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from the president…

Dave Zacek, President of Lohmann Animal Health International, Gainesville, Georgia, USA

Employees are the most valuable asset for any company. When the company is in the business of producing highly technical biotech products like LAHI, it becomes even more important that the correct people with the correct credentials and standards are "in the driver's seat." To ensure we have the right leadership, we have recently promoted three people in our company.

Wayne Collins, recently named Vice President, Sales, has been with the company more than five years. Prior to employment with LAHI, Wayne was global head of sales for an avian diagnostic supplier. Wayne's formal education is agriculture and microbiology. His experience is business, sales and marketing in the avian microbiological field. Wayne has charge of all sales, customer service and technical service for the Americas. In addition, Wayne contributes to global sales management via coordination of activities with our parent company LAH in Germany. Wayne's team of Area Managers, Veterinarians and Customer Service Coordinators work in Maine, New Jersey and Georgia. Wayne's passion for his task, experience and abilities are valuable assets for LAHI and for our customers.

Diana Rafuse, recently named Vice President Finance and Administration, has been with the company more than six years. Diana used the company-supported education system to gain her MBA credentials while working full time. She has moved from her IT beginnings into finance and now has a key role in oversight and management of company operations in America. One of Diana's groups plans vaccine production for the company to meet customer needs on time and in the correct form. Diana's work is linked directly to our parent company, LAH in Germany, and collaborates weekly with personnel at that site. One could say that Diana's focus is internal excellence while Wayne concentrates on external excellence.

Karen Heredia was very recently named Director of Regulatory and Quality Assurance. In her new leadership role, she will have the chance to apply many ideas she's developed through her seven years employment with the company. Karen's job is to assure that LAHI complies with national and international regulations. In addition, Karen must assure that company quality standards are met at every level in the organization. Karen and her team are directly involved in all phases of making and delivering LAHI avian vaccines to the marketplace in a form that exceeds both customer and company expectations.

We are very proud of these three corporate executives. All are on the company Management Team. This group directs the company operations in the Americas. These top people are here for you, our customers. So, remember, they are YOUR people, too.

Lohmann Animal Health International is a leading manufacturer and supplier of poultry biologics for the U.S. and international vaccine markets. Based in Gainesville, GA, LAHI maintains production facilities in Vineland, NJ, and Waterville, ME. LAHI is a member of the PHW Group, a 32-company enterprise that produces avian vaccines and poultry for the global market.

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Effect of Different Levels of Maternally Derived Antibodies on Protection Against Infectious Bursal Disease Virus

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Editors Note: This is an edited version of the complete article. For a copy of the full article and references please refer to Avian Diseases, which is reprinted with permission of the editor.

Infectious bursal disease (IBD) is an acute, highly contagious disease of young chickens, widespread in poultry producing areas around the world. It is considered of major economic significance to the poultry industry. The virus is able to destroy the precursors of antibody-producing cells in the bursa of Fabricius, particularly immunoglobulin B-bearing B lymphocytes. Subclinical infections recognized in susceptible chicks at less than 3 weeks old are characterized by bursal atrophy, resulting in severe immunosuppression.

Immunosuppression increases the susceptibility of chicks to opportunistic microorganisms in the environment and lowers responsiveness to vaccination. Biosecurity is an important measure in the prevention of avian diseases; however, IBD virus (IBDV) is highly resistant to adverse environmental conditions, resulting in its persistence in chicken houses; therefore, the control of this disease is based mainly on vaccination.

To ensure availability of maternal antibodies for the hatching chicks, breeder flocks are vaccinated with an oil emulsion vaccine after priming with live IBDV vaccines. This strategy is important in controlling the disease because maternal immunity can protect the newly hatched chicks during the first few weeks of life and thus prevent immunosuppression. However, different vaccination programs and management practices are used by different producers and growers. Therefore, flocks in different operations may have different antibody titers and, hence, different maternally derived antibodies in their progeny.

The main objective of this study was to determine how long different levels of maternally derived antibodies can protect chicks from experimental challenge with IBDV.

Materials and Methods

Fertile eggs were obtained from three different broiler breeder flocks. One originated from a non-vaccinated antibody-free university flock where eggs had no maternal antibodies (NAB). A second group of eggs had high levels of maternal antibodies identified (HAB). These eggs originated from hens that were vaccinated during the growing period with live IBD vaccines containing classic strains in the drinking water at 2, 4, and 6 weeks of age. In addition, the hens were vaccinated at 12 and 18 weeks of age with an inactivated vaccine containing classic and variant strains derived from tissue culture and bursal tissue. The third group of eggs had a medium level of maternal antibodies (MAB) and originated from a breeder flock that was vaccinated for IBD, but details of the vaccination program were unavailable. The eggs were incubated and hatched at our facilities.

The yolks of ten individual eggs from each flock were tested separately to determine their antibody content. Eggs were opened and the yolk was separated from the albumin. One milliliter of yolk was placed in a centrifuge tube and combined with either 1 ml of phosphate-buffered saline (PBS) or 1 ml of PBS and 1 ml of chloroform and mixed thoroughly. The mixture was allowed to set for 30 min. at room temperature, then centrifuged, and the upper aqueous clear layer was kept at 4 C for testing.

The IN variant serotype 1 IBDV, which was isolated at our laboratory and continually maintained in bursal tissue, was used for challenge. It was propagated in 3-week-old specific-pathogen-free chicks by the intranasal/intraocular route of inoculation. The bursas were harvested and homogenized. The dose of the challenge virus was adjusted to 10^2 EID_{50}/chick and given by intranasal/intraocular routes at 1, 2, and 4 weeks of age.

Ten chicks from each of the NAB, MAB, and HAB flocks were challenged with 10^2 EID_{50} of the challenge virus/chick at 1, 2, and 4 weeks of age, respectively. Ten chicks from the NAB flock served as negative controls at each challenge period. The chicks were bled at 1 day of age, prior to challenge, and at 5 and 11 days post-challenge for serology. The VN test was utilized to determine serum antibody levels. The procedure used a constant-virus-diluting-serum microtiter in BGM-70 cell culture with the IN virus as the antigen. The results were used to calculate the geometric mean titers (GMTs). Each group of chicks was necropsied at 5 and 11 days PC. The organ/body weight (BW) ratios were calculated and the bursas were evaluated histologically. After euthanasia, the bursa and spleen were

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removed from each chick and weighed. The bursa and spleen BW ratio was determined with the following formula: organ weight in grams x 1000/total body weight in grams. Bursas were fixed in 10% buffered formalin solution, tissues were processed, stained with hematoxylin and eosin stain and examined microscopically for histopathologic lesions. Each bursa was scored by a modification of the numerical scoring system described by Rosales *et al*. as follows: 0 = normal bursa (no lesions), 1 = mild scattered cell depletion in a few follicles, 2 = mild to moderate atrophy or cell depletion in 1/3 to 1/2 of the follicles, 3 = mild to moderate atrophy or cell depletion in > 1/3 of the follicles, and 4 = severe atrophy of all follicles.

Groups with a mean bursa/BW ratio and mean histologic score similar to that of the negative controls were considered to have full or "complete" protection. Groups with a mean bursa/BW ratio and a mean histologic score similar to the NAB-challenged chicks (fully susceptible positive controls) were considered unprotected.

Results

The GMT of VN yolk antibodies to IBDV in the three flocks tested were: NAB = <1:10, MAB = 1:975, and HAB = 1:3365, with a range of 1:200-1600 for the MAB and 1:1600-6400 for the HAB. There was no significant difference in these titers when the two methods of yolk extraction were used. The GMTs of VN serum antibodies to IBDV in the day-old chicks from the three flocks tested before challenge were <1:10, 1:875, and 1:3275.

The average organ/BW ratios at 5 and 11 days PC are shown in Table 1. When chicks were challenged at 1 week of age, the average bursa/BW ratios of the chicks with no maternally derived antibodies showed a statistically significant difference ($P < 0.05$) from the non-challenged controls at 5 and 11 days PC, whereas chicks with medium and high levels of maternally derived antibodies did not show any statistically significant difference from the non-challenged controls at 5 and 11 days PC. The spleen/BW ratios were similar to the controls at 5 and 11 days PC. When chicks were challenged at 2 weeks of age, the same response was seen as in those challenged at 1 week of age; only chicks with no maternally derived antibodies were significantly different from the non-challenged controls. The spleen/BW ratio was significantly different only in chicks with NAB at 5 days PC. In the chicks challenged at 4 weeks of age, those with HAB had average bursa/BW ratios that were significantly different from chicks with no maternal antibodies and those with medium level maternal antibodies, but similar to the controls at 5 and 11 days PC. The spleen/BW ratios were significantly different only in chicks with NAB at 5 and 11 days PC. There was no mortality in any of the challenged groups, and only birds from the NAB group were depressed after challenge.

The histopathologic lesion scores in the bursas at 5 and 11 days PC are presented in Table 1. Chicks with no maternal antibodies had a score of 4 at all ages tested. Chicks with medium antibodies had a score of 0 when examined at 1 and 2 weeks of age but not at 4 weeks of age, where they had a score of 4. Chicks with high levels of maternal antibodies had a score of 0 when examined at 1 and 2 weeks of age and a score of 0.3 (which indicates focal, mild scattered cell depletion in one to two out of five bursas) when examined at 4 weeks of age.

The GMTs of the VN antibodies for the three groups before and after challenge are shown in Figs. 2, 3 and 4, respectively. In chicks challenged at 1 and 2 weeks of age (Figs. 2, 3), chicks with high and medium levels of maternal antibodies had a decline in titer over time after challenge, whereas chicks with no maternal antibodies had a gradual increase in titer. In chicks challenged at 4 weeks of age, there was a decline in titer over time after challenge (Fig. 4), whereas chicks with medium or no maternal antibodies had a gradual increase in antibody titers.

Discussion

The results of this study indicated that the decay of maternally derived antibody is approximately linear. The calculated half-life for IBDV antibodies in white leghorn chickens was reported as 3 days, 6-8 days, 6.7 days, and 4 days in previous studies. In our study with broiler chickens, the half-life was calculated as 5.5 days.

Chicks with no maternal antibodies were susceptible to the virus at all ages tested, as was illustrated by the decrease in the bursa/BW ratios and the diffuse atrophy of all the bursal follicles. The challenge virus stimulated a VN titer that started to appear at 5 days PC.

Chicks with medium levels of maternal antibodies were refractory to infection at 1 and 2 weeks of age, as was demonstrated again in the average bursa/BW ratios, which were similar to the controls, the normal microscopic appearance of the bursal tissues, and the decline of the VN titers after 5 days PC. However, at 4 weeks of age, the maternal antibodies in those chicks had already declined, the VN titer was <1:10, and a response similar to that in chicks with no maternal antibody was observed.

Chicks with high levels of maternal antibodies were resistant to the virus when challenged at 1, 2, and 4 weeks of age, as indicated by the absence of bursal atrophy, the normal histology of the bursal tissues, and the continuous decline of the VN titers at 5 days PC.

These results indicated that high levels of maternal antibodies (GMT of VN = 1:3275) at hatch were protective for at least 5.5 weeks. Also, a GMT of the VN serum antibody of 1:200 before challenge at 4 weeks of age was protective. Comparison of the results of the bursa/BW and the spleen/BW ratios revealed that the bursa/BW ratios were sufficient for evaluating the change induced by the virus. One important feature of this study is the simultaneous inclusion of eggs originating from breeder flocks with different levels of antibodies.

In conclusion, these results demonstrated the important role of maternally derived antibodies and their titers in protecting the newly hatched chicks against IBDV when exposed to the virus at different ages. The level of VN maternal antibodies is a convenient tool to use for predicting when the chicks will be

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susceptible to the disease and to plan vaccination strategies accordingly. We opted to use the VN test in this study because results from that test are more reliable than the commonly used ELISA, because the VN test is specific for the type virus used in the test whereas the viruses used in the ELISA might not distinguish between serotypes of the virus.

Figures

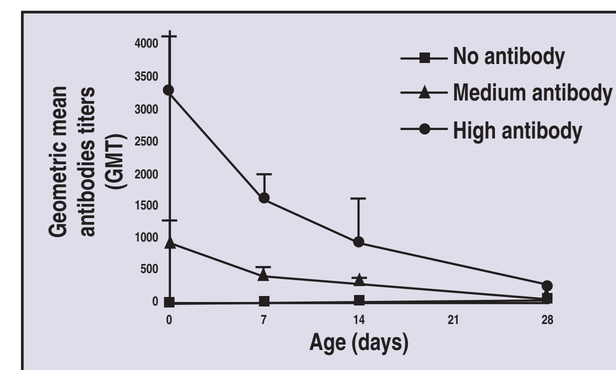

Fig. 1. Geometric mean titer of virus neutralizing antibodies in chicks from three flocks before challenge

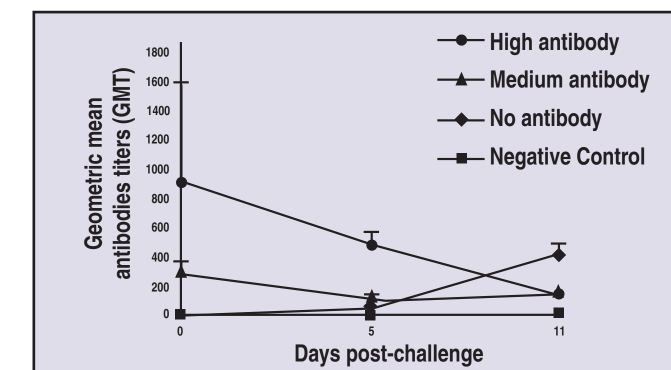

Fig. 3. Geometric mean titer of virus neutralizing antibodies for group 2 challenged at 2 weeks of age

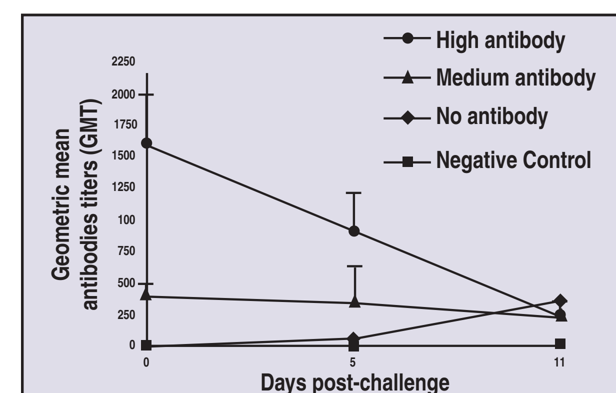

Fig. 2. Geometric mean titer of virus neutralizing antibodies for group 1 challenged at 1 week of age

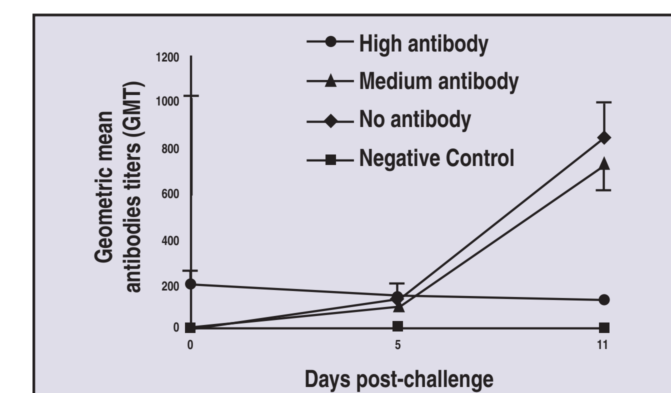

Fig. 4. Geometric mean titer of virus neutralizing antibodies for group 3 challenged at 4 weeks of age

Tables

Table 1. Response of chicks with different levels of maternally derived antibodies to challenge with IN variant strain of IBDV at different ages

		Average organ/BW ratio at days PC[C]					
		Bursa/BW		Spleen/BW		Bursal leasion score[D] at days PC	
Age[A] (wk)	Experimental Group[B]	5 days	11 days	5 days	11 days	5 days	11 days
1	NAB	1.08[b]	0.99[b]	1.26[c]	1.32[c]	4[a]	4[a]
	MAB	2.69[a]	2.54[a]	0.62[c]	0.76[c]	0[b]	0[b]
	HAB	2.44[a]	2.46[a]	0.70[c]	0.80[c]	0[b]	0[b]
	C	2.38[a]	2.66[a]	1.05[c]	0.90[c]	0[b]	0[b]
2	NAB	0.99[b]	0.73[b]	1.87[d]	1.13[c]	4[a]	4[a]
	MAB	2.76[a]	3.11[a]	0.78[c]	0.74[c]	0[b]	0[b]
	HAB	3.28[a]	3.08[a]	0.95[c]	0.80[c]	0[b]	0[b]
	C	2.70[a]	2.53[a]	0.91[c]	0.95[c]	0[b]	0[b]
4	NAB	1.08[b]	0.85[b]	1.74[d]	1.58[d]	4[a]	4[a]
	MAB	1.51[b]	0.88[b]	1.52[c]	1.18[c]	4[a]	4[a]
	HAB	2.77[a]	3.30[a]	0.93[c]	1.35[c]	0[b]	0.3[b]
	C	3.07[a]	2.60[a]	1.06[c]	1.06[c]	0[b]	0[b]

[A] Age when challenged.
[B] NAB = no maternal antibody (vn = <1:10); MAB = medium level maternal antibodies (VN = 1:200 – 1600);
HAB = high level maternal antibodies (VN = 1:1600 – 6400); C = NAB non challenged control negative.
[C] Values are average for five chickens. Values within a column followed by different lowercase superscript letters are significantly different ($P < 0.05$).
[D] Mean bursal lesion scores: 0 = no lesion; 1 = mild scattered cell depletion in a few follicles; 2 = mild to moderate atrophy or cell depletion in 1/3 to 1/2 of the follicles; 3 = mild to moderate atrophy or cell depletion in > 3/4 of the follicles; 4 = severe atrophy of all follicles.