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GENETIC AND ENVIRONMENTAL EFFECTS ON THE RESPONSE OF CHICKENS TO AVIAN ADENOVIRUS GROUP II INFECTION

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SUMMARY

The effects of husbandry procedures on an intravenous challenge with avian adenovirus group II were studied in lines of White Leghorns selected for high (HH) and low (LL) antibody response to sheep erythrocytes and their reciprocal crosses (HL and LH). Husbandry procedures were deprivation or no deprivation of water during the first week after hatching (ES), habituation or no habituation to humans throughout life, and flock stability or instability (LS) 24 hours prior to an intravenous challenge with avian adenovirus group II.

Spleens of chickens were weighed 6 days after challenge. HL chicks were most susceptible and LH chicks were most resistant to avian adenovirus group II. Chicks subjected only to ES or no stress responded similarly and were less affected than those subjected to LS or to both ES and LS. There were major genetic-environment interactions which affected spleen size.

INTRODUCTION

Genetic and environmental variables effect the resistance of chickens to infectious diseases. Lines of chickens selected for high (HH) or low (LL) antibody titres to sheep erythrocytes (Siegel and Gross, 1980) respond differently to infectious diseases (Gross and Siegel, 1980). Freeman (1987) suggested that measurement of pathological sequences was appropriate to investigations into stress. Generally more stressful environments enhance resistance to bacterial diseases and decrease resistance to viral diseases (Gross, 1984). The effects of a stressor during the first week post hatch (ES) may persist for at least 18 weeks. Social stressors and the feeding of diets containing corticosterone decrease antibody response to antigens (Gross and Siegel, 1973). Also human-animal relationships may influence animal productivity and physiological responses (Gonyou *et al.*, 1986; Hemsworth *et al.*, 1986). Socialization of chickens to their handlers increases antibody response to

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antigens and resistance to *E. coli* and decreases the effects of stressors (Gross and Siegel, 1979; Gross and Siegel, 1982). Also, the quality of long term environment will influence antibody responsiveness (Gross, 1986).

An avian adenovirus group II infection of chickens, characterised by splenomegaly and no other lesions, was identified by Domermuth *et al.*, (1979). Splenic lesions consist of reticuloendothelial hyperplasia and lymphocytic degeneration (Veit *et al.*, 1981). The infection was found to be common throughout the USA (Domermuth *et al.*, 1980). Subsequently it was shown that the presence of avian adenovirus group II infection in turkeys predisposes them to secondary *Escherichia coli* infection (Larsen *et al.*, 1985).

The purpose of this experiment was to measure the effects that stress early in life, social stress before challenge, chicken-handler relationships and the long term environment had on the severity of the response to avian adenovirus group II challenge infection in selected lines of chickens and their reciprocal crosses.

MATERIALS AND METHODS

Stocks and husbandry

The chickens were obtained from matings of White Leghorn lines selectively bred for 12 generations for either a high (H) or low (L) antibody response to sheep erythrocytes (Siegel and Gross, 1980). Matings included the parental lines (HH and LL) and their reciprocal crosses (HL and LH). (In denoting matings the line of the sire is given first followed by that of the dam.) The parents of the chicks were known to be free from avian adenovirus group II. Chicks were brooded in isolation in groups of 28 to 32 individuals until 4 weeks of age when they were transferred to Horsfall-Bauer type cages and maintained as groups of eight individuals. Each of these groups contained two individuals from each of the four mating combinations. (HH, HL, LH and LL) and approximately equal numbers of both sexes.

Adenovirus group II inoculum

The standard turkey vaccine isolate of avian adenovirus group II (AA) (Domermuth *et al.*, 1977) was stored at -40°C. The inoculum had an oral ID_{50} of $10^{4 \cdot 4}$ per ml. For these experiments 0.1 ml of a 10^{-2} dilution of the frozen material was administered via the intravenous route when chickens were 6 weeks of age. There are no signs of infection and the only observed lesion was splenomegaly. Six days after inoculation, which is the time of the maximal spleen size (Domermuth *et al.*, 1979), chicks were weighed and their spleens were removed and weighed. An enlarged spleen was considered as being >0.26% of body weight. Enlarged spleens were not seen in uninoculated chickens (0.16 ± 0.03% of body weight).

Environments

Upon hatching chicks from each stock were assigned at random into the environments. The 'good' environment consisted of gently handling and socialising the chicks to humans daily from day of hatch to 48 days of age. In the 'poor' environment chicks were subjected to quick movements by the handler and unusual sounds, never speaking to them and providing minimal human contact. In no case were the chicks physically mistreated.

There were two stressors of shorter duration. The presence or absence of stress (water withheld for 8 hours daily for 4 days) during the first week after hatching

(ES), and presence or absence of social stress one day before AA challenge (LS). Each experimental cage contained four pairs of chicks representing each of the four mating combinations (HH, HL, LH and LL). One chick from each pair was subjected to ES (total = 8 chicks per cage). The chicks in one group of 16 cages were subjected to the 'good' quality environment. The chicks in eight of these cages were subjected to LS one day before AA challenge while those in the other group of eight cages were not. In another group of 16 cages, chicks were subjected to the 'poor' quality environment. In eight of these cages chicks were subjected to LS 1 day before AA challenge while those in the other eight cases were not. There were three trials with a total of 768 chicks in a 4 x 2 x 2 x 2 factorial.

Statistical analysis

Spleen weights were expressed as a ratio body weight and transformed to $\sqrt{}$ arc-sin for normalising prior to analysis of variance. Preliminary analysis showed that trials and sexes were similar within the 'good' and 'poor' environments and these data were pooled for subsequent analyses. Interactions were significant (Table 1) and subsequent analyses were made to evaluate stocks and short term stressors within the long term environments (*i.e.* 'good' and 'poor'). When significance was found multiple means were separated by Duncan's multiple range test.

RESULTS

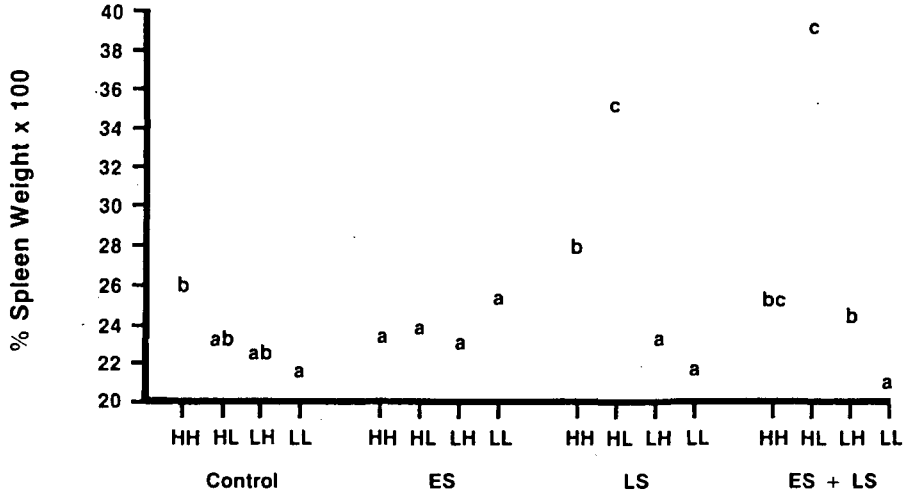
Table 1. Analysis of variance of spleen weights by long-term environment

Source of variation	df	Quality of environment	
		Good	Poor
Genotype (G)	3	0.0535**	0.0290**
Stressor (S)	3	0.1462**	0.0106**
G x S	9	0.0540**	0.0432**
Error	368	0.0035	0.0025

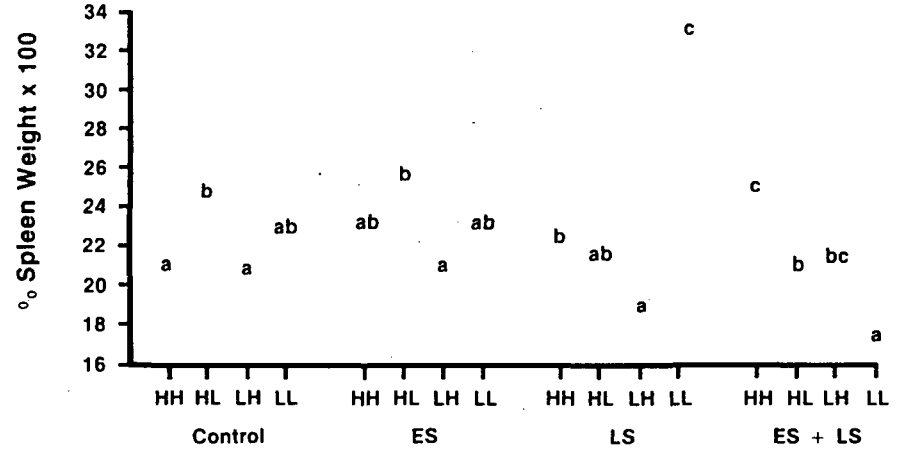
Data transformed to $\sqrt{}$ arc sin of ratios prior to analysis. ($P<0.01$).

Among chicks not subjected to ES or LS (Text-fig. 1), those from line HH had larger spleens than those from line LL (26% compared with 21%). Among those exposed to only ES there were no differences due to genotype. A LS before challenge resulted in larger spleens for HL than for HH chickens (35 *vs* 29). Spleens were smaller for LH and LL than HH chicks (23 and 22 *vs* 29). When subjected to both ES and LS before challenge, spleens for cross HL were larger than those for line HH (37 *vs* 26), while those for line LL were smaller than those of line HH (21 *vs* 26).

Spleens from chicks (Text-fig. 2) not subjected to ES or LS were larger for HL than HH and LH (25 *vs* 21). Among those subjected only to ES, spleens were larger for HL than for LH chicks (26 *vs* 19). When subjected to LS, spleens were larger for LL than HH chicks (33 *vs* 22). Under the same circumstances LH chicks had smaller spleens than those from line HH chickens (19 *vs* 22). When exposed to both ES and LS, spleens were larger for HH than HL chicks (25 *vs* 21). Under the same circumstances spleens were larger for LH than LL chicks (17 *vs* 21).



Text-fig. 1. Effect of combinations of stressors early in life (ES), stressors before challenge (LS) and genotype on spleen weight of avian adenovirus type II infected chickens (24/group) held in a long term 'good' quality environment. Letters (a, b, c) indicate mean spleen weights. Means within different environmental groupings with different letters are significantly different (P<0.05).



Text-fig. 2. Effect of combinations of stressors early in life (ES), stress before challenge (LS) and genotype on spleen size of avian adenovirus type II infected chickens (24/group) kept in a long term 'poor' quality environment. Letters (a, b, c) indicate mean spleen weights. Means within different environmental groupings with different letters are significantly different (P<0.05).

Spleen size of the four genotypes within both long term environments ('good' and 'poor' quality) are presented in Table 2. In the 'good' environment HL chicks had the largest spleens. Spleens of HH chicks were larger than those from line LL which did not differ from those of LH chicks. In the relatively 'poor' environment LH chicks had the smallest spleens while there were no differences among chicks of the other genotypes. Across both environments HL and LH chicks had the largest and smallest spleens respectively with the parental lines being intermediate and different from each other.

Table 2. Mean spleen weights (% of body weight x 100) and long term environment in chicks inoculated intravenously with avian adenovirus group II

Genotype	Environment					
	Good		Poor		Pooled	
	n	Mean	*n*	Mean	*n*	Mean
HL	96	30.2c	96	23.6b	192	27.1d
HH	96	25.8b	96	23.7b	192	24.5c
LL	96	22.3a	96	24.5b	192	23.3b
LH	96	23.5a	96	20.5a	192	22.1a

Means in a column followed by the same letter are not different ($P<0.05$).

Spleen size of the four combinations of short term stressors with both long term environments ('good' and 'poor' quality) are presented in Table 3. In the 'good' quality environment chicks exposed to LS the day before challenge had larger spleens than those remaining in socially stable flocks. In the 'poor' quality environment chicks exposed to either ES or LS alone had larger spleens than those exposed to both ES and LS. When exposed to both ES and LS chicks exposed to the 'good' quality environment had larger spleens than those exposed to the 'poor' quality environment. Among all experiments, only those exposed only to LS had spleens which were larger than those which were exposed to neither ES nor LS.

Table 3. Mean spleen weight (% of body weight x 100) by stress early in life (ES) stress before challenge (LS) and long term environment in chicks inoculated intravenously with avian adenovirus group II infected chickens

ES	LS	Long term environment					
		Good		Poor		Pooled	
		n	Mean	*n*	Mean	*n*	Mean
–	–	96	23.5a	96	22.8ab	192	23.2a
+	–	96	24.1a	96	23.5b	192	23.8ab
–	+	96	27.0b	96	24.0b	192	25.5c
+	+	96	27.3b	96	21.4a	192	24.4b

Means in a column followed by the same letter are not different ($P<0.05$).

DISCUSSION

Results of this experiment show that the magnitude of response of chickens to an AA infection, as measured by spleen size, is influenced by their genetic background and both the long and short term environmental history of the population. Experimental variables that were used could occur in flocks with or without the knowledge of the researcher. Under specific conditions a genotype may be especially susceptible, *e.g.* LL chickens exposed only to LS and a 'poor' quality long term environment or HL chickens exposed to LS and a 'good' quality long term environment. Although the chickens used in these trials were from experimental populations, it is not unreasonable to assume that similar genotypes exist in commercial stocks.

With regard to antibody responsiveness to sheep erythrocyte antigen, lines HH and LL develop the highest and lowest titres respectively, with the crosses being intermediate (Siegel and Gross, 1980). This finding suggests that mode of inheritance of antibody responsiveness to sheep erythrocytes cannot be used to predict a chicken's defence against AA. That the reciprocal cross HL and LH were the most susceptible and most resistant to the challenge respectively, provides evidence that differences between crosses cannot necessarily be predicted from the level of resistance of the parental lines. When reciprocal crosses bracket the parental lines, there is the possibility that sex-linked and maternal effects are working jointly for the particular mating combination to enhance resistance and susceptibility depending on the sire and dam line of the crosses. This thesis has been discussed in detail by Nordskog and Pevzner (1977) and demonstrates the need in commercial breeding programmes for comparing reciprocal crosses for differences in disease resistance. The pattern for one agent might not be the same as for another. For example, when these lines and crosses were challenged with *E. coli* HL was most resistant and LH was most susceptible with LL and HH being intermediate (W.B. Gross, unpublished data).

In spite of daily monitoring, the most difficult aspect of this experiment was maintaining the long term environments. Stress is a relative term and responses noted vary among genotypes and individuals. The results show that genotype-environment combinations may result in relatively high or low levels of resistance to specific disease producing agents. They demonstrate that knowledge of the genetic and environmental history of a population would be valuable to researchers in understanding experimental results.

REFERENCES

Domermuth, C.H., Gross, W.B., Douglas, C.S., DuBose, R.T., Harris, J.R. and Davis, R.B. (1977). Vaccination for hemorrhagic enteritis of turkeys. *Avian Diseases, 21: 557-565.*

Domermuth, C.H., Harris, J.R., Gross, W.B. and DuBose, R.T. (1979). Naturally occurring infection of chickens with a hemorrhagic enteritis/marble spleen disease type of virus. *Avian Diseases, 23: 479-484.*

Domermuth, C.H., Weston, C.R., Cowen, B.S., Colwell, W.M., Gross, W.B. and DuBose, R.T. (1980). Incidence and distribution of avian adenovirus group II splenomegaly of chickens. *Avian Diseases, 24: 592-594.*

Freeman, B.M. (1987). The stress syndrome. *World's Poultry Science Journal, 43: 15-19.*

Gonyou, H.W., Hemsworth, P.H. and Barnette, S.C. (1986). Effects of frequent interactions with humans on growing pigs. *Applied Animal Behaviour Science, 16: 269-278.*

Gross, W.B. (1984). Effect of social stress severity on *Escherichia coli* challenge infection. *American Journal of Veterinary Research, 45: 2074-2077.*

Gross, W.B. (1986). Effect of dose of antigen and social environment on antibody response of high and low antibody response chickens. *Poultry Science, 65: 687-692.*

Gross, W.B. and Siegel, P.B. (1973). Effect of social stress and steroids on antibody production. *Avian Diseases, 17: 807-815.*

Gross, W.B. and Siegel, P.B. (1979). Adaptation of chickens to their handlers and experimental results. *Avian Diseases, 23: 708-714.*

Gross, W.B. and Siegel, P.B. (1980). Effects of early environmental stress on chicken body weight, antibody response to RBC antigens, feed efficiency and response to fasting. *Avian Diseases, 24: 569-579.*

Gross, W.B. and Siegel, P.B. (1982). Socialization as a factor in resistance to infection, feed efficiency and response to antigen in chickens. *American Journal of Veterinary Research, 43: 2010-2012.*

Hemsworth, P.H., Gonyou, H.W. and Dzluk, P.J. (1986). Human communication with pigs: the behaviour responses of pigs to specific human signals. *Applied Animal Behaviour Science, 15: 45-54.*

Larsen, C.T., Domermuth, C.H., Sponenberg, D.P. and Gross, W.B. (1985). Colibacillosis of turkeys exacerbated by hemorrhagic enteritis virus. Laboratory studies. *Avian Diseases, 29: 729-732.*

Nordskog, A.W. and Pevzner, I.Y. (1977). Sex-linked versus maternal antibodies in the genetic control of disease. *World Poultry Science Journal, 33: 21-30.*

Siegel, P.B. and Gross, W.B. (1980). Production and persistence of antibodies to sheep erythrocytes 1. Directional selection. *Poultry Science, 59: 1-5.*

Veit, H.P., Domermuth, C.H. and Gross, W.B. (1981). Histopathology of avian adenovirus group II splenomegaly of chickens. *Avian Diseases, 25: 866-873.*

RESUME

Effet de la génétique et de l'environnement sur la réponse des poulets à l'infection par l'adénovirus aviaire de groupe II

Les effets des conditions d'élevage sur les conséquences d'une épreuve par voie intraveineuse réalisée avec un adénovirus aviaire de groupe II ont été étudiés chez des lignées de Leghorn blanches sélectionnées pour leur réponse en anticorps élevée (HH) et faible (LL) vis-à-vis des globules rouges de mouton ainsi que chez les croisements réciproques de ces lignées (HL et LH). Les conditions d'élevage ont consisté en la suppression ou non de l'eau durant la première semaine suivant l'éclosion (ES), l'habitude à la présence ou non d'humains pendant leur vie, la stabilité ou non du troupeau (LS), 24 heures avant l'épreuve intraveineuse avec l'adénovirus aviaire de groupe II.

Les rates des poulets ont été pesées six jours après l'épreuve. Les poulets HL ont été plus sensibles et les poulets LH ont été les plus résistants vis-à-vis de l'adénovirus aviaire de groupe II. Les poulets soumis uniquement au stress ES ou à l'absence de stress, ont répondu d'une manière similaire et ont été moins affectés que ceux soumis au stress LS ou à la fois ES et LS.

ZUSAMMENFASSUNG

Genetische und Umwelteffekte auf die Reaktion von Hühnern gegenüber einer Infektion mit der Gruppe II des aviären Adenovirus

Die Wirkung von Haltungsmethoden auf eine intravenöse Testinfektion mit einem aviären Adenovirus der Gruppe II wurde bei weißen Leghornhühnerlinien untersucht, die in Bezug auf hohe (HH) und geringe (LL) Antikörperreaktion gegen

Schaferythrozyten selektiert worden waren, sowie bei ihren reziproken Kreuzungen (HL und LH). Zu den Haltungsmethoden gehörte Wasserentzug oder nicht während der esten Woche nach dem Schlupf (ES), während des Lebens Gewöhnung an Menschen oder nicht, Herdenstabilität oder Unstabilität (LS) 24 Stunden vor der intravenösen Testinfektion mit aviärem Adenovirus der Gruppe II.

Die Milzen aller Küken wurden sechs Tage nach der Testinfektion gewogen. Die Küken der HL Linien waren die empfindlichsten und die der LH Linie am resistentesten gegen das aviäre Adenovirus der Gruppe II. Küken, die nur ES erfuhren oder keinem Streβ ausgesetzt waren, reagierten ähnlich und waren weniger gestört als solche die LS oder ES und LS ausgesetzt waren.

RESUMEN

Efectos geneticos y ambientales sobre la respuesta de los pollos a la infeccion por adenovirus aviares del grupo II

Se estudiaron los efectos de los métodos de manejo sobre una inoculación intravenosa con adenovirus aviar del grupo II en líneas de White Leghorns seleccionadas por su respuesta serológica alta (HH) y baja (LL) a los eritrocitos de oveja y sus cruces recíprocos (HL y LH). Los métodos de manejo incluyeron la privación o no del agua durante la primera semana tras la eclosión (ES), la adaptación o no a la especie humana a lo largo de su vida, y la estabilidad o inestabilidad (LS) del grupo 24 horas antes de una inoculación intravenosa con adenovirus aviar del grupo II.

Se pesaron los bazos de los pollos seis días después de la inoculación. Los pollos HL fueron los más susceptibles y los LH los más resistentes al virus inoculado. Los pollos sometidos únicamente a privación del agua o sin estrés respondieron de forma similar y fueron menos afectados que los sometidos a LS o a ES y LS a la vez.