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Short communication

Comparison of two commercial ELISA kits for serological monitoring of avian encephalomyelitis in a reproductive turkey flock

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Abstract

Avian encephalomyelitis (AE) is a viral disease of poultry. Although the disease has a milder clinical course in turkeys than in chickens, reproductive flocks of turkeys are vaccinated against AE. Commercial AE ELISA kits are specifically designed for chickens, which makes it difficult to implement these tests in serological monitoring of turkey flocks. The aim of the study was to compare the AE serological results provided by two ELISA kits from different producers when testing an AE-vaccinated flock of turkey hens and their progeny. We detected differences in the sensitivity of the ELISAs for testing specific anti-AE antibody levels in turkey serum samples.

Key words: reproductive turkey flock, ELISA, avian encephalomyelitis virus, serological monitoring

Introduction

Avian encephalomyelitis (AE) is an infectious disease of poultry caused by viruses belonging to the *Picornaviridae* family and *Tremovirus* genus. Infection with these viruses can occur either horizontally or vertically (Calnek 2008).

The avian species most susceptible to avian encephalomyelitis virus (AEV) infections are chickens and turkeys (Calnek 2008). The most severe clinical course of the disease is recorded in vertically infected chicks. In laying hens, AEV infection can lead to transient drops in egg production (Meroz 1990). High prevalence and problematic nature of AE has resulted in laying flocks being commonly vaccinated against AE with the use of live attenuated vaccines. Vaccination reduces production losses and virus transmission to eggs and stimulates the production of maternally derived

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Table 1. Avian encephalomyelitis (AE) serological results obtained with the use of two different ELISA kits for turkey hens serum samples collected from three turkey houses (W1, W2 and W4) at different time points.

Birds age (weeks)	Turkey house	IDEXX				BioChek			
		GMT	CV%	pos	neg	GMT	CV%	pos	neg
32	W1	802	94	18	5	3334	68	21	2
	W2	474	100	14	9	2587	69	20	3
	W4	619	89	16	7	3375	64	19	4
	Average	**631.66**	**94.33**	**16**	**7**	**3098.66**	**67**	**20**	**3**
38	W1	856	30	20	3	2122	20	20	3
	W2	1818	67	23	0	6075	27	23	0
	W4	1654	26	23	0	5606	13	23	0
	Average	**1442.66**	**41**	**22**	**1**	**4631**	**20**	**22**	**1**
42	W1	334	60	9	14	2116	68	23	0
	W2	225	66	6	17	1898	38	23	0
	W4	415	21	13	10	3152	14	23	0
	Average	**324.66**	**49**	**9**	**14**	**2388.66**	**40**	**23**	**0**

Table 2. AE serological results obtained with the use of IDEXX ELISA kit for turkey poults serum samples, with the calculation of the percentage of MDA transfer.

Birds age (weeks)	Turkey house	IDEXX					
		GMT	SD	pos	neg	hens GMT	MDA transfer (%)*
32	Poults W1	87	123	9	14	802	10.85
	Poults W2	68	122	7	16	474	14.35
	Poults W4	65	124	3	20	619	10.50
	Average	**73.33**	**123**	**6.33**	**16.66**	**631.66**	**11.90**
38	Poults W1	613	87	16	7	856	71.61
	Poults W2	383	111	10	13	1818	21.07
	Poults W4	796	96	16	7	1654	48.13
	Average	**597.33**	**98**	**14**	**9**	**1442.66**	**46.94**
42	Poults W1	44	120	11	12	334	13.17
	Poults W2	32	171	7	16	225	14.22
	Poults W4	31	127	8	15	415	7.47
	Average	**35.66**	**139.33**	**8.66**	**14.66**	**324.66**	**11.62**
						Mean transfer	**23.49**

* MDA transfer (%) was calculated with the folowing formula: (poults GMT/hens GMT) * 100%

antibodies (MDA) that protect offspring in the first weeks of their lives (Calnek 2008, Gharaibeh et al. 2008). Although the disease has a milder clinical course in turkeys, reproductive flocks of these birds are also regularly vaccinated against AE.

Vaccination effectiveness can be monitored serologically. Given that commercial AE-specific ELISA kits are specifically designed for chickens, there are difficulties in implementing these tests in serological monitoring of turkey flocks. An additional drawback of ELISA is that there are differences in the results obtained for turkeys when different commercial kits are used for the same samples, as the present authors have discovered.

The aim of the study was to evaluate the differences in the obtained results of mean AE-specific antibody titers in turkey hen serum samples collected at different stages of the laying period and analyzed with ELISA tests from two different manufacturers. Additionally, the experiment aimed to determine the level of transmission of MDA to the offspring.

Table 3. AE serological results obtained with the use of BioChek ELISA kit for turkey poults serum samples, with the calculation of the percentage of MDA transfer.

Birds age (weeks)	Turkey house	BioChek					
		GMT	SD	pos	neg	hens GMT	MDA transfer (%)*
32	Poults W1	2424	93	12	11	3334	72.71
	Poults W2	2069	111	12	11	2587	79.98
	Poults W4	702	111	9	14	3375	20.80
	Average	**1731.66**	**105**	**11**	**12**	**3098.66**	**57.83**
38	Poults W1	1344	107	15	8	2122	63.34
	Poults W2	3121	101	20	3	6075	51.37
	Poults W4	5321	80	22	1	5606	94.92
	Average	**3262**	**96**	**19**	**4**	**4631**	**69.88**
42	Poults W1	2051	98	15	8	2116	96.93
	Poults W2	1374	111	12	11	1898	72.39
	Poults W4	1585	110	13	10	3152	50.29
	Average	**1670**	**106.33**	**13.33**	**9.66**	**2388.66**	**73.20**
						Mean transfer	**66.97**

* MDA transfer (%) was calculated with the folowing formula: (poults GMT/hens GMT) * 100

Materials and Methods

Ethics Statement

According to information from the Local Ethics Committee in Olsztyn, no special approval was necessary for experiments performed under field conditions. Animal procedures and sample collection were performed as standard veterinary inspections.

Birds

Samples were collected from a reproductive flock of Hybrid Converter turkeys raised on a commercial farm in the Warmia and Mazury voivodeship, Poland. Hens were kept in 3 different houses (W1, W2 and W4) with approx. 2000 hens per house. The environmental and welfare conditions during entire production were in accordance with the guidelines of the manufacturer of the genetic line of the birds (Hybrid Turkeys, Poland). The flock was under constant veterinary supervision. Birds were vaccinated against AE with a live, attenuated Calnek 1143 AEV strain-based vaccine (Boehringer Ingelheim, Germany) at 11 weeks of age *via* drinking water, at a dose recommended by the vaccine producer. During rearing and the egg production cycle, the birds' health and production parameters were monitored daily.

Experimental layout

Blood for serological evaluation was collected from hens of each turkey house at 3 different time points (n=23). Samplings I, II and III were performed at 32, 38 and 42 weeks of life. Additionally, blood samples were collected from turkey poults hatched from eggs collected from the W1, W2 and W4 turkey houses at time points corresponding to the dates when hens were sampled. Twenty-three serum samples were collected from each batch of day-old poults.

Serological evaluation

Two commercial ELISA kits (IDEXX, USA and BioChek, Netherlands) were used to determine the levels of AE-specific IgY in serum samples. ELISAs were performed in accordance with producers instructions. Individual stages of the tests were performed with an epMotion 5075 LH automatic pipetting station (Eppendorf, Germany), an ELx405 automatic ELISA plate washer and an EL×800 ELISA plate reader (BioTek, USA). The mean geometric antibody titer (GMT), coefficient of variation (CV%) and the number of positive and negative samples were used for result presentation.

Results and Discussion

Production results in the analyzed flock didn't deviate from the standards specified for this genetic line of birds. No cases of infectious diseases were reported. Additionally, at the beginning of the laying period (at 32 weeks of age), samples were taken from birds for AEV testing and the results were negative (data not

shown). It allows to conclude that the recorded results should be considered physiological.

The serological results for the two different ELISAs from samples from laying hens and chicks, along with the calculation of MDA percentage transfer are summarized in Tables 1-3. We demonstrated higher mean antibody titers in turkey hens regardless of sampling date, and higher percentages of AE-positive samples in reproductive hens with the Biochek test than with the IDEXX equivalent (Table 1). Similar results were also obtained for turkey poults, in which higher AE-specific MDA titers and higher percentages of positive samples were detected with BioChek (Tables 2 and 3). Based on the results, we can conclude that the BioChek test offers higher sensitivity in the detection of AE-specific antibodies in turkey serum samples. The differences in the results obtained depending on the ELISA test used may be due to the use of different antigens for plate coating (which may have influenced the avidity of the antibodies in the tested serum samples), differences in the concentrations of antibodies in the conjugates used in these tests, and non-comparable specificities of binding of kit conjugates to turkey antibodies. Similarly disparate results obtained with the use of multiple ELISA kits have been observed under field practice conditions and documented on the basis of scientific studies (Marché and van den Berg 2010, Bauer et al. 2010). However, this does not change the fact that the publicly available data in this area are very few.

Interesting differences were noted in the level of MDA transfer calculated using the two ELISAs. The average AE MDA transfer was 23.49% estimated by the IDEXX assay and 66.97% using the BioChek test (Tables 2 and 3). The BioChek assay appears to be able to detect and quantify low levels of AE antibodies in turkey serum samples. Interestingly, we detected higher transfer of MDA in turkeys than Gharabeih et al. (2008) for chickens, who demonstrated 4.3% of AE MDA transfer from chickens to offspring. It is worth noting that cited authors used an ELISA from Synbiotics (USA) in their study. The present data show that in the conditions of veterinary practice, one should take into account the possibility of differences in the results of ELISA tests from different manufacturers, which may often be of key importance for the proper interpretation of these results.

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