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Immunoglobulin A in chickens. Comparison of total and immune-specific lacrimal and serum IgA levels in two lines of chickens immunized with Newcastle Disease Virus

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Introduction

Quantification of both total and immune specific IgA levels in saliva in humans has demonstrated negative correlations between perception of stress and IgA concentration (*Stone et al.* 1987, *Jemmott & Magloire* 1988).

There are few markers available for the assessment of prolonged stress and reduced welfare in animals and if secretory IgA levels were depressed in animals this finding might be used to develop non-invasive methods for quantifying long-term stress in animals.

We have initiated a series of studies analysing the generality of this correlation in other animal species. The intensive husbandry methods of chickens make it desirable to develop reliable non-invasive methods of assessing stress and low welfare in addition to behavioral studies, and the present study was designed to explore whether teardrops, which can be readily collected from chickens without the trauma of collecting blood, can be used as an alternative source of IgA.

IgA has been quantified in the serum and bile of chickens by radial immunodiffusion (*Leslie et al.* 1976) and ELISA (*Cihak et al.* 1992). In this work we used a specific and sensitive rocket immunoelectrophoretic method, which generally has a higher sensitivity than immunodiffusion assays and are characterized by small intra- and interassay cofficients of variation to quantify IgA. An advantage of this assay compared with ELISA assays is that when analysing samples from the saliva, serum and bile, the the environmemt of the antigen (IgA), is less likely to influence the results.

Virus-specific antibodies to infectious bronchitis virus (*Cook et al.* 1992) and to Newcastle Disease Virus (*Russell,* unpublished results) are higher in line C White leghorn chickens than in line 15I birds. We compared these two lines of birds for serum, biliary and lacrimal IgA after vaccination with Newcastle Disease Virus to demonstrate whether virus-specific IgA values correlated to total IgA values.

Materials and methods

Birds

White leghorn chickens of the Reaseheath C line with B4/4 alleles and the East Lansing 15I line with B15/15 alleles were bred from grandparent stock obtained from Houghton Poultry Research Station, England. Their parents were negative for antibodies to Newcastle disease virus and other infectious agents.

The chickens were housed in conventional animal rooms at a temperature of 2 +/– 2 C in a 12 hour light/12 hour dark light cycle. The animals were kept in single or double rabbit cages in group sizes declining with time to two birds per cage to comply with UK Home Office guidelines. The infected birds were kept in isolators under negative pressure. The birds had access to water and a commercial chicken feed *ad libitum.*

Tears were collected following application of a drop of glycerine which stimulated tear production and kept at –20 C until analysis.

Virus

Ulster 2C and Hitchner B1 strains of NDV were grown in the allantoic cavity of eggs to a titre of $10^{8.6}$ tissue culture infectious units (iu)/0.1 ml as assayed by an indirect immunoperoxidase monolayer assay (IPMA) with Hep-2 cells (*Russell* 1992). $10^{8.6}$ iu/0.1 ml represent about $10^{9.6}$ egg ID_{50}/0.1 ml.

Experiments

Line C and 15I birds of the same hatch were infected with $10^{9.4}$ iu of the Ulster-2C or Hitchner B1 strain of NDV. The virus was applied in 0.6 ml allantoic fluid between the eyes, nostril and mouth of line C and 15I birds of the same hatch. The birds were killed 10 days later when aged 65–75 days of age and the serum tears and cystic bile were collected for the determination of total IgA by rocket immunoelectrophoresis and for anti-NDV IgA by IPMA as previously described (*Russell* 1992).

Rocket immunoelectrophoresis

Rocket immunoelectrophoresis was performed as described by *Hau et al.* (1980). The antibody used was rabbit anti chicken IgA (Nordic, Tilberg, The Netherlands, Batch no. 3810) and the concentration in the gel was 1:100 (v/v). The sample wells received 5 µl samples of chicken tears, bile and serum.

Results

Lacrimal IgA which was specific to NDV was present in all the NDV-immune line C birds at a geometric mean titre of $10^{2.0+0.36}$ and was absent in the NDV-immune line 15I birds at $< 10^{1.5}$. Total lacrimal IgA values were marginally higher in NDV-immune line C compared to line 15I birds and significantly higher in the normal line C compared to line 15I birds (Table 1).

Serum IgA values did not differ significantly between lines or between immunized and non-immunized birds (Table 1).

If the lacrimal IgA value was divided by the corresponding serum IgA value in order to compensate for differences in systemic IgA, the relative values were significantly higher both for virus-immune line C compared to virus-immune line 15I birds and for normal line C compared to normal line 15I birds (Table 1). Line C birds therefore contained more lacrimal IgA than line 15I birds irrespective of infection with NDV (Table 1).

Bile was analyzed from the NDV-immune birds and neither its level of IgA nor its titre of IgA specific to NDV differed between line C and line 15I birds. Bile and serum IgA were therefore the same in both lines of birds whereas lacrimal IgA was not (Table 1).

Discussion

Both lines of White leghorn chickens had a similar immunospecific titre of IgA with specificities against NDV in the bile. However, line C also had a measurable titre in tears, whereas line 15I had not.

The differences in virus-specific lacrimal IgA between line 15I and line C birds were associated with similar differences in total lacrimal IgA. The lacrimal IgA concentration in line 15I birds being only half that of line C birds. This indicates that if lacrimal IgA is to be used as a measure of stress, birds of the same genotype need to be compared.

The differences in lacrimal IgA were not indicative of difference in circulating IgA because serum levels were similar in all groups of birds and the level of immunospecific IgA molecules with specificities against NDV was below the detection limit of the assay in all samples analysed. Serum IgA is actively secreted into the bile (*Peppard et al.* 1986), and the differences in immunospecific IgA in the tears of the two lines can not have been reflected in serum, albeit below the detection limit, bacause biliary IgA levels were not significantly different in the two lines.

The harderian gland (HG) of the nictitating membrane is the major source of lacrimal IgA (*Baba et al.* 1988). The difference in NDV-specific lacrimal IgA between line C and line 15I birds could not be reproducibly associated with differences in the number of

Table 1. The level of IgA in line C and line 15I birds and in NDV-immune line C and line 15I birds.

Exp.	Line of birds	Age of birds in days	Strain of NDV in inoculum[a]	Total IgA in arbitary units				Log 10 titre of IgA to NDV[b]		
				Tears	Serum	Bile	Tears/ Serum	Tears	Serum	Bile
1	C	65	Ulster	17	23	650	0.74	2.5	<1.5	3.0
	C	65	Ulster	17	17	850	1.00	2.0	<1.5	3.0
	C	65	Ulster	20	20	1300	1.00	2.0	<1.5	3.0
	C	72	Hitchner B1	10	26	850	0.39	1.5	<1.5	3.0
	C	72	Hitchner B1	20	20	450	1.00	2.0	<1.5	3.0
	C	72	Hitchner B1	37	20	750	1.90	2.0	<1.5	3.0
				20.2[d]	*21.0*	*775*	*0.99*[c]	*2.0*	*<1.5*	*3.0*
	15I	67	Ulster	1	23	750	0.04	<1.5	<1.5	3.0
	15I	67	Ulster	5	20	300	0.25	<1.5	<1.5	4.5
	15I	67	Ulster	10	20	750	0.50	<1.5	<1.5	3.0
	15I	75	Hitchner B1	5	32	1150	0.16	<1.5	<1.5	2.0
	15I	75	Hitchner B1	26	42	2050	0.62	<1.5	<1.5	2.0
	15I	75	Hitchner B1	15	29	1300	0.52	<1.5	<1.5	2.0
				10.3[d]	*21.7*	*1050*	*0.35*[c]	*<1.5*	*<1.5*	*2.8*
2	C	70	None	26	20		1.30	<1.5	<1.5	
	C	70	None	41	15		2.73	<1.5	<1.5	
	C	70	None	26	20		1.30	<1.5	<1.5	
				31.0[c]	*18.3*		*1.77*[c]	*<1.5*	*<1.5*	
3	15I	70	None	5	15		0.33	<1.5	<1.5	
	15I	70	None	8	15		0.53	<1.5	<1.5	
	15I	70	None	8	20		0.40	<1.5	<1.5	
	15I	70	None	5	20		0.25	<1.5	<1.5	
	15I	70	None	15	15		1.00	<1.5	<1.5	
				8.2[c]	*19*		*0.50*[c]	*<1.5*	*<1.5*	

[a] NDV, Newcastle the disease virus. NDV was inoculated 10 days earlier by the oculotopical route.
[b] IgA, measured by an indirect immunoperoxidase monolayer assay.
[c] Significance difference between line C and line 15I groups $p < 0.02$ by unpaired students 't' test.
[d] Marginal difference between line C and line 15I group by unpaired students 't' test $0.05 < p < 0.1$.

antibody forming cells secreting IgA to NDV in the HG (*Russell,* unpublished results) and so differences in the transport of IgA into the lacrimal fluid or its dilution by the lacrimal fluid may also contribute to the differences between line C and line 15I. Dilution by the lacrimal fluid is less likely because virus-specific IgG titres in the tears and serum were the same from the line C and 15I birds (*Russell,* unpublished results).

Cihak et al. (1992) found IgA level to be 43-fold higher in the bile compared to the serum and we also found an identical 43-fold difference for virus immune birds (Table 1). Biliary IgA would therefore be the most accurate measure of central IgA production in the chicken if birds were to be sacrified. Emptying of the gall bladder occurs during feeding and so bile collection may have to be standardised to 12 hours after starvation as utilised in a study of the ontogeny of IgA production (*Leslie et al.* 1976).

Cihak et al. (1992) showed that the depletion of T cells with the Vβ1 receptor from chickens led to a diminution of IgA but not IgG when biliary IgA decreased by 8,000 fold and serum IgA decreased by 15 fold. Stress is generally regarded to depress the cellular immune system more than the hu-

moral immune system (*Manser* 1992) and T cells may therefore be one link between stress level and IgA level.

In future experiments lacrimal and serum IgA will be compared in inbred birds stressed by housing conditions or inoculated with corticosteroids in place of stress to assess which cource of IgA is a better indicator of stress.

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Summary

In the human lower than normal concentrations of IgA in saliva have been found to be associated with stress.

In studies of animal welfare and prolonged stress there is a need for non-invasive methods and assays of secretory IgA would be simple to develop for many species. In the present study we studied IgA in the chicken and compared levels of IgA in tears, serum and bile. Two strains of chickens were compared. The concentration of IgA in the lacrimal fluid of line C chickens was two-fold higher than that of line 15I chickens. Levels of IgA in their serum and bile where the same. Following on from the replication of avirulent Newcastle disease virus (NDV) in the Harderian gland of the nictitating membrane line C chickens contained NDV – specific lacrimal IgA to a titre of 10^2 whereas line 15I chickens contained none at $10^{-1.5}$. Both lines contained NDV-specific IgA in their bile to a titre of 10^3. Line 15I chickens therefore have a selective deficiency in lacrimal IgA compared to biliary IgA.

Sammendrag

Studier af studenter har vist, at stress giver anledning til lavere end normale niveauer af IgA i spyt. I studier af dyrevelfærd og vedvarende stress hos dyr er der et behov for non-invasive metoder og assays til måling af sekretorisk IgA er forholdsvis enkle at udvikle for mange arter. I nærværende studie undersøgte vi IgA i kyllinger og sammenlignede niveauet i tårer, serum og galde. To stammer af kyllinger blev sammenlignet. Koncentrationen af IgA i tårevæsken af linie C kyllinger var to gange højere end i linie 15I. Niveauet af IgA i deres serum og galde var det samme. Efter immunisering med Newcastle disease virus (NDV) fandtes signifikant højere immunspecifik IgA titer i tårevæsken hos linie C sammenlignet med linie 15I. Begge linier havde samme koncentration i galdevæske.

Yhteenveto / K. Pelkonen

Ihmisessä on havaittu, että stressiin liittyy syljessä alentunut IgA:n määrä. Eläinten hyvinvoinnin ja pitkäaikaisen stressin tutkimuksessa olisi käyttöä ei-invasiivisille menetelmille ja olisi helppoa kehittää monille lajeille sopiva sekretorisen IgA:n määriyusmenetelmä. Tässä tutkimuksessa selvitettiin kanan IgA:n määriä ja verrattiin IgA:n tasoja kyynelnesteessä, seerumissa ja sapessa. Tutkimuksessa verrattiin kahta eri kanakantaa. C-linjassa IgN:n väkevyys kyynelnesteessä oli kaksi kertaa korkeampi kuin linjassa 15I. Seerumin ja sapen IgA-tasot olivat samoja. Harderian rauhasen avirulentin Newcastlen virus- (NDV)-infektion jälkeen C-linjen kanojen kyynelnesteessä NDV-spesifisen IgA:n tiitteri 10^2, mutta 15I-linjan kanoissa se oli alle $10^{-1.5}$. Molemmissa linjoissa NDV-specifisen IgA:n tiitteri sapessa oli 10^3. Näinollen linjan 15I kanoilla on kyynelnesteessä sappeen verrattuna selektiivinen IgA-puutos.

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