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IMMUNOPOTENTIATION OF INFECTIOUS BURSAL DISEASE (IBD) VACCINATION

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SUMMARY

Avian Apo-transferrin (AVAT) was prepared from pooled chicken serum collected from a poultry-processing plant. Employing polyacrylamide gel electrophoresis (PAGE), AVAT was identified as two very closely bands, approximately half the distance between the top of the gel and the albumin band. Using traditional immuno-electrophoresis (IE), counter IE, and rocket IE against anti-chicken whole serum it revealed one precipitation line. The total iron binding capacity (TIBC) of the prepared AVAT was 11.7 ug/mg, having a molecular weight of 76.500.

The protection against clinical signs, mortality and gross lesion development in vaccinated birds following IBDV challenge was positively modulated by both AVAT and Corynebacterium cutis lysate (CC) when given simultaneously with vaccination. Either AVAT or CC treatment potentiate the protective effects against mortality by 10 % and against gross lesions by 13.1 % and 29.6 % respectively. This effect proves their role as immunomodulators, which increased the immune potency.

PAGE analysis of sera of groups treated with AVAT or CC revealed increase in the level of immunoglobulins (Igs) and serum transferrin (Tf) as compared with non-treated vaccinated group at 3,7 and 14 days post vaccination.

A significant increase in relative bursal weight index was noticed in birds treated with CC over non-treated vaccinated and challenged control birds.

INTRODUCTION

Vaccination is generally considered the backbone of prophylaxis and control of viral diseases.

The potentiation of normal immune response in poultry occurs by alteration in any step involved in the host's immunologic reaction either in the classic humoral or in the cell-mediated system. This immunostimulation arises from the use of agents that increase non-specific resistance against infection. This work was planned to use the biological immunopotentiator avian Apo-transferrin (AVAT) prepared by Awaad et al. (1995) in comparison with the non-specific immunostimmulant biological modifier. Corynebacterium cutis lysate (CC) produced by Virbac Co. under the name of "Ultracorn" for immunopotentiation of IBD vaccination.

MATERIALS AND METHODS

Experimental chickens. A total of 100,22 day-old male egg type (L.C.L.) chickens proved to be free from maternally acquired antibodies against IBDV by agar gel precipitation test were used. Chickens were equally divided into 5 groups consisting of 20 each. Birds of each group housed in a separate, wire-floored pen and fed on a commercial balanced ration ad libitum.

IBDV strains. IBDV field virus was maintained by bird to bird passage (5 passages of infected bursal tissue). The virus was used as a 20 % suspension in phosphate buffered saline of the bursa of dead broilers in challenge experiments. IBDV vaccinal strain (Delvax Gumboro, Batch No. 76160 A) was used after titration.

Avian Apo-transferrin (AVAT). AVAT was prepared from chicken serum after Awaad et al. (1995) by a method involving precipitation with ammonium sulfate after Bezkorovainy (1963) and chromatography on diethyl amino ethyl cellulose (DEAE cellulose) after Sober et al. (1956). The dialyzed preparation was lyophilized after sterilization by passage through 0.22-mm membrane filter and stored at 4C until usage. The protein content of the preparation was determined according to Henry (1964) and Peters (1968). AVAT was characterized by determination of:

a- Spectral analysis; by measuring the absortption spectra in the visible and ultraviolet regions using Shimadzu Graphicard UV-240 spectrophotometer.

b- PAGE conducted after Maurer (1971).

c- Traditional IE (Graber and Williams, 1953), rocket IE (Laurell, 1972), and counter IE (Moody, 1968) were carried out against anti-chicken serum prepared in rabbits.

d- Determination TIBC; employing th emethod of Ramsay (1957). TIBC of the sample was given by the following equation:

$$\text{TIBC} = \text{Iron content of the supernate} \frac{\text{X } 0.5 \text{ X } 8 \text{ X } 200}{15} = \text{ug Fe/100}$$

Corynebacterium cutis lysate (CC). CC produced by Virbac Co., France, under the name of Ultracorn was used (Batch No. 803-523).

Experimental design. Chickens of groups 1-3 were vaccinated intraocularly against IBDV at 22 day-old. Birds of groups 1 and 2 were intramuscularly inoculated with AVAT and CC simultaneously with the vaccine in a dose of 10 mg/bird and 0.05 mg/bird respectively. At 37 day-old; chickens of groups 1-4 were challenged with 0.075 ml/bird of bursal homogenate suspension containing $10^{-5.73}$ EID_{50} / ml of virulent IBDV drooped on both eyes and cloaca. Birds of group 5 were kept as non-vaccinated, untreated, non-challenged controls. Individual blood samples were taken from the wing vein of chickens of groups 1,2,3 and 5 at 3,7 and 14 days post vaccination. Serum was separated and equal samples from each group were pooled for total protein determination and PAGE analysis after Maurer (1971) using the alternate method with gel system number 1a (pH 8.9-7%).

All chickens were kept for an observation period of 10 days post infection for clinical signs and / or mortalities. At the end of the observation period, survivors from all groups were individually weighted before being sacrificed for blood collection, bursa and spleen weighting, and gross lesion scoring after Nakamura et al. (1990) as follows:

0= no lesions.
1- Mild lesions.
2- Moderate lesions.
3- Severe lesions.

The severity index was calculated by adding the lesion scores of the tissues examined and dividing by the sum of the total number of chickens observed for macroscopic examination.

The relative bursal and spleen weight indices were figured out after Sharma et al. (1989) by the following equation:
Organ weight in grams X 1000 / total body weight in grams.

RESULTS

Results are shown in Tables 1-3 and Fig. 1.
AVAT had been successfully isolated and purified by the described techniques. The isolated AVAT had an isoelectric point of pH 3.7, TIBC of 467.3 Fe^3/100, iron content of 1.7 ug/mg protein and a molecular weight of 76.500 (calculated based on 2 moles of Fe^{3+} bound / mole of Tf). Fractionation on DEAE cellulose column chromatography is illustrated in Fig. 1. AVAT could be detected at 462 nm by spectrophotometric analysis. PAGE and IE had proved the purity and reactivity of the isolated AVAT.

Table 1: Protection and immunopotentiation percentages of IBD vaccinated chickens treated with Avian Apo-transferrin (AVAT) Apo-transferrin (AVAT) or Corynebacterium cutis lysate (CC) against challenge.

Group No.	IBD Vaccination	Treatment	IBDV challenge	Results of Challenge		Mean Gross Lesion Score*						Immunopo Tentiation %**	
				Deaths	Protection % against Mortality	Bursa Haemorrhage	Muscle Haemorrhage	Kidney Congestion	Proventriculus Haemorrhage	Sum of Lesion scores	% of Sum of Lesion scores***	Protection % against lesions	Against Mortality	Against Lesions
1	+	AVAT	+	0/20-	100	0.45	0.9	0.9	0.05	2.3	0.05	80.8	10	13.7
2	+	CC	+	0/20	100	0.25	0	0.05	0.1	0.4	0.1	96.7	10	29.6
3	+	-	+	2/20	90	1.05	1.4	1.2	0.3	3.95	0.3	67.1	-	-
4	-	-	+	19/20	5	3	2.95	2.9	0.2	9.05	0.2	24.6	-	-
5	-	-	-	0/20	100	0	0	0	0	0	0	100	-	-

* Mean gross Lesion score of birds that died or were sacrificed 10 days post IBDV challenge.

** Immunopotentiation % = Protection % of treated group - Protection % of non-treated group.

*** % = Sum lesion score x 100 / Total maximun lesion score (3x4 organs = 12).

\- Number of deathes / Total number of examined birds.

Table 2: Results of electrophoretic analysis of vaccinated biologically treated chickens challenged with IBDV.

Group No	Vaccination	Treatment	Electrophoretic analysis			
			Time*	Total protein	Igs** %	Tf** %
1	+	AVAT	3	4.8	30.0	20.0
			7	4.5	33.0	26.0
			14	4.0	36.0	29.0
2	+	CC	3	4.5	30.3	22.5
			7	4.9	34.2	26.0
			14	4.5	36.0	31.0
3	+	-	3	4.4	23.0	15.4
			7	4.2	26.5	13.8
			14	4.0	35.0	10.0
4	-	-	3	3.8	28.0	25.0
			7	4.8	29.8	16.0
			14	5.7	32.0	11.0

* Time of Sampling post-vaccination (days).
** Immunoglobulins (IgM and IgM)
*** Transferrin.

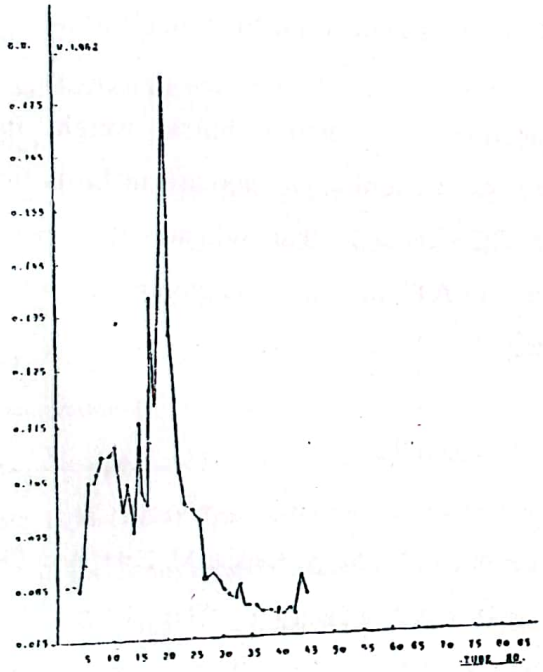

Fig 1: Demostration of fractions of chicken serum second precipitate using DEAE cellulose column chromatography.

Symptoms appeared 36 hours and mortality occurred 48 hours post challenge in non-vaccinated non-treated group (4). Mortality occurred after 4 days in vaccinated non-treated

Table 3: Results of Relative weight indices of bursa of Fabricus and spleen of vaccinated biologically treated chickens challenged with IBDV.

			Relative weight index		
Group No.	Vaccination	Treatment	No. of examined birds	Bursa of Fabricus	Spleen
1	+	AVAT	20	1.345 ± 0.228	1.557 ± 0.557
2	+	CC	20	1.790 ± 0.644*	1.377 ± 0.228
3	+	-	18	1.249 ± 0.356	1.372 ± 0.328
4	-	-	20	1.452 ± 0.356	1.702 ± 0.091

* Significantly different from vaccinated un-treated group ($p<0.05$).

group (3). Neither clinical signs nor mortality could be detected in the vaccinated, treated groups with AVAT or CC.

DISCUSSION

AVAT was prepared after Awaad et al. (1995) from pooled chicken serum collected from a poultry-processing plant. With PAGE, AVAT was identified as two very closely bands, approximately half the distance betweent he top of the gel and the albumin band. This is completely similar to the findings of Glick (1968) and Torres-Medina et al. (1971). AVAT revealed one precipitation line on using traditional IE, counter IE, and rocket IE against anti-chicken whole serum to chick its purity. The TIBC of the prepared AVAT was 1.7 ug/mg, having a molecular weight of 76.500 as calclated after Roberts et al. (1966). Torres-Medina et al. (1971) reported that chicken Tf is a protein of small molecular size and could elude from

Sephadex G-200 column with the albumin, suggesting a molecular weight similar to that of human Tf (73,000 - 76,000).

The protection against mortality and gross lesions development in vaccinated birds following challenge was positively modulated by either AVAT or CC when given simultaneously with vaccination. AVAT and CC treatment potentate the protective effect of groups 1 and 2 against mortality by 10% and against gross lesions by 13.1% and 29.6 % on IBDV challenge respectively (Table 1). This effect proves their role as immunomodulators, which increase the immune potency. Similar conclusion was previously observed in AVAT and CC by Awaad et al. (1995) on their usage in controlling IBD in chickens. Padany et al. (1980) and Soliman et al. (1991) previously reported similar conclusion in CC against Erysiplothrix rhusiopathiae and Newcastle disease virus respectively.

PAGE analysis of sera of groups 1-5 showed that the level of immunoglobulins (Igs) and serum Tf were higher in AVAT and CC treated groups (groups 1 and 2) as compared with non-treated vaccinated group (group 3) at 3,7 and 14 days post vaccination (Table 2). This increase of Igs in biologically treated groups may be attributed to activation of the immune response, i. e. a component of non-specific defense against antigen (formey et al., 1972 and Weir; 1985). This clarifies the paraimmunizing action of both AVAT and CC. As Tf plays a vital role in iron-binding and iron transport abilities (Roberts et al., 1966) and iron metabolism (Morgan, 1974), the increase of serum Tf in examined chickens may be to reduce the plasma iron level by its bounding to Tf. This supposes that Tf possess anti-infection properties where it suppers the growth of many pathogenic organisms by failure in using Tf-bound iron (Martin and Jandl, 1959). Results of CC as an immunomodulator is following those of Soliman et al. (1991).

Concerning the relative bursal weight index; there was a significant increase in birds treated with CC (Table 3) that indicates its superiority over AVAT in immunopotentiation of IBD vaccination.

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