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THE IMMUNE RESPONSE TO SEPARATE AND/OR COMBINED NEWCASTLE DISEASE AND INFECTIOUS BRONCHITIS VACCINES IN CHICKENS

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By

Maria N. Narimatsu

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ABSTRACT

THE IMMUNE RESPONSE TO SEPARATE AND/OR COMBINED NEWCASTLE DISEASE AND INFECTIOUS BRONCHITIS VACCINES IN CHICKENS

By

Maria N. Narimatsu

Infectious bronchitis and Newcastle disease have been constant problems for the poultry industry throughout the world for many years. Both infections spread with great rapidity, causing serious economic losses. In laying flocks, the major loss is decreased production and poor quality of eggs. In young chickens there may be appreciable mortality, particularly with Newcastle disease, and a loss in feed efficiency resulting in lowered weight gains.

Efficiency in the immune response to combined Newcastle disease and infectious bronchitis vaccine versus single vaccines was investigated using the hemagglutination-inhibition (HI) microtiter test to measure the specific antibody concentration in the sera.

The effect of two factors, timing and method of vaccination on the production of immunity, was analyzed. Vaccination of chicks at 10 days of age and 21 days of age via drinking water, intraocular or by a combination of the two routes (ND by eye drop and IB in the drinking water) did not show

a difference in immune response between separate and combined vaccines. Furthermore, vaccination at 10 days of age with revaccination at 15 days of age elicited a better immune response than one vaccination, either vaccination at 10 days or 21 days of age.

DEDICATION

To my parents, Tosiyuki and Kimie Narimatsu,
sisters and brothers in appreciation of
their love, guidance and perseverance.

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INTRODUCTION

Infectious bronchitis and Newcastle disease have been a constant problem for the poultry industry throughout the world for many years. Both infections spread with great rapidity, causing serious economic losses. In laying flocks, the major loss is decreased production and poor quality of eggs. In young chickens there may be appreciable mortality, particularly with Newcastle disease, and a loss in feed efficiency resulting from lowered weight gains.

The purpose of this thesis was to compare the efficacy of combined Newcastle disease and infectious bronchitis (ND/IB) vaccines versus single vaccines. Two different factors were considered -- time of vaccination and method of vaccination -- to analyze the effects of vaccination on the production of antibody to Newcastle disease (ND) virus in birds vaccinated with ND vaccine or IB vaccine alone or in combination (ND/IB). The use of combined vaccine as opposed to two single vaccines would help to reduce the cost of production and the stress on birds through less handling and manipulation.

The immune response of individual chickens of different ages exposed to different methods of vaccination against Newcastle disease and infectious bronchitis was measured by the hemagglutination-inhibition (HI) test. This is a

convenient procedure for measuring the specific antibody concentration in the sera, and the level of the latter test is known to reflect the immune status of the bird to some extent.

LITERATURE REVIEW

Newcastle Disease

Newcastle disease (ND) is an acute, highly contagious and destructive disease of chickens and occasionally of other fowls. It is characterized by respiratory distress and encephalitis. Humans are susceptible and, when infected, may develop conjunctivitis (Hanson and Brandly, 1958; Buxton and Fraser, 1977; Hanson, 1978).

The causative agent has been established as a RNA virus of the paramyxo group of viruses (Lancaster, 1976). There are several strains of the virus classified according to virulence of the strains: lentogenic, mesogenic and velogenic. All three types cause losses in egg production in laying birds (Hanson and Brandly, 1955; Grass, 1971; Utterback and Schwartz, 1973). The strain of Newcastle disease virus isolated in the current worldwide panzootic and the 1971-1973 epizootic in California was classified as velogenic viscerotropic (Utterback and Schwartz, 1973).

Nervous symptoms occur in some birds, especially young ones. These symptoms include paralysis of the legs or wings, and torticollis, resulting in a complete twisting of the neck. In laying flocks the major loss is decreased production of eggs and poor egg quality.

The incubation period is from two to five days. The morbidity rate is high, and the mortality rate varies with the age of chickens. The virus can be readily cultivated in chicken embryos inoculated via the allantoic sac. The virus has been grown in tissue cultures producing cytopathic effects (Buxton and Fraser, 1977).

An important property of the Newcastle disease virus is its capacity to agglutinate red cells. Avian erythrocytes are commonly used for hemagglutination studies; however, red cells of turkey and other avian species can also be used. Human, mouse, and guinea pig erythrocytes are also agglutinated by the virus (Buxton and Fraser, 1977; Hanson, 1978).

The hemagglutinating activity of Newcastle disease virus and the property of antiserum to specifically inhibit such hemagglutination were first demonstrated by Burnet (1942). The hemagglutination (HA) and the hemagglutination-inhibition (HI) tests have since proved to be of great value in diagnosis and research.

Infectious Bronchitis

Infectious bronchitis (IB) is an acute, highly contagious viral respiratory disease of young and adult chickens and is caused by the infectious bronchitis virus (IBV), a member of the coronavirus group (Cunningham, 1975; Hofstad, 1978). The disease is characterized by a bronchitis in young chickens with characteristic gasping and a sudden drop in egg production in layers. The disease was first identified in 1931 in North Dakota by Shalk and Hawn and soon became widespread (Hofstad, 1978).

Several distinct serotypes exist, such as Massachusetts, Connecticut, Beaudette, JMK, Florida strain, etc. The Massachusetts serotype is most common in poultry producing areas and is used as seed virus for most IBV vaccine. Antigenic variations among strains of bronchitis virus have been described by Hofstad (1961). Despite some antigenic difference among serotypes, they are closely related in regard to immunogenicity.

The incubation period for IB is from one to four days. The morbidity rate is high, and the mortality rate varies with the age group of chickens (Cunningham, 1952). Young birds are considerably more susceptible. The virus grows well in embryonating chicken eggs (Hofstad, 1978; Cunningham, 1975) and can be grown in cell cultures of the chicken embryo (Hofstad, 1978) and in embryonic turkey kidney cells (Coria and Peterson, 1971).

Normally the virus does not adsorb to the surface of erythrocytes, but modification of the virus by enzymatic treatment induces the hemagglutinating activity of the virus (Corbo and Cunningham, 1959).

Vaccine

Vaccination has proved to be a practical method of controlling Newcastle disease and infectious bronchitis (Luginbuhl et al., 1955; Winterfield and Seadale, 1956; Winterfield et al., 1957). Immunization has been carried out since the development of vaccines in 1940 (Phillips, 1973). A variety of vaccines, vaccination programs and

methods of administration have been introduced. It is important for poultrymen to use the most efficient vaccination program.

Mass immunization of poultry against Newcastle disease (ND) and infectious bronchitis (IB) either alone or in combination has been reported by many investigators using techniques such as aerosol or spray (Crawley and Fahey, 1954; Gough and Allan, 1973; Gough and Alexander, 1978; Yadin and Orthel, 1978), dust (Markham et al., 1955), or by adding vaccine to the drinking water (Luginbuhl et al., 1955; Winterfield et al., 1957; Jordan and Nassar, 1973; Gough et al., 1977).

Because of the necessity for vaccinating large numbers of birds, and of the time and expense involved in repeated vaccinations, the bronchitis vaccines have been combined with Newcastle disease vaccines without interference in the immune response from each vaccine (Markham et al., 1956). However, there have been conflicting reports with regard to these two viruses in certain combinations (Luginbuhl et al., 1955). Raggi and Lee (1964) reported that the IBV component of the vaccine interfered with the establishment of immunity to Newcastle disease. Winterfield (1968) has found some interference and a more prolonged reaction when bivalent vaccines were used. Thornton and Muskett (1973) reported a low rate of protection to artificial challenge with NDV in chickens inoculated simultaneously with commercially available monovalent ND and IB vaccines. Markham et al. (1956) showed an absence of interference when a combined

Newcastle disease and infectious bronchitis vaccine was given to birds under optimal conditions. Zygraich et al. (1973) reported no interference and no significant differences between birds vaccinated with the combined or the separate vaccine.

Methods of Vaccination

Aerosol methods have been increasingly used for the administration of Newcastle disease and infectious bronchitis vaccines, either alone or in combination. In the aerosol administration of the vaccine, a number of factors can influence successful vaccination, such as the particle size and distribution, virus concentration and stability (Markham et al., 1955; Gough and Allan, 1973; Yadin and Orthel, 1978).

Markham et al. (1955) reported that spray vaccine prepared from the B_1 strain of Newcastle disease virus and the DG strain of infectious bronchitis virus, either alone or in combination, could be successfully employed for mass vaccination when dispersed as dusts over the heads of birds. High titer of hemagglutination inhibition (HI) antibodies and good protection have been obtained in the field (Price et al., 1955). Gough and Allan (1973) have shown that the aerosol route of administration can elicit protection within three days in the absence of a detectable rise in antibody titer. Gough and Alexander (1979) found no major difference in the immune response following vaccination with live IB vaccine by aerosol, intraocular and drinking water routes.

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The mass vaccination technique in the drinking water has become a routine procedure with poultry farmers because it is labor saving, causes less stress, and generally produces satisfactory results in controlling the Newcastle disease and infectious bronchitis (Luginbuhl et al., 1955). The drinking water method is simple, fast, inexpensive, and handling of the birds is not required. It is an effective way of administering vaccine to all birds in a flock. Luginbuhl et al. (1955) demonstrated the practicability of immunizing chickens with IB and ND when these viruses were mixed and added to the drinking water.

Age for Vaccination

Newcastle disease. Buxton and Fraser (1977) described a standard program of immunization of replacement birds against ND which gave maximum protection: first vaccination at 21 days of age; revaccination at 8-10 weeks; again at 16-20 weeks; and every 5 months thereafter. Immunization of chickens at one day of age always resulted in a poor immune response. Chickens are revaccinated when they are under 4 weeks of age to insure the production of an adequate level of immunity. Allan (1973) reported that the vaccine is given at 1 to 7 days of age and revaccination at 14 days of age or later either by drinking water or aerosol.

Infectious bronchitis. The first vaccination in broilers is recommended at an early age (4 to 5 days of age) and again at 4 weeks. Replacement flocks should be vaccinated at 2 to

4 months (Hofstad, 1978). Davelaar and Kouwenhoven (1977) reported that they vaccinated broilers at 6 to 14 days of age either in the drinking water or by the spray method.

Effect of Passive Antibodies on Immune Response

It has been stated by several authors that congenital passive immunity may influence the immune response of young chickens to vaccination (Lancaster, 1966; Allan, 1971, 1974; Gough and Allan, 1976).

Brandly et al. (1946) reported that passively conferred immunity protected chicks against infection with ND virus but interfered with active immunization. Bankowski and Corstvet (1962) have shown that maternal immunity and residual immunity at time of vaccination with B_1 strain vaccine can affect the immunity induced. Holmes (1979) also found markedly suppressed antibody response when chickens with passively acquired antibody were vaccinated with live NDV vaccine. However, Raggi and Lee (1965) found that passive antibodies did not materially influence immune response to live virus vaccine as judged by challenge. Davelaar and Kouwenhoven (1977) have demonstrated that immunization against IB by vaccinating 1-day-old birds by the conjunctival and intranasal routes, despite the presence of high levels of circulating maternal antibody, was as effective as vaccination at an age of 15 days or later when passive protection has decreased.

Method to Detect Immunity

For determining flock immunity to Newcastle disease (ND) the most commonly used serological methods are the hemagglutination inhibition (HI) test (Allan and Gough, 1974; Spanoghe et al., 1977), and serum neutralization (SN) test (Bankowski and Corstvet, 1962; Beard, 1971) by measurement of antibody concentration in the sera. Another commonly used device to determine immunity is the challenge test (Spanoghe et al., 1977).

Under commercial conditions, high concentrations of serum antibody are generally accepted as a reliable indicator of flock immunity, but Levine and Fabricant (1950), Beard and Easterday (1967), and Allan (1975) have shown a lack of correlation between serum antibody concentration and resistance of the respiratory tract to challenge.

The usual method of detecting immunity to IBV following vaccination has been reported to be by serum neutralization (SN) test (Cunningham, 1973; Gough and Alexander, 1978; Hofstad, 1978), agar gel precipitin (AGP) tests (Gough and Alexander, 1978) and challenge of vaccinated fowls 3 to 6 weeks after vaccination (Winterfield and Fadly, 1971; Winterfield et al., 1972).

The serum neutralization (SN) test in eggs (Page and Cunningham, 1962; Cunningham, 1973) has been the method used most commonly, but it is time consuming, expensive, and it is often difficult to determine accurate endpoint titers. The disadvantages of the SN test led to the development of

a HI test for the detection of antibodies to infectious bronchitis virus.

Recently, many workers have shown the usefulness of the HA and HI tests in serological studies (Corbo and Cunningham, 1959; Biswal et al., 1966; Bingham et al., 1975; Alexander and Chettle, 1977; Bahl et al., 1977; Macpherson and Feest, 1978).

During the last few years several procedures for the production of hemagglutinating virus and for the HA and HI titrations for detecting antibodies to IBV have been reported. This hemagglutinating activity of the virus has been induced by enzymatic treatment of the virus or by chemical modification of the erythrocyte surface (Corbo and Cunningham, 1959; Brown et al., 1962; Bingham et al., 1975; Alexander et al., 1976; Alexander and Chettle, 1977; Bahl et al., 1977).

Corbo and Cunningham (1959) described a hemagglutination test for infectious bronchitis using a trypsin modified virus, but the hemagglutination was not specifically inhibited by immune serum. Recently Bingham et al. (1975) have reported that IBV Massachusetts, strain 41, when treated with phospholipase C (type 1), will agglutinate chicken red blood cells and that this hemagglutination (HA) could be inhibited by specific antisera. Alexander et al. (1976), in a preliminary examination of 9 strains of IBV, found 4 strains showing HA activity after treatment with phospholipase C (type 1). It was found that IBV M-41 strain possessed the best hemagglutinating properties for use in the HI test and that results

compared well with the IBV SN test. Later, Alexander and Chettle (1977) confirmed this work and developed a test system which was as reproducible as were the HA and HI tests for work with Newcastle disease (ND) virus.

Bahl et al. (1977) investigated the hemagglutinating ability of 2 strains of infectious bronchitis virus after the virus had been treated with phospholipase C (type 1) and found that Beaudette strain caused no detectable hemagglutination. However, Massachusetts strain 41 agglutinated chicken red blood cells (CRBC). This hemagglutination (HA) would be specifically inhibited by antisera.

Alexander et al. (1976) and Bahl et al. (1977) have shown the usefulness of the HI test for IBV as a rapid, simple, inexpensive and highly reproducible method of measuring antibodies against IBV.

MATERIALS AND METHODS

Time of vaccination was studied by comparing 3 groups: (1) vaccination at 10 days of age and revaccination at 15 days of age; (2) vaccination at 10 days of age; and (3) vaccination at 21 days of age. Groups (2) and (3) will allow for a comparison of effectiveness of early versus late vaccination, especially in view of the inhibiting effect of maternal immunity of the chick, while group (1) will allow for testing of the possibility to vaccinate early yet, through revaccination, compensate for the inhibitory effect of maternal immunity.

Method of vaccination was studied by comparing 4 groups (plus 3 control groups): (1) CONTROL group, (a) bled at 10 days, (b) bled postvaccination, (c) bled pre- and postvaccination; (2a) NEWCASTLE vaccinated with ND vaccine in the drinking water, (2b) BRONCHITIS vaccinated with IB vaccine in the drinking water; (3) COMB-WATER vaccinated with a combined (ND/IB) vaccine in the drinking water; (4) COMB-EYE vaccinated with a combined (ND/IB) vaccine by eye drop; (5) COMB-SEP vaccinated with a combined (ND/IB) vaccine, ND vaccine by eye drop and IB vaccine in the drinking water (the comparison between groups (1b and 1c) and groups (2) through (5) was done to establish if in fact antibody was produced in the latter groups, not to test

if vaccination produces antibody, which has been established sufficiently [Hanson, 1978; Hofstad, 1978]). The comparison between group (2) through (5) will test the relative effectiveness of producing antibody from the different methods of vaccination.

Experimental Chickens

A total of 235 White Leghorn male chickens from the same hatch were used. They were raised in the same battery until vaccination in 2 sets of 135 and 100 chickens, respectively.

Experimental Design

The objective of this study was to analyze the effect of 2 factors on the production of immunity: time of vaccination and method of vaccination. Thus, the following groups of animals were treated.

Factor 1. Time of vaccination was as follows: (1) vaccination at 10 days of age and revaccination at 15 days of age; (2) vaccination at 10 days of age only; and (3) vaccination at 21 days of age. It should be noted that subjects for groups (2) and (3) were taken from one set of a total of 135 animals, while group (1) was taken from a second set of a total of 100 birds.

Factor 2. Method of vaccination protocols for the 3 groups of Factor 1 (above) are presented in Table 1 (a, b, and c, respectively).

Table 1. Vaccination protocols

Group	Number of birds	Treatment	Route
1A: Vaccination at 10 days and revaccination at 15 days of age			
1a "CONTROL"	10	unvaccinated (bled at 10 days)	
1b "CONTROL"	15	unvaccinated (bled parallel to treated groups)	
2a "NEWCASTLE"	15	Newcastle disease	D.W.*
2b "BRONCHITIS"	15	Infectious bronchitis	D.W.
3 "COMB-WATER"	15	combined ND/IB	D.W.
4 "COMB-EYE"	15	combined ND/IB	I.O.**
5 "COMB-SEP"	15	combined ND/IB	ND=I.O. IB=D.W.
1B: Vaccination at 10 days of age			
1c "CONTROL"	15	unvaccinated (bled at 10 days and parallel to treated groups)	
2a "NEWCASTLE"	15	Newcastle disease	D.W.
2b "BRONCHITIS"	15	Infectious bronchitis	D.W.
3 "COMB-WATER"	15	combined ND/IB	D.W.
4 "COMB-EYE"	15	combined ND/IB	I.O.
5 "COMB-SEP"	15	combined ND/IB	ND=I.O. IB=D.W.

Table 1 (continued)

Group	Number of birds	Treatment	Route
	1C: Vaccination at 21 days of age		
2a "NEWCASTLE"	15	Newcastle disease	D.W.
2b "BRONCHITIS"	15	Infectious bronchitis	D.W.
3 "COMB-WATER"	15	combined ND/IB	D.W.

*D.W. = drinking water

**I.O. = intraocularly

Vaccines

Three commercially available vaccines, B_1 type LaSota strain live virus Newcastle disease; Massachusetts and Connecticut strains live virus bronchitis; and combined Newcastle-infectious bronchitis B_1 type, LaSota strain - Mass. & Conn. strains live virus recommended for primary vaccination of fowls by the manufacturers, were used.

Each group of chickens was vaccinated with one of the commercial vaccines administered by drinking water, eye drop or a combination of the two.

Feed Formula

The feed formula used to maintain the chicks is presented in Table 2.

Viruses

Newcastle antigen LaSota strain (10 HAU/0.025 ml), Newcastle disease virus antiserum (2/80 chicken), IBV Massachusetts antiserum #041679, and normal chicken serum (032880) were provided by USDA.*

The Massachusetts 41 (M-41) strain of infectious bronchitis virus (IBV) titer $10^{7.8}$ per ml #081277 was supplied by ASL.**

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*USDA - Veterinary Service Laboratory, Ames, Iowa.

**ASL - The American Scientific Laboratories, Madison, Wisconsin.

Table 2. MSU pullet starter 6148

Guaranteed Analysis		1/1/80
Crude protein not less than	20.0%	Variation LNS
Crude fat not less than	2.5	
Crude fiber not more than	10.0	

Ingredients: Grain products, plant protein products, animal protein products, forage products, cane molasses, vitamin B-12 supplement, ethoxyquin (a preservative), DL methionine, choline chloride, niacin, folic acid, vitamin A supplement, riboflavin supplement, vitamin E supplement, calcium pantothenate, D activated animal sterol, menadione sodium bisulfite (source of vitamin K activity), calcium carbonate, defluorinated phosphate, magnesium sulfate, potassium sulfate, salt, sodium selenite, manganous oxide, calcium iodate, copper oxide, zinc oxide. AG-6148

DIRECTIONS

Feed as the sole ration to starting pullets according to Michigan State University recommendations.

Manufactured by

Ralston Purina Co., Gen. Offices, St. Louis, MO 63188.

Preparation of Antigen

The infectious bronchitis antigen production was based on the procedure described by Alexander and Chettle (1977) and Bahl et al. (1977) using the M-41 strain of IBV as the seed virus to provide the antigen for both hemagglutination (HA) and the hemagglutination-inhibition (HI) tests, except that the virus was stored at -20°C after phospholipase C (PLC) treatment.

The Massachusetts 41 (M-41) strain of infectious bronchitis virus was propagated in embryonated chicken eggs, concentrated and treated with phospholipase C type 1 (PLC).

Ten-day-old embryonating chicken eggs were infected by inoculating 100 EID_{50} of M-41 in 0.1 ml into the allantoic sac.

Infected eggs were incubated at 37°C for 72-96 hours. Embryos that died up to 24 hours after inoculation were discarded as non-specific. The remaining eggs were chilled at 4°C overnight and the allantoic fluid was harvested. At all times during harvesting and subsequent preparation for enzyme treatment, the allantoic fluid was kept chilled in an ice bath. The allantoic fluid was clarified by low speed centrifugation. The virus was then centrifuged at 30,000 G to concentrate 100-fold by pelleting at 4°C for 1 hour in the SW-27 rotor of a Sorvall-OTD-2 (DuPont Company, Instruments Products, Biomedical Division, Newtown, CT 06470) ultracentrifuge. The pellet was resuspended in 0.01M TRIS/HCl buffer at pH 6.5. An equal volume of phospholipase C type 1 containing 1 unit of enzyme per ml was added to the

virus suspension and the mixture was incubated in a water bath for 2 hours at 37°C. This antigen was titrated (HA) and then dispensed into aliquots and stored at -20°C until use.

The Newcastle disease antigen was produced by the method described by Beard and Wilkes (1973) and modified by Schwartz (1980) using the commercial LaSota strain as the seed virus.

Nine- to 10-day-old embryonated chicken eggs were inoculated with 10^{-2} dilution of commercial LaSota strain vaccine in 0.1 ml into allantoic sac. Infected eggs were incubated at 37°C for 60 to 72 hours. Embryos that died up to 24 hours after inoculation were discarded as non-specific. The remaining eggs were chilled at 4°C overnight and the allantoic fluid was harvested and frozen. The fluids were thawed and 0.1% formalin added by volume, and held at 37°C for 36 hours. The 2% (w/v) NaCl and 10% (w/v) polyethylene glycol (molecular wt 6000) (all reagent grade chemicals) were added and held at 4°C for 2 hours.

The virus was then centrifuged in a refrigerated (4°C) Sorvall centrifuge at 4000 rpm for 1.5 hours, using a GSA head. The sediment (pellet) was reconstituted at 20X concentration in phosphate buffer. The concentrated antigen was then sonicated for 2 to 3 minutes to disperse finely and mix thoroughly. An equal volume of 100% glycerin was added to the virus suspension; the antigen was checked for HA titer and then dispensed in aliquots and diluted as needed, using saline.

Enzyme Preparation

Phospholipase C type 1 from *Clostridium perfringens (C. welchii)* (Sigma Chemical Company) was made up to contain 5 units per ml in phosphate buffered saline, pH 7.2 (PBS), divided in vials, stored at -20°C and used to treat virus in the manner described by Alexander et al. (1976), Alexander and Chettle (1977) and Bahl et al. (1977) at a final concentration of 1 unit of enzyme per ml.

Procedure

Control groups. A total of 40 birds served as controls, as indicated in Table 1 (A and B). Control group (1a) was bled at 10 days of age to establish maternal immunity level at the time of vaccination for the respective group (Lot 2); control group (1c) was bled at 10 days of age to establish the maternal immunity level for Lot 1A and at 21 days of age to establish the maternal immunity level for Lot 1B, as presented in Table 3. Control groups (1b) and (1c) were bled parallel to the experimental groups, 5 times, in weekly intervals, beginning at 22 days of age and 20 days of age, respectively.

Experimental groups. All chickens to be vaccinated were deprived of water for 4 hours immediately before vaccination. The vaccine was given in quantities of water that would be consumed in approximately 1 hour and at the manufacturer's recommended dose. After the drinking water vaccine was consumed, the waterers were filled with fresh water.

Table 3. Hemagglutination-inhibition (HI) titers to NDV and IBV in prevaccination control birds

Control (1a) 10 days of age			Control (1c) 10 days of age			21 days of age	
Bird #	NDV	IBV	Bird #	NDV	IBV	NDV	IBV
01	2*	512*	7777	0	8	0	2
02	2	128	7778	0	16	0	2
03	2	128	7796	0	16	0	8
04	2	128	7780	0	16	0	2
05	2	64	7783	0	8	0	2
06	2	64	7784	0	8	0	4
07	0	64	7785	0	16	0	8
08	0	64	7786	0	8	0	4
09	0	4	7787	0	16	0	4
10	0	4	7789	0	16	0	4
			7790	0	8	0	2
			7792	0	16	0	8
			7793	0	16	0	8
			7794	0	16	0	8
			7795	0	8	0	4

*Titers expressed as the reciprocal of the serum dilution.

The water used was sterile distilled. The waterers were sterile plastic water cups.

Seven days after the revaccination (Lot 2) and 10 days after vaccination for the other groups and at weekly intervals, 5 samples of serum were collected from the birds and tested individually for specific antibodies for Newcastle disease and infectious bronchitis (see Appendix A for raw data). The immunity was evaluated by the average HI antibody status measured weekly from serum samples as described by Cunningham (1966) and Bingham et al. (1975).

Serological Procedure for Newcastle Disease Virus and Infectious Bronchitis Antibodies

Hemagglutination (HA) and hemagglutination-inhibition (HI) tests. The immune response to infection was measured by hemagglutination-inhibition (HI) test for IBV (Alexander and Chettle, 1977; Bahl et al., 1977) using M-41 strain treated with phospholipase C type 1 as antigen and for NDV using LaSota strain as antigen (Beard and Wilkes, 1973; Schwartz, 1980).

Hemagglutination and hemagglutination-inhibition titers were carried out according to Cunningham (1966) and Bingham et al. (1975) performed in Microtiter "U" bottom plates using a manual 0.025 ml microtiter apparatus.* All dilutions of

*Cooke Engineering Company, 900 Slater Lane, Alexandria, Virginia.

virus or antisera were made in phosphate buffered saline, pH 7.2 (PBS). The HI test was routinely carried out at 4°C.

Hemagglutination (HA) test. Twenty-five microliters of virus suspension was serially diluted in 25 µl volume of PBS and an equal amount of 0.5% suspension of chicken erythrocytes was added to each well. The control contained 0.025 ml of PBS and 0.025 ml of RBC. The plate was shaken gently and incubated at 4°C for 45 to 60 minutes. Hemagglutination was determined by observing the pattern formed by the cells. Hemagglutination titers were expressed as the reciprocal of the highest dilution of virus at which 100% of the area agglutinated (Bahl et al., 1977). The titer of the antigen obtained was used to calculate the dilution necessary to give a solution (in PBS) containing 4 HA units in 0.025 ml for IB and 10 HA units in 0.025 ml for ND.

Hemagglutination-inhibition (HI) test. The beta-HI test, which uses constant antigen and varying serum concentration (Cunningham, 1966; Beard and Wilkes, 1973; Allan and Gough, 1974; Bingham et al., 1975) was used with 4 HA units (M-41 strain) for infectious bronchitis and 10 HA units (LaSota strain) for Newcastle disease as antigen dose.

Constant amounts of virus in 25 µl of antigen were added to each dilution (decreasing concentration) of serum, ranging from 1:2 through 1:2048. The serum-antigen mixture was incubated at 4°C for 15 minutes before adding 0.5%

suspension of chicken erythrocytes followed by further incubation at 4°C for 45 to 60 minutes. Individual HI titers were expressed as the reciprocal of the highest serum dilution (in 0.025 ml) causing a detectable inhibition of the agglutination.

Analysis of variance was used to express the average of each bleeding (HI titers) for the different groups of chickens, in order to compare the immune response to the different vaccines used separately or as a combination. The analysis of variance for repeated measures was performed using the BMDP2V program (Dixon, 1977). A further test used was Tukey's test to detect any difference between means, according to Gill (1978).

Chicken Erythrocytes

Blood was obtained from Single Comb White Leghorns by vein puncture. Red blood cells were collected in sterile Alsever's solution ("Manual of Microbiological Methods" in Society of American Bacteriologists, McGraw-Hill Book Co., Inc., New York, 1957).

The blood was centrifuged and the supernatant fluid removed. The cells were washed 3 times by centrifugation for 10 minutes at 1500 rpm in phosphate buffered saline (PBS). After the last wash the erythrocytes were suspended in PBS at a concentration of 0.5% for immediate use. A 0.5% cell suspension in PBS was used for hemagglutination and hemagglutination-inhibition tests.

Serum for Serology

Blood samples for serology were obtained by cardiac puncture. They were allowed to clot at room temperature and then stored overnight at 4°C, at which time the serum was transferred to sterile tubes. Before testing, sera were inactivated in a water bath at 56°C for 30 minutes prior to use in the HI test.

RESULTS

Study I: Newcastle Disease

Two different designs were followed to analyze the effects of vaccination on the production of antibody to Newcastle disease (ND) virus in birds vaccinated with ND vaccine alone or in combination (ND/IB). Both designs provided for a 2-factor analysis of variance with repeated measures, the 2 factors being (1) time of vaccination and (2) mode of vaccination.

Design 1. In the first design the factor "time of vaccination" was compared in 3 ways: "LOT 2" - 10 days after hatching with revaccination on day 15; "LOT 1A" - 10 days after hatching; and "LOT 1B" - 21 days after hatching. The second factor, "mode of vaccination", compared 2 different methods as follows: "NEWCASTLE" - vaccination with ND vaccine alone, and "COMB-WATER" - vaccination with a combined ND/IB vaccine in the drinking water. The dependent variable, amount of antibody produced, was determined from 5 bleedings at intervals of 7 days each, beginning 7 days after revaccination for "LOT 2" and 10 days after vaccination in the cases of "LOT 1A" and "Lot 1B."

The means for the amount of antibody are presented in Table 4.

An analysis of variance for repeated measures was performed using the BMDP2V program (Dixon, 1977). For the complete program, see Appendix B. The results of this analysis are presented in Table B3. The results indicated that there was only one significant difference ($P \leq 0.05$) in the amount of antibody produced, viz., on factor one, "time of vaccination", and, as can be seen from Table 4, "LOT 2" - vaccination on day 10 and revaccination on day 15 produced the highest level of antibody response among the 3 groups in contrast to "LOT 1A" - vaccinated at 10 days of age, and "LOT 1B" - vaccinated at 21 days of age. No significant difference over time was found; i.e., the relative amount of antibody remained approximately the same (Table B3).

Design 2. In the second design, the animals were vaccinated at 2 different times: "LOT 2" - 10 days of age with revaccination at 15 days of age, and "LOT 1A" - vaccinated at 10 days of age. Furthermore, 4 methods of vaccination were contrasted: "NEWCASTLE" - vaccination with ND vaccine alone in the drinking water; "COMB-WATER" - vaccination of combined ND/IB vaccine in the drinking water; "COMB-EYE" - vaccination of combined ND/IB vaccine intraocularly; and "COMB-SEP" - the vaccination of ND vaccine by eye drop and IB vaccine in the drinking water. As in Design 1, the dependent variable, i.e., the amount of antibody produced, was determined through 5 bleedings with intervals of 7 days

Table 4. Means of HI titer to NDV of chickens vaccinated at different ages with separate or combined vaccines

Treatment	Method	Means Titer Lot 2	Means Titer Lot 1A	Means Titer Lot 1B	Total Means
Newcastle disease vaccine	D.W.*	14.88	7.47	8.03	10.13 ±2.01
Combined ND/IB vaccine	D.W.	20.43	8.52	7.49	12.15 ±2.01
Total means		17.66_a ±2.41	8.00_b ±2.41	7.76_b ±2.41	

*D.W. = drinking water

^{a,b}Means not sharing the same letter are significantly different ($P \leq 0.05$). Tukey's test.

each, beginning 7 days after revaccination for "LOT 2" and 10 days after vaccination in the cases of "LOT 1A" and "LOT 1B."

The means for the amount of antibody are presented in Table 5.

The results for analysis of variance for repeated measures are presented in Table C3 (for complete program see Appendix C). There was a significant difference between time of vaccination ($P<0.05$), in the sense that condition "LOT 2", repeated vaccination, produced a higher level of antibody. Also, there was a significant effect for method of vaccination and for the interaction method vs. time ($P<0.05$). The means were compared within each lot. In lot 2, "COMB-WATER" and "COMB-EYE" gave higher values than "COMB-SEP" and "NEWCASTLE" alone. However, no significant difference was found between means when they were compared using Tukey's test. In lot 1A, the mean values for the different treatments were very similar and no significant difference was detected. Again, no differences over time were found; i.e., the amount of antibody detected during the 5 bleedings remained approximately the same.

Study II: Infectious Bronchitis

Two different designs were followed to analyze the effects of vaccination on the production of antibody to infectious bronchitis (IB) virus in birds vaccinated with IB vaccine alone or in combination (ND/IB). Both designs provided for a 2-factor analysis of variance with repeated

Table 5. Means of HI titers to NDV of chickens vaccinated at different ages and by different methods

Treatment	Method	Means Titer	
		Lot 2	Lot 1A
Newcastle	D.W.*	14.88±3.62	7.47±3.31
Combined ND/IB vaccine	D.W.	20.43±3.62	8.52±3.31
Combined ND/IB vaccine	I.O.**	23.64±3.62	6.32±3.31
Combined ND/IB vaccine	ND=I.O. IB=D.W.	16.50±3.62	6.93±3.31

*D.W. = drinking water

**I.O. = intraocularly

measures, the 2 factors being (1) time of vaccination and (2) mode of vaccination.

Design 1. In the first design the factor "time of vaccination" was compared in 3 ways: "LOT 2" - 10 days after hatching with revaccination on day 15; "LOT 1A" - 10 days after hatching; and "LOT 1B" - 21 days after hatching. The second factor, "mode of vaccination", compared 3 different methods as follows: "CONTROL" - unvaccinated birds; "BRONCHITIS" - vaccination with IB vaccine alone in the drinking water; and "COMB-WATER" - vaccination with a combined ND/IB vaccine in the drinking water. The dependent variable, i.e., the amount of antibody produced, was determined from 5 bleedings at intervals of 7 days each, beginning 7 days after revaccination for "LOT 2" and "LOT 1B."

The means for the amount of antibody are presented in Table 6.

That vaccination, in comparison to non-vaccination, will produce antibody is widely known (Hanson, 1978; Hofstad, 1978); thus, the reason for the introduction of the control group, i.e., unvaccinated birds, was to show that vaccination had indeed taken place. The objective of this thesis is to investigate whether time or method of vaccination would make a difference in the production of antibody. For this reason, the data from the control group are not included in the analysis of variance that follows.

Table 6. Means of HI titers to IBV of chickens vaccinated at different ages with separate or combined vaccines

Treatment	Method	Means Titer Lot 2	Means Titer Lot 1A	Means Titer Lot 1B	Total Means
Control (unvaccinated)		3.93	1.55	1.34	
Infectious bronchitis vaccine	D.W.*	33.92	13.06	17.13	21.37 ±3.13
Combined ND/IB vaccine	D.W.	35.34	10.22	12.36	19.31 ±3.13
Total Means		34.63_a ±3.78	11.64_b ±3.78	14.75_b ±3.78	

*D.W. = drinking water

^{a,b}Means not sharing the same letter are significantly different ($P \leq 0.05$). Tukey's test.

The results of this analysis of variance are presented in Table D3 (for complete program, see Appendix D) and indicate that there is only one significant difference ($P \leq 0.05$), namely on factor one: "time of vaccination." As may be noted from Table 6, "LOT 2", vaccination on day 10 and revaccination on day 15, produced the highest level of antibody response among the 3 groups in comparison with "LOT 1A", vaccinated at 10 days of age, and "LOT 1B", vaccinated at 21 days of age. No differences over time were found; i.e., the relative amount of antibody remained approximately the same.

Design 2. In the second design, the animals were vaccinated at 2 different times: "LOT 2" - 10 days of age with revaccination at 15 days of age, and "LOT 1A" - vaccinated at 10 days of age. Furthermore, 5 methods of vaccination were compared: "CONTROL" - unvaccinated birds; "BRONCHITIS" - vaccination with IB vaccine alone in the drinking water; "COMB-WATER" - vaccination of combined ND/IB vaccine in the drinking water; "COMB-EYE" - vaccination of combined ND/IB vaccine intraocularly; and "COMB-SEP" - vaccination of ND vaccine by eye drop and IB vaccine in the drinking water. As in Design 1, the dependent variable, the amount of antibody produced, was determined from 5 bleedings at intervals of 7 days each, beginning 7 days after revaccination for "LOT 2" and 10 days after vaccination in the cases of "LOT 1A" and "LOT 1B."

The means for the amount of antibody are presented in Table 7. Again, the control group (unvaccinated birds) was not included in the analysis of variance.

The results of the analysis of variance for repeated measures are presented in Table E3 (for complete program, see Appendix E). As may be noted, there was only one significant difference ($P \leq 0.05$), viz., on factor one, "time of vaccination" and, as can be seen from Table 7, "LOT 2", repeated vaccination, produced a higher level of antibody. Again, no difference over time was found; i.e., the relative amount of antibody remained approximately the same.

Table 7. Means of HI titers to IBV of chickens vaccinated at different ages and by different methods

Treatment	Method	Means Titer Lot 2	Means Titer Lot 1A	Total Means
Bronchitis	D.W.*	33.92	13.06	23.49±4.92
Combined ND/IB vaccine	D.W.	35.34	10.22	22.78±4.92
Combined ND/IB vaccine	I.O.**	34.13	11.27	22.70±4.92
Combined ND/IB vaccine	ND=I.O. IB=D.W.	34.20	8.56	21.39±4.92
Total Means		34.40_a ±3.48	10.78_b ±3.48	

*D.W. = drinking water

**I.O. = intraocularly

^{a,b}Means not sharing the same letter are significantly different ($P \leq 0.05$). Tukey's test.

DISCUSSION

The assessment of immunity would have been best measured by challenging vaccinated birds with an ND or IB virus of known virulence. As stated above, this procedure was not readily performable; for this reason, the immune response was assessed by the titer of antibodies in the serum from 5 bleedings at 1-week intervals. These repeated tests allowed for a more accurate assessment of the antibody levels which to some extent reflect protection.

The objective of the present study was to analyze the effect of two factors, (1) time and (2) method of vaccination, on the production of antibody in chicks vaccinated against Newcastle disease and infectious bronchitis vaccine, either combined or separately.

The results reported above suggest that a combined ND/IB vaccine administered in 10-day-old and 21-day-old chicks via the drinking water, intraocularly, or by combining two methods (ND by eye drop and IB in the drinking water) will produce the same immune response as separate applied vaccine, both ND and IB. Similar observations had previously been made by Zygraich et al. (1973).

Furthermore, vaccination at 10 days of age with revaccination at 15 days of age was found to produce better

immunity than either vaccination at 10 days or 21 days of age.

Newcastle Disease

Considering the first factor studied, time of vaccination, it was found in both designs that vaccination at 10 days of age and revaccination at 15 days of age produced the highest level of antibody, regardless of the method of vaccination. When comparing this time of vaccination with both vaccination at 10 days of age and at 21 days of age, no difference between the two latter times was found. Furthermore, the level of antibody produced was numerically different over the time of the 5 successive bleedings. However, there were no significant differences between the repeated tests.

Infectious Bronchitis

Similar to the findings of ND, revaccination was found to produce higher antibody levels than either vaccination at 10 days of age or at 21 days of age. However, comparing the latter two times, vaccination at 21 days of age indicated a higher antibody titer, which may have been due to interference as a result of the very low level of maternal antibodies at the time of vaccination. Similar observations were made by Brandly et al. (1946), Levine and Fabricant (1950) and Zygraich et al. (1973).

At any rate, the results suggest that it may be possible to immunize the birds at a younger age, i.e., 10 days of age, and thus to counteract the inhibitory effect of the

relatively higher maternal immunity level at this age with revaccination at 15 days of age, rather than risk waiting until 21 days of age for the first vaccination while still producing less protection.

Given that revaccination appears to be preferable, a combination of both ND and IB in the drinking water appears to be the most effective method.

Even considering the results of study design two of Newcastle disease, which produced a significant interaction effect, the intraocular application was more effective for revaccination, and drinking water application for vaccination at 10 days of age. Also as a result of infectious bronchitis vaccination with further labor costs from large scale with application, it may be argued that combined vaccine in the drinking water application of ND and IB at 10 days of age with revaccination at 15 days of age is the most effective and efficient manner of vaccination.

Conclusion

Given the results of the present study, it may be concluded that combined (ND/IB) vaccination, applied orally via drinking water at 10 days of age with a revaccination at 15 days of age, was the most effective procedure and produced the higher level of antibody for the two diseases.

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APPENDICES

APPENDIX A

Table A-1. Hemagglutination-inhibition (HI) titers in group "CONTROL" - unvaccinated birds

A	B	C	D	E	F
7976	07	016	1	1	8
7976	14	008	1	1	4
7976	21	000	1	1	2
7976	28	000	1	1	2
7976	35	000	1	1	0
7977	07	008	1	1	32
7977	14	004	1	1	8
7977	21	000	1	1	4
7977	28	000	1	1	2
7977	35	000	1	1	0
7978	07	008	1	1	32
7978	14	004	1	1	4
7978	21	004	1	1	2
7978	28	000	1	1	2
7978	35	000	1	1	0
7979	07	008	1	1	8
7979	14	004	1	1	4
7979	21	002	1	1	2
7979	28	000	1	1	2
7979	35	000	1	1	0
7980	07	000	1	1	8
7980	14	000	1	1	4
7980	21	000	1	1	0
7980	28	000	1	1	0
7980	35	000	1	1	0
7981	07	008	1	1	16
7981	14	004	1	1	4
7981	21	002	1	1	0
7981	28	000	1	1	0
7981	35	000	1	1	0
7982	07	004	1	1	16
7982	14	002	1	1	4
7982	21	000	1	1	2
7982	28	000	1	1	2
7982	35	000	1	1	0
7983	07	4	1	1	8
7983	14	4	1	1	2
7983	21	2	1	1	4
7983	28	0	1	1	2
7983	35	0	1	1	0
7984	07	16	1	1	4
7984	14	8	1	1	4
7984	21	2	1	1	2
7984	28	0	1	1	0
7984	35	0	1	1	0
7985	07	4	1	1	8
7985	14	2	1	1	2
7985	21	2	1	1	2
7985	28	0	1	1	0
7985	35	0	1	1	0
7986	07	2	1	1	4
7986	14	8	1	1	2
7986	21	2	1	1	4
7986	28	0	1	1	0
7986	35	0	1	1	0
7987	07	16	1	1	4
7987	14	4	1	1	2
7987	21	0	1	1	0
7987	28	0	1	1	0
7987	35	0	1	1	0
7988	07	000	1	1	16
7988	14	2	1	1	4
7988	21	2	1	1	2
7988	28	0	1	1	2
7988	35	0	1	1	0
7989	07	4	1	1	8
7989	14	4	1	1	4
7989	21	2	1	1	4
7989	28	0	1	1	2
7989	35	0	1	1	0
7990	07	16	1	1	16
7990	14	8	1	1	4
7990	21	2	1	1	4
7990	28	0	1	1	2
7990	35	0	1	1	0

A = bird's number; B = days of bleeding; C = HI titer to NDV; D = Lot 2, E = group "CONTROL" - unvaccinated chicks; F = HI titer to IBV

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Table A-2. Hemagglutination-inhibition (HI) titers in group "NEWCASTLE" - vaccinated with a single Newcastle disease vaccine or "BRONCHITIS" - vaccinated with a single infectious bronchitis vaccine

A	B	C	D	E	F
7901	07	16	1	2	8
7901	14	32	1	2	16
7901	21	16	1	2	16
7901	28	8	1	2	64
7901	35	8	1	2	16
7902	07	16	1	2	16
7902	14	32	1	2	32
7902	21	16	1	2	32
7902	28	8	1	2	16
7902	35	8	1	2	4
7903	07	16	1	2	16
7903	14	32	1	2	32
7903	21	16	1	2	32
7903	28	8	1	2	32
7903	35	4	1	2	16
7904	07	8	1	2	128
7904	14	8	1	2	64
7904	21	16	1	2	16
7904	28	8	1	2	32
7904	35	4	1	2	8
7905	07	32	1	2	128
7905	14	64	1	2	64
7905	21	32	1	2	16
7905	28	16	1	2	8
7905	35	8	1	2	8
7906	07	8	1	2	128
7906	14	16	1	2	64
7906	21	16	1	2	32
7906	28	8	1	2	32
7906	35	4	1	2	8
7907	07	8	1	2	32
7907	14	16	1	2	16
7907	21	16	1	2	64
7907	28	8	1	2	64
7907	35	4	1	2	16
7908	07	16	1	2	16
7908	14	32	1	2	32
7908	21	16	1	2	16
7908	28	8	1	2	16
7908	35	4	1	2	8
7909	07		1	2	32
7909	14		1	2	16
7909	21		1	2	16
7909	28		1	2	16
7909	35		1	2	8
7910	07	8	1	2	128
7910	14	16	1	2	64
7910	21	32	1	2	16
7910	28	16	1	2	32
7910	35	16	1	2	32
7911	07	32	1	2	32
7911	14	8	1	2	16
7911	21	16	1	2	16
7911	28	8	1	2	32
7911	35	4	1	2	16
7912	07	8	1	2	
7912	14	8	1	2	
7912	21	32	1	2	
7912	28	16	1	2	
7912	35	4	1	2	
7913	07	32	1	2	32
7913	14	8	1	2	32
7913	21	16	1	2	16
7913	28	8	1	2	16
7913	35	4	1	2	8
7914	07	8	1	2	16
7914	14	8	1	2	8
7914	21	32	1	2	
7914	28	16	1	2	16
7914	35		1	2	4
7915	07	8	1	2	64
7915	14	16	1	2	64
7915	21	32	1	2	16
7915	28	8	1	2	16
7915	35	-	1	2	8

A = bird's number; B = days of post-revaccination; C = HI titer to NDV; D = Lot 2 - vaccinated at 10 and 15 days of age; E = group "NEWCASTLE" or "BRONCHITIS" - vaccinated in the drinking water; F = HI titer to IBV

Table A-3. Hemagglutination-inhibition (HI) titers in group "COMB-WATER" - vaccinated with a combined ND/IB vaccine

A	B	C	D	E	F
7931	07	16	1	3	8
7931	14	32	1	3	16
7931	21	16	1	3	16
7931	28	8	1	3	16
7931	35	4	1	3	8
7932	07	16	1	3	128
7932	14	32	1	3	64
7932	21	8	1	3	16
7932	28	8	1	3	32
7932	35	4	1	3	16
7933	07	32	1	3	16
7933	14	64	1	3	32
7933	21	8	1	3	8
7933	28	16	1	3	16
7933	35		1	3	
7934	07	16	1	3	64
7934	14	64	1	3	32
7934	21	16	1	3	16
7934	28	8	1	3	32
7934	35	8	1	3	16
7935	07	16	1	3	64
7935	14	32	1	3	32
7935	21	16	1	3	8
7935	28	8	1	3	64
7935	35	8	1	3	32
7936	07	16	1	3	128
7936	14	32	1	3	64
7936	21	16	1	3	16
7936	28	8	1	3	16
7936	35	4	1	3	8
7937	07	32	1	3	64
7937	14	32	1	3	32
7937	21	16	1	3	16
7937	28	8	1	3	32
7937	35	4	1	3	16
7938	07	16	1	3	128
7938	14	32	1	3	32
7938	21	16	1	3	16
7938	28	8	1	3	16
7938	35	4	1	3	16
7939	07	16	1	3	128
7939	14	32	1	3	32
7939	21	16	1	3	8
7939	28	8	1	3	16
7939	35		1	3	
7940	07	32	1	3	64
7940	14	32	1	3	32
7940	21	32	1	3	32
7940	28	16	1	3	32
7940	35	16	1	3	16
7941	07	16	1	3	64
7941	14	32	1	3	32
7941	21	16	1	3	32
7941	28	8	1	3	32
7941	35	4	1	3	8
7942	07	16	1	3	64
7942	14	32	1	3	32
7942	21	32	1	3	64
7942	28	16	1	3	32
7942	35	8	1	3	8
7943	07	32	1	3	64
7943	14	32	1	3	64
7943	21	32	1	3	32
7943	28	8	1	3	32
7943	35	4	1	3	8
7944	07	32	1	3	128
7944	14	64	1	3	64
7944	21	16	1	3	32
7944	28	8	1	3	32
7944	35	4	1	3	16
7945	07	16	1	3	128
7945	14	32	1	3	16
7945	21	16	1	3	16
7945	28	8	1	3	32
7945	35	8	1	3	16

A = bird's number; B = days of post-revaccination; C = HI titer to NDV; D = Lot 2 - vaccinated at 10 and 15 days of age; E = group "COMB-WATER" - vaccinated in the drinking water; F = HI titer to IBV

Table A-4. Hemagglutination-inhibition (HI) titers in group "COMB-EYE" - vaccinated with a combined ND/IB vaccine

A	B	C	D	E	F
7946	07	64	1	4	32
7946	14	128	1	4	64
7946	21	64	1	4	16
7946	28	16	1	4	32
7946	35	128	1	4	16
7947	07	32	1	4	32
7947	14	32	1	4	64
7947	21	16	1	4	16
7947	28	64	1	4	64
7947	35	32	1	4	32
7948	07	32	1	4	16
7948	14	32	1	4	32
7948	21	16	1	4	32
7948	28	16	1	4	32
7948	35	16	1	4	16
7949	07	16	1	4	8
7949	14	32	1	4	16
7949	21	16	1	4	16
7949	28	32	1	4	32
7949	35	16	1	4	16
7950	07	32	1	4	64
7950	14	64	1	4	32
7950	21	64	1	4	32
7950	28	32	1	4	64
7950	35	32	1	4	32
7951	07	32	1	4	64
7951	14	64	1	4	16
7951	21	32	1	4	16
7951	28	32	1	4	32
7951	35	32	1	4	32
7952	07	16	1	4	128
7952	14	16	1	4	64
7952	21	16	1	4	16
7952	28	32	1	4	32
7952	35	32	1	4	32
7953	07	16	1	4	32
7953	14	64	1	4	32
7953	21	32	1	4	32
7953	28	32	1	4	32
7953	35	16	1	4	16
7954	07	32	1	4	64
7954	14	64	1	4	32
7954	21	64	1	4	32
7954	28	32	1	4	32
7954	35	16	1	4	32
7955	07	16	1	4	64
7955	14	32	1	4	64
7955	21	32	1	4	16
7955	28	32	1	4	32
7955	35	32	1	4	32
7956	07	16	1	4	16
7956	14	64	1	4	16
7956	21	16	1	4	32
7956	28	16	1	4	32
7956	35	32	1	4	16
7957	07	32	1	4	64
7957	14	32	1	4	64
7957	21	32	1	4	32
7957	28	32	1	4	32
7957	35	32	1	4	32
7958	07	32	1	4	64
7958	14	32	1	4	32
7958	21	16	1	4	16
7958	28	32	1	4	32
7958	35	32	1	4	16
7959	07	32	1	4	128
7959	14	128	1	4	128
7959	21	32	1	4	32
7959	28	32	1	4	64
7959	35	32	1	4	64
7960	07	16	1	4	256
7960	14		1	4	
7960	21		1	4	
7960	28		1	4	
7960	35		1	4	

A = bird's number; B = days of post-revaccination; C = HI titer to NDV; D = Lot 2 - vaccinated at 10 and 15 days of age; E = group "COMB-EYE" - vaccinated by eye drop; F = HI titer to IBV

Table A-5. Hemagglutination-inhibition (HI) titers in group "COMB-SEP" - vaccinated with a combined ND/IB vaccine

A	B	C	D	E	F
7961	07		1	5	
7961	14		1	5	
7961	21		1	5	
7961	28		1	5	
7961	35		1	5	
7962	07	16	1	5	64
7962	14	32	1	5	32
7962	21	16	1	5	8
7962	28	8	1	5	16
7962	35	8	1	5	8
7963	07	16	1	5	128
7963	14	32	1	5	64
7963	21	16	1	5	32
7963	28	8	1	5	32
7963	35	8	1	5	16
7964	07	16	1	5	16
7964	14	32	1	5	32
7964	21	32	1	5	16
7964	28	16	1	5	32
7964	35	16	1	5	16
7965	07	16	1	5	32
7965	14	16	1	5	8
7965	21	8	1	5	8
7965	28	8	1	5	16
7965	35	8	1	5	8
7966	07	16	1	5	256
7966	14	16	1	5	32
7966	21	8	1	5	16
7966	28	16	1	5	32
7966	35	8	1	5	8
7967	07	8	1	5	64
7967	14	16	1	5	32
7967	21	32	1	5	32
7967	28	16	1	5	32
7967	35	8	1	5	16
7968	07	16	1	5	256
7968	14	32	1	5	64
7968	21	16	1	5	16
7968	28	16	1	5	32
7968	35	8	1	5	32
7969	07	32	1	5	128
7969	14	16	1	5	64
7969	21	32	1	5	32
7969	28	16	1	5	32
7969	35	8	1	5	8
7970	07	8	1	5	32
7970	14	8	1	5	16
7970	21	16	1	5	16
7970	28	8	1	5	32
7970	35	4	1	5	8
7971	07	16	1	5	64
7971	14	32	1	5	16
7971	21	16	1	5	16
7971	28	16	1	5	32
7971	35	8	1	5	16
7972	07	32	1	5	64
7972	14	32	1	5	64
7972	21	16	1	5	32
7972	28	16	1	5	32
7972	35	8	1	5	16
7973	07	8	1	5	64
7973	14	16	1	5	32
7973	21	32	1	5	32
7973	28	8	1	5	32
7973	35	16	1	5	8
7974	07	32	1	5	16
7974	14	32	1	5	32
7974	21	32	1	5	32
7974	28	8	1	5	32
7974	35	8	1	5	16
7975	07	16	1	5	32
7975	14	64	1	5	16
7975	21	16	1	5	16
7975	28	8	1	5	32
7975	35	8	1	5	16

A = bird's number; B = days of post-revaccination; C = HI titer to NDV; D = Lot 2 - vaccinated at 10 and 15 days of age; E = group "COMB-SEP" - vaccinated with ND by eye drop and IB in the drinking water; F = HI titer to IBV

Table A-6. Hemagglutination-inhibition (HI) titers in group "CONTROL" - unvaccinated chicks

A	B	C	D	E	F
7777	10		2	1	2
7777	17		2	1	2
7777	24		2	1	2
7777	31		2	1	0
7777	38		2	1	0
7778	10		2	1	2
7778	17		2	1	2
7778	24		2	1	0
7778	31		2	1	0
7778	38		2	1	0
7794	10		2	1	8
7794	17		2	1	4
7794	24		2	1	2
7794	31		2	1	0
7794	38		2	1	0
7780	10		2	1	4
7780	17		2	1	2
7780	24		2	1	0
7780	31		2	1	0
7780	38		2	1	0
7783	10		2	1	4
7783	17		2	1	2
7783	24		2	1	0
7783	31		2	1	0
7783	38		2	1	0
7784	10		2	1	4
7784	17		2	1	0
7784	24		2	1	0
7784	31		2	1	0
7784	38		2	1	0
7785	10		2	1	8
7785	17		2	1	4
7785	24		2	1	2
7785	31		2	1	0
7785	38		2	1	0
7786	10		2	1	4
7786	17		2	1	0
7786	24		2	1	0
7786	31		2	1	0
7786	38		2	1	0
7787	10		2	1	4
7787	17		2	1	2
7787	24		2	1	0
7787	31		2	1	0
7787	38		2	1	0
7789	10		2	1	4
7789	17		2	1	2
7789	24		2	1	2
7789	31		2	1	0
7789	38		2	1	0
7790	10		2	1	4
7790	17		2	1	2
7790	24		2	1	0
7790	31		2	1	0
7790	38		2	1	0
7792	10		2	1	8
7792	17		2	1	4
7792	24		2	1	2
7792	31		2	1	0
7792	38		2	1	0
7793	10		2	1	8
7793	17		2	1	4
7793	24		2	1	2
7793	31		2	1	0
7793	38		2	1	0
7794	10		2	1	8
7794	17		2	1	4
7794	24		2	1	2
7794	31		2	1	0
7794	38		2	1	0
7795	10		2	1	4
7795	17		2	1	2
7795	24		2	1	2
7795	31		2	1	0
7795	38		2	1	0

A = bird's number; B = days of bleeding; C = HI titer to NDV; D = Lot 1A; E = group "CONTROL" - unvaccinated chicks; F = HI titer to IBV

Table A-7. Hemagglutination-inhibition (HI) titers in group "NEWCASTLE" - vaccinated with a single Newcastle disease vaccine or "BRONCHITIS" - vaccinated with a single infectious bronchitis vaccine

A	B	C	D	E	F
7701	10	4	2	2	4
7701	17	4	2	2	4
7701	24	4	2	2	8
7701	31	4	2	2	32
7701	38	4	2	2	16
7702	10	4	2	2	8
7702	17	4	2	2	8
7702	24	4	2	2	16
7702	31	4	2	2	32
7702	38	4	2	2	16
7703	10	4	2	2	16
7703	17	4	2	2	8
7703	24	4	2	2	16
7703	31	4	2	2	32
7703	38	4	2	2	16
7704	10	4	2	2	16
7704	17	4	2	2	16
7704	24	8	2	2	16
7704	31	16	2	2	32
7704	38	16	2	2	32
7705	10	4	2	2	16
7705	17	8	2	2	4
7705	24	4	2	2	4
7705	31	4	2	2	16
7705	38	4	2	2	16
7706	10	8	2	2	16
7706	17	8	2	2	4
7706	24	16	2	2	8
7706	31	32	2	2	8
7706	38	64	2	2	8
7707	10	4	2	2	8
7707	17	4	2	2	4
7707	24	4	2	2	8
7707	31	8	2	2	32
7707	38	16	2	2	8
7708	10	4	2	2	16
7708	17	4	2	2	8
7708	24	8	2	2	16
7708	31	16	2	2	16
7708	38	8	2	2	8
7709	10	4	2	2	8
7709	17	4	2	2	4
7709	24	4	2	2	16
7709	31	4	2	2	32
7709	38	4	2	2	32
7710	10	4	2	2	8
7710	17	4	2	2	8
7710	24	4	2	2	4
7710	31	4	2	2	16
7710	38	4	2	2	8
7711	10	4	2	2	8
7711	17	4	2	2	8
7711	24	16	2	2	8
7711	31	16	2	2	16
7711	38	16	2	2	8
7712	10	4	2	2	8
7712	17	4	2	2	4
7712	24	8	2	2	8
7712	31	8	2	2	16
7712	38	32	2	2	16
7713	10	8	2	2	4
7713	17	8	2	2	4
7713	24	16	2	2	4
7713	31	32	2	2	8
7713	38	32	2	2	8
7714	10	32	2	2	8
7714	17	4	2	2	4
7714	24	4	2	2	8
7714	31	4	2	2	16
7714	38	4	2	2	8
7715	10	4	2	2	8
7715	17	4	2	2	4
7715	24	4	2	2	16
7715	31	8	2	2	32
7715	38	8	2	2	16

A = bird's number; B = days of postvaccination; C = HI titer to NDV; D = Lot 1A - vaccinated at 10 days of age; E = group "NEWCASTLE" or "BRONCHITIS" - vaccinated in the drinking water; F = HI titer to IBV

Table A-8. Hemagglutination-inhibition (HI) titers in group "COMB-WATER" - vaccinated with a combined ND/IB vaccine

A	B	C	D	E	F
7731	10	2	2	3	8
7731	17	[illegible]	2	[illegible]	[illegible]
7731	24	[illegible]	2	[illegible]	[illegible]
7731	31	[illegible]	2	[illegible]	[illegible]
7731	38	[illegible]	[illegible]	[illegible]	[illegible]
7732	10	[illegible]	[illegible]	[illegible]	16
7732	17	16	[illegible]	[illegible]	[illegible]
7732	24	[illegible]	[illegible]	[illegible]	[illegible]
7732	31	[illegible]	[illegible]	[illegible]	16
7732	38	[illegible]	[illegible]	[illegible]	[illegible]
7733	10	[illegible]	[illegible]	[illegible]	[illegible]
7733	17	[illegible]	[illegible]	[illegible]	[illegible]
7733	24	[illegible]	[illegible]	[illegible]	[illegible]
7733	31	[illegible]	[illegible]	[illegible]	[illegible]
7733	38	[illegible]	[illegible]	[illegible]	[illegible]
7734	10	[illegible]	[illegible]	[illegible]	16
7734	17	[illegible]	[illegible]	[illegible]	[illegible]
7734	24	[illegible]	[illegible]	[illegible]	[illegible]
7734	31	[illegible]	[illegible]	[illegible]	[illegible]
7734	38	[illegible]	[illegible]	[illegible]	[illegible]
7735	10	[illegible]	[illegible]	[illegible]	[illegible]
7735	17	[illegible]	[illegible]	[illegible]	[illegible]
7735	24	[illegible]	[illegible]	[illegible]	16
7735	31	[illegible]	[illegible]	[illegible]	16
7735	38	[illegible]	[illegible]	[illegible]	[illegible]
7736	10	[illegible]	[illegible]	[illegible]	[illegible]
7736	17	[illegible]	[illegible]	[illegible]	[illegible]
7736	24	[illegible]	[illegible]	[illegible]	16
7736	31	[illegible]	[illegible]	[illegible]	16
7736	38	[illegible]	[illegible]	[illegible]	[illegible]
7737	10	[illegible]	[illegible]	[illegible]	[illegible]
7737	17	[illegible]	[illegible]	[illegible]	[illegible]
7737	24	[illegible]	[illegible]	[illegible]	[illegible]
7737	31	[illegible]	[illegible]	[illegible]	[illegible]
7737	38	[illegible]	[illegible]	[illegible]	[illegible]
7738	10	[illegible]	[illegible]	[illegible]	[illegible]
7738	17	[illegible]	[illegible]	[illegible]	[illegible]
7738	24	[illegible]	[illegible]	[illegible]	[illegible]
7738	31	[illegible]	[illegible]	[illegible]	[illegible]
7738	38	[illegible]	[illegible]	[illegible]	[illegible]
7739	10	[illegible]	[illegible]	[illegible]	[illegible]
7739	17	[illegible]	[illegible]	[illegible]	[illegible]
7739	24	[illegible]	[illegible]	[illegible]	[illegible]
7739	31	[illegible]	[illegible]	[illegible]	[illegible]
7739	38	[illegible]	[illegible]	[illegible]	[illegible]
7740	10	[illegible]	[illegible]	[illegible]	[illegible]
7740	17	[illegible]	[illegible]	[illegible]	[illegible]
7740	24	[illegible]	[illegible]	[illegible]	[illegible]
7740	31	[illegible]	[illegible]	[illegible]	[illegible]
7740	38	[illegible]	[illegible]	[illegible]	[illegible]
7741	10	[illegible]	[illegible]	[illegible]	[illegible]
7741	17	[illegible]	[illegible]	[illegible]	[illegible]
7741	24	[illegible]	[illegible]	[illegible]	[illegible]
7741	31	[illegible]	[illegible]	[illegible]	[illegible]
7741	38	[illegible]	[illegible]	[illegible]	[illegible]
7742	10	[illegible]	[illegible]	[illegible]	[illegible]
7742	17	[illegible]	[illegible]	[illegible]	[illegible]
7742	24	[illegible]	[illegible]	[illegible]	[illegible]
7742	31	[illegible]	[illegible]	[illegible]	[illegible]
7742	38	[illegible]	[illegible]	[illegible]	[illegible]
7743	10	[illegible]	[illegible]	[illegible]	[illegible]
7743	17	[illegible]	[illegible]	[illegible]	[illegible]
7743	24	[illegible]	[illegible]	[illegible]	[illegible]
7743	31	[illegible]	[illegible]	[illegible]	[illegible]
7743	38	[illegible]	[illegible]	[illegible]	[illegible]
7744	10	[illegible]	[illegible]	[illegible]	[illegible]
7744	17	[illegible]	[illegible]	[illegible]	[illegible]
7744	24	[illegible]	[illegible]	[illegible]	[illegible]
7744	31	[illegible]	[illegible]	[illegible]	[illegible]
7744	38	[illegible]	[illegible]	[illegible]	[illegible]
7745	10	[illegible]	[illegible]	[illegible]	[illegible]
7745	17	[illegible]	[illegible]	[illegible]	[illegible]
7745	24	[illegible]	[illegible]	[illegible]	[illegible]
7745	31	[illegible]	[illegible]	[illegible]	[illegible]
7745	38	[illegible]	[illegible]	[illegible]	[illegible]

A = bird's number; B = days of postvaccination; C = HI titer to NDV; D = Lot 1A - vaccinated at 10 days of age; E = group "COMB-WATER"-vaccinated in the drinking water; F = HI titer to IBV

Table A-9. Hemagglutination-inhibition (HI) titers in group "COMB-EYE" - vaccinated with a combined ND/IB vaccine

A	B	C	D	E	F
7746	10	4	4	4	8
7746	17	8	4	4	8
7746	24	8	4	4	8
7746	31	32	4	4	16
7746	38	8	4	4	16
7747	10	2	4	4	
7747	17	2	4	4	4
7747	24	4	4	4	8
7747	31	4	4	4	16
7747	38	4	4	4	8
7748	10	2	4	4	16
7748	17		4	4	
7748	24		4	4	
7748	31		4	4	
7748	38		4	4	
7749	10		4	4	4
7749	17	8	4	4	8
7749	24	4	4	4	8
7749	31	8	4	4	16
7749	38	8	4	4	8
7750	10	4	4	4	8
7750	17	8	4	4	4
7750	24	4	4	4	8
7750	31	8	4	4	16
7750	38	4	4	4	8
7751	10	2	4	4	4
7751	17	2	4	4	8
7751	24	4	4	4	8
7751	31	4	4	4	16
7751	38	2	4	4	8
7752	10	2	4	4	16
7752	17	8	4	4	32
7752	24	8	4	4	8
7752	31	4	4	4	16
7752	38	4	4	4	8
7753	10	4	4	4	4
7753	17	4	4	4	8
7753	24	4	4	4	8
7753	31	4	4	4	16
7753	38	4	4	4	16
7754	10	8	4	4	4
7754	17	4	4	4	8
7754	24	4	4	4	8
7754	31	4	4	4	8
7754	38	4	4	4	4
7755	10		4	4	
7755	17	4	4	4	8
7755	24	4	4	4	4
7755	31	4	4	4	16
7755	38	4	4	4	8
7756	10	8	4	4	8
7756	17	8	4	4	8
7756	24	16	4	4	16
7756	31	8	4	4	8
7756	38	8	4	4	4
7757	10	4	4	4	16
7757	17	8	4	4	8
7757	24	8	4	4	8
7757	31	32	4	4	16
7757	38	16	4	4	8
7758	10	4	4	4	8
7758	17	4	4	4	16
7758	24	8	4	4	16
7758	31	8	4	4	32
7758	38	4	4	4	16
7759	10	2	4	4	16
7759	17	2	4	4	8
7759	24	4	4	4	8
7759	31	4	4	4	16
7759	38	4	4	4	8
7760	10	4	4	4	4
7760	17	4	4	4	8
7760	24	4	4	4	4
7760	31	32	4	4	16
7760	38	8	4	4	8

A = bird's number; B = days of postvaccination; C = HI titer to NDV; D = Lot 1A - vaccinated at 10 days of age; E = group "COMB-EYE" - vaccinated by eye drop; F = HI titer to IBV

Table A-10. Hemagglutination-inhibition (HI) titers in group "COMB-SEP" - vaccinated with a combined ND/IB vaccine

A	B	C	D	E	F
7761	10				
7761	17				16
7761	24				
7761	31				
7761	38				
7762	10				
7762	17				
7762	24				
7762	31				
7762	38				
7763	10				
7763	17				
7763	24				
7763	31				16
7763	38				16
7764	10				
7764	17				
7764	24				
7764	31				
7764	38				
7765	10				
7765	17				
7765	24				
7765	31				
7765	38				
7766	10				
7766	17				
7766	24				
7766	31				16
7766	38				
7767	10				
7767	17				
7767	24	1			16
7767	31				32
7767	38				
7768	10				
7768	17				
7768	24				
7768	31				
7768	38				
7769	10				
7769	17				16
7769	24				
7769	31				
7769	38				
7770	10				
7770	17				16
7770	24				
7770	31				
7770	38				
7771	10				
7771	17	16			
7771	24				
7771	31				
7771	38				
7772	10				
7772	17				
7772	24				
7772	31				
7772	38	16			
7773	10				
7773	17				16
7773	24				
7773	31				
7773	38				
7774	10				
7774	17				
7774	24				
7774	31				
7774	38				
7775	10				
7775	17				
7775	24				
7775	31				
7775	38				

A = bird's number; B = days of postvaccination; C = HI titer to NDV; D = Lot 1A - vaccinated at 10 days of age; E = group "COMB-SEP" - vaccinated with ND by eye drop and IB in the drinking water; F = HI titer to IBV

Table A-11. Hemagglutination-inhibition (HI) titers in group "CONTROL" - unvaccinated chicks

A	B	C	D	E	F
7777	10			1	2
7777	17			1	0
7777	24			1	0
7777	31			1	0
7777	38			1	0
7778	10			1	0
7778	17			1	0
7778	24			1	0
7778	31			1	0
7778	38			1	0
7796	10			1	0
7796	17			1	0
7796	24			1	0
7796	31			1	0
7796	38			1	0
7780	10			1	4
7780	17			1	0
7780	24			1	0
7780	31			1	0
7780	38			1	0
7783	10			1	0
7783	17			1	0
7783	24			1	0
7783	31			1	0
7783	38			1	0
7784	10			1	0
7784	17			1	0
7784	24			1	0
7784	31			1	0
7784	38			1	0
7785	10			1	4
7785	17			1	4
7785	24			1	2
7785	31			1	0
7785	38			1	0
7786	10			1	0
7786	17			1	0
7786	24			1	0
7786	31			1	0
7786	38			1	0
7787	10			1	0
7787	17			1	0
7787	24			1	0
7787	31			1	0
7787	38			1	0
7789	10			1	0
7789	17			1	0
7789	24			1	0
7789	31			1	0
7789	38			1	0
7790	10			1	0
7790	17			1	0
7790	24			1	0
7790	31			1	0
7790	38			1	0
7792	10			1	0
7792	17			1	0
7792	24			1	0
7792	31			1	0
7792	38			1	0
7793	10			1	0
7793	17			1	0
7793	24			1	4
7793	31			1	0
7793	38			1	0
7794	10			1	0
7794	17			1	0
7794	24			1	0
7794	31			1	0
7794	38			1	0
7795	10			1	0
7795	17			1	0
7795	24			1	0
7795	31			1	0
7795	38	--		1	0

A = bird's number; B = days of bleeding; C = HI titer to NDV; D = Lot 1B; E = group "CONTROL" - unvaccinated chicks; F = HI titer to IBV

Table A-12. Hemagglutination-inhibition (HI) titers in group "NEWCASTLE" - vaccinated with a single Newcastle disease vaccine or "BRONCHITIS" - vaccinated with a single infectious bronchitis vaccine

A	B	C	D	E	F
7801	10	4	3	2	16
7801	17	4	3	2	32
7801	24	4	3	2	32
7801	31	4	3	2	16
7801	38	4	3	2	32
7802	10	4	3	2	32
7802	17	4	3	2	16
7802	24	8	3	2	16
7802	31	8	3	2	32
7802	38	4	3	2	8
7803	10	8	3	2	32
7803	17	16	3	2	32
7803	24	8	3	2	16
7803	31	8	3	2	16
7803	38	4	3	2	8
7804	10	8	3	2	16
7804	17	16	3	2	16
7804	24	16	3	2	16
7804	31	16	3	2	16
7804	38	8	3	2	8
7805	10	4	3	2	16
7805	17	4	3	2	32
7805	24	4	3	2	32
7805	31	4	3	2	32
7805	38	4	3	2	16
7706	10	16	3	2	16
7806	17	32	3	2	64
7806	24	16	3	2	64
7806	31	16	3	2	32
7806	38	8	3	2	16
7807	10	8	3	2	8
7807	17	8	3	2	8
7807	24	8	3	2	8
7807	31	4	3	2	8
7807	38	4	3	2	16
7808	10	16	3	2	16
7808	17	16	3	2	16
7808	24	16	3	2	16
7808	31	16	3	2	16
7808	38	8	3	2	8
7809	10	8	3	2	8
7809	17	8	3	2	16
7809	24	8	3	2	16
7809	31	8	3	2	8
7809	38	4	3	2	4
7810	10	4	3	2	32
7810	17	4	3	2	16
7810	24	4	3	2	16
7810	31	4	3	2	16
7810	38	4	3	2	8
7811	10	4	3	2	32
7811	17	4	3	2	16
7811	24	8	3	2	16
7811	31	4	3	2	16
7811	38	4	3	2	8
7812	10	4	3	2	32
7812	17	32	3	2	32
7812	24	32	3	2	16
7812	31	16	3	2	16
7812	38	8	3	2	8
7813	10	4	3	2	8
7813	17	4	3	2	32
7813	24	8	3	2	16
7813	31	4	3	2	16
7813	38	2	3	2	16
7814	10	4	3	2	32
7814	17	4	3	2	16
7814	24	4	3	2	16
7814	31	4	3	2	16
7814	38	4	3	2	8
7815	10	4	3	2	8
7815	17	16	3	2	4
7815	24	16	3	2	4
7815	31	4	3	2	4
7815	38	2	3	2	2

A = bird's number; B = days of postvaccination; C = HI titer to NDV; D = Lot 1B - vaccinated at 21 days of age; E = group "NEWCASTLE" or "BRONCHITIS" - vaccinated in the drinking water; F = HI titer to IBV

Table A-13. Hemagglutination-inhibition (HI) titers in group "COMB-WATER"-vaccinated with a combined ND/IB vaccine

A.	B	C	D	E	F
7831	10	4	3	3	16
7831	17	4	3	3	16
7831	24	4	3	3	16
7831	31	4	3	3	8
7831	38	2	3	3	4
7832	10	32	3	3	16
7832	17	16	3	3	16
7832	24	32	3	3	16
7832	31	16	3	3	16
7832	38	8	3	3	4
7833	10	4	3	3	8
7833	17	4	3	3	4
7833	24	4	3	3	16
7833	31	4	3	3	8
7833	38	4	3	3	8
7834	10	4	3	3	8
7834	17	8	3	3	16
7834	24	4	3	3	32
7834	31	4	3	3	8
7834	38	2	3	3	4
7835	10	4	3	3	16
7835	17	4	3	3	8
7835	24	32	3	3	32
7835	31	16	3	3	8
7835	38	8	3	3	8
7836	10	4	3	3	16
7836	17	4	3	3	8
7836	24	4	3	3	16
7836	31	16	3	3	8
7836	38	4	3	3	8
7837	10	4	3	3	16
7837	17	4	3	3	16
7837	24	4	3	3	32
7837	31	8	3	3	16
7837	38	2	3	3	8
7838	10	8	3	3	8
7838	17	32	3	3	16
7838	24	32	3	3	16
7838	31	16	3	3	16
7838	38	8	3	3	4
7839	10	4	3	3	32
7839	17	4	3	3	16
7839	24	8	3	3	16
7839	31	8	3	3	8
7839	38	4	3	3	4
7840	10	4	3	3	8
7840	17	4	3	3	16
7840	24	4	3	3	32
7840	31	4	3	3	8
7840	38	2	3	3	8
7841	10	4	3	3	16
7841	17	8	3	3	16
7841	24	4	3	3	8
7841	31	4	3	3	8
7841	38	4	3	3	4
7842	10	8	3	3	16
7842	17	4	3	3	8
7842	24	8	3	3	8
7842	31	4	3	3	16
7842	38	2	3	3	8
7843	10	4	3	3	4
7843	17	4	3	3	16
7843	24	8	3	3	16
7843	31	4	3	3	8
7843	38	2	3	3	4
7844	10	4	3	3	16
7844	17	8	3	3	8
7844	24	16	3	3	16
7844	31	16	3	3	16
7844	38	8	3	3	8
7845	10	4	3	3	8
7845	17	4	3	3	16
7845	24	4	3	3	16
7845	31	4	3	3	16
7845	38	2	3	3	8

A = bird's number; B = days of postvaccination; C = HI titer to NDV; D = Lot 1B - vaccinated at 21 days of age; E = group "COMB-WATER"-vaccinated in the drinking water; F = HI titer to IBV

APPENDIX B

Table B-1. Statistical analysis program

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BMDP2V - ANALYSIS OF VARIANCE AND COVARIANCE INCLUDING REPEATED MEASURES                PROGRAM REVISED NOVEMBER, 1976
HEALTH SCIENCES COMPUTING FACILITY                                                      MANUAL DATE - 1977
UNIVERSITY OF CALIFORNIA, LOS ANGELES
COPYRIGHT (C) 1977, THE REGENTS OF THE UNIVERSITY OF CALIFORNIA

IN THIS VERSION OF BMDP2V                                          "DISTINCT" = "NEWCASTLE"
   -- COMPUTATIONS ARE PERFORMED IN DOUBLE PRECISION.              "COMBAGUA" = "COMB-WATER"

  PROGRAM CONTROL INFORMATION

        /PROBLEM        TITLE="ANOVA NEWCASTLE 2 GRUPOS BY 3 TEMPOS".
        /INPUT          VARIABLES ARE 13.
                        FORMAT IS "(F4.0,4X,F3.0,2X,2F2.0,3X,F3.0/4(8X,F3.0,9X,F3.0/))".
                        CASES ARE 195.
        /VARIABLE       NAMES ARE SID,ND07,LOTE,GRUPO,IB07,ND14,IB14,ND21,IB21,ND28,IB28,
                                  ND35,IB35.
                        USE ARE LOTE,GRUPO,ND07,ND14,ND21,ND28,ND35.
                        MIN IS (2)0,1,2,2*0.
                        MAX IS (2)520,3,3,9*520.
                        BLANK IS MISS.
        /GROUP          CODE(3) IS 1,2,3.
                        CODE(4) IS 2,3.
                        NAME(3) IS LOT2,LOT1A,LOT1B.
                        NAME(4) IS DISTINCT,COMBAGUA.
        /DESIGN         GROUP IS 3,4.
                        DEPEND IS 2,6,8,10,12.
                        LEVEL IS 5.
        /END


  PROBLEM TITLE . . . . . .ANOVA NEWCASTLE 2 GRUPOS BY 3 TEMPOS

  NUMBER OF VARIABLES TO READ IN. . . . . .      13
  NUMBER OF VARIABLES ADDED BY TRANSFORMATIONS. .       0
  TOTAL NUMBER OF VARIABLES . . . . . .      13
  NUMBER OF CASES TO READ IN. . . . . .     195
  CASE LABELING VARIABLES . . . . . .
  LIMITS AND MISSING VALUE CHECKED BEFORE TRANSFORMATIONS
  BLANKS ARE. . . . . .  MISSING
  INPUT UNIT NUMBER . . . . . .       5
  REWIND INPUT UNIT PRIOR TO READING. . DATA. . .      NO

  INPUT FORMAT
        (F4.0,4X,F3.0,2X,2F2.0,3X,F3.0/4(8X,F3.0,9X,F3.0/))


  VARIABLES TO BE USED
          3  LOTE          4  GRUPO          2  ND07          6  ND14          8  ND21
         10  ND28         12  ND35

DESIGN SPECIFICATIONS

        GROUP =    3   4
        DEPEND =   2   6   8  10  12
        LEVEL =    5
```

Table B-2. Means and standard deviations - effect of time and method of vaccination in the production of antibody to Newcastle disease vaccine

GROUP STRUCTURE

LOT	GROUP	COUNT
LOT2	DISTINCT	10.
LOT2	COMBAGUA	11.
LOT1A	DISTINCT	13.
LOT1A	COMBAGUA	10.
LOT1B	DISTINCT	12.
LOT1B	COMBAGUA	11.

"DISTINCT" = "NEWCASTLE"
"COMBAGUA" = "COMB-WATER"

CELL MEANS FOR 1-ST DEPENDENT VARIABLE

LOT GROUP R	= LOT2 = DISTINCT	LOT2 COMBAGUA	LOT1A DISTINCT	LOT1A COMBAGUA	LOT1B DISTINCT	LOT1B COMBAGUA	MARGINAL
ND17 1	16.80000	23.63636	6.15385	7.[illegible]000	7.[illegible]333	8.36364	11.40299
ND14 2	12.30000	18.13182	6.00000	6.60000	7.66667	9.45455	9.97015
ND21 3	15.20000	17.45455	6.46154	5.80000	9.00000	6.36364	9.51045
ND28 4	16.40000	19.27273	8.46154	9.[illegible]000	8.00000	5.81818	11.10448
ND35 5	13.20000	23.63636	10.30769	13.40000	7.66667	7.45455	12.44776
MARGINAL	14.80000	20.43636	7.47692	8.52000	8.03333	7.49091	10.96716
COUNT	10	11	13	10	12	11	67

STANDARD DEVIATIONS FOR 1-ST DEPENDENT VARIABLE

LOT GROUP R	= LOT2 = DISTINCT	LOT2 COMBAGUA	LOT1A DISTINCT	LOT1A COMBAGUA	LOT1B DISTINCT	LOT1B COMBAGUA
ND17 1	18.26229	17.20042	4.50641	5.[illegible]802	8.46204	8.66340
ND14 2	9.39047	9.52700	3.65148	4.00555	5.44949	11.31692
ND21 3	10.11529	10.00364	4.48359	4.04969	5.42720	4.96533
ND28 4	11.36420	18.91609	7.92270	5.69216	5.11682	2.09893
ND35 5	8.01110	35.57323	10.64099	10.75174	4.33450	9.08145

Table B-3. Results of 2-factor analysis of variance with repeated measures

ANALYSIS OF VARIANCE FOR 1-ST DEPENDENT VARIABLE - ND07 ND14 ND21 ND28 ND35

	SOURCE	SUM OF SQUARES	DEGREES OF FREEDOM	MEAN SQUARE	F	TAIL PROBABILITY
	MEAN	41205.17145	1	41205.17145	307.89	0.0000
	L	6855.49756	2	3432.74878	25.65	.0000
	G	338.39851	1	339.39851	2.53	.1170
	LG	542.04932	2	271.02466	2.03	.1408
1	ERROR	8163.58145	61	133.82920		
	R	310.11123	4	77.52781	.65	.6247
	RL	573.13327	8	71.64166	.60	.7740
	RG	234.13535	4	58.53384	.49	.7404
	RLG	123.57952	8	15.44744	.13	.9979
2	ERROR	28930.95981	244	118.56951		

L = lot: time of vaccination

G = group: method of vaccination

R = repetition

APPENDIX C

Table C-1. Statistical analysis program

```
BMDP2V - ANALYSIS OF VARIANCE AND COVARIANCE INCLUDING REPEATED MEASURES          PROGRAM REVISED NOVEMBER, 1978
HEALTH SCIENCES COMPUTING FACILITY                                               MANUAL DATE - 1977
UNIVERSITY OF CALIFORNIA, LOS ANGELES
COPYRIGHT (C) 1977, THE REGENTS OF THE UNIVERSITY OF CALIFORNIA

IN THIS VERSION OF BMDP2V                                          "DISTINCT" = "NEWCASTLE"
   -- COMPUTATIONS ARE PERFORMED IN DOUBLE PRECISION.              "COMBAGUA" = "COMB-WATER"
                                                                   "COMBOLHO" = "COMB-EYE"

  PROGRAM CONTROL INFORMATION

        /PROBLEM      TITLE IS "ANOVA NEWCASTLE 4 GRUPOS COM 2 TEMPOS".
        /INPUT        VARIABLES ARE 13.
                      FORMAT IS "(F4.0,4X,F3.0,2X,2F2.0,3X,F3.0/4(8X,F3.0,9X,F3.0/))".
                      CASES ARE 195.
        /VARIABLE     NAMES ARE SID,ND07,LOTE,GRUPO,IB07,ND14,IB14,ND21,IB21,ND28,IB28,
                                ND35,IB35.
                      USE ARE LOTE,GRUPO,ND07,ND14,ND21,ND28,ND35.
                      MIN IS (2)0,1,2,9*0.
                      MAX IS (2)520,2,5,9*520.
                      BLANK IS MISS.
        /GROUP        CODE(3) IS 1,2.
                      CODE(4) IS   2,3,4,5.
                      NAME(3) IS LOT2,LOT1A.
                      NAME(4) IS         DISTINCT,COMBAGUA,COMBOLHO,COMBSEP.
        /DESIGN       GROUP IS 3,4.
                      DEPEND IS 2,6,8,10,12.
                      LEVEL IS 5.
        /END


  PROBLEM TITLE . . . . . .ANOVA NEWCASTLE 4 GRUPOS COM 2 TEMPOS

  NUMBER OF VARIABLES TO READ IN. . . . . .      13
  NUMBER OF VARIABLES ADDED BY TRANSFORMATIONS. .       0
  TOTAL NUMBER OF VARIABLES . . . . . .      13
  NUMBER OF CASES TO READ IN. . . . . .     195
  CASE LABELING VARIABLES . . . . . .
  LIMITS AND MISSING VALUE CHECKED BEFORE TRANSFORMATIONS
  BLANKS ARE. . . . . .  MISSING
  INPUT UNIT NUMBER . . . . . .       5
  REWIND INPUT UNIT PRIOR TO READING. . DATA. . .      NO

  INPUT FORMAT
        (F4.0,4X,F3.0,2X,2F2.0,3X,F3.0/4(8X,F3.0,9X,F3.0/))


  VARIABLES TO BE USED
         3  LOTE          4  GRUPO         2  ND07          6  ND14          8  ND21
        10  ND28         12  ND35

DESIGN SPECIFICATIONS

        GROUP =    3   4
        DEPEND =   2   6   8  10  12
        LEVEL =    5
```

Table C-2. Means and standard deviations - effect of time and method of vaccination in the production of antibody to Newcastle disease vaccine

'GROUP STRUCTURE

LOTE	GRUPO	COUNT
LOT2	DISTINCT	10.
LOT2	COMBAGUA	11.
LOT2	COMBOLHO	9.
LOT2	COMBSEP	11.
LOT1A	DISTINCT	13.
LOT1A	COMBAGUA	10.
LOT1A	COMBOLHO	11.
LOT1A	COMBSEP	12.

"DISTINCT" = "NEWCASTLE"
"COMBAGUA" = "COMB-WATER"
"COMBOLHO" = "COMB-EYE"

CELL MEANS FOR 1-ST DEPENDENT VARIABLE

LOTE / GRUPO	R	LOT2 DISTINCT	LOT2 COMBAGUA	LOT2 COMBOLHO	LOT2 COMBSEP	LOT1A DISTINCT	LOT1A COMBAGUA	LOT1A COMBOLHO	LOT1A COMBSEP	MARGINAL
ND07	1	16.80000	23.63636	20.44444	18.18182	6.15385	7.00000	4.36364	8.00000	12.71264
ND14	2	12.80000	18.18182	20.00000	17.09091	6.00000	6.60000	5.09091	6.66667	11.21839
ND21	3	15.20000	17.45455	27.55556	17.45455	6.46154	5.80000	6.00000	6.66667	12.32184
ND28	4	16.40000	19.27273	27.55556	13.09091	8.46154	9.80000	8.00000	6.33333	13.10345
ND35	5	13.20000	23.63636	22.66667	16.72727	10.30769	13.40000	8.18182	7.00000	14.04598
MARGINAL		14.88000	20.43636	23.64444	16.50909	7.47692	8.52000	6.32727	6.93333	12.68046
COUNT		10	11	9	11	13	10	11	12	87

STANDARD DEVIATIONS FOR 1-ST DEPENDENT VARIABLE

LOTE / GRUPO	R	LOT2 DISTINCT	LOT2 COMBAGUA	LOT2 COMBOLHO	LOT2 COMBSEP	LOT1A DISTINCT	LOT1A COMBAGUA	LOT1A COMBOLHO	LOT1A COMBSEP
ND07	1	18.26029	17.20042	17.93817	11.36502	4.50641	5.09902	1.96330	4.17786
ND14	2	8.39047	9.52700	12.00000	8.40779	3.65148	4.00555	2.42712	3.55050
ND21	3	10.11929	10.00364	16.54623	10.00364	4.48359	4.04969	2.36643	3.55050
ND28	4	11.38420	18.91608	22.66667	7.39533	7.92270	5.69210	8.19756	2.05971
ND35	5	8.01110	35.57323	18.11077	8.35573	10.64099	10.75174	8.78428	3.46410

Table C-3. Results of 2-factor analysis of variance with repeated measures

ANALYSIS OF VARIANCE FOR 1-ST DEPENDENT VARIABLE - ND07 ND14 ND21 ND28 ND35

	SOURCE	SUM OF SQUARES	DEGREES OF FREEDOM	MEAN SQUARE	F	TAIL PROBABILITY
	MEAN	73699.17307	1	73699.17307	560.06	0.0000
	L	14350.20877	1	14350.20877	109.05	0.0000
	G	1182.10561	3	394.03520	2.99	.0357
	LG	1420.26331	3	473.42110	3.60	.0171
1	ERROR	10385.80165	79	131.59243		
	R	378.07951	4	94.51988	.73	.5701
	RL	251.93626	4	62.98406	.49	.7443
	RG	993.28734	12	82.77395	.64	.8060
	RLG	350.91818	12	29.24318	.23	.9971
2	ERROR	40759.67901	316	128.98633		

L = lot: time of vaccination

G = group: method of vaccination

R = repetition

APPENDIX D

Table D-1. Statistical analysis program

```
BMDP2V - ANALYSIS OF VARIANCE AND COVARIANCE INCLUDING REPEATED MEASURES PROGRAM REVISED NOVEMBER,
HEALTH SCIENCES COMPUTING FACILITY                                      MANUAL DATE - 1977
UNIVERSITY OF CALIFORNIA, LOS ANGELES
COPYRIGHT (C) 1977, THE REGENTS OF THE UNIVERSITY OF CALIFORNIA

IN THIS VERSION OF BMDP2V                          "DISTINCT" = "BRONCHITIS"
   -- COMPUTATIONS ARE PERFORMED IN DOUBLE PRECISION. "COMBAGUA" = "COMB-WATER"

  PROGRAM CONTROL INFORMATION

        /PROBLEM      TITLE IS "ANOVA BRONCHITIS 3 GRUPOS COM 3 TEMPOS".
        /INPUT        VARIABLES ARE 13.
                      FORMAT IS "(F4.0,4X,F3.0,2X,2F2.0,3X,F3.0/4(4X,F3.0,9X,F3.0/))".
                      CASES ARE 195.
        /VARIABLE     NAMES ARE SID,ND07,LOTE,GRUPO,IB07,ND14,IB14,ND21,IB21,ND28,IB28,
                                ND35,IB35.
                      USE ARE LOTE,GRUPO,IB07,IB14,IB21,IB28,IB35.
                      MIN IS (2)0,2*1,9*0.
                      MAX IS (2)520,3,3,9*520.
                      BLANK IS MISS.
        /GROUP        CODE(3) IS 1,2,3.
                      CODE(4) IS   2,3.
                      NAME(3) IS LOT2,LOT1A,LOT1B.
                      NAME(4) IS         DISTINCT,COMBAGUA.
        /DESIGN       GROUP IS 3,4.
                      DEPEND IS 5,7,9,11,13.
                      LEVEL IS 5.
        /END

  PROBLEM TITLE . . . . . .ANOVA BRONCHITIS 3 GRUPOS COM 3 TEMPOS

  NUMBER OF VARIABLES TO READ IN. . . . . .      13
  NUMBER OF VARIABLES ADDED BY TRANSFORMATIONS. .       0
  TOTAL NUMBER OF VARIABLES . . . . . .      13
  NUMBER OF CASES TO READ IN. . . . . .     195
  CASE LABELING VARIABLES . . . . . .
  LIMITS AND MISSING VALUE CHECKED BEFORE TRANSFORMATIONS
  BLANKS ARE. . . . . .  MISSING
  INPUT UNIT NUMBER . . . . . .       5
  REWIND INPUT UNIT PRIOR TO READING. . DATA. . .      NO

  INPUT FORMAT
        (F4.0,4X,F3.0,2X,2F2.0,3X,F3.0/4(4X,F3.0,9X,F3.0/))


  VARIABLES TO BE USED
          3  LOTE          4  GRUPO         5  IB07          7  IB14          9  IB21
         11  IB28         13  IB35

DESIGN SPECIFICATIONS

        GROUP =    3   4
        DEPEND =   5   7   9  11  13
        LEVEL =    5

                    BEFORE TRANSFORMATION                                    INTERVAL RANGE
 VARIABLE          MINIMUM      MAXIMUM      MISSING     CATEGORY   CATEGORY   GREATER     LESS THAN
 NO.  NAME         LIMIT        LIMIT        CODE        CODE       NAME       THAN        OR EQUAL TO
 ---------------------------------------------------------------------------------------------------
   3   LOTE        1.00000      3.00000                  1.00000   LOT2
                                                         2.00000   LOT1A
                                                         3.00000   LOT1B

   4   GRUPO       1.00000      3.00000                  2.00000   DISTINCT
                                                         3.00000   COMBAGUA

  NUMBER OF CASES READ. . . . . .     162
     CASES WITH DATA MISSING OR BEYOND LIMITS . .      17
        REMAINING NUMBER OF CASES . . . . . .     145
     CASES WITH GROUPING VALUES NOT USED. . . . .      80
        REMAINING NUMBER OF CASES . . . . . .      65
```

Table D-2. Means and standard deviations - effect of time and method of vaccination in the production of antibody to infectious bronchitis vaccine

GROUP STRUCTURE

LOT2	GRUPO	COUNT
LOT2	CONTROL	13.
LOT2	DISTINCT	10.
LOT2	COMBAGUA	11.
LOT1A	CONTROL	12.
LOT1A	DISTINCT	12.
LOT1A	COMBAGUA	9.
LOT1B	CONTROL	13.
LOT1B	DISTINCT	12.
LOT1B	COMBAGUA	11.

"DISTINCT" = "BRONCHITIS"
"COMBAGUA" = "COMB-WATER"

CELL MEANS FOR 1-ST DEPENDENT VARIABLE

LOTE / GRUPO / R	LOT2 CONTROL	LOT2 DISTINCT	LOT2 COMBAGUA	LOT1A CONTROL	LOT1A DISTINCT	LOT1A COMBAGUA	LOT1B CONTROL	LOT1B DISTINCT	LOT1B COMBAGUA
1807 1	4.92308	51.20000	37.81818	1.00000	12.33333	8.33333	.92308	20.00000	11.63636
1814 2	3.69231	32.60000	39.27273	1.83333	16.33333	12.00000	.76923	18.00000	12.72727
1821 3	2.30769	28.00000	34.90909	1.83333	13.66667	9.77778	.45154	19.66667	14.18182
1828 4	2.76923	25.60000	23.27273	1.83333	13.00000	9.77778	1.69231	13.00000	9.45455
1835 5	6.00000	32.00000	41.45455	1.83333	10.00000	10.22222	3.07692	15.00000	13.81818
MARGINAL	3.93846	33.92000	35.34545	1.66667	13.06667	10.22222	1.38462	17.13333	12.36364
COUNT	13	10	11	12	12	9	13	12	11

MARGINAL

R	
1807 1	15.68932
1814 2	14.56311
1821 3	13.26214
1828 4	10.64078
1835 5	14.17476
MARGINAL	13.66602
COUNT	103

STANDARD DEVIATIONS FOR 1-ST DEPENDENT VARIABLE

LOTE / GRUPO / R	LOT2 CONTROL	LOT2 DISTINCT	LOT2 COMBAGUA	LOT1A CONTROL	LOT1A DISTINCT	LOT1A COMBAGUA	LOT1B CONTROL	LOT1B DISTINCT	LOT1B COMBAGUA
1807 1	5.57467	47.39620	22.65312	1.34840	8.08293	4.00000	1.32045	16.18080	5.20140
1814 2	4.00990	22.76840	34.58639	2.62274	12.23507	9.16515	1.53590	9.10544	4.67099
1821 3	2.13638	20.39608	20.00727	2.32900	9.86577	4.94413	.87706	9.86577	7.45410
1828 4	3.21854	10.53249	8.35573	3.01008	9.51554	4.94413	4.46065	4.55272	4.48026
1835 5	9.66092	35.17575	45.79162	2.48022	4.67099	8.96908	8.74130	9.04534	10.01817

Table D-3. Results of 2-factor analysis of variance with repeated measures

ANALYSIS OF VARIANCE FOR 1-ST DEPENDENT VARIABLE - IH07 IB14 IB21 IB28 IB35

	SOURCE	SUM OF SQUARES	DEGREES OF FREEDOM	MEAN SQUARE	F	TAIL PROBABILITY
	MEAN	133100.70223	1	133100.70223	422.83	0.0000
	L	32762.27729	2	16381.13865	52.04	.0000
	G	342.20981	1	342.20981	1.09	.3014
	LG	544.14480	2	272.07240	.86	.4266
1	ERROR	18572.48808	59	314.78793		
	R	2287.72304	4	570.93076	1.69	.1529
	RL	2938.19289	8	367.27411	1.09	.3725
	RG	1057.21426	4	264.30356	.78	.5374
	RLG	1036.93845	8	129.61731	.38	.9286
2	ERROR	79608.80880	236	337.70682		

L = lot: time of vaccination

G = group: method of vaccination

R = repetition

APPENDIX E

Table E-1. Statistical analysis program

```
BMDP2V - ANALYSIS OF VARIANCE AND COVARIANCE INCLUDING REPEATED MEASURES
HEALTH SCIENCES COMPUTING FACILITY
UNIVERSITY OF CALIFORNIA, LOS ANGELES                          PROGRAM REVISED NOVEMBER, 1
'COPYRIGHT (C) 1977, THE REGENTS OF THE UNIVERSITY OF CALIFORNIA   MANUAL DATE - 1977

IN THIS VERSION OF BMDP2V

   -- COMPUTATIONS ARE PERFORMED IN DOUBLE PRECISION.


  PROGRAM CONTROL INFORMATION

        /PROBLEM      TITLE IS "ANOVA BRONCHITIS 5 GRUPOS COM 2 TEMPOS".
        /INPUT        VARIABLES ARE 13.
                      FORMAT IS "(F4.0,4X,F3.1,2X,2F2.0,3X,F3.0/4(8X,F3.0,9X,F3.0/))".
                      CASES ARE 195.
        /VARIABLE     NAMES ARE SID,ND07,LOTE,GRUPO,IB07,ND14,IB14,ND21,IB21,ND28,IB28,
                            ND35,IB35.
                      USE APE LOTE,GRUPO,IB07,IB14,IB21,IB28,IB35.
                      MIN IS (2)0,2*1,9*0.
                      MAX IS (2)520,2.5,9*520.
                      BLANK IS MISS.
        /GROUP        CODE(3) IS 1,2.
                      CODE(4) IS   2,3,4,5.
                      NAME(3) IS LOT2,LOT1A.
                      NAME(4) IS         DISTINCT,COMBAGUA,COMBOLHO,COMBSEP.
        /DESIGN       GROUP IS 3,4.
                      DEPEND IS 5,7,9,11,13.
                      LEVEL IS 5.
        /END


  PROBLEM TITLE . . . . . .ANOVA BRONCHITIS 5 GRUPOS COM 2 TEMPOS

  NUMBER OF VARIABLES TO READ IN. . . . . .      13
  NUMBER OF VARIABLES ADDED BY TRANSFORMATIONS. .       0
  TOTAL NUMBER OF VARIABLES . . . . . .      13
  NUMBER OF CASES TO READ IN. . . . . .     195
  CASE LABELING VARIABLES . . . . . .
  LIMITS AND MISSING VALUE CHECKED BEFORE TRANSFORMATIONS
  BLANKS ARE. . . . . .  MISSING
  INPUT UNIT NUMBER . . . . . .       5
  REWIND INPUT UNIT PRIOR TO READING. . DATA. . .      NO

  INPUT FORMAT
        (F4.0,4X,F3.1,2X,2F2.0,3X,F3.0/4(8X,F3.0,9X,F3.0/))


  VARIABLES TO BE USED
            3  LOTE          4  GRUPO         5  IB07          7  IB14          9  IB21
           11  IB28         13  IB35

DESIGN SPECIFICATIONS

        GROUP =    3   4
        DEPEND =   5   7   9  11  13
        LEVEL =    5

                    BEFORE TRANSFORMATION                                  INTERVAL RANGE
 VARIABLE        MINIMUM     MAXIMUM     MISSING     CATEGORY  CATEGORY    GREATER     LESS THAN
 NO.  NAME       LIMIT       LIMIT       CODE        CODE      NAME        THAN        OR EQUAL TO

  3   LOTE       1.00000     2.00000                 1.00000   LOT2
                                                     2.00000   LOT1A

  4   GRUPO      1.00000     5.00000                 2.00000   DISTINCT
                                                     3.00000   COMBAGUA
                                                     4.00000   COMBOLHO
                                                     5.00000   COMBSEP

  NUMBER OF CASES READ. . . . . .     182
     CASES WITH DATA MISSING OR BEYOND LIMITS . .      17
        REMAINING NUMBER OF CASES . . . . . .     145
     CASES WITH GROUPING VALUES NOT USED. . . . .      61
        REMAINING NUMBER OF CASES . . . . . .      84
```

"DISTINCT" = "BRONCHITIS"
"COMBAGUA" = "COMB-WATER"
"COMBOLHO" = "COMB-EYE"

Table E-2. Means and standard deviations - effects of time and method of vaccination in the production of antibody to infectious bronchitis vaccine

GROUP STRUCTURE

LOTE	GRUPO	COUNT
LOT2	CONTROL	13.
LOT2	DISTINCT	10.
LOT2	COMBAGUA	11.
LOT2	COMBOLHO	9.
LOT2	COMBSEP	12.
LOT1A	CONTROL	12.
LOT1A	DISTINCT	12.
LOT1A	COMBAGUA	9.
LOT1A	COMBOLHO	11.
LOT1A	COMBSEP	10.

"DISTINCT" = "BRONCHITIS"
"COMBAGUA" = "COMB-WATER"
"COMBOLHO" = "COMB-EYE"

CELL MEANS FOR 1-ST DEPENDENT VARIABLE

LOTE =	LOT2	LOT2	LOT2	LOT2	LOT2	LOT1A	LOT1A	LOT1A	LOT1A
GRUPO =	CONTROL	DISTINCT	COMBAGUA	COMBOLHO	COMBSEP	CONTROL	DISTINCT	COMBAGUA	COMBOLHC
R									
IB07 1	4.92308	31.20000	37.81818	27.55556	35.33333	1.00000	12.33333	9.33333	9.09091
IB14 2	3.61231	32.60000	39.27273	24.00000	46.66667	1.83333	16.33333	12.00000	12.00000
IB21 3	2.31769	28.80000	34.90909	36.44444	31.33333	1.83333	13.66667	9.77778	10.90909
IB28 4	2.75923	25.60000	23.27273	52.44444	20.16667	1.83333	13.00000	9.77778	12.00000
IB35 5	6.01000	32.01000	41.45455	30.22222	37.50000	1.83333	10.00000	10.22222	12.36364
MARGINAL	3.93846	33.92000	35.34545	34.13333	34.20000	1.66667	13.06667	11.22222	11.27273
COUNT	13	10	11	9	12	12	12	9	11

LOTE =	LOT1A	MARGINAL
GRUPO =	COMBSEP	
R		
IB07 1	10.81000	19.41200
IB14 2	10.80000	19.72477
IB21 3	7.61000	17.13702
IB28 4	6.41000	15.81651
IB35 5	7.21000	18.51371
MARGINAL	8.50000	18.12121
COUNT	11	169

Table E-2 (continued)

STANDARD DEVIATIONS FOR 1-ST DEPENDENT VARIABLE

LOTE GRUPO	=	LOT2 CONTROL	LOT2 DISTINCT	LOT2 COMBAGUA	LOT2 COMBOLHO	LOT2 COMBSEP	LOT1A CONTROL	LOT1A DISTINCT	LOT1A COMBAGUA	LOT1A COMBOLHO
IB07	1	5.57467	47.39620	22.05312	16.54623	19.13271	1.34840	8.08290	4.00000	4.76350
IB14	2	4.05990	22.76841	34.98639	22.97825	67.61029	2.62274	12.23507	9.16515	7.79744
IB21	3	2.13638	20.39608	20.00727	35.57777	17.54648	2.32988	9.86577	4.94413	4.03620
IB28	4	3.21854	10.53240	8.45573	45.45083	11.26405	3.01008	9.51554	4.94413	7.79744
IB35	5	9.06092	35.17575	46.79162	21.45797	43.57752	2.48022	4.67099	8.96909	5.20140

LOTE GRUPO	=	LOT1A COMBSEP
IB07	1	8.65127
IB14	2	8.65127
IB21	3	3.50238
IB28	4	2.06559
IB35	5	3.67575

Table E-3. Results of 2-factor analysis of variance with repeated measures

ANALYSIS OF VARIANCE FOR 1-ST DEPENDENT VARIABLE - I807 I814 I821 I828 I835

	SOURCE	SUM OF SQUARES	DEGREES OF FREEDOM	MEAN SQUARE	F	TAIL PROBABILITY
	MEAN	211882.47570	1	211882.47570	415.22	0.0000
	L	57907.44833	1	57907.44833	113.48	0.0000
	G	253.70125	3	84.56708	.17	.9192
	LG	390.20510	3	130.06837	.25	.8576
1	ERROR	38782.45657	76	510.29548		
	R					
	RL	843.11346	4	235.77837	.42	.7956
	RG	702.97235	4	175.74309	.31	.8702
	RLG	7661.50339	12	638.45862	1.13	.3334
2	ERROR	6473.77914	12	539.48159	.96	.4908
		171474.29899	304	564.06019		

L = lot: time of vaccination

G = group: method of vaccination

R = repetition