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Efficacy of infectious bronchitis virus vaccines against heterologous challenge

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Twenty-four-week-old white Leghorn layers were inoculated subcutaneously with a killed Newcastle disease-infectious bronchitis (Massachusetts type) virus (MIBV) vaccine. Twenty-eight weeks after vaccination, the birds were challenged intraocularly with the Arkansas strain of infectious bronchitis virus (AIBV) to determine the effects of heterologous virus exposure on egg production, egg quality and serum antibody response of the birds. The challenged hens laid significantly ($P<0\cdot005$) fewer eggs than the unchallenged layers. Eggs laid by the unchallenged groups weighed significantly more ($P<0\cdot005$) than those laid by the challenged groups. Further, the internal quality (Haugh units) and shell quality of eggs laid by the AIBV-challenged hens was significantly ($P<0\cdot005$) inferior to those from the unchallenged hens. In addition, the AIBV-challenged hens laid more soft-shell, misshapen and small eggs than the unchallenged hens. The Arkansas serum haemagglutination inhibition (AIBV-HI) titres of AIBV challenged birds increased up to four weeks after challenge. The corresponding MIBV haemagglutination-inhibition (MIBV-HI) titres decreased during the same period. The study indicates that killed MIBV vaccine offered no protection to birds exposed to heterologous AIBV.

AVIAN infectious bronchitis (IB) of domestic chickens was first reported from the United States (Schalk and Hawn 1931), but by the early 1960s IB had been identified all over the world. The IB virus (IBV), a coronavirus, is known to cause pathological changes in the respiratory tract, reproductive tract and kidneys in both laying hen and broiler chicken flocks (Cunningham 1970). The disease continues to be an economically significant problem despite the widespread use of vaccines (Hofstad 1981). The IB outbreaks occur both in vaccinated and unvaccinated flocks. These outbreaks are attributed to different strains of the causal virus, emergence of new antigenically distinct strains (Koch et al 1986), alteration in antigenic characteristics possibly as a result of prolonged exposure to live IBV vaccines (Cook 1983), lack of availability of a broad spectrum vaccine giving protection against many of the strains of IBV, decline of sufficient immunity to resist reinfection with similar IBV strain (Cunningham 1970) and poor correlation of humoral neutralising antibody titres to protection against reinfection with IBV (Raggi and Lee 1965). However, Gough and Alexander (1977) reported that the HI titres of 1:128 did provide resistance to a Massachusetts challenge evaluated by virus recovery from the trachea. The effects of IBV on the reproductive tract of laying chickens are economically significant. The effects observed are in the form of a drop in egg production, laying of eggs of unequal sizes and inferior internal and shell quality (McDougall 1968).

The Arkansas strain of infectious bronchitis virus (AIBV) is antigenically dissimilar to most of the endemic IBV types such as Massachusetts, Connecticut and JMK (Gelb et al 1983). However, a recent report by Hunton (1987) suggests that good antibody titres to Newcastle disease resulting from the use of LaSota strain vaccine and for bronchitis using the Massachusetts followed by a Holland strain vaccine, will give good protection against the pigeon paramyxovirus and the $Arkansas_{99}$ IB strain.

The purpose of the present study was to assess some of the previous reports on protection against various IBV strains and the capability of laying hens vaccinated with a killed Massachusetts infectious bronchitis virus (MIBV) vaccine to resist heterologous AIBV challenge at a time when they were still laying at a high level and the MIBV humoral antibody titres in vaccinated birds were significantly different from birds which did not receive killed MIBV vaccine as adults.

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Materials and methods

Experimental birds and vaccination

One hundred 24-week-old white Leghorn hens, previously vaccinated with a live Newcastle disease-infection bronchitis (Massachusetts type) vaccine at the age of two and six weeks, and laying at an average rate of approximately 50 per cent were obtained from the poultry science department, University of Minnesota. The chickens were randomly divided into four groups, each consisting of 25 birds. Each hen in two of the four groups was inoculated subcutaneously with 0·5 ml of a killed, oil emulsion Newcastle disease-infectious bronchitis (Massachusetts type) virus vaccine. The vaccine was manufactured by Intervet America and was stored at 4°C until used according to the manufacturer's instructions. The hens in the other two groups were not given this killed MIBV vaccine. The birds of one of the killed-vaccine inoculated groups, and one of the unvaccinated groups were challenged with AIBV, while those in other groups served as unchallenged controls. The chickens of all groups were housed four per cage (30×45 cm) up to 50 weeks old and were provided identical conditions of light, temperature and ad libitum feed and water. The four groups were housed separately. To determine prevaccination serum antibody titres against MIBV and AIBV, 10 birds from each of the four groups were randomly bled and their individual serum samples analysed by the haemagglutination-inhibition (HI) test using both Massachusetts and Arkansas antigens. The post-inoculation sera from vaccinated and unvaccinated birds were collected at intervals of seven, 14, 22, 52, 172, 194, 201, 208, 215, 222 and 231 days. The group-geometric mean serum antibody HI titres (GMT) for both MIBV and AIBV were determined.

The HI test was conducted in microtitre U bottom plates obtained from Gibco Laboratories. The HI test was carried out according to the procedure described by Lashgari and Newman (1984). Briefly, 25 μl of HI buffer was delivered to each well of the microtitre plate. A 25 μl amount of serum was added to the first well. Using a 25 μl multichannel microdiluter, serial twofold dilutions of the serum were carried out from wells 1 to 10. Well 11 was used as the antigen control and well 12 was used as an erythrocyte control. Twenty-five μl of IBV (Massachusetts or Arkansas type) antigen obtained from Spafas Laboratories containing 8 haemagglutination units per 50 μl was added to wells 1 to 11 and the plates were incubated at 37°C for one hour. A 50 μl amount of 0·5 per cent chicken erythrocytes was added to each well, and the results were recorded after incubating the plates at room temperature for minutes. The HI titre was the reciprocal of the highest serum dilution inhibiting haemagglutination.

Challenge virus

The challenge virus was the Arkansas type, DPI strain of IBV, obtained from Intervet America. The virus (ninth embryonic passage) was suspended in normal saline solution containing antibiotics (penicillin 100 iu ml^{-1} and streptomycin 100 μg ml^{-1}), immediately before challenging the experimental birds.

Infection of experimental birds

The experimental birds were housed in an isolation unit at 50 weeks old. The birds were placed in electrically heated rooms having identical conditions of light (16 hours per 24 hours), space and temperature. The hens in each group were randomly housed two per cage in wire (30×45 cm) cages and provided feed and water ad libitum. The birds were allowed to adjust for 15 days. Twenty-eight weeks after vaccination the birds in two of the four groups received an intraocular instillation of 0·05 ml challenge-virus suspension which had a titre of $10^{5·8}$ EID50 ml^{-1}. The AIBV challenged and unchallenged birds were kept in separate rooms.

Evaluation of AIBV challenge

The parameters used to evaluate the AIBV challenge were development of clinical signs and the effects on egg production, egg weight, eggshell weights, internal egg quality and post-challenge serological response.

Two birds in each group were examined twice daily for 15 days for the development of any signs of illness.

Egg production was recorded daily from the time of transfer of layers to isolation unit until five weeks after AIBV challenge. All the eggs laid by each group of the experimental layers were counted regardless of their shell quality.

The egg quality was evaluated on the basis of size, shape and internal quality. The egg shell quality was based on texture and dry shell weight. The dry shell weight was determined by the procedure described by Hamilton (1978). Briefly each day 15 eggs (excluding soft shelled and misshapen) from each group were randomly picked, labelled and broken. The eggshells with shell-membrane were washed with water, dried in an oven at 105°C for six hours and weighed.

The egg surface area was calculated from the egg weight according to the method described by Carter (1975), $(3·9782)(W^{0·7056})$, where W is the weight of eggs in grams. Shell weight (mg) per square cm of egg surface area (SWUSA) was then calculated.

The internal egg quality was determined on the basis of Haugh (1937) unit score by using a Haugh meter. The egg and shell weight and internal quality evaluation for all treatment groups were carried out at

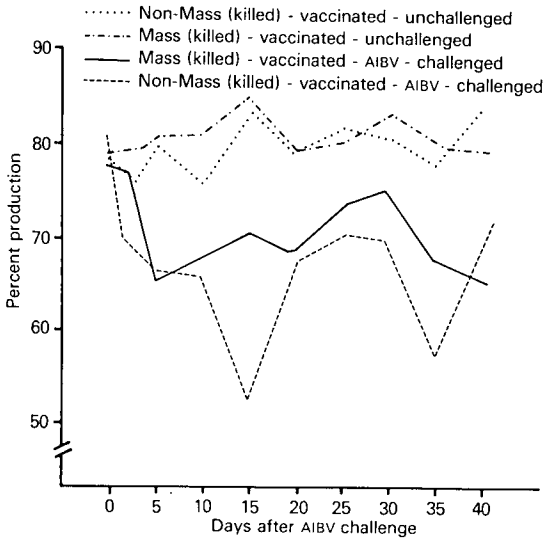

FIG 1: Egg production from various treatment groups challenged with AIBV

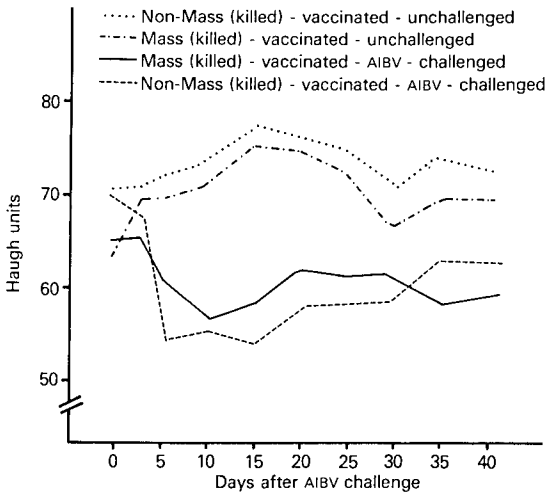

FIG 3: Internal quality of eggs from various treatment groups challenged with AIBV

the same time. The data for production, egg and eggshell weights and Haugh unit scores were pooled in five-day increments until day 40 after AIBV challenge. The data were analysed by analysis of variance.

The serum samples from 10 birds in each group were collected on days 0, 7, 15, 22, 29 and 37 after AIBV challenge and analysed using the HI test.

Results

No appreciable signs of illness could be detected in AIBV-challenged or unchallenged birds, except the presence of slight nasal and ocular discharges on day 8 after challenge in four unvaccinated AIBV-challenged birds. These signs were not seen after day 10 after AIBV challenge.

The data on effects of AIBV challenge on egg production, egg weight, eggshell weight and internal egg quality are presented in Figs 1 to 4. An overall comparison of percentage egg production, average egg weights, eggshell weights, SWUSA and Haugh unit scores of eggs from birds of various treatment groups is summarised in Table 1.

Statistically significant ($P<0{\cdot}005$) differences in

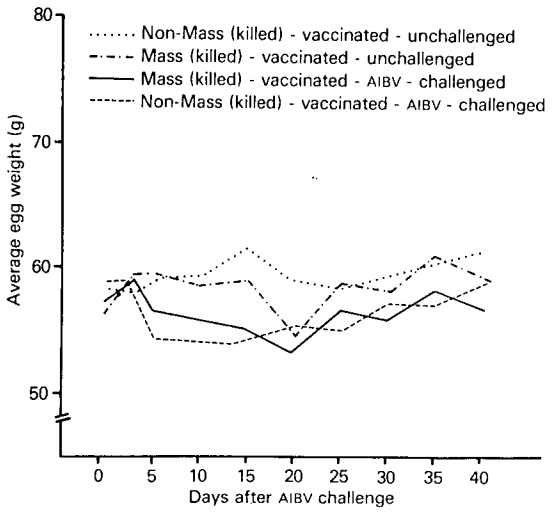

FIG 2: Average egg weights from various treatment groups challenged with AIBV

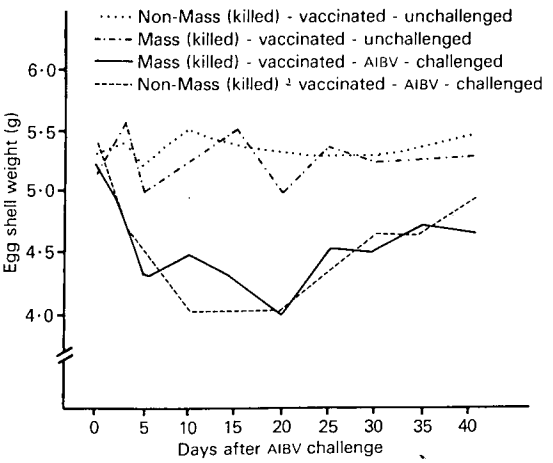

FIG 4: Average shell weights of eggs from various treatment groups challenged with AIBV

TABLE 1: Overall* comparison of average egg production, egg weight, shell weight, SWUSA† and Haugh units of eggs from AIBV challenged and unchallenged layers‡

Treatment group	Percentage production	Egg weight (g)	Shell weight (g)	SWUSA	Haugh units (% score)
1§	80·84 ± 1·98[a]	58·61 ± 1·69[a]	5·29 ± 0·1[a]	74·79 ± 3·06[a]	70·45 ± 3·71[a]
2	79·94 ± 2·66[a]	59·48 ± 1·16[a]	5·38 ± 0·09[a]	75·59 ± 1·35[a]	72·84 ± 2·57[a]
3	72·81 ± 5·73[b]	56·77 ± 1·67[b]	4·59 ± 0·35[b]	66·62 ± 4·59[b]	61·16 ± 3·03[b]
4	67·59 ± 7·80[b]	56·67 ± 1·96[b]	4·55 ± 0·41[b]	66·17 ± 5·15[b]	60·60 ± 3·05[b]

* Overall total 40 days study period
† Shell weight (mg) per egg surface area (cm^2)
‡ Mean and standard deviation data
§ 1 Massachusetts killed virus vaccinated unchallenged
2 Unvaccinated (Massachusetts killed virus) unchallenged
3 Massachusetts killed virus vaccinated AIBV challenged
4 Unvaccinated (Massachusetts killed virus) AIBV challenged
[a,b] Any two means carrying the same superscript in the same column are not significantly different from each other

production occurred between AIBV challenged and unchallenged birds from day 15 after challenge onwards. The birds in both the vaccinated and unvaccinated groups had significant drops in production throughout the post-challenge period (Fig 1). In addition the AIBV challenge in both vaccinated and unvaccinated groups caused laying of soft-shelled, misshapen and small eggs. Quite a large number of eggs (3·9 per cent) laid by the AIBV-challenged birds were either soft-shelled, small or had calcareous papule-like deposits on the egg shells. These types of effects were negligible (0·75 per cent) in the unchallenged birds.

The differences in mean egg weights are presented in Fig 2. Mean egg weights from unchallenged and AIBV challenged groups were also significantly different.

Although all the birds receiving AIBV challenge showed a decrease in egg shell weight (Fig 3), the birds that were vaccinated as adults and challenged had relatively lower effects on shell weights up to day 15 after challenge than those challenged birds not receiving MIBV vaccine as adults.

The SWUSA of unchallenged birds remained more or less at similar levels throughout the study period, whereas a trend towards a decrease in SWUSA score was noted in challenged birds. The average SWUSA scores of eggs from unchallenged birds were significantly higher ($P<0·005$) than those of challenged birds. The mean egg surface area (cm^2) and SWUSA scores of eggs from birds in various treatment groups are presented in Table 2.

The Haugh unit scores are presented in Fig 4 and the overall percentages of egg production by grades are summarised in Table 3. The Haugh unit scores of eggs from AIBV challenged groups dropped significantly ($P<0·005$) on day 10 after AIBV challenge and remained less than those from the unchallenged groups throughout the study.

The HI test did not detect any significant antibody

TABLE 2: Comparison of mean egg surface area (cm^2) and mean shell weight cm^{-2} egg surface area (SWUSA) of eggs from various treatment groups challenged with AIBV

Days after AIBV challenge	Treatment groups 1* cm^2	1* SWUSA	2 cm^2	2 SWUSA	3 cm^2	3 SWUSA	4 cm^2	4 SWUSA
0	68·47	75·50	70·09	76·17	69·15	76·99	67·47	77·15
5	70·93	70·21	70·63	73·62	68·78	62·22	67·04	68·01
10	70·43	74·74	70·80	78·31	68·16	66·28	66·93	61·57
15	70·76	78·15	72·69	74·14	68·02	64·09	66·87	62·51
20	67·26	76·70	70·78	75·86	65·84	60·91	67·81	59·86
25	70·63	76·30	70·20	76·20	69·26	66·99	67·26	65·20
30	70·16	75·67	70·98	74·94	68·00	66·61	69·45	67·81
35	72·17	72·32	71·88	75·54	70·48	67·53	69·23	64·56
40	71·11	73·53	72·55	75·52	69·19	67·92	70·97	68·89
Mean	70·21	74·79	71·18	75·59	68·54	66·62	68·11	66·17
SD	1·47	3·06	0·96	1·35	1·28	4·59	1·43	5·15

* 1 Massachusetts killed virus vaccinated unchallenged
2 Unvaccinated (Massachusetts killed virus) unchallenged
3 Massachusetts killed virus vaccinated AIBV challenged
4 Unvaccinated (Massachusetts killed virus) AIBV challenged

TABLE 3: Overall grading of eggs from various treatment groups challenged with AIBV

Haugh units	Grade	Percentage eggs in each grade* 1†	2	3	4
⩾79	AA	33·33	38·66	18·00	18·66
55-78	A	60·66	60·66	57·33	54·00
31-54	B	5·33	0·66	17·33	19·33
0-30	C	0·66	0·00	7·33	8·00

* Data based on egg production and Haugh unit scores of eggs collected for 40 days after AIBV challenge

† 1 Massachusetts killed virus vaccinated unchallenged
2 Unvaccinated (Massachusetts killed virus) unchallenged
3 Massachusetts killed virus vaccinated AIBV challenged
4 Unvaccinated (Massachusetts killed virus) AIBV challenged

titres to AIBV or MIBV in the prevaccination serum samples taken from adult birds. The prevaccination sera from birds had GMT titres of 20, or less, and 24, or less, for AIBV and MIBV, respectively.

The killed MIBV vaccine proved to be quite effective in elaborating the humoral antibody response of the birds as a rise in MIBV GMT-HI titres in birds receiving killed MIBV vaccine was noted from day 14 to day 22 after vaccination. The highest MIBV GMT-HI titres (GMT>723) were observed in killed MIBV-vaccinated birds on day 22 after vaccination. A consistent drop in MIBV GMT-HI titres was noted from day 22 after vaccination to the end of the study. Very low MIBV GMT-HI titre (GMT<20) were detectable on day 231 after killed MIBV vaccination in unchallenged birds. The unvaccinated and unchallenged birds had quite negligible HI titres (MIBV or AIBV) throughout the study period (GMT<28). The MIBV GMT-HI titres for post-challenge period are presented in Fig 5. The serum samples from unvaccinated and unchallenged birds revealed almost similar levels of GMT titres

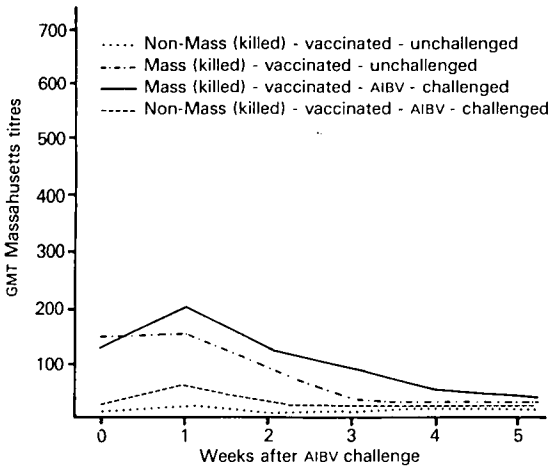

FIG 5: Post AIBV challenge geometric mean AIBV HI titres from various treatment groups

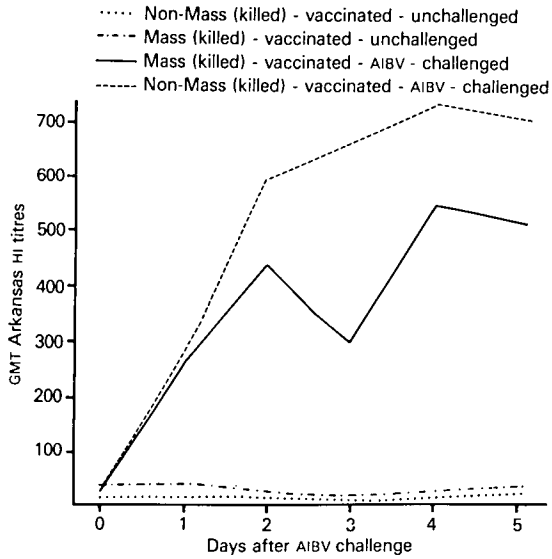

FIG 6: Post AIBV challenge geometric mean AIBV HI titres from various treatment groups

throughout the study. A transient rise in MIBV GMT-HI titres (GMT = 208) in MIBV-vaccinated and challenged birds was noted one week after challenge. These titres, however, dropped 15 days later. The vaccination of chickens with killed MIBV also elicited a short ranged antibody response against AIBV. The highest AIBV GMT-HI titre (GMT = 79) was noted on day 14 after vaccination. These titres dropped very soon compared with those against the homologous MIBV type which were maintained at considerable levels up to 208 days after adult MIBV vaccination. The MIBV GMT-HI titres in two MIBV vaccinated groups on the day of AIBV challenge were 145 and 123 and in two unvaccinated groups were 18 and 24, respectively. This difference in titres between the vaccinated and unvaccinated birds was statistically significant. None of the groups had antibody titres over 30 against Arkansas virus. Serum samples from both MIBV vaccinated and unvaccinated birds showed an increase in AIBV GMT-HI titres following challenge with AIBV. The highest AIBV GMT-HI titres following challenge with AIBV in both MIBV vaccinated (GMT = 549) and unvaccinated (GMT = 789) birds were observed on day 28 after challenge (Fig 6).

Discussion

Many factors such as age, natural feed ingredients and drug intake can affect the egg quality of laying chickens (Sherwood 1958). In the present work the experimental birds were at the prime of their laying period, they were not on any kind of medication and

birds in all the four groups received the same ration. Therefore it is unlikely that the above factors would have contributed to the drop in egg production or other qualitative changes. The authors' studies with the AIBV indicate that this virus strain contributes towards a decrease in egg production and quality.

The thickness of an egg shell depends on the amount of shell present relative to the egg surface area. The egg surface area is dependent on the size of the egg and can be calculated from the egg weight (Carter 1975). For an increase in shell thickness, either the shell weight must increase, egg surface area must decrease or a combination of both. The relative SWUSA can be calculated from the surface area by dividing the shell weight by its surface area. The authors' work has demonstrated that the AIBV challenge definitely influences egg surface area (cm^2) and SWUSA.

This study has highlighted the effects of AIBV exposure in layers with regard to egg production, quality and the serological response to AIBV challenge. The frequency of such heterologous exposures is probably great under field conditions.

The data on egg production shows that AIBV challenge significantly lowers egg production and increases the laying of soft-shelled, small sized and misshapen eggs. In addition the AIBV challenge resulted in considerable loss of internal egg quality, which is evidenced by the laying of more B and C grade eggs by the AIBV-challenged hens. The data obtained through the present work suggest that challenge with AIBV may have the adverse effects indicated above on birds which showed a good antibody production response against a killed Massachusetts type vaccine and had significant levels of antibodies at the time of AIBV exposure. Furthermore titres in response to the Massachusetts vaccine which were lower than reported in this study did confer immunity against a homologous virus challenge (data not presented). There is a need to vaccinate the birds against AIBV to provide protection against this virus. The study also stresses the need for development of a polyvalent IBV vaccine which could confer immunity against multiples of IBV types.

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