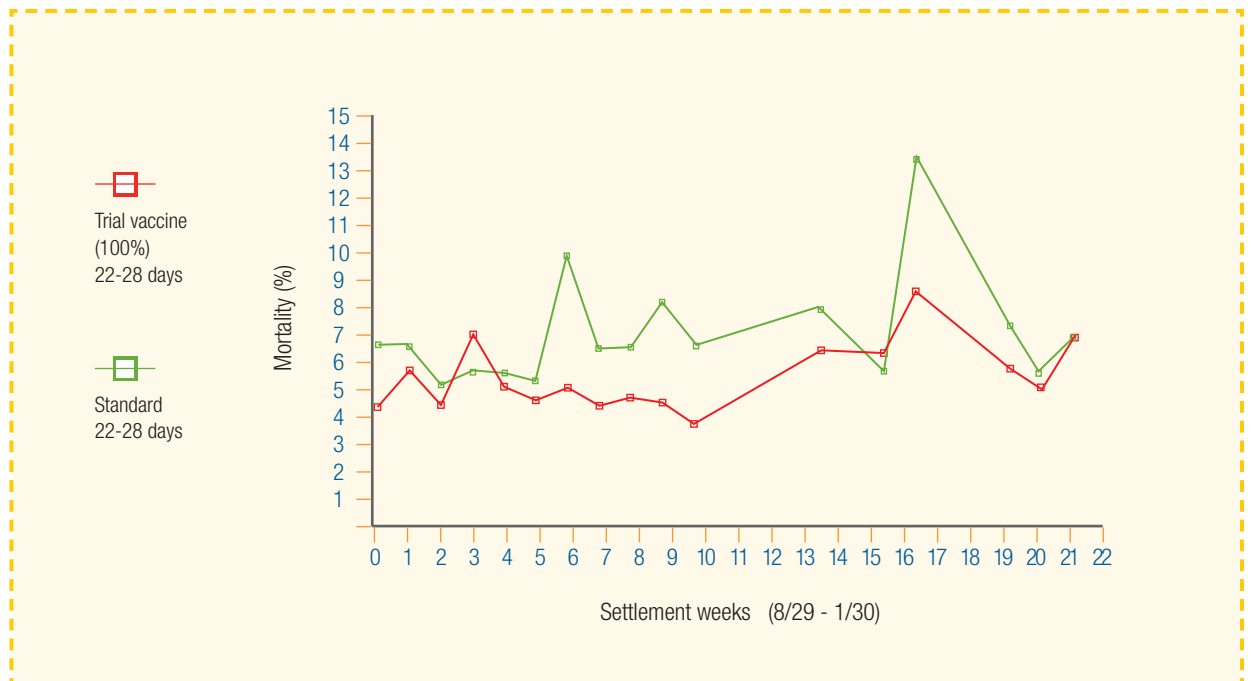


Figure 2. Average total mortality by placement week was lower in the trial group of broilers from toxoid-vaccinated hens than in standard broilers from unvaccinated hens.



were used to divide flocks placed in the warm season (29 August 05 to 03 October 05) and the cooler season (10 October 05 to 30 January 06). The odds for mortality were not different during the warm season for the 22- to 28-day period or overall. However, during cooler weather conditions, there was a difference in the odds for mortality in the 22- to 28-day period, with the standard group 73% higher than the trial group (1.80% versus 1.05%, $P=0.0005$).

Over the life of the flock, the odds for mortality were 39.7% higher for the standard group (7.92%) compared to the trial group (5.81%, $P<0.0001$).

Discussion

Field evaluation of a disease that is sporadic and episodic in nature can be difficult. A company growing antibiotic-free chickens was chosen because it would provide a large numbers of farms for evaluation and because it had a history of NE-associated mortality during cooler months. This provided an opportunity to determine the efficacy of Netvax, the *C. perfringens* type A toxoid breeder vaccine, by using mortality to approximate the losses in flocks due to NE.

A review of the data found progeny from toxoid-vaccinated hens to have a significantly lower overall mortality compared to standard flocks. These differences in mortality were even greater for the trial group in the high risk period (22 to 28 days) compared to the standard group.

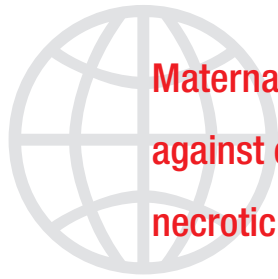
Mortality differences were likely underestimated based on the proportional contribution of vaccinated and unvaccinated hens to the population of chickens evaluated in this field study. The strict criteria for flock placement in the trial group (100% progeny of vaccinated hens) versus the standard group (<100%) may add to an underestimation of mortality differences between the two groups. In addition, flocks experiencing increased mortality were treated with antibiotics, which limited the mortality associated with NE breaks. This would further contribute to an underestimation of the mortality differences between progeny in the trial and standard groups.

Conclusion

The study results indicate that passive immunity derived from Netvax, a *C. perfringens* type A toxoid breeder vaccine, was effective in reducing the incidence or severity of NE in a company growing antibiotic-free chickens.

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Maternal immunization with Netvax against clinical and subclinical necrotic enteritis in broiler chickens

Summary

KEY POINTS



In trials conducted in two European countries, Netvax, an innovative *Clostridium perfringens* type A toxoid for control of necrotic enteritis (NE), was administered to hens to determine if they would convey protective immunity to their progeny.

- **NE lesions were not observed in any of the broilers from hens vaccinated with the toxoid, but were observed in controls.**
- **In addition, mortality was lower in broilers from hens vaccinated with Netvax compared to controls.**

Necrotic enteritis (NE) is a costly, multifactorial disease of poultry characterized by pathologic lesions. The clinical form of NE can lead to high mortality, while the subclinical form results in poor flock performance.

Because *Clostridium perfringens* is an important cause of NE, Netvax, an innovative *C. perfringens* type A toxoid for control of the disease, was investigated. It is a water-in-oil emulsion vaccine that is administered twice to breeders.

Experimental data from this trial demonstrates that immunization of hens with the toxoid vaccine conveys passive antibodies against NE to their broiler progeny. Moreover, results from clinical field observations have confirmed the safety and efficacy of the vaccine.

Introduction

C. *perfringens* type A in low numbers is a normal inhabitant of the chicken intestine. Under certain circumstances, however, the bacteria proliferate, secreting alpha-toxins that produce necrotic patches on epithelial surfaces, representing the disease known as necrotic enteritis.¹

Clinical NE is associated with high mortality, while milder, subclinical forms of the disease result in depressed flock growth rates.² Intestinal mucosal necrosis may be associated with cholangiohepatitis and necrotic dermatitis, which both lead to downgrading at the processing plant.

NE represents a globally important animal welfare and economic problem. It has been estimated that the subclinical form alone may cost poultry producers up to USD \$0.05 per broiler chicken.^{3,4}

Antibiotic growth promoters have been used to control the disease. However, interest in immunizing against NE has risen in recent years due to strong worldwide restrictions or even bans on the routine use of in-feed antibiotic growth promoters.

Generally speaking, passive protection in poultry still has several obvious shortcomings, most notably a limited duration of only 2 to 3 weeks and the absence of maternal antibodies from milk. However, some authors^{5,6} have reported that maternal antibodies, either from natural exposure or following vaccine stimulation, may represent a valid alternative for protecting offspring

against the pathologic effects of *C. perfringens*.

More recently, a water-in-oil emulsion vaccine for NE has been investigated in poultry and it is now available for commercial use.

Materials and methods

A “Good Clinical Practice” (GCP) trial was conducted in two European countries to evaluate Netvax, the innovative *C. perfringens* type A toxoid vaccine, which was administered to broiler breeders with the aim of protecting their offspring against NE for the first 3 weeks of life.

The study was conducted at two sites and included 11,234 vaccinated and 9,304 control hens. Clinical observation of their progeny, consisting of 5 batches of broiler

chickens, was used to test the safety and efficacy of the test vaccine.

Results and discussion

Vaccination of breeder hens induced a significant antibody response against *C. perfringens* alpha-toxin. Significantly higher levels of *C. perfringens* alpha-toxin antibodies were observed in eggs

collected from vaccinated hens (Figure 1). This confirms active transfer of specific antibodies from hen serum into their eggs.

Progeny chicks hatched from eggs of vaccinated hens also demonstrated significantly higher levels of *C. perfringens* alpha-toxin antibodies. The chicks were from eggs collected on site 1 when hens were 30, 35 and 45 weeks of age and on site 2 when the hens were 27 and 32 weeks.

No *C. perfringens*-associated gut lesions were observed in chickens from vaccinated hens. In contrast, 5.7% of controls from one site and 17.8% of controls from the other displayed lesions and *C. perfringens* was isolated in gut samples taken when NE was suspected.

Throughout the entire study, mortality in broilers was slightly higher among controls

Progeny chicks hatched from eggs of vaccinated hens... demonstrated significantly higher levels of *C. perfringens* alpha-toxin antibodies.

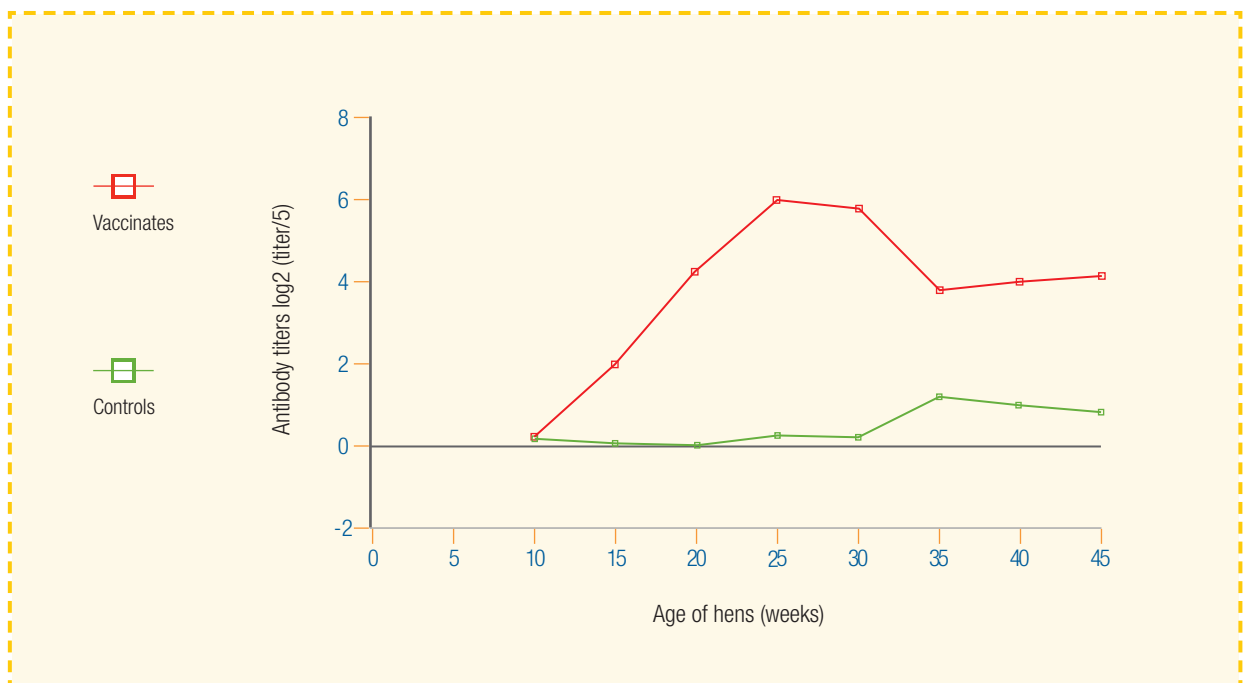


Figure 1. Mean serum *C. perfringens* alpha-toxin antibody titers in hens.

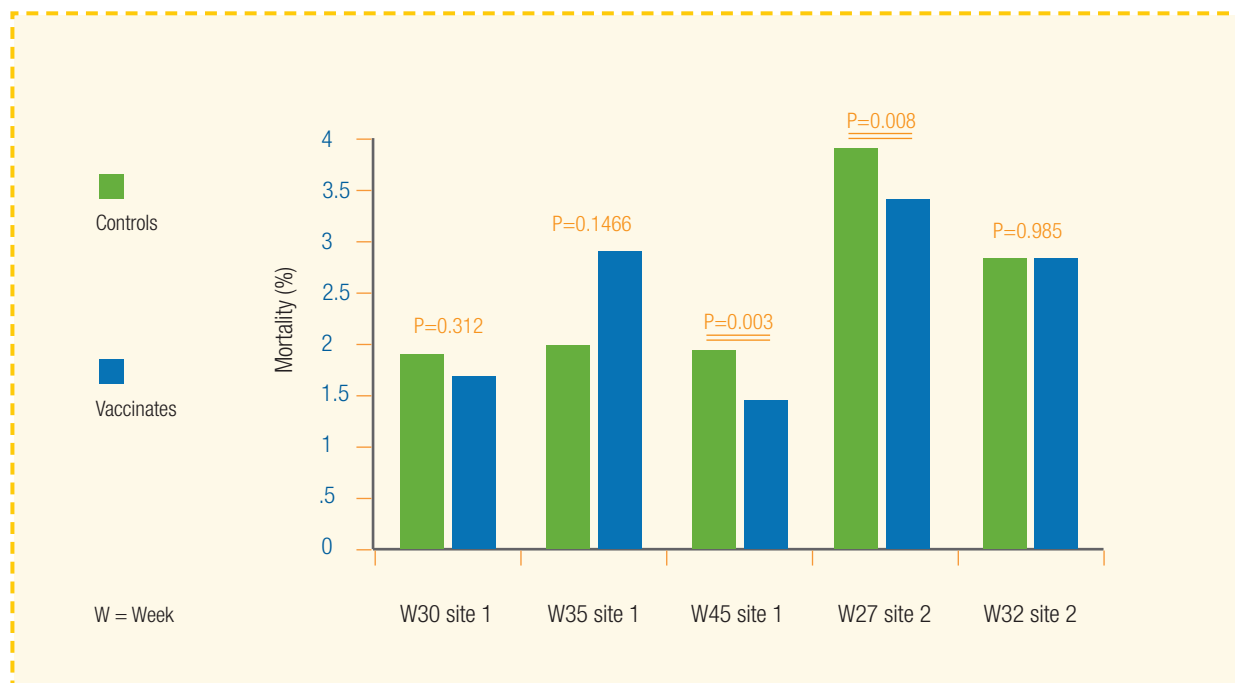


Figure 2. Mortality, shown below at various weeks (W) throughout the study, was somewhat higher among controls compared to birds from hens vaccinated against *C. perfringens*.

compared to the vaccine group, except for hatch-week 35 at site 1 due to yolk sac infection (Figure 2). In addition, one hatch of birds from vaccinated hens had a statistically higher mean weight compared to the control group.

No systemic reactions or unanticipated reactions were observed in vaccinated pullets/hens. Some minor local reactions were observed, but they were not serious enough to raise concerns about the welfare of the animals.

The safety and efficacy of Netvax was therefore confirmed under field conditions and the study surely provides data supporting the wide use of *C. perfringens* type A toxoid immunization by broiler producers.

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Canadian broiler performance using Netvax, a *Clostridium perfringens* type A toxoid for necrotic enteritis control under commercial production

Summary

KEY POINTS



Necrotic enteritis has been a continuing problem for Canadian producers trying to raise antibiotic-free chickens.

- **The use of Netvax, a *Clostridium perfringens* type A toxoid administered to hens, provided immunity to their progeny chickens, enabling them to be reared without NE under commercial conditions.**
- **Maternal antibody protection against *C. perfringens* type A toxins is an important new tool for reducing the incidence of NE in broilers raised without antibiotics.**

Commercial broiler production without the aid of antibiotic growth promoters or ionophores is a challenging endeavor in Canada. Mortality and performance loss due to necrotic enteritis (NE) is the limiting factor, exacerbated by Canadian wheat-based rations and local environmental conditions. The severity of loss — despite the implementation of dietary and management interventions — has caused multiple processors to attempt but then abandon antibiotic-free (ABF) broiler production due to continuing problems with NE.

Here we describe Netvax, a novel *Clostridium perfringens* type A toxoid that was administered to hens, which in turn conveyed immunity to their progeny. The vaccine enabled chicks to be successfully reared without clinical NE under commercial ABF production conditions.

Performance, as measured by daily weight gain, varied according to the field management and the type of ration used. Nevertheless, this success has enabled one processor to move ahead with an ABF production program, and others are in the early stages of initiating ABF production as well. Maternal antibody protection against the *C. perfringens* type A toxins is an important new tool in the arsenal of

interventions to reduce NE in broilers raised without antibiotics.

Introduction

Necrotic enteritis, caused by the enterotoxins of *C. perfringens*,¹ induces severe mucosal necrosis and mortality. The incidence of NE is greater in broilers reared without antibiotics and in flocks fed wheat-based rations.²

In addition to the wheat-based rations common in Canada, Canadian producers also face local environmental conditions that appear to exacerbate NE, but are not well-documented in the literature. These include high pH limestone soils in Ontario, high environmental humidity and rainfall in British Columbia and the use of wheat straw poultry litter in many provinces. The combination of these factors has made Canada a difficult country in which to grow antibiotic-free poultry without a high incidence of NE.

Netvax, a novel vaccine for necrotic enteritis protection, was introduced to a limited number of broiler breeders in 2007. The vaccine, a *C. perfringens* type A toxoid, was administered to breeders to induce serological titers and to transfer maternal antibody against the toxins

produced by *C. perfringens* type A to the broiler progeny.

The project was initiated by a processor who had been interested in rearing broilers without the use of antibiotics. Initial attempts to use live coccidiosis vaccines in lieu of in-feed anticoccidial medication had resulted in heavy losses due to NE, despite the support of in-feed growth promotional antibiotics.

The processor had first attempted to raise broilers without anticoccidials on a single, carefully managed and monitored farm to test the feasibility of ABF production. For the initial flocks, growth promotants were still used in the feed at full level. Six flocks in succession were raised using a non-attenuated coccidiosis vaccine, and all six flocks developed clinical NE *twice*: the first outbreak occurred at about 19 days of age and the second at 26 to 31 days of age.

The experimental coccidiosis vaccination program was discontinued for three flocks until progeny with maternal antibody against the *C. perfringens* type A toxins were available.

Methods

Breeder replacement pullets were vaccinated with a *C. perfringens* type A toxoid (Intervet/Schering-Plough Animal Health) via intramuscular or subcutaneous injection at 10 to 12 weeks of age and again at 17 to 18 weeks of age. Serological titers were verified by a hemolysis-inhibition test that used doubling dilutions. The mean titer of hens tested was 1:467, with titers of non-vaccinated test control serum of 0 or 1:2.

The processor initially placed two sequential, completely ABF flocks on the same farm that had produced the NE mortality pattern described above. The broilers were progeny of toxoid-vaccinated Ross 708 hens. The producer used the same live, non-attenuated coccidiosis vaccine for coccidiosis control (CocciVac-B, Intervet/Schering-Plough Animal Health) as before. The initial two flocks used a highly digestible starter feed formulation followed by a wheat-based broiler ration that included animal proteins. Farm management was closely monitored by the consulting veterinarian, with daily weights monitored by in-house scales. No antibiotics, including ionophores, were used in either flock.

The project was initiated by a processor who had been interested in rearing broilers without the use of antibiotics.

Following the successful implementation of the ABF program on the test farm, the processor initiated an on-going experimental program, placing one Ross 308 ABF flock per week in commercial broiler houses. The on-going program uses an all-vegetable ration formulation and the live coccidiosis vaccine, compared to the standard production flocks which still receive in-feed anticoccidials, growth promotional antibiotics and animal

proteins. Strict management procedures have been initiated on the ABF farms to reduce stressors that might induce NE.

Results

The initial two ABF flocks raised on the test farm did not break with clinical NE. The daily mortality followed a normal pattern after high early mortality in one of the two ABF flocks (Figure 1). Early mortality in one flock was due to a mistake in formulation of the enhanced starter ration, resulting in rickets. Despite the early nutritional stress, there was no sign of clinical NE in the affected flock.

The weight gain of the ABF flocks exceeded the expected growth curve for a Ross 708 broiler and also exceeded the weight gain of conventional medicated program birds grown simultaneously on the same farm (Figure 2). Neither the use of a non-attenuated coccidiosis vaccine nor the exclusion of antibiotics appeared to adversely affect the weights at any point during the growth cycle.

The mortality pattern of the on-going Ross 308 ABF test program reveals slightly elevated mortality from day 19 through slaughter. Only one flock broke with clinical NE. That flock, upon further investigation, was composed of progeny that were *not* from toxoid-vaccinated hens. It had been an error in chick placements. This single flock had been fed the same ABF ration formulation and was subjected to the same intensive management program as the other flocks, but the broilers lacked the additional support of anti-toxin antibody (Figure 3).

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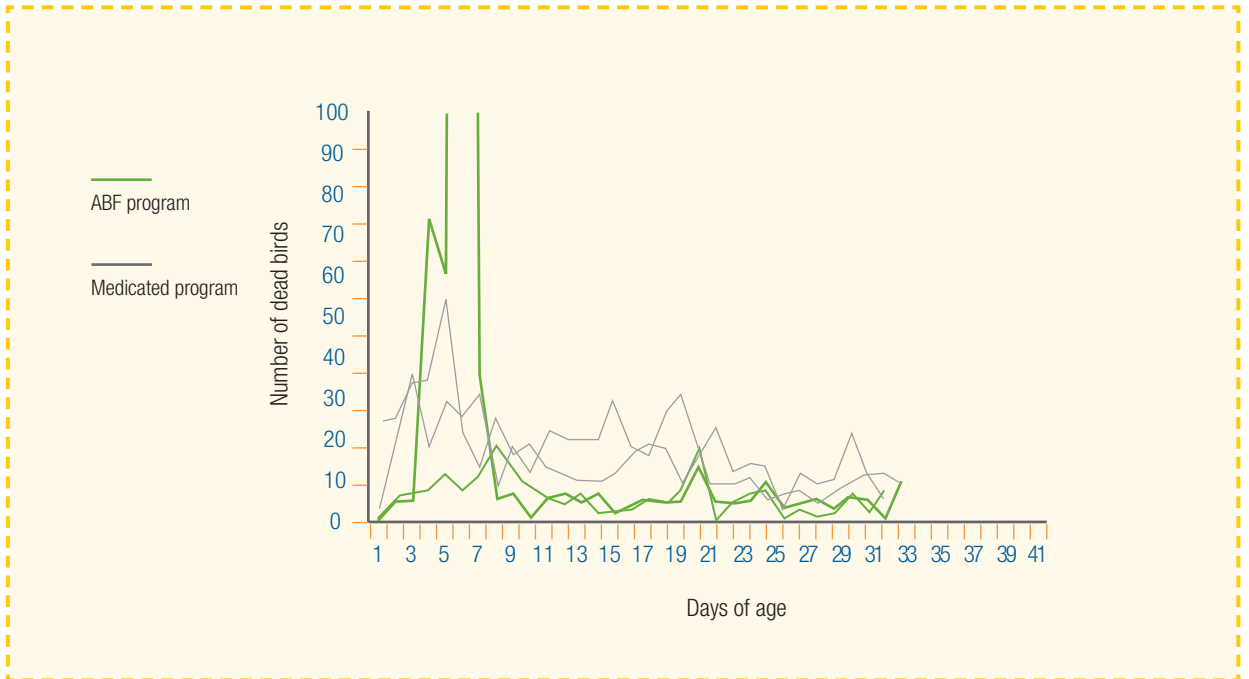


Figure 1. Daily mortality of ABF toxoid-progeny flocks vs. conventional medicated feed flocks grown on the same farm in side-by-side houses during the same time period.

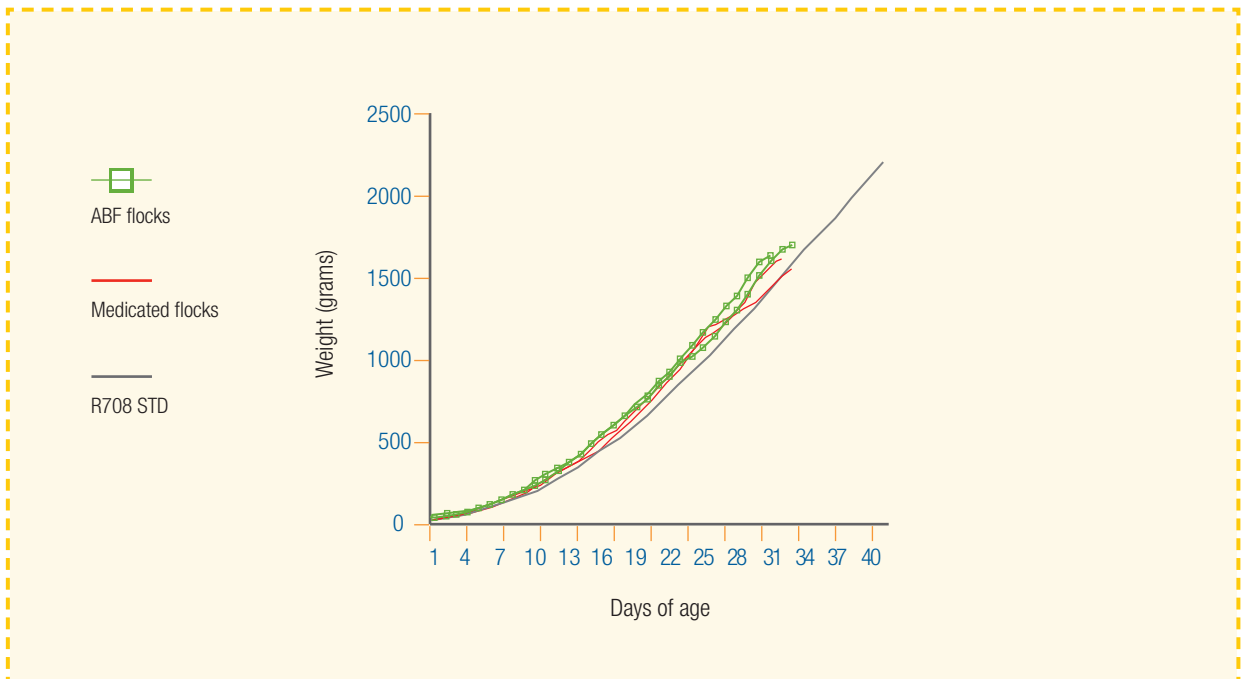


Figure 2. Weight gain of ABF toxoid-progeny flocks vs. medicated program flocks grown on the same farm in side-by-side houses during the same time period.

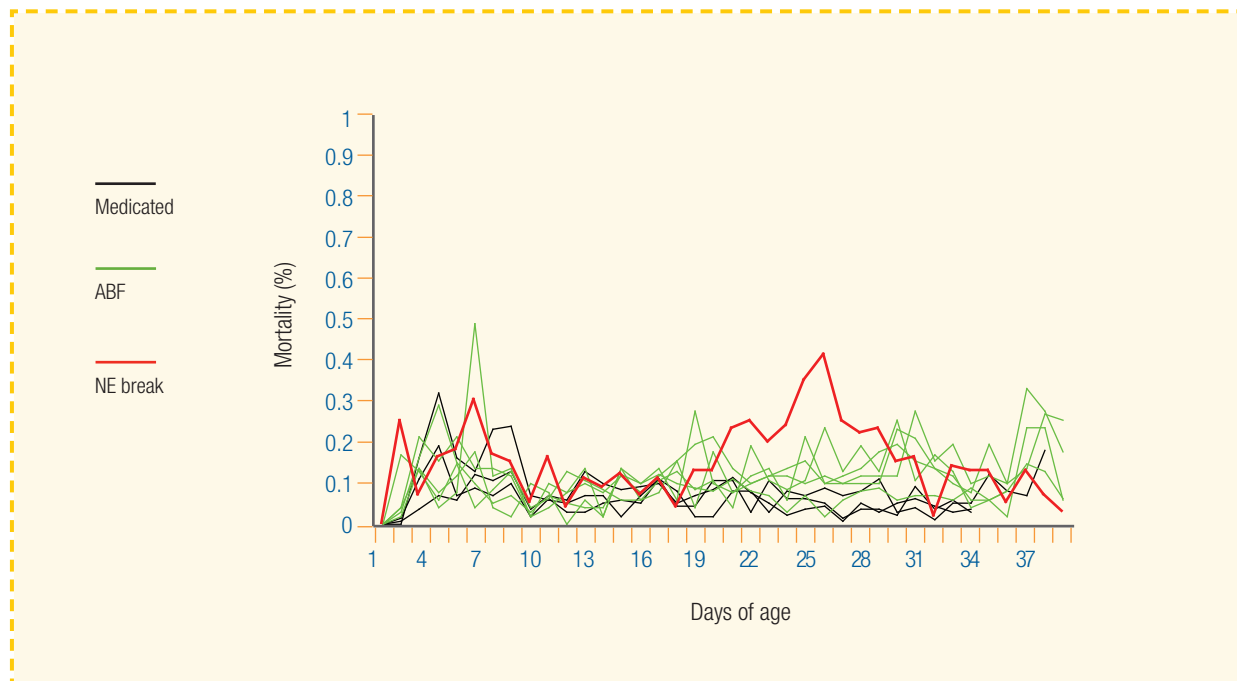


Figure 3. The daily mortality pattern of toxoid-progeny ABF flocks vs. conventional medicated flocks grown on the same farms. The NE break occurred because non-toxoid progeny were accidentally placed in an ABF house.

The weights of the Ross 308 ABF test flocks on the all-vegetable ration formulation are slightly lower for age and feed conversions are slightly higher compared to conventional flocks being processed at the same time.

Discussion

Maternal antibodies against *C. perfringens* type A toxins appeared to provide protection against clinical NE in these test flocks. Mortality and performance results were best on a carefully controlled and monitored farm. The moderate performance deficits on the commercial farms may be the result of subclinical NE, or it may be a

side-effect of the all-vegetable ration. The all-vegetable ration has produced markedly more litter moisture, which has been difficult to control. The veterinarian has detected no clinical or subclinical lesions of NE on these farms.

Netvax appears to provide additional support to ABF production. It is another tool in the arsenal to support broilers in the absence of in-feed antibiotics. It does not completely replace the support provided by in-feed medication, however, and must be used in combination with good management and ration formulation as part of a complete program to raise broilers without antibiotics.

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Association of *Clostridium perfringens* type A alpha-toxin with lesions of necrotic enteritis evaluated by monoclonal antibody test strips and immunohistochemistry

Summary

KEY POINTS



A test kit was evaluated for its ability to detect *Clostridium perfringens* organisms and alpha-toxin in feces and lesions of broilers with necrotic enteritis (NE).

- **There was a good correlation between lesion score and the detection of alpha-toxin and high lesion scores correlated with positive test strip results for *C. perfringens* and alpha-toxin at the site of NE lesions. Immunohistochemistry was used to confirm that antigens within NE lesions in the intestine were recognized by anti-*C. perfringens* type A toxoid vaccine (Netvax).**
- **The results of this study strongly demonstrate the involvement of *C. perfringens* type A alpha-toxin in the disease of necrotic enteritis.**

A test kit was evaluated for detecting *Clostridium perfringens* organisms and alpha-toxin in feces and lesions of broilers with necrotic enteritis (NE) following experimental challenge. There was a good correlation between lesion score and the detection of alpha-toxin, with higher lesion scores resulting in greater detection of *C. perfringens* A and alpha-toxin using the test strip kit.

Immunohistochemistry of NE lesions clearly demonstrated a positive binding of antibodies developed against the Netvax vaccine, one component of which is *C. perfringens* type A alpha-toxoid.

The results of this study strongly demonstrate the involvement of *C. perfringens* type A alpha-toxin in the disease of necrotic enteritis.

Introduction

Intervet/Schering-Plough Animal Health currently holds a conditional license from the US Department of Agriculture for a *C. perfringens* type A toxoid vaccine (Netvax) for administration to breeder hens and protection of progeny chicks against necrotic enteritis (NE).

Clinical NE, characterized by enteric lesions and spikes in mortality at 2 to 5 weeks of age, especially during cold weather months, is a widely recognized and growing disease concern for commercial broiler chicken producers. The deleterious effects of subclinical NE on growth and performance of broiler flocks has recently been recognized as a significant problem.¹

Enzyme-linked immunoabsorbent assay (ELISA) testing has been developed as a tool for detecting antibody to alpha-toxin for the purpose of diagnosing subclinical NE in broiler chickens.² Similarly, a commercial test strip diagnostic kit has been developed for NE based on monoclonal antibody bound to a paper strip.³ When exposed to chicken feces, one line develops color in the presence of approximately 1×10^5 *C. perfringens* type A and a second line develops color in the presence of alpha-toxin.

Immunohistochemistry has been widely used to detect the presence of disease agents in tissues. The objective of this study was to evaluate the utility of the

Score	Description
0	No lesions
1	Non-specific enteritis or subclinical NE. Thin and flaccid intestinal wall; excess or thickened mucus; no loss of mucosa; mild reddening or congestion of serosal blood vessels
2	Single or few multifocal areas of ulceration or necrosis of the intestinal mucosa
3	Moderate to severe multifocal to coalescing areas of ulceration or necrosis of the intestinal mucosa ± significant hemorrhage
4	Fibrinous necrotic debris on the mucosal surface adhering to the intestinal wall (Turkish towel) or dead animal with NE gross lesions scored 3 or above

Table 1. Scoring for gross lesions of necrotic enteritis.

test kit for detecting *C. perfringens* organisms and alpha-toxin in feces and lesions of broilers with NE following experimental challenge, and to demonstrate antigens recognized by antibody raised against Netvax, the *C. perfringens* type A toxoid vaccine, within NE lesions.

Materials and methods

Fifty-two commercial run broiler chicks were received as day-old chicks and placed in floor pens at an Intervet/Schering-Plough facility in Elkhorn, Nebraska. Thirty-five chicks were housed in one hut and the remaining chicks were housed as negative controls in another hut.

Chicks were fed a non-medicated starter ration for the first 5 days and switched to a high protein diet for the remainder of the study. When chicks were 19, 20 and

21 days of age, a *C. perfringens* type A challenge was performed by oral gavage. At 23 days of age, chickens were euthanized by cervical dislocation.

Following euthanasia, fecal material was collected from the caudal rectum/cloaca of each chicken and tested according to the kit instructions. Three replicate strips were tested for each sample. Each strip was recorded as positive or negative for *C. perfringens* organisms and positive or negative for the presence of alpha-toxin. Chickens were subsequently scored for lesions of NE.⁴ Using the gross lesion score as the true prevalence (Table 1), the sensitivity and specificity of the test strips were calculated.

Tissue sections of gross NE lesions were fixed in 10% buffered formalin. Formalin-fixed specimens were processed by routine histological methods. Sections were cut at four microns from paraffin blocks for immunohistochemical staining. Immunohistochemical staining followed an indirect procedure. The primary antibody incubation was with optimally

diluted rabbit polyclonal antisera raised against Netvax vaccine. Biotinylated secondary antibody was applied followed by alkaline phosphatase-conjugated streptavidin and BioRed-Fast red chromagen substrate. The slides were counter-stained with hematoxylin and coverslipped.

Results

Prevalence of *C. perfringens* by gross pathology. Necropsy was performed on 24 surviving, challenged chickens and 15 unchallenged negative controls. Severe lesions (Score 3 or 4) were observed in 50% of the challenged group and 71% of these were positive for NE (Score > 2) (Table 2).

Reproducibility, sensitivity and specificity of the test strips. There was 95% reproducibility of the test strip results between the three replicate tests. The overall sensitivity of the diagnostic test kit for accurately detecting *C. perfringens* in the feces of chickens positive for NE was 47%. *C. perfringens*



Lesion score	Challenged (%)	Negative control (%)
0	4	33
1	25	67
2	21	0
3	25	0
4	25	0

Table 2: Prevalence of gross lesion scores by group.

Gross lesion score	Prevalence of <i>C. perfringens</i> (%)	Prevalence of alpha-toxin (%)
0	33	0
1	18	0
2	19	0
3	60	37
4	88	71

Table 3. Prevalence of *C. perfringens* and alpha-toxin by fecal test.

was detected with a specificity of 78%. The rate of false positive tests (test strip positive in birds with lesion scores of 0 or 1) was 22%.

Prevalence of *C. perfringens* by fecal test. The overall prevalence of positive tests for *C. perfringens* was 33% of birds with lesion score 0 (6/18); 18% of birds with lesion score 1 (14/78); 19% of birds with lesion score 2 (9/48);

61% of tests among birds with lesion score 3 (11/18); and 88% of tests among birds with lesion score 4(16/18) (Table 3).

Prevalence of alpha-toxin by fecal test. Alpha-toxin was not detected by the test strips until lesion scores reached 3 or 4. Therefore, positive test results were only observed in the challenged group. The test kit was able to detect alpha-toxin in 37% (7/18) of tests among chickens with lesion

scores of 3 and 71% (13/18) of tests among chickens with lesions scores of 4 (Table 3).

Comparison of fecal and lesion scraping test strip results. In a few chickens, testing was performed on a sample of fecal material from each bird and on lesion scrapings from the same

Gross lesion score	Positive for <i>C. perfringens</i>	Positive for alpha-toxin (%)
1 or 2	0% (0/7)	0% (0/7)
3	67% (6/9)	0% (0/9)
4	67% (6/9)	56% (5/9)

Table 4. Comparison of fecal sample or lesion scraping test.

bird. Comparison of lesion scraping scores to the gross lesion scores is shown below (Table 4). When testing lesion scrapings, the test kit detected *C. perfringens* in 67% of birds with lesion scores of 3 and 4. Alpha-toxin was detected in 0% or 56% of lesions in the chickens with lesion scores of 3 or 4, respectively. The test strips did not detect *C. perfringens* or alpha-toxin in any birds with lesion scores of 1 or 2.

Histopathology-immunohistochemistry.

Histopathologic examination of intestinal lesions showed severe diffuse necrosis of the intestinal epithelium with fibrin and cellular debris adherent to the necrotic mucosa. Numerous rod-shaped bacteria were present at the necrotic interface. Positive staining was found within NE lesions, demonstrating a specific binding of Netvax antibody in the tissue.

Discussion

There was a good correlation between lesion score and the detection of alpha-toxin, with higher lesion scores resulting in greater detection of alpha-toxin.

In addition, we found that high lesion scores were coupled with positive test strip results for *C. perfringens* and alpha-toxin at the site of NE lesions. This supports the hypothesis that the severity of the gross lesions is directly proportional to the number of *C. perfringens* present and amount of alpha-toxin produced.

However, the prevalence of *C. perfringens* as measured in the feces was similar for challenge and control groups (33% and 38%, respectively), which may suggest that while an overgrowth of *C. perfringens* is linked to NE, the perturbation of the intestinal peristalsis induced by enteritis may act to transfer *C. perfringens* into the upper intestine instead of flushing the bacteria out of the intestine, where it would be detected in the feces. This hypothesis is also congruent with our observation that most NE lesions were found in the small intestine.

Immunohistochemistry of NE lesions clearly demonstrated a positive binding of antibodies developed against the Netvax vaccine, one component of which is *C. perfringens* type A alpha-toxoid.

The results of this study strongly demonstrate the involvement of *C. perfringens* type A alpha-toxin in the disease of necrotic enteritis.

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Transforming coccidiosis-mediated lesion scores into production and calorific cost

Summary

KEY POINTS



In controlled studies designed to quantify the impact of coccidia-mediated intestinal lesion scores on bird performance and energetics, birds challenged with coccidia had lesion scores that were inversely correlated with performance parameters such as average daily gain.

- **The results indicate that the impact of coccidiosis on performance and energetics is significant and this impact may be estimated using mass and lesion score-based polynomials.**
- **The adverse impact of coccidiosis on performance increased with broiler age and mass: early challenge had minor consequences with time for compensatory gain, while late challenge had the most severe negative impact on performance.**

Coccidia-mediated intestinal lesion scores provide a method of tracking coccidiosis timing and relative severity, but they lack quantitative inference to bird performance and energetics.

To expand upon information gleaned from intestinal lesion scoring, broilers were reared in calorimetry chambers with and without coccidia challenge so that the consequences of coccidiosis on broiler performance and energy metabolism might be simultaneously studied.

Variables describing performance, such as weight gain and feed efficiency, were modeled so that metabolic maintenance energy (MME) and any added excreta energy (AEE) over that anticipated by dietary metabolizable energy content might be determined. All variables were examined for predictability using polynomial equations comprised of bird mass and lesion score transformations.

Results from these studies indicate that the impact of coccidiosis upon performance and energetics is significant and this impact may be estimated using mass and lesion score-based polynomials. The results also indicate that the timing and severity of intestinal lesion scores is critical to optimizing broiler production.

Though coccidiosis challenge (CC) occurring early in the production cycle had performance and metabolic costs, a challenge late in the growth curve dramatically exacerbated the consequences on growth and feed conversion. Performance declines were quantitatively explained by energetic changes mediated by a reduced appetite, elevated energy cost of metabolic maintenance, added excreta energy and reduced retained energy. Optimization of metabolizable energy utilization requires immunological approaches minimizing energy wastage, especially in the last half of the bird's growth curve.

Introduction

Coccidiosis is a protozoan disease of the genus *Eimeria* affecting the intestinal tract of poultry and is among the top diseases of economic importance. The disease is characterized by a quick onset of bloody diarrhea and reduced weight gain, coupled with an elevated feed conversion ratio and mortality.

Coccidiosis causes intestinal lesions that may be scored visually and/or by microscopic technique¹ to judge disease timing and/or relative severity. Of the seven protozoan species identified as having ability to

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parasitize broilers, *E. acervulina*, *E. maxima*, and *E. tenella* are of particular concern.² Though a variety of products are available to reduce coccidiosis, disease avoidance requires constant vigilance coupled with appropriate management and therapeutic response.

Many factors impacting broiler energy metabolism are critical to successful broiler production. For example, managerial issues as ventilation, stocking density, the lighting program and feed processing³ have received considerable study for their calorific impact. In contrast, the calorific costs of coccidiosis have not been quantified, even though coccidiosis is well known to adversely affect broiler performance. Indeed, since coccidiosis increases feed passage, body temperature and blood loss, its quantification upon energy balance is warranted.

Methods

Experiments using 1,200 Cobb X Cobb broilers were conducted to quantify the impact of coccidiosis on bird performance and energy balance. Birds were evaluated during five 6-day time periods along with a coccidial challenge at 14, 21, 28, 35, and 42 days of age within the 48 day growth curve. General bird management in floor pens and metabolic chambers as well as dietary ration specifications have been previously described.⁴

The challenge, designed to mimic the production environment, consisted of an oral dose of sterile saline or a mixture of three *Eimeria* species, which were *E. maxima*, *E. acervulina*, and *E. tenella*; they were administered initially at 20,000; 50,000; and

30,000 oocysts per bird and increased to 55,000; 105,000; and 50,000 oocysts per bird, respectively, at 42 days of age.

Though a variety of products are available to reduce coccidiosis, disease avoidance requires constant vigilance coupled with appropriate management and therapeutic response.

The variables examined 6 days after challenge included gross (upper small intestine, mid small intestine) and microscopic lesion scores for *E. maxima*, *E. tenella* and *E. acervulina*, with scores as 0=none and 4=high. Performance variables included average daily gain (ADG), feed consumption (ADF) and feed efficiency (ADG/ADF). Bird heat production (Kcal/day) was measured continuously by indirect calorimetry and body composition via x-ray analysis.⁵ Partitioning of energy into maintenance energy (ME), retained energy, and ME consumption has been described.^{6,7}

Bird live weights (g), metabolic weights (live weight^{0.75})⁸, visual lesion scores, and microscopic lesion scores were examined as main and interactive combinations of linear, quadratic and cubic transformations for inclusion in polynomial equations predicting variable responses. Variables used in the model selection were examined in forward stepwise regression.⁹ Factors were added to the regression model until three conditions were met: 1) adding

factors to the model did not result in a substantial increase in the model R² (R² improvement > 2 %); 2) factors in the model were significant (P ≤ 0.1); and 3) the resulting model matched known properties of the independent variables.

Results and discussion

The experiments were successfully conducted. Study results are displayed in Table 1 for three ages of the five evaluation intervals examined. As the 21 and 35 day data were intermediate to the 14, 28 and 42 day results, they were not shown to conserve writing space, but were used in all modeling.

Bird daily gain and feed efficiency ratio (FER) for unchallenged birds (CUC) and CC birds housed in the floor pens and metabolic chambers were similar to previous observations with CC markedly reducing performance. Lesion scores for CUC birds did not differ from zero (P>0.10) throughout the testing period, while CC scores exceeded 0 and were inversely correlated (P<0.01) with ADG, feed efficiency ratio (FER), maintenance energy consumption (MEC), heat production (HP) and energy gain (EG).

Although CC induced measurable cost at all ages, the cost of infection 6 days post-challenge disproportionately increased with progression along the broiler growth curve. As a result, if compensatory gain occurs, it would be anticipated that CC of birds early in the production cycle would have less impact at processing.

Energy modeling in this study occurred as two approaches, with the first directed at

Study interval and fixed initial weight		Lesion score**				
		0	0.5	1	1.5	2
(Age, days) Initial live weight (g)		Average daily gain (g)				
(14-20)	904	76.5	70.2	60.6	48.7	40.7
(28-34)	2096	92.6	72.2	54.3	38.2	27.3
(42-48)	3398	97.3	61.0	32.7	10.0	-7.0
		Maintenance cost (Kcal/day)				
(14-20)	904	124	148	151	154	281
(28-34)	2096	187	215	218	222	308
(42-48)	3398	281	311	308	304	315
		ME_n consumption/day (Kcal)				
(14-20)	904	386	364	342	318	300
(28-34)	2096	562	516	477	444	420
(42-48)	3398	701	628	570	522	482
		Added excreta (Kcal/day)				
(14-20)	904	16	5	22	37	35
(28-34)	2096	24	30	57	81	86
(42-48)	3398	38	57	94	122	130
		Retained energy (Kcal/day)				
(14-20)	904	188	170	149	121	100
(28-34)	2096	274	210	162	119	87
(42-48)	3398	305	191	110	49	-0.9
		Feed efficiency (gain/feed consumed)				
(14-20)	904	0.64	-	0.60	-	0.38
(28-34)	2096	0.54	-	0.37	-	-0.04
(42-48)	3398	0.43	-	0.10	-	-0.49

Table 1. Coccidiosis mediated lesion score effects upon broiler average daily gain, maintenance energy (ME) expenditure, ME consumption, added energy lost in excreta, retained energy and feed efficiency at equalized initial weights* during 6 days after coccidial challenge.

*Values created using predictive models ($R^2 > .95$) and standardized initial weights.

**Mixed lesion scores were utilized for all variables except gain/feed, where homogenous arrays of 0, 1, 2, 3 and 4 were applied.

classical applications of energy relationships as a form of experimental validation. Classical expressions of energy balance were accurately repeated in the reported studies as MEC equaled the summation of HP, determined by indirect calorimetry, and EG determined by dual-energy X-ray absorptiometry (DXA) scan to within $\pm 4\%$ of determined dietary MEC value for CUC birds. As such, the deviation of the HP + EG sum from dietary gross energy equals apparent excreta energy. Barring offsetting errors associated with HP and EG, MEC may be further partitioned into heat production associated with maintenance + activity (HPMPA) and HP attributable to tissue synthesis (HPTS) by the following expressions where K_p at 0.67 and K_f at 0.87 are the metabolic efficiencies of protein and lipid accretion, respectively:

$$(1) \text{ HPTS Kcal/day} = (\text{g protein gain} \times 5.65 \text{ kcal/K}_p) + (\text{g fat gain} \times 9.3 \text{ kcal/gram/K}_f) - \text{EG}$$

$$(2) \text{ HPMPA} = \text{HP Kcal/day} - \text{HPTS}$$

The second modeling approach created polynomial equations relating response variables with bird mass and lesion score transformations for the 6-day periods. Classical bird relationships were sufficient for CUC birds, while CC birds necessitated lesion score inclusion to improve accuracy.

The results indicated that lesion scores were positively correlated with ME cost and excreta energy loss. In some cases, CC birds were observed eating less and producing similar to greater amounts of heat, suggesting that the metabolic effects of coccidiosis have great metabolic impact.

Further, the consequence of coccidial challenge was observed to be more pronounced later in the growth curve. Table 1 data indicates that bird maintenance cost increases with bird size, classically so for unchallenged birds, while the cost for coccidial-challenged birds increased linearly with lesion score.

If challenged birds exhibited higher body weights, then the elevated maintenance would be expected. However, as discussed, the live weight and FER of challenged birds was reduced ($P < .01$). Indeed, the elevated maintenance cost occurred with less energy consumed and lower body weight ($P < .01$). In addition, calories added to excreta rose over that anticipated by dietary metabolizable energy, with lesion score and bird age, averaging 12% on day 20 and exceeding 26% at 48 days. Whether the adverse excreta response represents blood or feed loss is moot, since the calorific cost must be paid.

Conclusion

The combination of lowered energy consumption coupled with elevated MME and AEE makes it critical to avoid a coccidiosis challenge. If some coccidiosis challenge is going to occur, the data suggests that early exposure will have fewer overall consequences on energy utilization.

Bird daily retained energy fell to 0 for lesion score 2 birds at 48 days in contrast to the 100 Kcal at 20 days despite the lesion score 2 birds consuming 300 and 482 Kcal of energy, respectively. Birds with subclinical coccidiosis (0.5 lesions score) had 18 less Kcal retained energy at 20 days in contrast to 114 less Kcal at 48 days. Feed efficiency responses paralleled

energy responses with the consequences of coccidial challenge becoming more profound late in the growth curve.

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Calorific cost of immunity development to coccidiosis

Summary

KEY POINTS



Calorimetry chambers were used to quantify the performance and energetic effects of coccidiosis.

- **Coccidiosis has significant consequences that worsen as the bird ages.**
- **Immunity against coccidiosis that is developed later in the production cycle is more costly than immunity development earlier in the bird's life, such as that achieved by vaccination at hatch.**

Experiments were conducted in calorimetry chambers to quantify the performance and energetic consequences of coccidiosis. Birds were orally dosed with sterile saline or a mixture of three *Eimeria* species oocysts at 14, 21, 28, 35 and 42 days of age. Six days later, necropsies were performed.

At necropsy, visual and microscopic intestinal lesion scores were tallied, as well as bird performance and energy metabolism. Lesion scores for coccidiosis non-challenged (CNC) birds did not differ from zero ($P > 0.10$) throughout the testing period, suggesting that they were reared in a “coccidiosis free” environment. In contrast, coccidiosis challenged (CC) birds exhibited a range of lesion scores that were subsequently grouped into subclinical (SLC; score ≤ 1.0) or clinical lesion scores (CLS; score > 1.0). Birds were also evaluated by lesion grouping for performance variables.

The findings demonstrate that there are significant consequences to a coccidiosis challenge — and an interaction with age ($P < .01$). Birds that developed coccidiosis immunity later during the growth curve exhibited much higher costs compared to those that developed immunity earlier on the growth curve.

Introduction

Coccidiosis, mediated by protozoa of the genus *Eimeria*, is among the major disease challenges facing the world's poultry industries. Though a variety of therapeutics are available to minimize the incidence and severity of coccidiosis, birds will normally develop immunity during the production cycle. Immunity development is critical since drugs are usually not fed continuously throughout the bird's life.

Vaccination at hatch speeds immunity. The timing of immunity development can be critical to performance because challenges during the late growth curve have costs for birds lacking immunity.

Methods

Two populations of unchallenged Cobb X Cobb broilers (1 vaccinated with the live oocyst vaccine Coccivac-B) were maintained in a coccidiosis-free environment prior to housing in metabolic chambers for a 6-day study.

Birds were assessed during five, 6-day periods, spaced throughout a 48-day growth curve. General bird management in floor pens and metabolic chambers have been described.¹

continued

The challenges were designed to mimic the production environment and were administered at 14, 21, 28, 35, and 42 days of age. They consisted of an oral dose of sterile saline or a mix of three *Eimeria* species, which were *E. maxima*, *E. acervulina*, and *E. tenella* administered initially at 20,000; 50,000; and 30,000 oocysts per bird and increasing to 55,000; 105,000; and 50,000 oocysts per bird, respectively, at 42 days.

Variables examined 6 days after challenge included gross and microscopic lesion scores,² with scores as 0=none and 4=high. Several performance variables were considered including average daily gain (ADG), feed consumption (ADF) and feed efficiency. Bird heat production (Kcal/h) was measured continuously by indirect calorimetry and body composition via x-ray analysis. Modeling techniques for maintenance energy (ME), retained energy, accretion energy, added excreta energy and predicted ME consumption have been described.³

Results and discussion

Bird populations were used to study the development of immunity to coccidia. Mean lesion scores differed between the CNC, SLC and CLS groups ($P<.01$), averaging 0, 0.37 and 1.94, respectively.

Viewed by lesion grouping, ADG, feed efficiency, maintenance cost, added excreta calories and total energy loss

per Kcal ME_n consumed averaged: 81, 69, 26 g; 0.575, 0.518, 0.249; 157, 171, 192 Kcal/day; 18, 11, 71 Kcal/day; 0.36, 0.40, 0.67 Kcal/Kcal ME consumed, respectively.

The CNC birds lacked intestinal lesions and were consequently assumed to have no immunological response to coccidia (score=0; lesion group 0).

The energy needed for immunity development was just 5% higher compared to 28% for coccidiosis. This cost becomes disproportionately elevated as birds age.

Birds with lesion scores were placed in two groups: One group with subclinical lesion scores (SLC; score ≤ 1.0 ; lesion group 1), which reflects birds that are developing immunity and a second group with clinical lesion scores (CLS; score > 1.0 ; lesion group 2), which reflects birds that are developing immunity and coping with full blown coccidiosis. Groupings were used to track coccidiosis consequences throughout the growth curve.

A summary of bird lesion scores as they relate to the growth curve is presented in Figure 1. These 3 groupings differed ($P<.01$). Bird ADG, displayed in Figure 2, reveals that the ADG consequence during

immunity development is relatively mild, with the exception of the last week, where gain was depressed for group 1 versus group 0, ($P<.01$). Bird ADG was uniformly depressed ($P<.01$) for group 2 chicks having coccidiosis versus groups 0 and 1. Feed efficiency for birds generally paralleled ADG (Figure 3).

Reductions in bird performance are explained by immunity development and the effects of coccidiosis that are related to bird energy metabolism. Maintenance increases for unchallenged lesion 0 birds as they mature (Figure 4). Classical energy relationships indicate that this maintenance will increase in proportion to metabolic body size.⁴ Bird maintenance needs are elevated slightly for immunity development and markedly so for coccidiosis. Indeed, the energy needed for immunity development was just 5% higher compared to 28% for coccidiosis. This cost becomes disproportionately elevated as birds age.

Additional costs of coccidiosis are feed passage and excreta blood loss. Data displayed in Figure 5 shows bird response to the proportion of consumed calories lost in the excreta. In this study, control birds averaged just 3.6% energy loss over that anticipated with the dietary ME provided. Birds developing immunity were similar to the controls, while birds with full coccidiosis averaged over a 50 Kcal additional each day in excreta.

continued

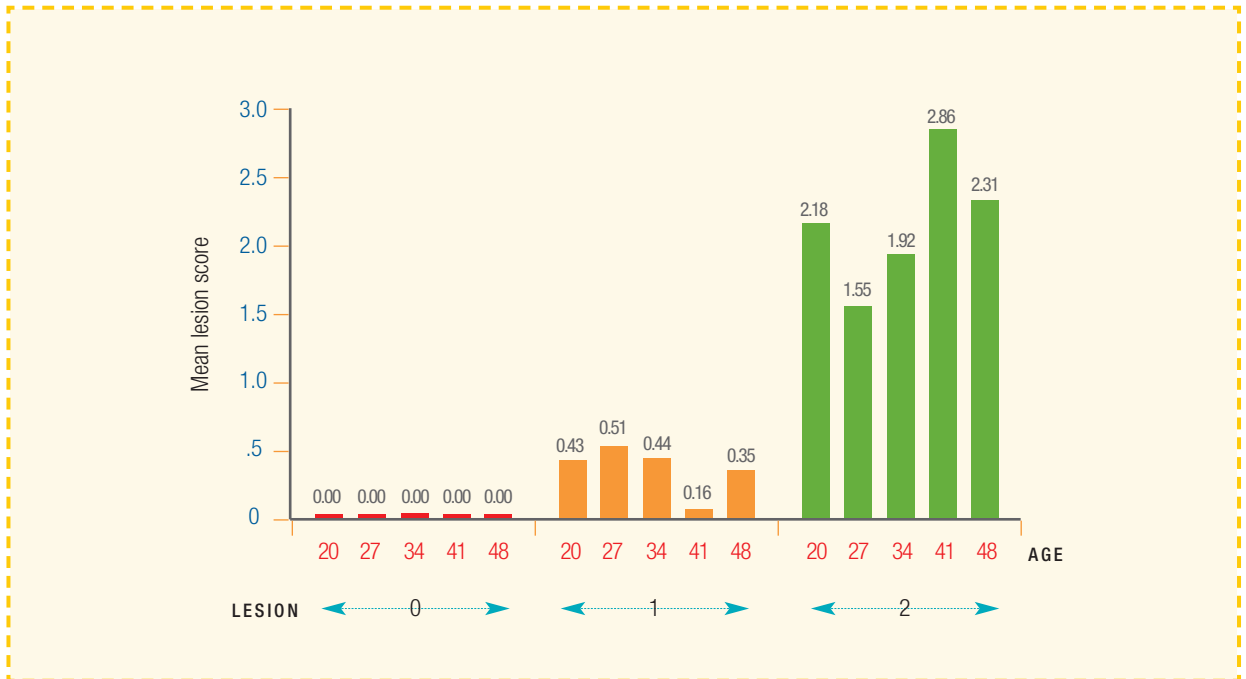


Figure 1. Mean lesion score of coccidiosis free (lesion group 0) , subclinical (lesion group 1) and clinically infected groups (lesion group 2).

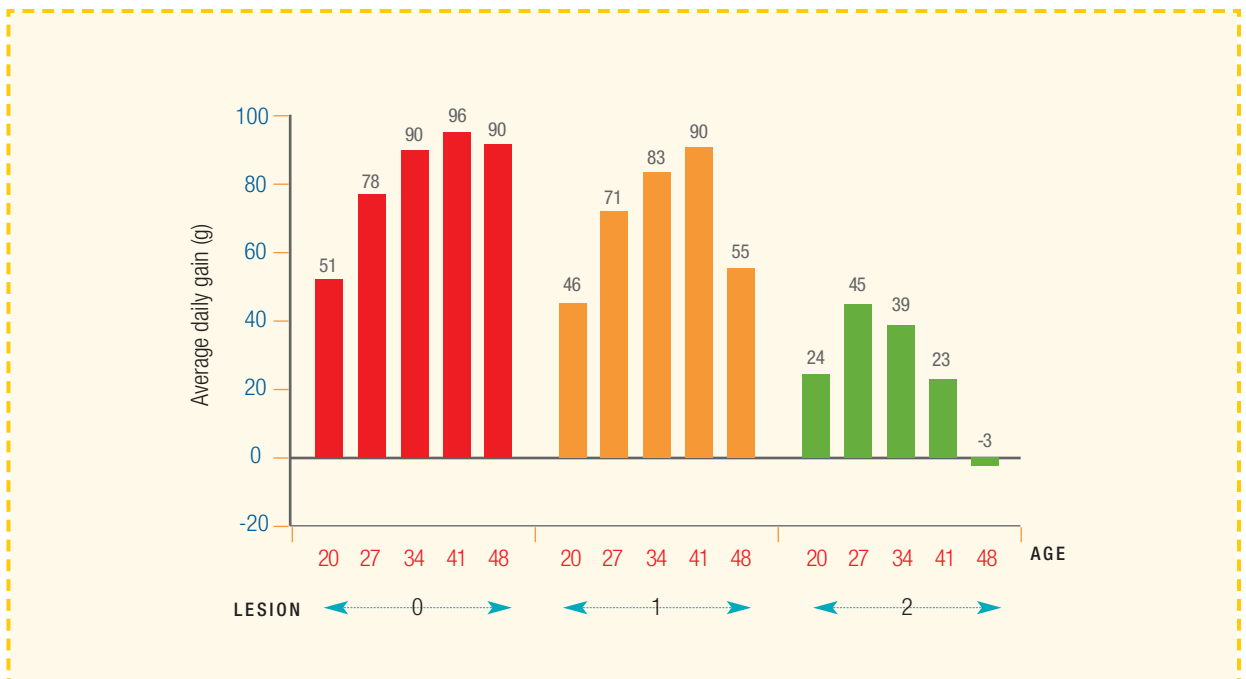


Figure 2. Daily weight gain of coccidiosis free (lesion group 0), subclinical (lesion group 2) and clinically infected (lesion group 2) groups.

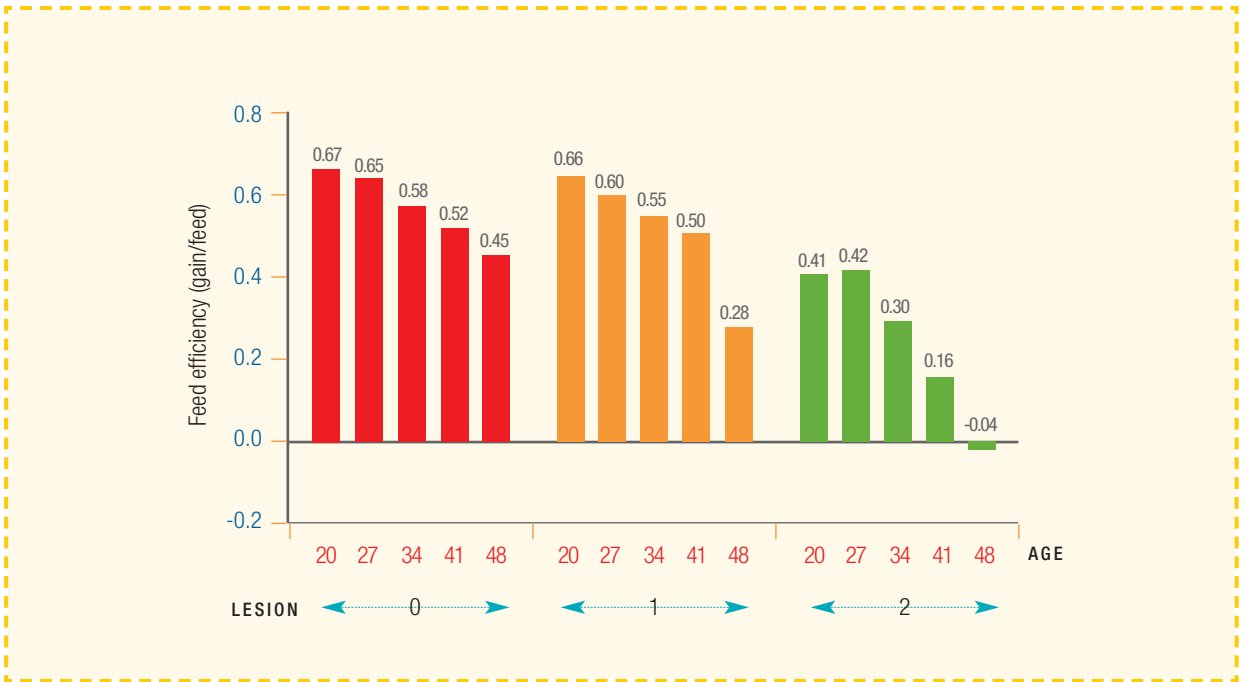


Figure 3. Feed efficiency of coccidiosis-free (lesion group 0), subclinical (lesion group 1) and clinically infected (lesion group 2) groups.

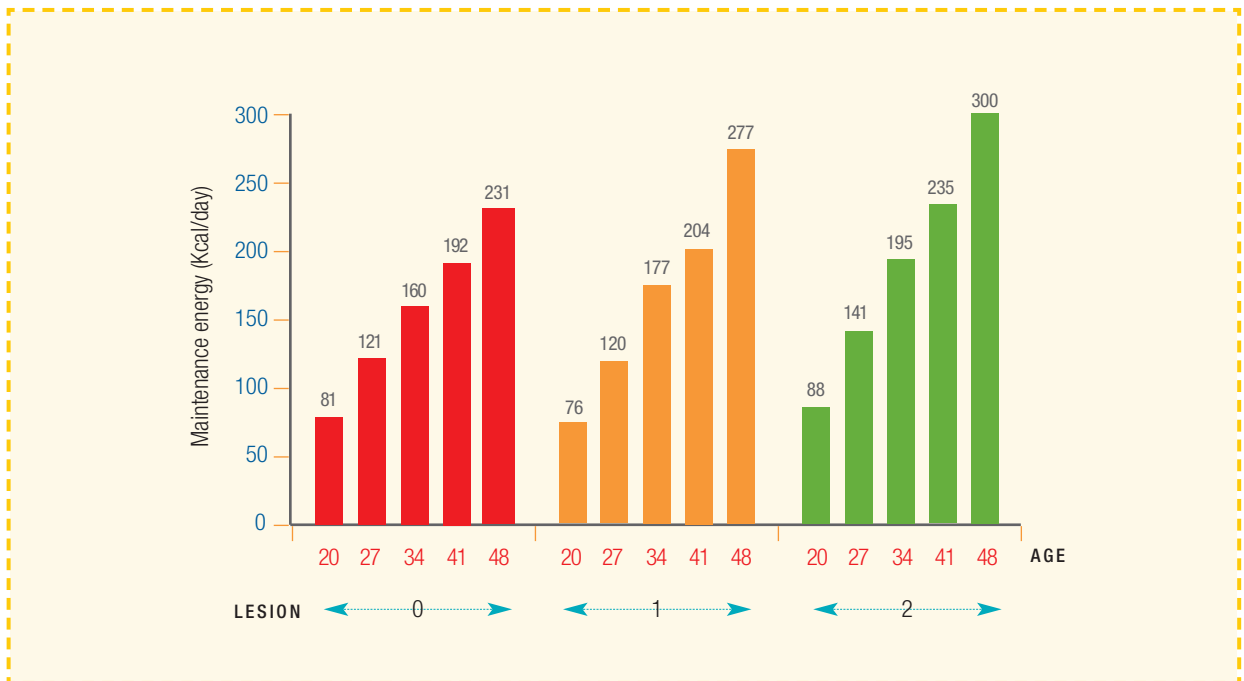


Figure 4. Daily maintenance energy expenditure of coccidiosis free (lesion group 0), subclinical (lesion group 1) and clinically infected (lesion group 2) groups.

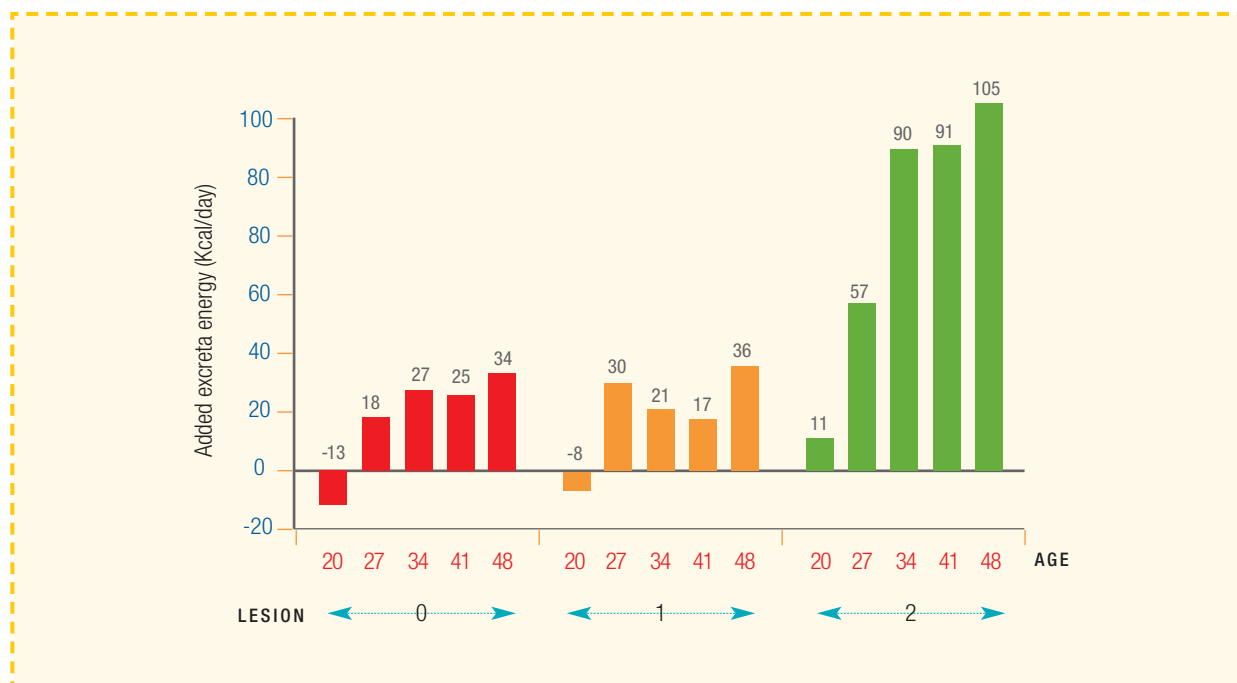


Figure 5. Energy added to excreta over that anticipated by dietary metabolizable energy value for the coccidiosis free (lesion group 0), subclinical (lesion group 1) and clinically infected (lesion group 2) groups.

Conclusion

In conclusion, the energy costs associated with the development of immunity against coccidiosis are minimized early in the growth curve. The costs are markedly elevated when immunity development occurs with full blown coccidiosis and may exceed 70 additional excreta Kcal.

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The effect of different management conditions on the productivity and health of broiler birds vaccinated with the anticoccidial vaccine Paracox-5

Summary

KEY POINTS



An extensive clinical trial on commercial broiler farms was conducted in Northern Italy to determine the influence of management on birds vaccinated against coccidiosis.

- **The best results were achieved on farms that applied good management practices, which also curtailed the incidence of problems such as necrotic enteritis (NE).**
- **Poultry producers who are well advised about how to properly vaccinate and manage will be most likely to find economically profitable ways to raise healthy birds without conventional in-feed anticoccidials.**

Anticoccidial vaccines enable poultry producers to cease using anticoccidials from the diet of broiler chickens, which consequently eliminates or greatly reduces the risk of drug residues in poultry meat.

An extensive clinical trial, conducted in meat-type chickens grown under commercial broiler rearing conditions in Northern Italy, has shown that to achieve optimal results, vaccination needs to be coupled with good bird management, including high quality nutrition and brooding care that fosters healthy intestinal tract development at an early age. This approach helps ensure that poultry producers get a positive return on their investment. Data are presented for different management conditions.

Introduction

One of the most serious, widespread and costly diseases in poultry is coccidiosis, which is the result of infection with the protozoan parasite *Eimeria* spp. Traditionally, coccidiosis has been controlled with the use of in-feed drugs. Today, more producers are turning to vaccination for control of the disease, which enables birds to naturally build immunity against the parasite.

Vaccination against coccidiosis in European broiler chickens was first introduced in 2000 and rapidly became common practice in several Western European production units; coccidiosis vaccination has also become common in many production units in North America.^{1,2,3}

Since then, numerous studies and extensive field experience have shown that use of the anticoccidial vaccine Paracox-5 in Europe and Coccivac-B in North America (both from Intervet/Schering-Plough Animal Health) are not only safe for birds, but that birds receiving the vaccines perform as just as well or even better than birds raised with in-feed anticoccidials.

To achieve the best results, however, proper use of the vaccines, along with good management conditions throughout the full growout operation are necessary.

Materials and methods

An extensive clinical trial was conducted in a major poultry production area of Northern Italy. The study was carried out at several sites from the same integrated company and involved more than 2 million broiler chickens.



The vaccine used in the trial was Paracox-5, a live, attenuated anticoccidial vaccine. It is based on precocious genetic lines of *Eimeria acervulina*, *E. maxima* (two lines), *E. mitis* and *E. tenella*. The vaccine was administered in the hatchery with a spray cabinet to all birds in the trial at 1 day of age.

Different parameters and operational conditions were considered and judgments about vaccine efficacy were based on clinical observations such as mortality, culling rate and coccidiosis outbreaks, as well as performance data such as daily weight gain (DWG) and feed conversion ratio (FCR). Weight gain following natural coccidial challenge provides definitive evidence of immunity to coccidiosis, which is further supported by the FCR.

The bird management conditions considered in the trial were as follows:

- uniform vs. uneven vaccine distribution with the hatchery spray cabinet
- good or poor bird management practices during full growout
- the inheritability factor, since some genetic lines appear to have a greater risk for developing sub-clinical necrotic enteritis (NE) and associated poor growth and increased FCR
- tactical antibiotic therapy

Results

It is apparent from the trial results (Table 1) that management can have a deep impact on vaccination results.

The best results were achieved on farms with good management, which requires proper vaccine administration procedures that result in uniform vaccine application and good litter conditions that ensure oocyst survival and better recycling, particularly during the brooding phase. Another important factor that contributed to positive results was knowledge of good field management.

Even on farms using antibiotic medication to control secondary infections with *Clostridium perfringens*, performance results were below those in birds on farms with “best” management practices.

continued

Parameter/condition	Final live body weight (Kg)	FCR	Mortality (%)	“Breaks”
Hatchery code L-24	2.502	1.867	5.20	<i>E. ace. +; E. max. ++; E. ten. (rare)</i>
Hatchery code V-01	2.530	1.822	4.50	None
Management “best 10”	2.490	1.853	5.00	=
Management “worst 10”	2.394	1.930	6.50	=
Breed/line “A”	1.698	1.769	3.01	=
Breed/line “B”	1.741	1.701	2.80	=
Antibiotic therapy “yes”	2.419	1.874	6.57	=
Antibiotic therapy “no”	2.348	1.956	7.48	=

Table 1. Numerical effects of management, bird genetics and antibiotic therapy on bird health status and flock performance.

Discussion

Anticoccidial vaccines control coccidiosis in the field when good management practices are applied and, in fact, they yield performance that is similar to that achieved with in-feed anticoccidials.

Accurate vaccine administration is necessary to ensure complete primary immunization of “all” birds, and recycling of *Eimeria* oocysts provides a booster effect. If vaccination is not properly and uniformly applied, resulting in incomplete primary immunization, birds will only be exposed to oocyst shedding and will develop immunity later into the production cycle, putting them at risk for coccidiosis breaks, demonstrated by the clinical signs of coccidiosis such as poor weight gain and intestinal lesions.

Flock management and feeding have a strong relationship with the surge of a multifactorial disease like subclinical NE. To minimize performance loss due to NE, it is necessary to identify and manage all factors contributing to an increase in the incidence of the disease.

There is a strong correlation between sub-clinical NE, an increased FCR and retarded growth in certain poultry lines, which either have a higher vulnerability to secondary invaders such as *C. perfringens* or to increased food refusal and litter ingestion.

In instances where *C. perfringens* leads to NE, antibiotic medication may reduce associated performance losses, but not always.

The tactical use of antibiotics — usually 3 days of an antibacterial compound in the water — to reduce performance loss, however, is seldom recommended to control bacterial overgrowth when it is at its peak in commercial broiler units. Sometimes it helps, and other times it is only of limited benefit in minimizing the deleterious effects of NE.

A comprehensive approach
to vaccine management is
needed to achieve success
over the long term.

Conclusion

From the data presented, compelling evidence is provided on two critical points:

- Knowledge of field management is essential to adopt Paracox-5 vaccination and make it work under all rearing conditions and for all types of meat-producing birds.
- When producers provide vaccinated birds with good management, including a sound nutritional plan and particularly brooding care that fosters healthy development of the intestinal tract at an early age, other serious problems such as clostridial enteritis can also be curtailed or prevented.

Hence, there are many and varied relationships between flock management and achieving full efficacy with the anticoccidial vaccine. A comprehensive approach to vaccine management is needed to achieve success over the long term.

Moreover, poultry producers must be duly advised on critical points for vaccination success so they can find economically profitable ways to raise healthy birds without standard in-feed anticoccidial medication.

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Unexpected coccidiosis multiplication patterns under various broiler management and coccidiosis control programs

Summary

Sequential fecal *Eimeria* spp. oocyst counts were conducted on multiple broiler farms using a variety of coccidiosis control and farm management programs.

Surprisingly, the highest counts came from farms that were completely cleaned out with a mandatory down time of at least two weeks. Earlier and lower counts came from high density flocks placed on litter that was reused for at least 5 years and also from flocks using year-round live coccidiosis vaccination.

These results indicate that in-feed anticoccidial medications no longer “control” coccidiosis in the same way they did 25 years ago, when many of those products were introduced. Instead, coccidiosis control is achieved through immunity, regardless of whether the coccidiosis control program uses in-feed anticoccidials or intentional vaccination.

Total cleanout and low density delay the development of immunity with in-feed anticoccidials and can result in heavy subclinical coccidiosis loads during the late broiler growth period, just before slaughter. The problem is magnified by rapid growth and a young slaughter age because unmedicated withdrawal feed

coincides with peak coccidial challenge. The final weeks of growth account for most of the feed consumed and most of the broiler meat yield.

Coccidiosis control programs should be evaluated and adjusted to avoid late subclinical challenge if producers are to achieve the maximum genetic potential for broiler performance.

Introduction

Broiler production depends largely upon in-feed anticoccidial medication — either ionophore antibiotics or chemicals — to control coccidiosis infection throughout the growing period. The commonly used products were developed in the 1970s and 1980s, with a only a couple of introductions in the 1990s. Despite responsible attempts to keep these products effective through rotation and shuttle programs, the sensitivity of field *Eimeria* populations is waning according to laboratory tests.^{1,2}

However, laboratory sensitivity studies can be debated: the challenge severity, methodology and the applicability to real-world performance may vary according to the researcher.

continued

KEY POINTS



Sequential fecal *Eimeria* spp. oocyst counts were conducted on multiple broiler farms that were using a variety of coccidiosis control and farm management programs.

- **Surprisingly, the highest counts came from farms that were completely cleaned out and had a mandatory down time, while earlier and lower counts came from high density flocks on reused litter or from flocks vaccinated against coccidiosis year-round.**
- **Poultry producers who want to maximize genetic performance potential of their flocks must adjust coccidiosis control programs to reduce late intestinal damage.**

Another way to look at the *practical* efficacy of the coccidiosis control programs is via sequential fecal oocyst counts conducted throughout the life of the flock. The timing and quantity of oocyst shedding can be used as an indicator of the efficacy of a coccidiosis control program under local real-world management and challenge conditions.

Methods

Fecal samples were collected from multiple broiler farms every 3 days beginning at day 7 of age. The farmers or field service representatives collected fresh fecal samples by one of two methods: Either three large sheets of paper were placed at random in the house for two or more hours to harvest feces or 10 to 20 fresh individual fecal/cecal dropping samples were collected at random by hand. Samples were refrigerated and submitted to the laboratory for counting. The oocysts per gram (OPG) of fecal material were estimated using a McMaster chamber.

Results

A benchmark for highly effective coccidiosis control was established by collecting samples from US farms using clopidol in 2007. Clopidol was reintroduced to the US broiler market in late 2005 after 15 to 20 years of rest. Four farms were sampled, yielding only very low levels of oocysts during the growout period (Figure 1). Unfortunately, this level of control is only temporary, since resistance builds quickly to chemical anticoccidials.

Samples were also collected in 2006 and again in 2007 from three farms located in the US Delmarva Peninsula that had moderate-density (14 birds/m²) and belonged to a small bird broiler integrator (Figure 2). This poultry-growing region is unique because of constraints on litter availability and environmental regulations prohibiting litter disposal. Litter is never completely cleaned out. Many farms have used the litter for five or more years with only the removal of caked litter from the surface.

The 2006 samples came from a nicarbazin-narasin combined product program fed from 1 day of age until five days before slaughter. The 2007 samples came from a salinomycin program fed from 1 day of age until 5 days before slaughter.

The patterns of oocyst shedding were unexpected: Clean litter and low density resulted in the highest and latest coccidia challenge...

Samples taken in 2006 to 2007 from Canadian barns yielded very different results. The Canadian broiler industry does a complete cleanout and disinfection between flocks. Broiler production is supply-managed, with a defined quota period and mandatory down time of two or more weeks between flocks. Despite cleaning, disinfection and down time, flocks had much higher and later oocyst peaks than those reared on reused litter.

Similar results were obtained from clean litter US farms.

Samples taken in 2007 from US broilers placed at low stocking-density (10 birds/m²) on litter reused for three sequential flocks also yielded later and higher oocyst peaks than those from the heavily reused litter (Figure 4). In this example, salinomycin was used from day 1 of age until 5 days before slaughter, and is compared to the higher stocking density salinomycin sample from Figure 1.

Flocks vaccinated year-round with a live, non-attenuated coccidiosis vaccine (Coccivac-B, Intervet/Schering-Plough Animal Health) produced an early oocyst peak when placed at moderate density (13 birds/m²) on litter reused for three sequential flocks (Figure 5). The pattern was similar to the moderate density peak on heavily reused litter.

Flocks vaccinated with the same vaccine, but raised with full-house brooding on clean litter in Canada produced oocyst peaks one week later, but the levels were still earlier and lower than comparable anticoccidial programs.

Discussion

The patterns of oocyst shedding were unexpected: Clean litter and low density resulted in the highest and latest coccidial challenge, while heavily reused litter, higher density and non-attenuated vaccines resulted in earlier and *lower* coccidial challenge. These results indicate that in-feed anticoccidials rely very heavily upon the development of immunity to protect flocks.

However, cleanout and low density provide insufficient natural challenge to stimulate

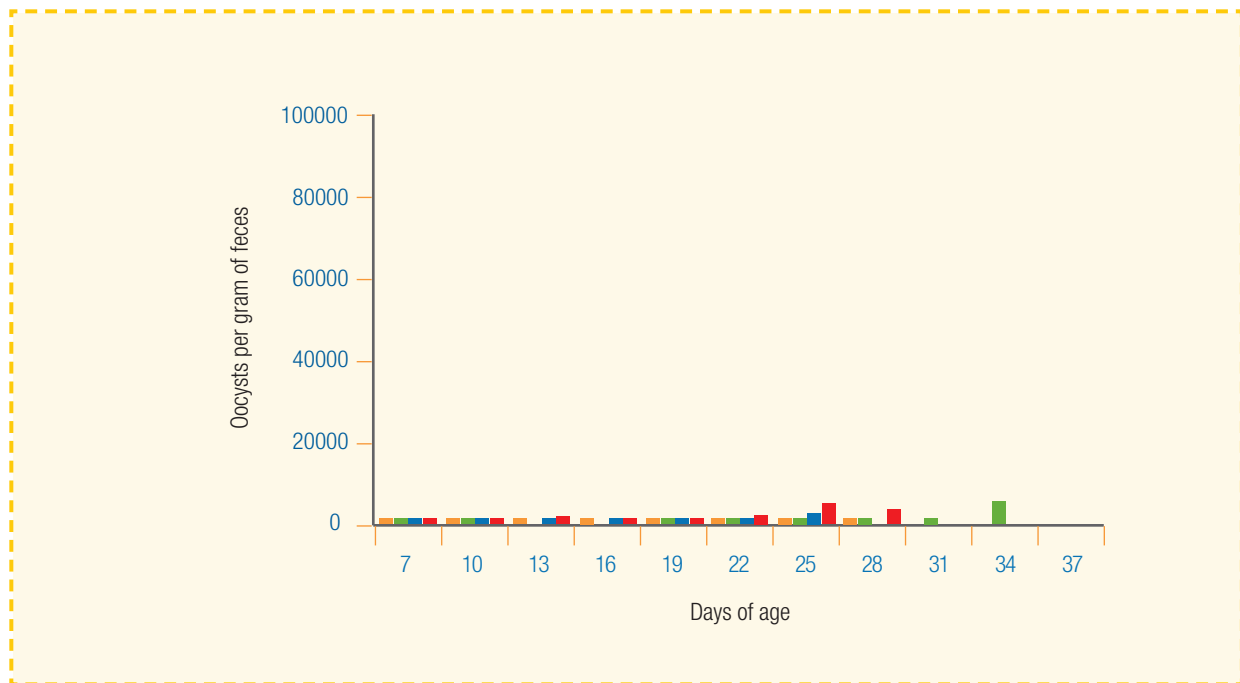


Figure 1. Sequential fecal oocyst counts taken in 2007 based on samples taken after the US reintroduction of clopidol demonstrate true coccidiosis control.

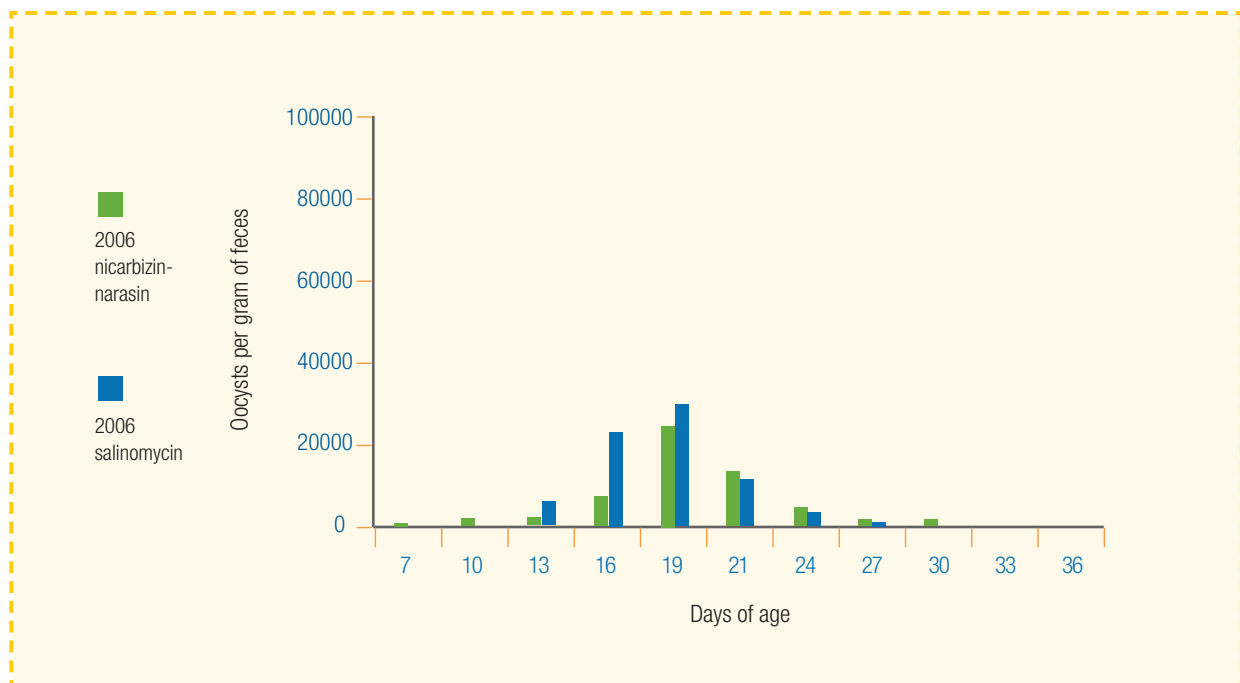


Figure 2. Average fecal oocyst counts 2006 (nicarbazin-narasin) vs. 2007 (salinomycin) on five-year reused litter. Note the early 19-day peak, regardless of the program.

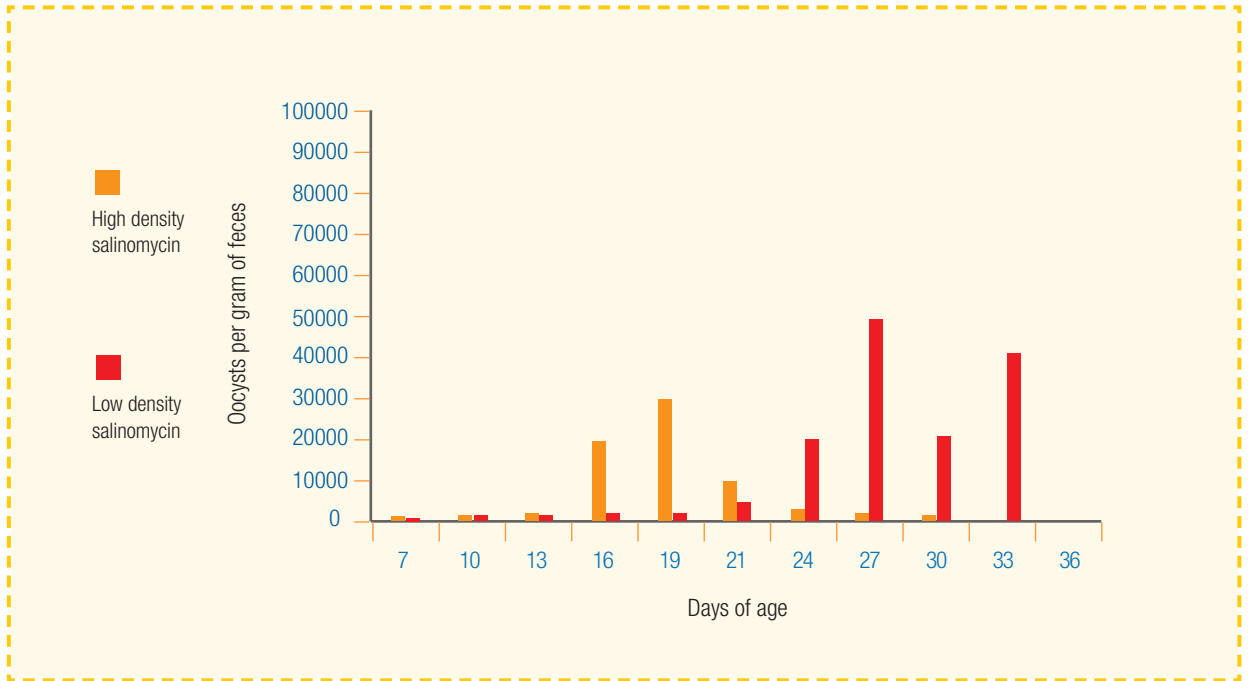


Figure 3. Sequential fecal oocyst counts from moderate broiler density vs. low broiler density on reused litter. Higher density produced lower/earlier oocyst peaks.

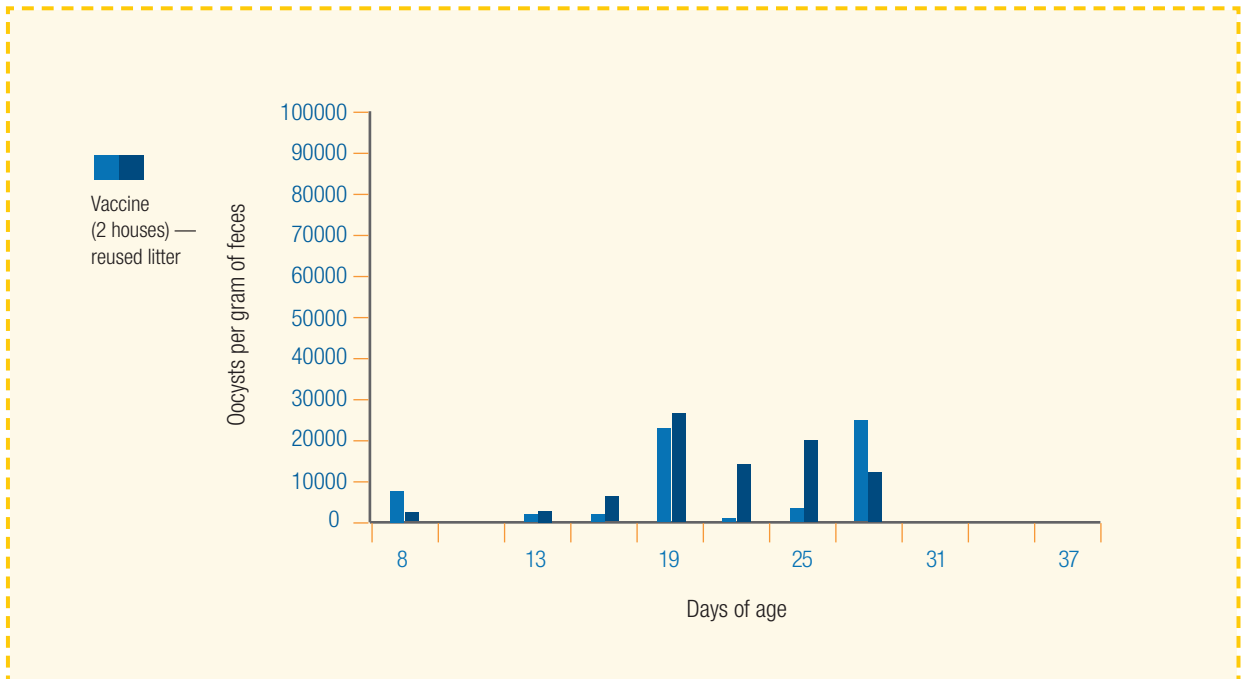


Figure 4. Sequential fecal oocyst counts from long-term live, non-attenuated coccidiosis vaccine use.



early immunity. The inability of anticoccidials to effectively control the infection without the aid of immunity results in high and late oocyst peaks in these flocks.

Twenty-five years ago, coccidiosis control with in-feed medication was more effective. Immunity still developed with ionophores at around four weeks of age, but the degree of coccidial leakage was less and the flocks took longer to reach processing weights: in other words, there was time for compensatory gain. Today, flocks are slaughtered at much younger ages, which are close to the peak of coccidiosis infection. The current weakness of drugs against natural infection means

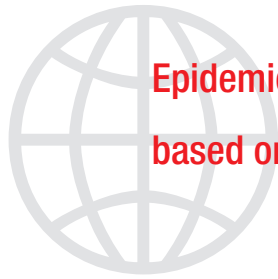
greater damage at a time when such damage can have the maximum negative effect on performance — without time for compensatory gain.

Conclusion

Sequential fecal oocyst shedding curves can be used to measure the level and timing of coccidiosis challenge in broiler flocks. Integrators seeking to maximize genetic performance potential must adjust coccidiosis control programs to reduce late intestinal damage.

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Epidemiology of coccidiosis in vaccinated turkeys based on oocyst shedding patterns

Summary

KEY POINTS



Coccidial oocyst shedding patterns were compared in turkeys that were sprayed or orally dosed with a coccidiosis vaccine.

- **Major peaks in oocysts per gram (OPG) occurred at 6 to 8 days after oral dosing and at 15 to 18 days in spray-vaccinated birds and in both groups, were followed by a much lower OPG peak.**
- **This epidemiological information can be used as a reference for field levels of oocysts in vaccinated turkey houses.**

The objective of the study was to compare the coccidial oocyst shedding patterns of turkeys that were sprayed or orally dosed with the commercial turkey coccidial vaccine Coccivac-T.

Vaccination was performed on day of hatch. Fresh fecal droppings were collected every two days and oocysts per gram (OPG) were determined.

In the orally-challenged birds, a major OPG peak occurred 6 to 8 days post-vaccination with a secondary peak at 15 to 18 days. In the spray-vaccinated birds, the major OPG peak was around 15 to 18 days, then declined. The decline indicated that coverage was adequate to induce substantial coccidial immunity.

The difference in OPG peaks between the two administration programs is related to initial percent coverage. While 100% of the orally-dosed birds were vaccinated, the spray-vaccinated birds had less initial coverage.

Introduction

Coccidiosis control in turkeys relies on anticoccidial drugs or live coccidial

vaccination. Coccidial immunity plays an important role with both programs.

In turkeys, anticoccidial drugs are generally only fed during the first 8 to 10 weeks of production. Thus, immunity is important for sustained coccidiosis control after protection from the drug is removed.¹ Vaccination is given early in the growout, either by spray applied at the hatchery or by administration in feed or water soon after poults arrive at the farm.

Oocyst cycling is vital for immunological protection to develop.² Fitz-Coy has found that in the United States, the most common *Eimeria* species in turkeys are *E. meleagritidis*, *E. gallopavonis*, *E. dispersa*, and *E. adenoides*. All poults must at some time receive adequate and repeated numbers of *Eimeria* oocysts of each of these major species to develop a broad spectrum of complete immunity.

The best vaccine protection with the least chance of performance loss occurs when a high percentage of poults are exposed to oocysts as early as possible.³ Spray vaccination of chicks at the hatchery has been shown to increase the initial percent coverage. Oocyst shedding patterns are a good indicator of coverage and immunity development.⁴

The objective of this study was to compare the coccidial oocyst shedding patterns of turkeys sprayed or orally dosed with a commercial turkey coccidial vaccine, Coccivac-T, which was administered on day of hatch.

Methods

The experiment consisted of 8 pens of 40 turkey hens per pen. The treatments were replicated 4 times, but were not randomized. The pens were the experimental units and each pen was kept empty between replicated treatments.

Commercial strain poultlets either received Coccivac-T administered by spray cabinet (Spraycox machine) or were orally dosed individually. Sprayed birds were allowed to

preen (remove oocysts) for approximately 1 hour before they were placed in pens.

The floor pen house was a modified poultry house with dirt floors and curtain sidewalls. The birds were kept in pens with fresh pine shavings as bedding. The stocking density, after subtracting out for equipment, was 0.35 m²/turkey-hen. Commercial rearing conditions were mimicked as closely as possible and the birds were raised humanely. Nonmedicated feed and water were provided *ad libitum*.

During the study, ten fresh fecal droppings were collected from each pen every two days and oocyst numbers and the ratios of species were determined. Ten grams of the sample were removed and added to 100 ml of water. The container with the mixture was labeled, swirled, covered and

then refrigerated overnight. The mixture was next filtered through a screen and the liquid suspension (10 ml) was poured into labeled tubes and centrifuged at 1500 rpm for 5 minutes.

After centrifugation, the liquid was poured off, and 10 ml of saturated salt solution were added to the tube and thoroughly mixed. A portion of the salt/oocyst suspension was loaded into a McMaster chamber. The observed oocysts were counted in all six lanes of the chamber. The oocysts/ml was calculated by the following formula: Count X 6.67 X 10 (dilution) = Oocysts/ml.

Results and discussion

Orally-dosed birds had a major peak in oocyst shedding between days 6 to 8 days

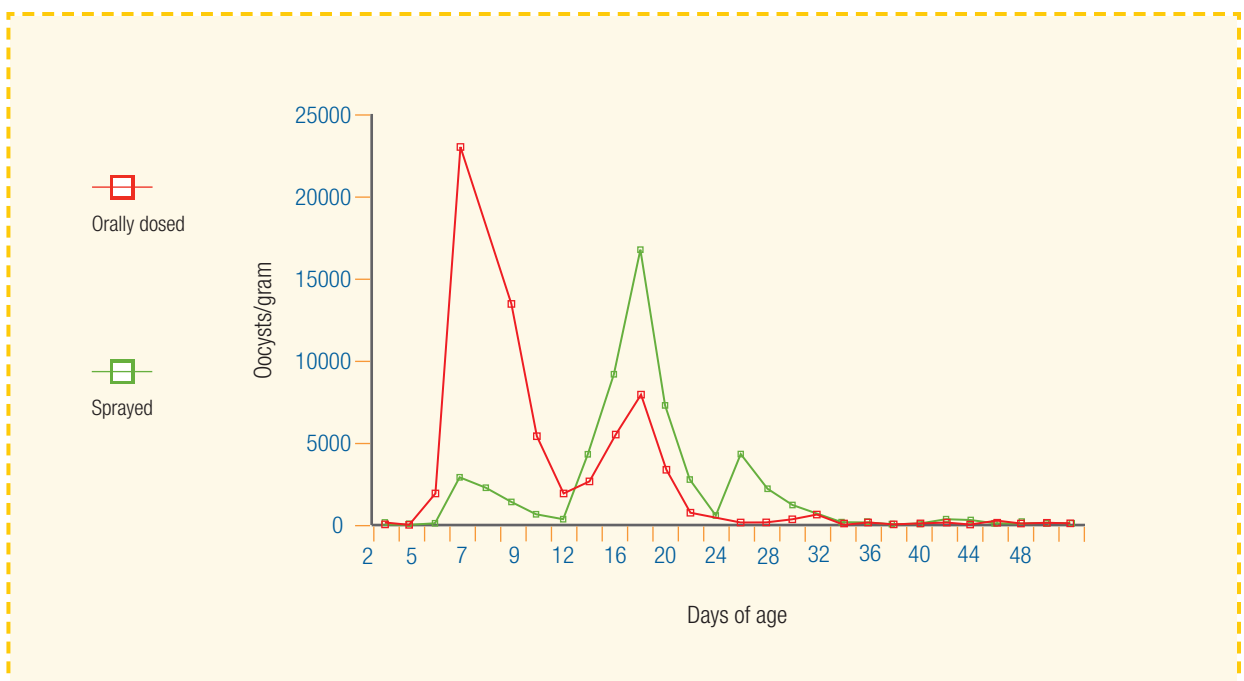


Figure 1. Oocyst shedding patterns in turkeys either orally dosed or sprayed with the coccidial vaccine Coccivac-T.

post challenge (Figure 1). This major OPG peak occurred with the first coccidia cycle after vaccination. This large amount of oocysts occurred because 100 % of all birds were dosed.

The first OPG peak with the spray-applied vaccine was also between 6 to 8 days (Figure 1). This peak was lower than observed with the orally administered vaccine, indicating less than 100 % coverage. The major OPG peak in poult that were sprayed was between 15 to 18 days and coincided with a second cycle of infection.

The second cycling peak occurred during this time because the first oocyst shedding occurred 6 to 8 days post vaccination and these shed oocysts took 2 or 3 days to become infective (sporulate), when they were consumed by birds and continued with the normal 7 to 8 day pre-patent period.

The substantial peak at 15 to 18 days after challenge indicated that even though spray application of the vaccine did not initially provide 100% coverage, it did provide enough oocysts to infect a large number of birds, resulting in a sizeable second cycle.

Sharp reductions in OPGs after the major peak indicate that coccidial immunity had developed, reducing the number of parasites reproducing within the bird.

A much lower OPG peak after the major peak was observed with both dosing methods. The minor peak in the orally-dosed poult occurred at the same time that the 15 to 18 day peak occurred in sprayed birds. The minor peak in sprayed birds occurred around day 24 to 26.

Sharp reductions in OPGs after the major peak indicate that coccidial immunity had developed.

Eimeria species that infect turkeys have a generally shorter pre-patent period than *Eimeria* species that infect chickens; OPG peaks in chickens last about one day less than they do in turkeys.⁵ This epidemiological information can be used as a reference for field levels of oocysts in vaccinated turkey houses.

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Newcastle disease (ND) efficacy in broilers vaccinated at 1 day of age with the recombinant HVT/F(ND): Innovax-ND-SB challenged by the intramuscular and ocular routes with the Mexican virulent ND virus (NDV) genotype V Chimalhuacan strain

Summary

KEY POINTS



Innovax-ND-SB is a recombinant vaccine for protection against virulent Newcastle disease virus (NDV) and very virulent Marek's disease virus (MDV) when administered to birds with high maternal immunity at one day of age.

- **Birds vaccinated with the recombinant vaccine with or without the addition of a conventional live vaccine were challenged at different times with Mexican virulent NDV (Chimalhuacan strain) administered by the intramuscular or ocular/intranasal route.**
- **Data from the trial were still being analyzed at the time these proceedings were published, but initial results showed that vaccinated birds had complete protection against virulent NDV.**

Innovax-ND-SB is a live recombinant vaccine for protection against Newcastle disease virus (NDV) and very virulent Marek's disease virus (MDV).

The current study, conducted in Mexico, evaluated the effects of NDV maternal antibodies and protection achieved against the recent, virulent Mexican Chimalhuacan NDV strain of the genotype V. Protection was determined based on mortality, morbidity and virus shedding.

Data from the trial were still being analyzed at the time these proceedings were published, but by approximately 30 days after vaccination, birds that had received the recombinant vaccine at one day of age showed complete protection when challenged with the virulent Mexican NDV strain by the intramuscular or ocular/intranasal route.

This recombinant vaccine also has a lot of advantages compared with conventional live and inactivated ND vaccines.

Introduction

The recombinant vaccine Innovax-ND-SB for protection against NDV and very virulent MDV is the first turkey herpes

virus (HVT)-vectored ND vaccine to reach the market.

Innovax-ND-SB has the ND virus F (fusion protein) gene inserted. The F protein induces F antibodies, providing protection against the ND virus. The HVT/ND is combined with the MDV vaccine SB1.

In previous studies, the recombinant vaccine provided similar protection in chickens against very virulent MDV compared to the conventional HVT/SB1 vaccine and excellent protection against the Texas GB virulent NDV in birds with low to moderate ND virus maternal antibody levels.^{1,2,3}

In this study, the effect of high NDV maternal antibodies and the protection achieved against the virulent Mexican NDV strain of the genotype V were evaluated based on mortality, morbidity and virus shedding.

Methods

Different groups of broilers with very high maternal antibodies for ND virus were

vaccinated subcutaneously at one day of age with the HVT/ND recombinant vaccine, with or without the addition of a live ND vaccination.

Birds from each group were then challenged at various ages with the virulent Mexican NDV administered by the intramuscular or intranasal/ocular route.

Results

Preliminary results showed that by about 30 days after vaccination, birds that had received the recombinant vaccine at one day of age showed complete protection after challenge with the Mexican virulent ND genotype V virus, although the challenge virus could be isolated from the trachea of the clinically protected birds for about 10 days post-challenge. Similar protection has been obtained in birds vaccinated with an inactivated NDV vaccine.

In birds simultaneously vaccinated at one day of age with a live ND vaccine, protection was obtained from the first week of age onwards and there was a significant reduction in virus re-isolation.

Discussion

Most HVT recombinant/vector vaccines, like the recombinant HVT/F ND vaccine, will provide complete protection against the foreign gene protein insert 3 to 4 weeks post vaccination. Because complete protection after administration of the HVT/ND recombinant vaccine is delayed, a safe, live, conventional ND vaccine should also be administered, preferably at one day of age, in exotic ND virus-endemic areas.

Birds that received the recombinant vaccine at one day of age showed complete protection after challenge with the Mexican virulent ND genotype V virus.

The main benefits of the recombinant HVT/ND Innovax-ND-SB are:
No vaccine reaction as with live ND vaccines, no local reaction/systemic distress as observed with inactivated vaccines and no interference with ND maternal antibodies.

The recombinant vaccine also has an extended duration of immunity, which reduces the need to administer additional live or inactivated ND vaccines.

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A novel HVT-based recombinant vaccine (Innovax-ILT) to simultaneously control infectious laryngotracheitis and Marek's disease in chickens

Summary

KEY POINTS



Innovax-ILT, a commercially available, recombinant vaccine based on the herpesvirus of turkeys (HVT), was developed to help prevent infectious laryngotracheitis (ILT) and Marek's disease in chickens.

- **The vaccine is safe, easy to administer, does not spread from vaccinated to susceptible chickens and is expected to induce life-long immunity.**
- **The positive features of Innovax-ILT make it an ideal vaccine to replace current modified live ILT vaccines and their drawbacks, such as the spread of the vaccine virus to non-vaccinates.**

Infectious laryngotracheitis (ILT) is a serious, costly and highly contagious respiratory disease in chickens. Modified live vaccines are widely used to prevent ILT outbreaks, but have several drawbacks such as the spread of the vaccine virus to non-vaccinates.

Innovax-ILT is a relatively new, commercially available recombinant vaccine based on the herpesvirus of turkeys (HVT), which has already been used extensively for preventing Marek's disease.

Controlled studies with the vaccine administered either *in ovo* or subcutaneously to chicks show that Innovax-ILT protected 97% of chicks from ILT. The vaccine was also found safe to use and did not spread from vaccinates to non-vaccinates. A study in progress is expected to show that Innovax-ILT induces life-long immunity.

Introduction

Infectious laryngotracheitis (ILT) is an acute, highly contagious respiratory disease of chickens caused by an alphaherpesvirus. It is an economically important disease of poultry that may

cause severe losses in meat and egg production.

Currently, ILT vaccines based on modified live viruses are widely used to prevent disease outbreaks. Although they are highly efficacious, attenuated live ILT vaccines have often been associated with a variety of adverse effects, including spread of the vaccine virus to non-vaccinates, increased virulence as a result of bird-to-bird passage and occurrence of latently infected carriers.

Vaccines based on the herpesvirus of turkeys (HVT) have already been extensively used for the prevention of Marek's disease. Here, a commercially available, recombinant HVT vaccine developed to help prevent Marek's disease as well as ILT is described. The vaccine expresses two protective glycoprotein genes of ILT virus (Innovax-ILT).

Study results

The safety profile of Innovax-ILT has been shown to be identical to the safety of commonly used HVT vaccine strains.

continued

A novel HVT-based recombinant vaccine (Innovax-ILT) to simultaneously control infectious laryngotracheitis and Marek's disease in chickens



Figure 1. Efficacy of Innovax-ILT against Marek's disease (MD) and infectious laryngotracheitis (ILT), single *in ovo* administration.

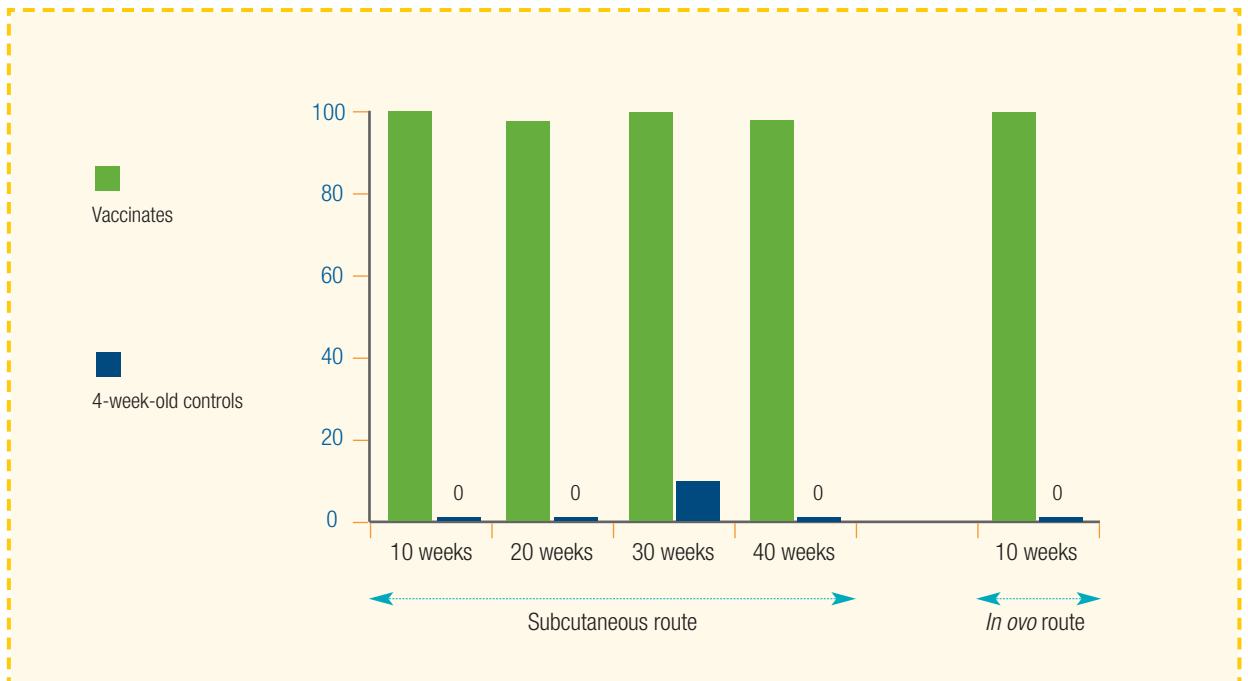


Figure 2. Duration of immunity of Innovax-ILT, subcutaneous and *in ovo* administration.



Innovax-ILT was also genetically stable in more than ten serial cell culture passages and remained fully infectious in chickens. However, Innovax-ILT could not be isolated from sentinel chickens during a contact exposure period of four weeks, demonstrating that it does not spread horizontally from vaccinated to susceptible chickens.

A single *in ovo* administration of the vaccine protected 97% of the chickens against ILT challenge for at least 10 weeks (Figures 1 & 2) — the longest period of time tested in the study after *in ovo* administration.

In addition, a single subcutaneous administration protected 97% of vaccinated chickens against an ILT challenge that caused clinical signs or death in more than 90% of the control chickens (Figures 1 & 2).

Discussion

Because the vaccine persists without being shed, it is expected to induce life-long immunity against ILT and experiments to confirm at least one year duration of immunity are in progress.

No statistically significant difference in Marek's protection provided by Innovax-ILT and the parent HVT strain was observed, indicating that insertion of foreign sequences into the HVT genome did not compromise the ability of HVT to protect against Marek's disease.

The safety profile of Innovax-ILT has been shown to be identical to the safety of commonly used HVT vaccine strains.

Overall, the safety, efficacy, ease of administration, the expected ability to induce long-lasting immunity and its non-spreading nature are some of the features that make Innovax-ILT an ideal vaccine to replace current modified live ILT vaccines and avoid the drawbacks associated with them. Field experience and additional laboratory data with Innovax-ILT since its recent introduction in the USA will be discussed.



Future poultry vaccines: A 15-year perspective

Summary

KEY POINTS



Better understanding of the avian immune system and avian microorganisms coupled with technological advances has raised expectations for a new generation of poultry vaccines.

- **Although there is cause for optimism, the complex requirements for new and improved poultry vaccines mean that only a fraction of research ideas are likely to make it to market.**

Knowledge of the avian immune system and avian microorganisms has increased tremendously and that, along with technological advances, particularly in molecular biology, has raised expectations for new generations of poultry vaccines. The requirements for new poultry vaccines, however, are complex. Poultry vaccines, in addition to being safe and highly effective, must be easily and rapidly administered. These requirements have to be met while there are economic constraints and changing regulatory political, environmental and disease pressures. In addition to all this, the challenges are compounded by the fact that most current vaccines work well. For this reason, whilst research endeavors for new poultry vaccines are plentiful, only a fraction of them will make it to market.

Introduction

Understanding of the avian immune system and the pathogenesis of avian microorganisms has increased considerably over the past few decades. That coupled with technological advances in molecular biology, genomics, proteomics, bioinformatics and vaccine delivery systems has understandably

raised expectations for new generations of poultry vaccines.

Our expectations, however, should be moderated to some extent since the pace of change is likely to be slower than hoped for, for many reasons. Poultry vaccines have more complex requirements compared to vaccines for other species.

Of course, new poultry vaccines should be safe and efficacious, but they should also be quick and easy to apply, they should be in correct combinations and, whenever possible, they should be broad spectrum to reduce the number of vaccinations and/or handling of birds. All these requirements need to be achieved within the constraints of a strict economic framework while the regulatory, political, environmental and disease pressures are constantly shifting. In addition to all this, the challenges are compounded by the fact that most current vaccines work extremely well. For these reasons, we should adopt a balanced, cautious optimism for future advances.

Advanced knowledge

So what have we learned that can assist us over the next 15 years? Of particular



note is the advance in our knowledge of avian immunology, including the innate immune response, the role of chemokines/cytokines, antigen processing and presentation, cell mediated and mucosal immunity.

From studies of the innate immune response, we now better understand the way in which immune responses are initiated, with the manner in which pathogens are recognized being crucially important. This knowledge has to some extent shifted the emphasis on adjuvant research to target the innate immune response. By developing adjuvants that initiate the immune response more optimally, we may be able to lower antigen doses, to give fewer doses, to obtain faster onset of immunity whilst maintaining a long duration of immunity.

From the chemokines/cytokines field we have learned that chickens express many of the regulatory proteins that mammals do. Much effort is on-going to augment immune responses or even alter a bird's capacity to respond to vaccines by delivering these proteins as vaccine constituents. Avian dendritic cells are now being characterized and the research suggests that these cells, like their mammalian counterparts, are the key antigen presenting cell in the initiation of a robust immune response. Targeting dendritic cells with vaccines is now a realistic target for many research groups.

Finally, increasing knowledge of the cell-mediated and mucosal immune responses may help us analyze and target these vital components in order to optimize vaccine efficacy.

No technology has promised more for a new generation of poultry vaccines than molecular biology, and finally we are beginning to see the fruits of this work. Fowlpox and turkey herpesvirus recombinant vaccines targeting five different diseases plus *Salmonella* and *E. coli* deletion mutants have regulatory approval in the USA. In the future, we can expect different types of vectors to become available, especially now we can manipulate RNA viruses as readily as DNA viruses. Increased knowledge should allow more marker vaccines and the commercial optimization of various antigen expression systems should help improve vaccine design plus play a role in manufacturing logistics, reducing our reliance on the use of egg-based production systems.

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Significant advances have also been made in our knowledge of avian microorganisms. It is not surprising to find that many of these have evolved mechanisms to either avoid or regulate the host immune response. This knowledge should enable us to counter the strategies of such organisms and novel antigen discovery technologies such as 'reverse vaccinology' will assist this field. However, here we

should acknowledge that our information is far from complete and of course the organisms are constantly evolving.

Although the poultry industry already has the most varied application routes of all species, there remains scope for further changes and refinements. Vaccine delivery devices are still being improved and there are plenty of opportunities for more vaccines to be delivered via *in-ovo* vaccination. Advances in vaccine and drug -delivery technologies may also filter into the poultry vaccine field, broadening our options.

Conclusion

In summary, knowledge of science and technology that can influence the development of new poultry vaccines is increasing rapidly. This brings great optimism for the future, but the complex requirements of a successful poultry vaccine suggests that only a fraction of research ideas will make it through to market.

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