POULTRY HEALTH TODAY

HIGHLIGHTS OF A ROUNDTABLE DISCUSSION

Optimizing immunity

in 'no antibiotics ever' and 'reduced use' broiler flocks

JULY 2017 • INDIANAPOLIS, INDIANA

POU-00094

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HIGHLIGHTS OF A ROUNDTABLE DISCUSSION

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WELCOME

The growing trend toward "no antibiotics ever" and "reduced use" production systems has prompted poultry companies to rethink their traditional disease-management practices.

When flocks are raised with few or no antibiotics, they're naturally more susceptible to diseases caused by primary or secondary infections. This has presented a huge challenge for poultry veterinarians.

Alternative therapies have shown potential, but reports from the field — both scientific and anecdotal — show they've also been inconsistent.

Making refinements in nutrition, stocking rates and housing may help to reduce disease pressure. But in the end, finding ways to optimize immunity and give broilers more "staying power" could be the best strategy for maintaining the health and welfare of these birds.

To help the poultry industry meet this goal, we brought together an all-star team of experts with expertise in three diseases affecting the broiler's immune system — IBD, Marek's and reovirus — to talk about what producers can do now to raise the bar for protection and flock welfare.

This booklet presents highlights from that lively and informative discussion. Special thanks to the participants for sharing their insights and expertise.

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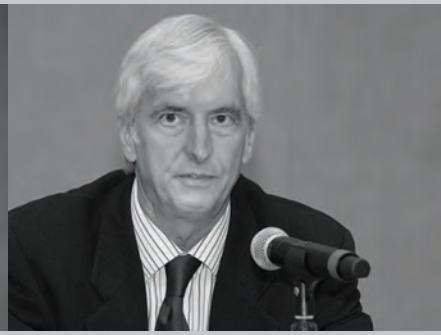

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POULTRY
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BUILDING A STRONGER BIRD

> "IBD is one of those diseases that will be here long after all of us are gone. It's never going to go away."
>
> GUILLERMO ZAVALA, DVM

SANDER

Dr. Zavala, would you tell us a bit about how control of immunosuppressive diseases such as infectious bursal disease (IBD), reovirus and Marek's can help us produce a more resilient bird with a stronger immune system?

ZAVALA

IBD is one of those diseases that will be here long after all of us are gone. It's never going to go away. It affects B cells and impairs their ability to produce specific antibodies that have high affinity for very specific disease agents.

Protecting birds against Marek's disease is absolutely critical if, for example, we're to reduce condemnations due to neoplasia in the processing plant. It's also critical for protecting the immune system.

Reovirus is a little bit more inconsistent — some strains are immunosuppressive and some are not. Immunosuppressive reoviruses, like IBD, may affect B cells. They cause bursal atrophy, for example, and reduce the ability of the bird to produce immunoglobulins against specific disease agents.

Marek's disease also affects B cells, impairing the bird's ability to produce sufficient antibodies to a number of different infectious disease agents. It can impair the ability of T cells to function properly, which help coordinate the bird's immune responses.

A fourth very, very critical immunosuppressive agent is chicken infectious anemia virus (CAV). It can interact with any or all of these other viruses and predispose birds to secondary opportunistic infections. It can infect and destroy bone marrow cells. The result is increased bacterial infection and increased severity of any infectious disease.

Control of these viruses is therefore critical — and we have the tools we need to protect against the immunosuppressive diseases they cause. We don't always protect birds against them 100% the way we'd like to, but for the most part, the vaccines we have for use in the field are good tools.

SANDER

Can vaccination against viral diseases help reduce the incidence of secondary bacterial infections and, in that way, reduce the need for antibiotics?

JACKWOOD

When we're talking about vaccines for immunosuppressive diseases like IBD, reovirus and Marek's, I think we can do a better job than what we're doing now. If we can prevent birds from becoming immune-suppressed and getting secondary bacterial infections, we may be able to get away with using fewer antibiotics.

continued

BUILDING A STRONGER BIRD

> “...it’s important to manage our secondary bacterial levels, and even bronchitis.”
>
> KALEN COOKSON, DVM

SANDER

Should efforts to build immunity in broilers start with the breeder program?

SMITH

Absolutely. The key to controlling diseases like IBD or reovirus, at least under US conditions, is hyper-immunization of the hen using strains that are as close as possible to your resident challenge strains.

DALE

If the bird is immune-compromised or overwhelmed by conditions in the environment, you can use the best vaccines in the world but they’re not going to elicit an appropriate response, and a bacterial challenge will break through pretty easily.

COOKSON

I’ll just share a study we performed a few years ago at Auburn University because I think it illustrates the interaction between virally induced immune suppression and susceptibility to bacterial challenge.[1] In this study, we picked a broiler flock we knew had low reovirus titers and challenged half of them with reovirus at 3 days of age and then IBD virus and CAV at 7 days. And the results of that, from a viral standpoint, is that we got about 10% weight suppression, probably from the reovirus challenge, and 50% of the birds were infected with AL-2 by 2 weeks of age. So IBD virus was also probably a factor.

On top of that, we challenged all the birds with a pathogenic *Escherichia coli* using either a high dose of about 8 logs or a low dose of 5 logs. Well, there wasn’t much difference in the high-dose *E. coli* birds — as if even the immune-intact birds were just as overwhelmed by the high *E. coli* challenge. But in the low-dose *E. coli* birds, we still had significant levels of *E. coli* disease in the virally challenged birds and no *E. coli* in the birds that weren’t immune suppressed. So I think there’s information in that, that can kind of fit into our paradigms that we’re facing today. You know, how it’s important to manage our secondary bacterial levels, and even bronchitis. It’s still very important that we manage our immune health programs, as well.

SANDER

Dr. Dale, according to published reports, Pilgrim is the second largest producer in the US, and about 24% of your production is now “no antibiotics ever.” Have you seen a relationship between the management of these three diseases and the staying power of your flocks, and does it differ in antibiotic-free production systems versus conventional production systems?

DALE

The management of those three diseases is just as important in our conventional systems. These problems lead to morbidity and mortality in both types of systems. However, it’s not as easy to prevent secondary bacterial issues due to immunosuppression in antibiotic-free production systems.

Careful management throughout the entire production process is needed to ensure we get the vaccine reaction we need to elicit adequate immunity, whether that’s in breeders, at the hatchery or in the broiler house.

INFECTIOUS BURSAL DISEASE

INFECTIOUS BURSAL DISEASE

SANDER

Dr. Jackwood, can you expand on IBD and tell us more about how it contributes to immune suppression?

JACKWOOD

The virus infects immature B cells in the bursa of Fabricius. Ultimately, what you end up with is a bird with a diminished ability to produce antibodies.

However, the severity of compromise depends on the timing of infection. Let's say the bird is 14 days or older. You'll get a depopulation of bursa B cells and you'll get immune suppression, but it's a transient suppression. Those birds will recover because the bursa will repopulate with lymphocytes and go on to mount an immune response. Is it 100%? Maybe not, but the birds certainly do recover.

If birds are infected before 14 days of age — 14 days seems to be the cut-off — you don't have B cells reaching the secondary lymphoid organs yet. They're concentrated in the bursa, and if they're destroyed, you don't have any more cells to repopulate — resulting in permanent immune suppression where antibodies can't be produced.

It's important to note that the T-cell population is also affected by IBD so you also see some cellular immune suppression in these birds. (T cells are critical for fighting bacterial infections) .

SANDER

Can vaccination of breeders help ensure broilers will have access to maternal antibodies during those first 14 days?

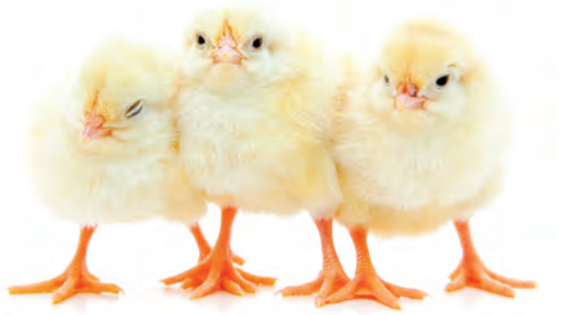
JACKWOOD

Yes, breeder vaccination is the key to preventing permanent or severe immune suppression due to IBD.

The key to successful breeder vaccination is having breeder birds that aren't immune-suppressed to begin with and that have a fully functioning immune system so they can respond to vaccines. You need to get the titers of antibodies very high so breeders pass those antibodies on to broiler chicks through the yolk.

It's been shown fairly well that if you have high serum antibodies in your breeders, you will have high antibodies in the broilers when they hatch. The issue with vaccination of breeders, as Dr. Smith mentioned, is that you want to make sure the vaccines you use in your breeders match the antigenic type of virus in the field that will challenge the broilers.

It doesn't matter how high your maternal antibody titers are in broilers — if they're not the right antibody, the broilers are going to get infected early on in life and you're going to have permanent immune suppression.



> "Vaccinating broilers before those maternal antibodies drop too low is really the key."
>
> DARAL JACKWOOD, PHD

SANDER

Let's build on that. Some poultry companies seem to believe IBD protection starts and ends with the breeder program and they don't vaccinate broilers against IBD. Others believe that both breeders and the broilers need to be vaccinated. Why does this decision vary and what goes into making that decision?

JACKWOOD

Let's talk about antibodies present in the broilers when they hit the farm. During the first 4 days, their immunoglobulin antibody stays fairly constant because the birds are still reabsorbing yolk. Afterwards, metabolism and growth start eroding antibody titers.

The half-life — the amount of time it takes for half of the population of immunoglobulin antibodies in that bird to be reduced by 50% — ranges depending on the assay used to detect those antibodies and the type of chicken, but by 7 days, broilers have probably lost half of the antibodies they started with. By 10 days, they've lost another half, so they're down to 25%, and by 13 to 15 days of age, they're down to 12.5%.

By the time broilers are 18 to 20 days of age, the titers are getting pretty low. That's when the IBD virus is going to have the opportunity to infect these birds. At about 3 weeks of age, we've got maternal antibodies that are low enough for the birds to become infected with IBD field virus unless they have active immunity. Vaccinating broilers before those maternal antibodies drop too low is really the key.

IBD VACCINE DECISIONS

SANDER

How do you decide which IBD vaccine to give broilers and when?

JACKWOOD

You've got to give one that matches the field virus, otherwise it's not going to work. Next you have to decide *when* to give the vaccine — we're talking about a live, attenuated virus — and how well it can break through maternal immunity.

Some of the very mild viruses struggle to do that, so you have to wait until maternal antibodies are pretty low before you can use those vaccines, and you have to remember that once you give the vaccine immunity isn't immediate. It will take 10 to 12 days before you see any kind of antibodies showing up in those birds.

The intermediate, intermediate-plus or hotter vaccines will break through maternal immunity a little bit better, so you can give them earlier when maternal antibodies are relatively high. The timing depends on the individual vaccine and how virulent or attenuated it is. The trade off with these hotter vaccines is that if they are given when maternal immunity is very low they can produce some damage to the bursa and cause a transient immune suppression.

SANDER

What about overall flock immunity? How does it factor in?

JACKWOOD

It's important. You have to consider the percentage of birds that have lower or higher titers. You want to try and vaccinate the flock when you have somewhere around 70% to 75% of birds with titers low enough to take with this vaccine. If it's less than that, you're just not going to get a really good immune response from your vaccine. For example, if you've got only

> "Besides making sure the correct strain is chosen, it's important to make sure the vaccine is actually getting into breeders."
>
> MARK BURLESON, DVM

40% or 50% of your birds reacting to the vaccine, you've got half a flock that's still susceptible.

If flock titers are already too low (i.e., you waited too long to vaccinate), you end up with a population that's very susceptible to the field virus, and they're going to get infected and start shedding field viruses. Then you've got a flock that's already getting immune-suppressed when you're trying to administer a vaccine.

So, I'll reiterate — population immunity is very important when you're deciding when to give those vaccines. There are a lot of variables involved in making the decision of when to vaccinate.

SANDER

How can the breeder-vaccination program for IBD be set up to help ensure it protects broiler chicks?

COOKSON

First, the breeder pullet must be adequately primed. Until the advent of recombinant vaccines, it was virtually standard practice to use an intermediate vaccine at around 18 days of age, usually followed by one live intermediate-plus or strong vaccine at around 5 to 6 to weeks of age. Historically, that program worked very well for priming the immune system so pullets could respond well to the killed vaccines they got later on.

There've been changes since the emergence of recombinants, which certainly have a place. The recombinants will protect pullets most of the time. In fact, they may do a better job than live vaccines when the challenge is in the 4- to 6-week period and immunity from the recombinant is in full effect. However, because a recombinant may actually reduce the circulating field challenge is all the more reason to continue to keep a strong, live vaccine in your program. Live-priming helps improve the titer response to the killed vaccines.

DALE

I also believe in live-priming followed by inactivated vaccines for breeders. But we wrestle with finding appropriate commercial vaccines that have homologous strains that match the field challenge.

This need creates an ongoing cycle of autogenous vaccine use. We have to use autogenous vaccines to meet specific regional challenges and make sure the breeders are hyper-immunized for strains the broilers will be exposed to.

BURLESON

Besides making sure the correct strain is chosen, it's important to make sure the vaccine is actually getting into breeders. Sometimes companies establish a strong IBD vaccination program and assume it's being administered correctly, but I've seen first-hand that they don't always follow up. If you're using killed vaccines, you have to be auditing vaccination crews and monitoring antibody titers to ensure every breeder is getting injected.

SMITH

I think the substrate used to grow the virus for killed IBD vaccines makes a difference. With current technologies, the vaccines produced in bursal tissue are superior.

I like to use a couple of high-quality bursal-derived vaccines with a variety of viral antigens, and I'll add an autogenous vaccine on top of that.

JACKWOOD

I'll agree with Dr. Smith about the use of bursal-derived killed vaccines. When you start growing bursal disease virus in eggs or in cell culture, mutations have to occur in order for that virus to infect cells and replicate to a fairly high titer. Those mutations are subtle but can sometimes affect the antigenicity of those viruses.

In addition, the amount of high-quality antigen you get with bursal-derived vaccines is much higher than you can get with egg- or cell-cultured vaccines. That's why the bursal-derived vaccines work better.

ZAVALA

I agree it's really critical to prime birds. I don't typically see recombinant vaccines used as a sole vaccine prior to the use of killed vaccines in many, many places.

Wherever producers have attempted to use only recombinant vaccines followed by killed vaccines, they typically go back to the more traditional programs. Not every country around the world has access to bursal-derived products, but I think it has been our common experience that if you have the ability to use tissue-culture substrate vaccines and chicken-embryo substrate vaccines, in addition to bursal-derived vaccines, you not only have a better chance of increasing your titers but you broaden the diversity of antigens you're actually injecting in those birds. There's a much higher likelihood of success.

SANDER

Recombinant IBD vaccines are in greater use, but you can't use more than one. What do you do if you also need to vaccinate against Newcastle disease (ND) or infectious laryngotracheitis (ILT)?

DALE

T hat decision, for me, is always made on a case-by-case basis for any individual complex and its challenge. It's all about mitigating risk.

There are times I will prioritize using a recombinant vaccine for something else — for example, ILT.

COOKSON

If you're going to use a recombinant ND vaccine, it's likely going to be in the wintertime when the challenge with respiratory diseases such as bronchitis is the highest. Many producers like to focus their live-vaccine program on bronchitis in the winter for this reason.

For producers who use herpesvirus of turkey (HVT)-ND vaccines in the winter but still want IBD vaccine coverage, immune-complexed live IBD vaccines are a good alternative. Then the recombinant IBD vaccine can be reintroduced in off-winter months.

Of course, if you have ILT, that's the highest priority and the recombinant HVT-ILT vaccine appears to be the most effective.

AUTOGENOUS IBD VACCINATION

SANDER

How do you decide which IBD strains to use if an autogenous vaccine is needed?

SMITH

I've found serology to test for IBD is a little less helpful and not as clear-cut as serology for, say, bronchitis or Newcastle.

continued

I rely more on examination of bursas and I do that two ways. One is during routine postmortems. I look at the bursa without fail on every postmortem and compare it to the standard for that age and bird — essentially continuous monitoring of the bursal status in the operation.

The other thing I do is arrange for periodic bursal surveys. Most companies have a list that ranks their growers by performance. I'll get their list and select farms with reasonably good managers who are still struggling with performance or disease problems and may have immune suppression going on due to IBD virus. I don't want farms on the bottom of the list because those growers are not taking care of their chickens; I want the farms that are low on the list in spite of reasonably good management.

I'll typically sample those selected flocks, certainly at least two and sometimes three times, usually between 2 to 4 weeks of age, taking five to six bursas per sampling. We measure the bursas, although the value of that measurement is likely limited, or calculate a bursa-to-bodyweight ratio.

More valuable is histopathology. We split the bursa into thirds, put one-third into formalin and freeze the other two pieces. The five to six bursas from a given farm are pooled, so we have one vial with five to six sections for histopathology, and two frozen whirl-packs, each with five to six pooled fragments. Then we have a pathologist evaluate the formalin-fixed bursas from each sample to determine which samples should be tested for IBD virus by polymerase chain reaction (PCR). An experienced pathologist can very accurately predict which bursas have active infection and are good candidates for viral detection.

We submit one of the two frozen sample pools recommended by the pathologist for PCR and sequencing of VP2. Now it gets more complicated. How do you pick the IBD strains that will be the best autogenous vaccine candidates? Basically, we look for significant changes in the hypervariable regions of VP2, and frankly we rely on the advice of experts like Drs. Jackwood, Sellers and Cookson. Once it's decided which strains to use, we retrieve the final frozen bursa pools in the freezer to submit to the vaccine manufacturer to make the autogenous vaccine.

SANDER

Should there be a limit on the number of strains in an autogenous IBD vaccine?

SMITH

I don't know of a good rule of thumb for this, but there's a limited amount of space in that emulsion for antigens. I typically try to limit an autogenous vaccine to about three different antigens. This is where I'd like to hear the experts talk more about the selection process. Obviously, if we could set up a challenge, that would be the gold standard, but that's difficult to get done.

> "In an ideal world, it would be really nice to do the animal studies and not to be restricted to having to use isolates from your farm or your flock."
>
> HOLLY SELLERS, PHD

IBD DIAGNOSTIC AND REGULATORY CHALLENGES

SANDER

Dr. Sellers, what do you see in your laboratory that would be helpful regarding diagnostics and the decision about what type of vaccine to use?

SELLERS

What we see coming in from bursal surveys, representing the highest number of IBD cases submitted to the lab — at least from the US — fall into a category where they are very similar once we sequence the VP2 gene, which codes for the major antigenic protein of the IBD virus.

They're similar to variant viruses, but how similar is the question. We're not necessarily looking at the *percentage* of similarity, but we're looking to see *where* those changes occur within the VP2 protein. We then determine whether or not a particular group of viruses, having amino acid changes in the hypervariable region of VP2, are viruses appropriate for an autogenous vaccine.

You can't really just stop there. You might have a field isolate that's 98.5% similar to the Delaware E strain, but amino acid changes in VP2 are in critical regions and you're not going to get enough protection with the commercial vaccine.

We need to perform challenge studies to determine if commercial vaccines would provide adequate protection and to know what kind of IBD titers your birds need to be able to use a commercial vaccine versus an autogenous.

Unfortunately, time is the issue. You're taking samples, you're 2.5 months into the bursal survey and you've got your histopathology report. Virus isolation may take up to 3 weeks if multiple passages are needed to isolate the virus. Now you've got this data and you need to make decisions quickly. So maybe there's not time or animal facilities available for protection studies in birds.

So, we've done a lot of work up to a point, but now we are out of time or there aren't resources to do the type of work that needs to be done to evaluate the biological properties of these variant viruses that we're pulling out of birds. In an ideal world, it would be really nice to do the animal studies and not to be restricted to having to use isolates from your farm or your flock. You've got a virus that matches one that's already been described. These are killed vaccines. So, we're just running into time issues.

SMITH

I've got to jump in here because this has touched a nerve. The autogenous regulations found in Title 9 Code of Regulations (CFR) §113.113[2] really hamstring us. We're trying to use these vaccines for the right purpose. We're trying to ensure the health and welfare of our birds and decrease our reliance on antibiotics, and the regulations are inhibiting us.

Dr. Sellers mentioned time. The clock starts when you isolate the virus, then we've got all these other steps we have to do before we can make an informed decision and get the IBD virus into the hands of the manufacturer, and they've usually got a lead time of several months from receipt of the isolate(s) and production and release of the first serial.

The production of an autogenous product is restricted to 15 months from the date of

> "It goes back to timing when you're trying to decide which field viruses to put into your breeder program."
>
> DARAL JACKWOOD, PHD

isolation, or 12 months from the date of harvest of the first serial of product, whichever comes first. The administrator may authorize production of additional serials under special permit, and with considerable documentation, including an assessment of the continued involvement of the flock with the originally isolated organism, diagnostic work to support this assessment and evidence of efficacy of the previously produced product.

Simultaneously demonstrating continued involvement with the agent and efficacy of the previously used product is a bit of a catch-22 and can be problematic, although the agency generally has been accommodating in this regard.

The seed cannot be maintained by the establishment beyond the time of authorized use in production (12 months plus any extension). The expiration date of the product must not exceed 18 months from the date of harvest. So, with an initial term of 12 months, a secondary term of 12 months (although the length of the permitted extension is not specifically stated in Title 9 (CFR) §113.113) and an expiration date of 18 months, the maximum period of use could be up to 42 months (3.5 years). These temporal provisions of the regulation appear to be fairly inflexible and can be problematic.

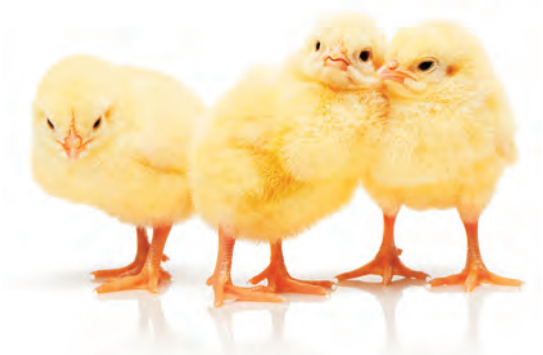
We're eating into the time we're able to use that isolate, and when it expires, you're done with it. You've got to throw it out and start over. It doesn't matter if it's still in the field, you've got to go and find it and get it again.

There's another issue. The seed must be isolated from sick or dead animals in the "herd of origin" only, but there are provisions for use in adjacent herds at risk and in non-adjacent herds with special permission — that requires formal application and considerable specific information regarding the adjacent and non-adjacent herds. Again, the agency has been fairly liberal and reasonable in the application of these extensions to adjacent and non-adjacent flocks in the poultry industry, but in view of the structure of our industry and our objectives in using these vaccines, this process of geographical extension should almost be a given and could be streamlined considerably.

If a neighbor has it, I think most of us have been very collegial in giving permission to use our isolates. We get a little relief there, but there still are a lot of issues with the regulations that really need to be examined, because they are inhibiting the proper and judicious and beneficial use of this technology. Thank you for letting me get that off my chest!

DALE

I'm glad Dr. Smith said that. A huge part of our frustration in the field is making a correct decision regarding which isolate to use, based on the available information we have, in a timely manner. When you're talking about using an autogenous



vaccine in your breeders, you're a year out before you see protection in your broilers. Too often by that time, the field challenge may have changed.

It's difficult to know whether you have the correct isolate. We see diversity sometimes even within a complex where only a certain percentage of the farms are affected, and you're trying to choose one to put in your whole production system.

JACKWOOD

It goes back to timing when you're trying to decide which field viruses to put into your breeder program.

When did you isolate that virus? Did you find the virus at 35 days or 14 days? If it's a 14-day virus, that's the one breaking through maternal immunity, but you've really got to look closely at the sequence to be sure.

When you start to generate a database and get to know what viruses are there, you can start to work with a farm. You can say, "Okay, we've seen this sequence before and it's never been a problem, so what else might be going on with your breeder program?" Or, "Hey, this is a new virus and it's breaking through in broilers at 10 days of age. You've got to get this strain in your breeder program." I don't care where the mutations are — the timing is telling me this is a problem virus.

Now, let's go back to mutations. As Dr. Sellers said, these viruses can be 98.5% alike and still be antigenically different enough due to the location of the mutations. One or two amino acids in the right place can make a big difference with these viruses, particularly in the field. If it makes a difference in the laboratory setting with vaccine-challenge studies, it's going to make an even bigger difference in the field.

COOKSON

I'm going to go out on a limb and guess that the vast majority of the autogenous formulations for IBD have the variant AL2 in them.

We conducted a survey about 5 years ago that included data from hundreds of farms from California to the Delmarva Peninsula. We had over 100 IBD isolates. About half were exactly AL2.

I want to reiterate what Dr. Sellers said. You can't look at percent homology per se; you have to look at all of the amino acid mutations, especially in the hyper-variable region — the four peaks. And especially in Peak B. That's where I think 80%, at least, of the antigen expression is being driven, from Peak B.

If you want an autogenous vaccine, you probably want AL2 in it, and if you haven't isolated AL2, which is unlikely, I would say keep pulling samples until you do.

If you're not going to rely on an autogenous vaccine, you probably want to build a program that gives you solid protection against the Delaware E strain of IBD and try to get AL2 cross protection as good as you can from your conventional program.

JACKWOOD

To expand on what Dr. Cookson just said — when you start looking at surveys across the US, you'll see we don't have what we had years and years ago, and that's one virus that dominates the entire US.

Based on samples coming into my lab, we oftentimes see pockets of viruses that have evolved because they are geographically isolated. For example, in Ohio, which I know the best, we've got several small broiler companies that really don't interact much with the rest of the broiler industry because they're in Ohio, and they have some unique bursal disease viruses in their flocks that we don't see anywhere else in the world.

When you start looking across the US at a lot of the smaller farms, you'll see a huge variety of IBD viruses.

SANDER

Challenge studies would be the ideal way to demonstrate the potential efficacy of vaccines, but since they are unrealistic for most producers, what else can be done to determine if their IBD vaccine program is working?

BURLESON

The bursal surveys as Dr. Smith discussed are helpful for assessing bursal health. I reserve challenge studies for when I want to change the IBD program for breeders.

JACKWOOD

Bursal surveys are an excellent way to assess bursal health. Here in the US, the IBD viruses infecting our flocks are causing subclinical disease — so there aren't any clinical signs. The only way you'll know you have IBD is by examining the bursa to see how large they are.

Tracking overall flock performance can be helpful. When you start seeing things like uneven flocks or poor feed efficiency, slower growth — those kinds of things — that's a sign that it's time to consider immune suppression and bursal surveys.

DALE

Before we even initiate a bursal survey, we look at overall performance. Obviously, there are a lot of other conditions to rule out. If performance starts slipping, we're having late mortality or increased condemnations at the plant due to opportunistic, secondary-type infections and I see colibacillosis or a secondary-type respiratory infection, I look for underlying bursal issues and conduct a bursal survey.

RAY OF HOPE

SANDER

Does anyone anticipate an improvement in the situation with autogenous vaccines?

JACKWOOD

Let me offer a ray of hope. The USDA's Center for Veterinary Biologics (CVB) now has a new licensing category for vaccines — it's called a "conditional license" for platform vaccines (VS Memorandum No. 800.213). These are genetically engineered vaccines. Most of the time they're virus-like particles or they're

> "Before we even initiate a bursal survey, we look at overall performance."
>
> ELIZABETH DALE, DVM

combinations of proteins that are assembled together, and they can be very, very effective vaccines because they match exactly what the virus looks like.

They are essentially the outer shell of the virus and have no genetic material inside; they are non-replicating and behave like a killed viral vaccine.

If you find that virus on a farm and you make a virus-like particle to it, you can then take that back to the farm and use it in your breeder program. You can also take that to a farm in another state and use it in their breeder program if you found that same viral sequence there. You're not limited by time on these, either.

Once you've found a unique IBDV strain and you're using that conditionally licensed vaccine, the CVB memorandum states that you can use it indefinitely in your breeders.

This is something I think is going to make a big change in the broiler industry, particularly in the killed-vaccine industry for breeders. Hopefully, we'll get away from autogenous vaccines.

SANDER

What about the efficacy of these vaccines?

JACKWOOD

Based on what I've seen in not only our lab but in other research labs, vaccines produced with *in vitro* protein-expression systems are just as good as the bursa-derived killed products.

In some ways, they're better because you don't have to kill them with something, which can alter proteins and antigens. They're not live vaccines and they don't have to be treated with any chemicals, yet you have an antigen that exactly matches the field virus.

SANDER

When might these vaccines be commercially available?

JACKWOOD

That depends on how quickly the CVB can approve the first one coming through. Some of these have already been approved and are on the market for swine and horses. It's the exact same licensing procedure for poultry.

REOVIRUS

> "Humoral immunity is the cornerstone for protection against reovirus."
>
> HOLLY SELLERS, PHD

SANDER

Let's turn our focus to reovirus. Dr. Sellers, how does this disease affect the immune system? What are the consequences of infection, and are they worse in flocks raised with no or fewer antibiotics?

SELLERS

Humoral immunity is the cornerstone for protection against reovirus. However, we don't know the extent of immunosuppression that various reoviruses cause. Some pathogenic chicken reoviruses cause incredible lymphocyte depletion in the bursa. Other versions of the virus cause viral arthritis and tenosynovitis, yet the bursas look okay. Therefore, I don't know we can say that all reoviruses have the ability to affect the humoral immune system equally.

I don't have any scientific evidence about whether or not the consequences of reovirus infection are worse in flocks raised with or without antibiotics. We do know these viruses replicate in the gastrointestinal tract before they cause viremia; if gut health is poor in flocks that are raised without any antibiotics and a pathogen comes in, it may take a higher toll, but there's no direct evidence there.

SANDER

Dr. Sellers, I understand that, in 2011, you saw an increase in clinical cases of viral arthritis or tenosynovitis due to reovirus. Why do you think that occurred?

SELLERS

Everyone asks that and I don't have an answer. We know we had an increase in reovirus among both chickens and in turkeys in the US, and an increase was also seen in Asia, South America, Europe, parts of Africa and Canada.[3]

Some of the reoviruses we isolated from the US matched some of the viruses characterized in other countries and in other situations. There are viruses unique to geographic regions, but I don't know where they came from. It just appears that they all came at the same time.

SANDER

Dr. Zavala, you do a lot of international travel. Are you seeing any kind of difference in reovirus isolations in your travels?

ZAVALA

I'll second what Dr. Sellers just said. It's interesting — I was still at the University of Georgia around 2011 to 2013, which was my last year there and when we saw a lot of reovirus cases from many companies.

In the beginning, we thought it might be breed related, but then we also saw it in other breeds or breed crosses. I then started traveling quite a bit more to other countries including Canada, Mexico, Peru, Ecuador, Chile and Brazil, and lo and behold, there were increased problems with reovirus. Countries in North-Central Europe started having problems with it all of a sudden, even including countries that never reported having clinical reovirus tenosynovitis before.

continued

> “...there was a huge serological shift, and all of a sudden, we’ve got multiple new serotypes.”
>
> JOHN SMITH, DVM

We never evaluated immunosuppression. But I found it fascinating that when we started submitting clinical samples to Dr. Sellers’ lab, at least from a molecular point of view, the isolates were extremely different from what we were used to seeing, and they were extremely similar to the variants she’d been identifying in the US, Canada and other countries.

When you have a problem that occurs in multiple countries at the same time, you’ve got to think about what things are common among those affected. I’m just going to leave it at that.

COOKSON

Vertical transmission of reovirus. That’s one thing that’s very different about this virus versus IBD virus, and it’s what makes reovirus even more challenging for us to deal with, both from an epidemiological standpoint and for control with vaccination.

ZAVALA

This applies to other disease agents as well. Around 2006, we started seeing a lot more ILT in the southeastern US and in some other countries after 15, perhaps 18, years of not having seen a single case of ILT.

How did it get there? It’s one of those mysteries I don’t have an explanation for, but theoretically you could, little by little, have problems with disease agents that can be transmitted vertically or transported indirectly by contamination of egg shells or chick boxes.

SMITH

It’s remarkable and unusual that reovirus was stable for decades, and the same vaccine worked very well. Then there was a huge serological shift, and all of a sudden, we’ve got multiple new serotypes. Something has fundamentally changed and I have no idea what it is.

JACKWOOD

Bursal disease did the same thing. Both reoviruses and IBD viruses are double-stranded RNA viruses. They mutate but not like a bronchitis or avian influenza virus, which are single-stranded RNA viruses that can mutate very fast compared to the double-stranded RNA viruses.

Now, all viruses mutate and it’s random. What’s different is the selection. You get random mutations but selection for just certain ones. The selections are for mutations that give that virus the ability to survive in the environment or out-compete its ancestors, which are basically its cousins. That’s what happened with IBD virus in the 1980s when very virulent IBD virus became a problem. This is probably what happened with reovirus.

REOVIRUS VACCINATION

SANDER

Dr. Burleson, please describe the reovirus vaccination plan you currently use.

BURLESON

I want to take care of the classic reoviruses first, using commercially available products. We typically give our live vaccines on day 1, and then at 2 weeks and 6 weeks of age. We then follow that with a killed IBD-reovirus vaccine at 12 and 21 weeks of age.

We also have a routine program with an autogenous reovirus vaccine that has

multiple variant reovirus serotypes. Whenever it comes time to make a new autogenous vaccine, I'll typically test the waters to see what contemporary isolates are out there to include in our autogenous vaccine in an effort to stay proactive.

As mentioned, when you have a problem, reovirus is bad, and it's bad for a long time until you get adequate protection. You have to stay on the offensive with autogenous vaccines.

We've also found we want to inject pullets as early as possible with the autogenous vaccine since the virus can be vertically transmitted. We want protection of that breeder bird as soon as possible.

SELLERS

I agree with Dr. Smith that our commercial vaccines have really been a success story because they have been so successful for decades. Commercial reovirus vaccines worked well and protection achieved by priming with a live, attenuated vaccine, followed by a boost with a homologous, inactivated vaccine. The number of live and inactivated vaccinations varies by company, but the strategy of live-prime/boost provided good protection.

What's different now is that we are missing the homologous, live-vaccine component (to help protect against and prime for variant strains).

If you couple that with the timing of vaccination — I'm aware of vaccination programs with three commercial live vaccinations followed by one commercial plus autogenous. Some programs include just two live vaccinations followed by two or three commercial plus autogenous in various combinations. What you're trying to do is extend the duration of immunity so that you can prevent vertical transmission, and we know there's a lot of that going on out there. You also want to be sure that chicks have adequate levels of maternal-derived antibodies to protect during the first week or so when they are most susceptible.

We also need to look at the production of reovirus autogenous vaccines. Perhaps there's a method of producing a reovirus vaccine that's superior, similar to what's been said about the bursa-derived vaccines for IBD.

The 1133 is great because it grows in fibroblasts. The variants don't grow very well in fibroblasts, and if they do, the titers are very low. Replication of the variant viruses in fibroblasts requires adaptation, and you can't predict how long this process will take. Again, this requires time and you're already at a deficit because the clock started ticking 3 months prior when the virus was isolated.

There are just a lot of variables making this even more painful for the industry. Couple that with having so many different viruses in the autogenous vaccine. It's really like a rogues' gallery. The number of reoviruses included in autogenous vaccines varies,

generally from two to four in any given serial. Selection of the appropriate isolate/isolates for inclusion in an autogenous vaccine is not always straightforward — unless you have clinical disease in a complex and all the reoviruses isolated from those farms are the same. The difficulty is when there are four or five different isolates, how do you pick the right one?

It's really complicated. And I don't really see a change in the number of samples we're receiving from clinically affected flocks over the course of the past 5 or 6 years. What I'm seeing is a change in the genetic and antigenic profile of the viruses. It's shifting pretty rapidly, and it's hard to predict or to make recommendations for what you need to use. It's really a best guess, absent putting those viruses back into birds, and then we come back to the race against time.

BURLESON

We're not willing to take out the autogenous vaccine now. We've found if we can prevent two houses of broilers from breaking with reovirus, we can pay for a year's worth of autogenous vaccine. So, we're going to leave it in there, but I don't know for how long.

ZAVALA

It's interesting to see how the industry has dramatically sero-converted, so to speak, in the last few years. However, things are starting to get back to normal, and I think the industry is starting to abandon, little by little, the use of autogenous vaccines.

Reovirus vaccination programs for broiler breeders is probably one of the most variable types of programs out there. There are companies that use only one live vaccine given in water, followed by one killed vaccine at about 15 to 16 weeks.

Others give three live and two killed plus one autogenous. The autogenous vaccine will contain two or three resident strains, which I think is probably one of the very best things that you can do. Of course, there's cost involved, but the expense is in the broiler, so you don't save money with your breeder program.

You really have to be as aggressive as possible, hoping you have strains in the vaccine that are as close as possible to what you have on broiler farms. If you don't, your other resource is to use as many vaccines as possible and to vaccinate as much as possible, and do something that hasn't been said yet — which is really work with your crews, supervise them and conduct serologic surveillance. That sounds very, very basic, but a lot of times we don't have time to do that and we just leave it up to them.

REOVIRUS SURVEILLANCE

SANDER

How are companies monitoring for reovirus status in their flocks? What are the best practices that can help ensure you're making your investment worthwhile?

ZAVALA

Serology is probably the most practical way to follow your flocks and determine if your vaccines are performing, since challenge work often isn't realistic. If you follow your flocks serologically, follow their performance and the health status of your progeny birds and you don't have a significant frequency of reovirus-like problems in the field, I think for the most part that gives you a good assessment.

> It helps a lot to take as much wind out of the variant's sails as possible before giving the killed vaccine.
>
> KALEN COOKSON, DVM

COOKSON

I'd want to look at seroconversion to reovirus in my flocks or just prior to the first killed injection to see what the live response is. You should have a good sense of what to expect from your pullets from your live-vaccine program.

If you have any outliers — a variant reovirus challenge — it's pretty clear you'll have a really big bump in titer response. That will give you an idea of what's out there and how early it's out there. Then it's time to reconsider your program. It might be too light on the live-vaccine side.

Granted, all we have are conventional, classic vaccines. But if you're just using the milder vaccines that are mass-applied, you're not really touching that immune system much. It's not really creating much effect and resistance to variant strains.

It helps a lot to take as much wind out of the variant's sails as possible before giving the killed vaccine. We've seen that even flocks infected at 6 to 8 weeks of age with these variant reoviruses can end up shedding in lay unless you have two really solid autogenous vaccines in there. I think that's why there's still a big opportunity for live-priming.

BURLESON

The good thing about reovirus diagnostics is that it's pretty classic. You know if your vaccination program is working or not because you typically have lesions — versus maybe IBD, which is a bit tougher — but with reovirus the tenosynovitis is usually obvious.

ZAVALA

That's right. Serology in broilers, however, hasn't been mentioned, but that's another good barometer of what might be circulating. It doesn't reflect things 100%, but when you have a problem, seroconversion tends to be a very magnified version of what you normally have. It's very quickly clear.

DALE

I've approached it from both ends at times. We look at post-live prime titers and also at post-killed vaccine titers before birds go into production. Sometimes you can catch on to something early, as Dr. Zavala alluded to earlier.

Those titers are interesting. Often your first sign of it is in the broilers or at the plant, even. We also look at broiler titers and sometimes actually start with broiler titers, then start looking at common source flocks and go back from there and try and find it.

This becomes a really maddening issue in production, and part of that reason is because it's sporadic in terms of where you're affected and in different regions. And it seems to continue to change.

I'll second Dr. Burleson's comment that usually you can diagnose reovirus in the field if you start looking at the right ages — between about 2 and 4 weeks of age in your broilers. However, I've found recently this is also changing. I've gotten some isolates from complexes where there are no obvious gross lesions or tenosynovitis at the time of active infection, but we've seen weight suppression and then tendon ruptures at older ages. It's getting harder to find the correct samples to take in broilers because now we're seeing more of these variants with much more subtle lesions during active infection. I don't know that we have looked explicitly at immunosuppression; we're not necessarily seeing secondary infections.

MAREK'S DISEASE

> "The dose of Marek's vaccine that will protect against tumor expression may not be enough to protect the immune system."
>
> GUILLERMO ZAVALA, DVM

SANDER

Turning to Marek's — I'd like Dr. Zavala to review how this disease affects the immune system.

ZAVALA

It's interesting that during the very first days post-infection in the field, the virus is going to start replicating very actively in birds that aren't properly protected. First, B cells and their ability to produce antibodies are affected. Initially, immunosuppression is transient in a way, but then it becomes more of a permanent inability to produce immunoglobulins.

After a few rounds of replication, the virus is going to infect T cells. They aren't going to be destroyed as effectively as B cells, but their function can be at least partially impaired.

T cells are critical. They can coordinate other actions of the immune system, such as T helper cells of different kinds, and they also can act as effector cells. In other words, they can directly attack either infected cells or pathogens themselves or even tumor cells.

Generally speaking, that's how Marek's affects the ability of the immune system to respond to just about any pathogen. If you affect B cells and T cells, you're effectively damaging almost the entire ability of the immune system.

SANDER

What about the consequences of Marek's virus in flocks raised without antibiotics or with fewer antibiotics?

ZAVALA

As far as flocks raised in systems with fewer or no antibiotics, it becomes more critical than ever to protect the immune system because that's the only thing you can do to help that baby chick and growing bird to cope with just about any pathogen, whether that be a mycoplasma, a bacterium, protozoa, etc.

I should note here that recent research confirms something we've suspected for a long time: The dose of Marek's vaccine that will protect against tumor expression may not be enough to protect the immune system. In other words, when you fractionate doses of Marek's disease vaccine, you may be avoiding tumors at the plant, but you may not be protecting adequately against immunosuppression.

SANDER

Are you seeing variation in the problems that Marek's disease causes in different regions or different areas?

ZAVALA

Historically in the US, we've had geographic pockets or states where Marek's tends to be more of a problem based on condemnations. That's probably the most effective barometer we have, if you will, industry-wide. Granted, Marek's isn't the only disease that leads to condemnations, and condemnations are also affected by differences in the inspection process at different plants.

Worldwide, Marek's tends to be more of a problem when companies either have technical problems in the way they apply the vaccines or the way they store, reconstitute or apply the vaccines versus true vaccine failures.

The other problem occurs when companies fractionate doses to save

> "Because of the efficacy of available vaccines, we've gotten to the point where Marek's is a non-issue in our company."
>
> MARK BURLESON, DVM

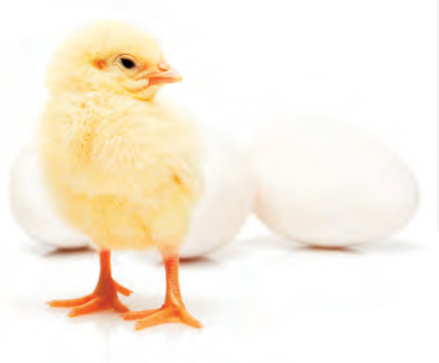
money. You can fractionate doses to a certain extent without hurting protection against neoplastic changes or condemnations due to tumors, but it's very difficult to assess at what point you are hurting yourself in terms of affecting the immune system.

SANDER

I'd like to hear your experience with classic Marek's vaccination versus recombinants and the degree of protection you get with each.

DALE

Most of my experience is with the recombinants. As Dr. Zavala noted, I'd advise making sure the full dose is used. In addition, there are times in higher challenge regions when SB1 needs to be added to the HVT. That seems to give good overall coverage.



BURLESON

Because of the efficacy of available vaccines, we've gotten to the point where Marek's is a non-issue in our company. We have a system in place where we administer only HVT in half of our operations; in the other half of the operations, we use both HVT and SB1. We just don't deal with Marek's condemnations anymore.

SMITH

We've likewise found the recombinants to be effective for control of Marek's disease.

ZAVALA

I'll share something interesting. Before I left the University of Georgia, we conducted a relatively small pilot study to see if we could find more septicemia/toxemia and cellulitis in birds that had less detectable HVT vaccine in the spleen. There was a very clear trend.

Birds with cellulitis and condemnations due to septicemia/toxemia tended to have a lower concentration of HVT vaccine DNA in the spleen. Birds that were healthy tended to have a higher copy number of HVT genomes in the spleen.

I'm not sure what that means exactly and I'm not sure that finding could be repeated, but it was an interesting observation. It would be interesting to find out exactly what dose is needed to protect the immune system so you don't have a variation in your condemnation rate.

JACKWOOD

There was an interesting study from Washington State University published back in 1980[4] about how bursal disease virus infection affects antibodies produced to an HVT vaccine. When birds received a full dose of an HVT vaccine and were then challenged with bursal disease virus at 1 day of age or 21 days of age, it didn't matter — the amount of antibody produced to Marek's disease was lower in birds that got bursal disease virus. So, if antibody titers to HVT are being lowered by bursal disease and not available to stop the Marek's disease infection, that could be one of the things that might be going on. In other words, even a full dose of HVT may not be protective if the bird is challenged — immune suppressed — with IBD virus by 21 days of age.

WRAPPING UP

WRAPPING UP

SANDER

Please sum up the impact the trend toward antibiotic-free production and reduced use of antibiotics has had on the industry?

BURLESON

It's caused poultry veterinarians to be more proactive in our approach. As an example, take gentamicin. When it's in your hatchery, you tend to be more reactive. If mortality at 7 days is high — say 1% or 1.5% — you work your way back through the system, find the problem, fix it and you move on.

Without gentamicin, the same problem will be much worse in terms of mortality — as high as 2% to 3%. In that situation, you find yourself thinking through issues and putting stronger standard operating procedures (SOPs) in place so it won't happen again. So, I'd say for us, removing antibiotics has caused us to be more proactive versus reactive.

DALE

My experience has been similar and I agree; the push toward antibiotic-free production has really prompted us to be more meticulous in every aspect of the entire process. Even though our area of the profession has always focused on preventative health, this trend makes us even more so.

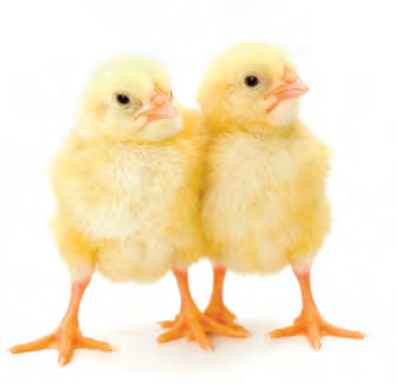
It's interesting that removing antibiotics seems to have a cumulative impact. By taking gentamicin out of the hatchery, for example, everyone's very focused on 7-day mortality. But I believe I've seen more of a difference later in life regarding bacterial infections and flock livability.

In our case, it's improved our hatcheries greatly. It's been surprising that a couple of complexes raising birds without antibiotics are top-performing in their production class. We've seen the same thing in our organic complex. It's beating our conventional birds of the same size. It has a lot to do with the management that's put into place when you are raising birds without antibiotics.

ZAVALA

I've worked with a number of poultry companies in the US and other regions of the world and have found one of the first things they realize is that transitioning to antibiotic-free production is not like turning a switch to get instant results. It's a process that will require sometimes years of hard work and standardization of their procedures.

Companies realize very, very quickly that with the aid of antimicrobial drugs, they work with whatever birds they get, whether it's in the hatchery or the broiler house. They don't ask questions. Withdrawing antibiotics has forced the industry to be much more stringent about their process and requirements in general. You can't operate with floor eggs the way you used to. You cannot overlook hygienic issues in nests the way you used to.



> "...if we're honest, abandoning the use of antibiotics puts more pressure on us to further reduce stress."
>
> JOHN SMITH, DVM

SANDER

Beyond protecting flocks from the immunosuppressive diseases we've discussed, what else can be done to build strong immunity in flocks, especially those that are raised without antibiotics? Are there other management practices we need to consider more closely?

BURLESON

Remember that it starts at the breeder farm. As I've mentioned, we establish SOPs that build in attention to detail. This would include egg-handling procedures at the farm as well as embryo temperatures, tray wash sanitation and rectal temperatures of chicks coming out of the hatchers. The SOPs that are established should be aimed at ensuring good chick quality by making sure people do the same thing every time, the right way. That way, you know that if you put out a good chick, it already has a good head start.

SMITH

That's all true. There's no magic bullet. It's elbow grease and hard work and attention to detail.

It's ventilation. It's litter management. It's lighting programs and water sanitation. Just the nuts and bolts of raising a healthy chicken, including a high-quality diet.

Something that I think gets overlooked is hatchery maintenance. The need for cleanliness should be obvious, but you've also got to have the correct air flows, temperature and humidity in the hallways so the machines don't have to work as hard. The machines have to be maintained. That includes the fan blades, shrouds and the turning mechanisms.

The times birds are pulled need to be just right to make sure optimal chicks are placed.

I've found built-up litter is actually protective. I examined new versus built-up litter as risk factors, and it's quite clear that the risk is greater with new litter.[5]

Breed can have a tremendous impact. So can grower selection.

We know that stress is immunosuppressive, so you have to do everything you can to reduce stress for the bird. We'd like to think we're always doing that, but if we're honest, abandoning the use of antibiotics puts more pressure on us to further reduce stress.

DALE

It absolutely starts on the breeder farm, and it's all the things Dr. Smith mentioned. If you're putting out a good-quality chick, it makes the whole rest of the process in the life of that bird easier.

I'll echo that and reiterate Dr. Zavala's point that the transition to antibiotic-free production is a process. Where we have been successful thus far can be attributed to starting the process in advance of making the transition, with attention to those things Dr. Smith mentioned are necessary for raising a healthy chicken: ventilation, litter depth and so on.

It's true that even in a conventional complex that's been running on short down time, especially for a long period of time, I can isolate viruses all day long. All these management considerations are needed and help ensure vaccines are able to do their job.

WRAPPING UP

> “The choice between antibiotic-free production or reduced antibiotic use and conventional production should remain.”
>
> ELIZABETH DALE, DVM

If you're transitioning to antibiotic-free production, start early and make the investment on the front end in your hatcheries. Make sure all other aspects of management are in good order. If you do that well in advance, it should be a smoother transition.

ZAVALA

I would like to remind everyone that antibiotic-free production is the way the market is going and there's no way back, whether we agree or disagree. We all know that.

It's not, however, because the industry was producing chickens with antibiotics, because there's a withdrawal period that ensures there are no residues in the poultry products the public gets. The industry was not selling chicken that wasn't wholesome. There are many other reasons why it was decided that this is the avenue to go.

DALE

That's really important, and I'll add one more thing. The choice between antibiotic-free production or reduced antibiotic use and conventional production should remain. There shouldn't be shaming within the industry regarding the different production types. At my company, we have all three types of systems. Let's not have animosity among companies for whichever production system they choose. That choice, hopefully, will be maintained in the marketplace as well.

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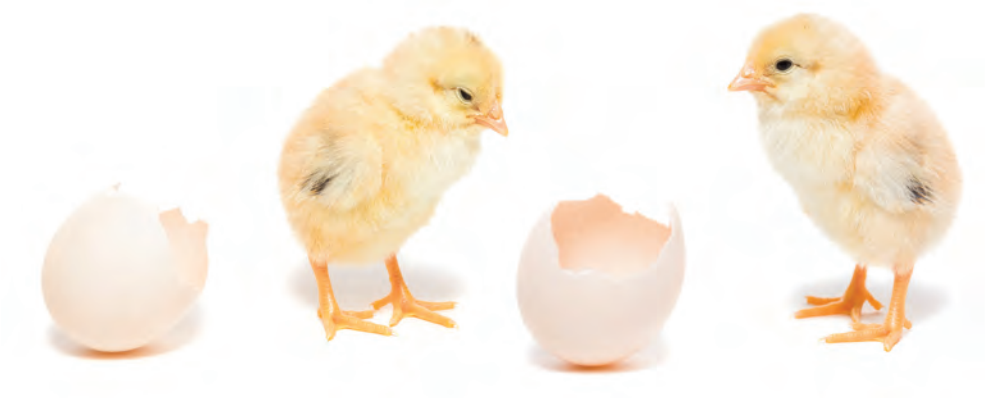

“There’s no magic bullet.
It’s elbow grease and hard work
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JOHN SMITH, DVM

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