Onderstepoort Journal of Veterinary Research,
Volume 25, *Number* 4, *December,* 1952.
The Government Printer, Pretoria.

NEWCASTLE DISEASE VACCINATION: THE USE OF LIVE VIRUS AFTER INACTIVATED VACCINE.

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INTRODUCTION.

During the summer of 1950 the Newcastle disease epizootic was at its height in the Western Province of the Cape. Mortality was heavy and efforts to bring the outbreak under control by the usual slaughter and quarantine measures had failed, due to the somewhat peculiar local conditions. Infection continued to spread and several large poultry establishments, one of which regularly maintains some 70,000 birds, were in imminent danger of being exterminated.

No vaccine for the immunization of these birds was available in the country, so the South African Poultry Association, in collaboration with the Department of Agriculture, arranged the importation and distribution of large quantities of inactivated vaccine from the United States of America.*

The judicious use of this vaccine undoubtedly saved many thousands of birds. Its application was confined chiefly to the large commercial plants and infection continued to smoulder in the small " back-yard " flocks frequently maintained on open range under the most appalling hygienic conditions.

The short duration of adequate protection produced by the inactivated vaccine made repeated immunization a necessity. Not only was this costly but repeated handling of the birds had an adverse effect upon egg production. It was decided, therefore, that full control could be obtained economically only by the use of an attenuated live virus to produce a durable high grade immunity.

No facilities were available for the production of such a vaccine locally and it was considered inadvisable to introduce any Newcastle disease virus into Onderstepoort where the large poultry plant maintained to produce the fertile eggs required for the preparation of bluetongue, fowl pox and dog distemper vaccines was fully susceptible. It was, therefore, decided to establish and equip a laboratory in Cape Town to prepare the live virus vaccine and to carry out the research work necessary to determine its effect under South African conditions.

Meanwhile use of the inactivated vaccine continued so that it became a matter of some importance to determine when and how a change could be made to a single application of live virus vaccine to produce a durable immunity and the effect, if any, of the previous treatment. During the course of these investigations it was found that whereas the use of live virus on fully susceptible laying hens resulted in a marked drop in egg production and in certain isolated instances might result in fairly heavy mortality due to that condition known generally as

* Imported from Fort Dodge Laboratories, Fort Dodge, Iowa, U.S.A.

Received for publication on 17th July, 1951.—Editor.

" egg yolk peritonitis ", no such drop in egg production and no mortality occurred among hens previously treated with inactivated vaccine. Moreover, the substantial rise in haemagglutination inhibition (H.I.) titre of the serum was indicative of a response to the live virus vaccine.

The results obtained from this series of experiments form the basis of this report.

Methods and Materials.

Except as indicated in Table No. 1 groups g and h, where a Cape virulent virus was employed, the live virus used in all cases was the avirulent Roakin strain (Beaudette *et al.*, 1949) generation 5, propagated in the allantoic sac of nine-day embryonated hens' eggs at 37° C. The pooled allantoic fluid could be relied upon to have an LD 50 for embryos of not less than 10^{-8}.

The inactivated vaccine, in all cases except one, was an imported * aluminium hydroxide formalinized vaccine of egg embryo origin used in a single dosage of 1·0 c.c. intramuscularly. The exception is mentioned in Table No. 6, flock 6, where a locally produced similar vaccine was used.

The method of determining whether there had been a response to the live virus was based on the development of high titre haemagglutination-inhibition (H.I.) antibodies in the serum. The H.I. tests were done according to Fabricant's (1949) modification of the United States Bureau of Animal Industry method, with the addition of a control serum-red cell test for each serum. In a flock which reacted satisfactorily, more than 50 per cent. showed titres of 1/1,280 or higher persisting for long periods. A titre of 1/160 was regarded as inconclusive, since fowls showing such low titres usually became negative in four to six weeks. Fowls which had been vaccinated with the inactivated vaccine never showed a titre higher than 1/40 one month after treatment.

In order to determine the response in a large flock 10–20 blood samples of various selected groups were tested. It was found that by this method a representative picture of the entire flock was obtained. More than 1,000 H.I. tests were carried out. In small flocks all birds were tested.

In order to facilitate observations on the distribution of the live virus after application, crystal violet was added to the virus-saline-glycerine mixture to a final concentration of 1/8,000. This coloured the vaccine brightly and had no harmful effect on the viability of the virus at room and lower temperatures.

Past experience has shown that it is most desirable to conduct experiments of this nature on as large a number of birds from as many different flocks as possible to obtain statistically significant results owing to variations in reactions amongst birds maintained under different conditions. The co-operation of a number of poultry breeders in the Western Province was obtained for the extensive field trials to observe the reaction of the live virus on fowls previously vaccinated with the inactivated vaccine.

Experimental.

A series of experiments was conducted to determine the most effective route of application of the live virus, its dosage and its effect on egg production. These experiments are detailed below.

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* Imported from Fort Dodge Laboratories, Fort Dodge, Iowa, U.S.A.

1. *Preliminary comparison of various routes of application of live virus on immunized birds.*

27 Cockerels which has received 1 c.c. of inactivated vaccine three weeks previously were divided into nine groups of three each, six groups (a–f, Table No. 1) to be immunized with Roakin live virus as indicated in Table No. 1, and two groups (g–h, Table No. 1) to receive a Cape virulent strain of virus. This latter virus was infected allantoic fluid with an embryo Ld 50 of 10^{-9}.

TABLE No. 1.

Preliminary comparison of various routes of application of live virus.

Group.	Route of Application of Live Virus.	Dose of Virus.	Result (H.I. Titres).
		Roakin Strain.	
a	Wing-web.....	Moistened point of double needle allantoic fluid Ld 50 10^{-8}	No reaction.
b	Intramuscularly....	0·1 c.c. allantoic fluid Ld 50 10^{-3}.....	No reaction.
c	Intramuscularly....	0·1 c.c. allantoic fluid Ld 50 10^{-8}.....	No reaction.
d	Intranasally.....	One drop allantoic fluid Ld 50 10^{-8}.....	Positive reaction.
e	Intra-ocularly.....	One drop allantoic fluid Ld 50 10^{-8}.....	Positive reaction.
f	Intratracheally.....	One drop allantoic fluid Ld 50 10^{-8}.....	Positive reaction.
		Cape Virulent Strain.	
g	Wing-web.....	Moistened point of double needle allantoic fluid Ld 50 10^{-9}	Positive reaction.
h	Intramuscularly....	0·1 c.c. allantoic fluid Ld 50 10^{-9}	Positive reaction.

" No reaction " indicates H.I. titre of 1/40 or lower in all three.

" Positive reaction " indicates H.I. titre of 1/160 or higher in all three.

Groups a–f received avirulent Roakin strain live virus.

Groups g–h received Cape virulent virus.

It is seen from Table No. 1 that only those birds which were treated intra-ocularly, intranasally or intratracheally with Roakin strain live virus reacted as indicated by the development of high titre H.I. antibodies, while birds treated by intramuscular injection or by wing-web stab did not react. Those birds which received virulent virus (groups g and h), although showing no evidence of a clinical reaction, developed extremely high titre H.I. antibodies, 1/50,000 or higher in all six birds.

Conclusion.

Three weeks after treatment with inactivated vaccine, there exists sufficient immunity fully to protect against infection by intramuscular injection or wingweb stab in the case of the avirulent Roakin strain but the virulent strain was able to produce high titre antibodies, without signs of clinical illness.

The favourable response with the Roakin strain by the intra-ocular, intranasal or intratracheal routes, prompted further investigation.

2. *Comparison of intra-ocular and intranasal routes of application of the live virus.*

The intratracheal route was considered impractical and was not further considered.

One hundred adult laying hens which had been immunized four months previously with inactivated vaccine, were divided into two groups of 50 each to be treated with infected allantoic fluid (embryo LD 50 10^{-8}) diluted 1/10 in crystal violet glycerine-saline. The birds received one drop of this diluted allantoic fluid which was applied with an eye-dropper.

TABLE No. 2.

Comparison of the routes of application of the live virus.

Route.	No. of Fowls in Experiment.	Percentage of Birds showihg H.I. Titre Response.
Intra-ocular.....	50	96
Intranasal.....	50	80

Result.

From the data in Table No. 2 it is seen that 96 per cent. of the birds which received the live virus vaccine intraocularly developed high titre H.I. antibodies as compared with 80 per cent. that were treated intranasally.

Discussion.

The intranasal route has been popularised in the United States of America by the use of the BI strain (Hitchner and Johnson 1948). In our experience the intraocular instillation with an eye-dropper is easier and quicker. A fowl usually keeps its eye wide open and there is no difficulty in introducing the vaccine. A few moments after its application into the eye, the coloured vaccine could be observed in the nasal passages and buccal cavity. The intranasally applied vaccine remained in the nasal passages and did not spread. Frequently it was ejected from the nostrils by normal breathing. In addition many nostrils were blocked with exudate due to intercurrent coryza infection, so that the virus could not contact the naso-pharyngeal mucosa. Consequently the intranasal route was considered unreliable and intra-ocular instillation was regarded as the method of choice.

3. *The effect of intra-ocular instillation of various dilutions of virus.*

The fowls used were adult laying hens which had received inactivated vaccine four months previously. The virus used was the same as in the previous experiment, diluted as indicated below in Table No. 3.

TABLE No. 3.

The effect of intra-ocular instillation of dilutions of virus.

No. of Fowls in Experiment.	Dilutions of Allantoic Fluid.	Percentage of Birds showing Serum H.I. Titre Response.
50	1/100	70
50	1/20	90
50	1/10	96
50	1/5	96

Result.

It is evident that the stronger virus mixtures produced better H.I. titres, the 1/10 virus equalling the 1/5 dilution.

4. *The effect of the interval between inactivated vaccine and intra-ocular instillation of live virus vaccine.*

The numbers of fowls available for this experiment were variable and in some cases inadequate. The virus used was the same as in experiment 2.

TABLE No. 4.

Effect of the time interval between inactivated vaccine and live virus intraocularly.

No. of Fowls in Experiment.	Interval.	Dilution of Allantoic Fluid.	Percentage of Birds showing Serum H.I. Titre Response.
2,000	8 weeks	1/100	20
8	10 weeks	(*a*) 1/30	25
8	—	(*b*) 1/10	50
4	12 weeks	(*a*) 1/30	75
4	—	(*b*) 1/10	100
30	16 weeks	1/10	90

" Interval " refers to the interval between the use of the inactive and live virus vaccines.

Result.

The longer the interval between the inactive vaccine and the application of the live virus intra-ocularly, the better is the response.

Conclusion.

For the use of the 1/10 virus dilution, the interval should be at least twelve weeks.

5. *Effect of the interval between inactive vaccine and live virus per wing-web.*

The numbers of birds again in this experiment were variable and in many cases inadequate. As it is known that the response of fowls from different flocks may vary within wide limits, the results of this experiment are unfortunately reduced in value since the samples were drawn from different flocks. The day-old chickens referred to originated from eggs laid by fully susceptible hens. These day-old chickens received 0·5 c.c. inactivated vaccine intramuscularly when one day old. The live virus used was undiluted allantoic fluid, embryo LD 50 10^{-8}, applied with a two-pronged needle.

TABLE No. 5.

The effect of the time interval between inactivated vaccine and live virus per wing-web.

No. of Birds in Experiment.	Interval.	Percentage of Birds showing Serum H.I. Titre Response.
23	4 weeks	10
10	8 weeks	10
8	10 weeks	0
18	12 weeks	20
4	14 weeks	50
8	16 weeks	25
(*a*) 8	20 weeks	25
(*b*) 8	—	88
8	24 weeks	88
20	32 weeks	90
*8	8 weeks	80
*10	8 weeks	90
*10	8 weeks	20

* Denotes chickens which received 0·5 c.c. inactivated vaccine intramuscularly when a day old.

Result.

Those birds which received live virus within five months of the inactive vaccine showed poor response but those vaccinated per wing-web with live virus with intervals of 20, 24 and 32 weeks respectively, showed a considerable increase in H.I. antibodies, yet at the same time showing no evidence of clinical reaction or a drop in egg production. This differed from those flocks which had never been vaccinated before, and where a marked drop in egg production usually followed live virus immunization per wing-web one week later.

Conclusion.

An interval of at least five months must elapse before the application of live virus per wing-web after an inactivated vaccine. The response to the vaccination was marked by the absence of a drop in egg production, which usually occurs in fully susceptible hens.

6. *The effect on egg production on a field scale.*

The effect of the live virus on the serum antibody H.I. titres and the egg production of fowls previously vaccinated with a full dose, i.e. 1 c.c., of inactivated vaccine (Table No. 6, flocks 1–5), a half dose (flock 6), and in fully susceptible

fowls (flocks 7 and 8) was examined on a field scale. Eight privately owned, well managed commercial flocks were selected for these experiments. These flocks were highly representative of the average commercial flocks in the Western Province of the Cape. The daily egg records could be relied upon as being accurate. The total daily egg records, for the entire flocks concerned in these experiments were closely followed, commencing shortly before the use of live virus up to 5–6 weeks after its administration. The particular emphasis of this experiment was on the effect of the live virus on egg production, but in order to determine whether a reaction had occurred, ten serum samples were tested from each different group in each flock. This gave a representative picture of the reaction in the entire flock. The results are tabulated in Table No. 6. The virus used was the same as in experiment 2 and it was applied with an eye-dropper.

Result.

As could be expected from previous experiments these flocks were responsive to the live virus applied intra-ocularly, as proved by a high percentage serum H.I. titre response in all cases. No drop in egg production occurred in flocks 1–6. Flock 6 which had received a half dose of locally prepared inactivated vaccine showed a very satisfactory response, and the results obtained merit further consideration of the use of reduced doses of inactivated vaccine at shorter intervals. The fully susceptible flocks 7 and 8 experienced a drop in egg production comparable with that usually obtained when live virus is used per wing-web in fully susceptible birds.

The reaction that occurs is evidently a systemic one, for in no case was evidence of local inflammation of the eye obtained.

Conclusion.

Those flocks which had a partial immunity as a result of the decline of the immunity which resulted from inactivated vaccine, while responsive to the live virus as indicated by a rise in serum antibodies, differed markedly in the absence of a drop in egg production which was so marked in the fully susceptible flocks.

Discussion.

It is well-known among poultrymen that when birds are handled, disturbed or exposed to changes of environment or food, their reaction as far as egg production is concerned, depends largely on the time of the year. When laying hens are handled from July to September, the usual hatching season in South Africa, it is seldom that a drop in egg production occurs. Should they be handled or disturbed at other times, especially in late summer, they are very prone to go off lay and may even be thrown into a moult. A drop in egg production can, therefore, be ascribed to many causes. Most of our experiments and observations were made from July to November 1950. It is felt that where a drop in egg production followed vaccination at this time of the year, it was due to the reaction to vaccination. This occurred in the two small flocks (Table No. 6, flocks 7 and 8). It was also the usual reaction to be expected in fully susceptible laying hens vaccinated through the wing-web, at all times of the year.

Live virus after killed vaccine has been used on several occasions. Burnet and Anderson (1946) showed that fowls could be immunized with a virulent live virus after a formalinized one. Likewise Le Dosseur and Lissot

TABLE NO. 6.

The effect on egg production on a field scale.

Flock No.	Interval.	No. of Birds.	Breed.	Age and Description of Flock.	Percentage Egg Production before l.v.i.	Percentage Production 3 Weeks after l.v.i.	Percentage of Birds showing Serum H.I. Titre Response.
1	4 Months.....	500	B.A.	Adult laying hens.....	68	68	90
2	5 Months.....	4,000	W.L.	Adult laying hens.....	70	63	96
		20,000	W.L.	Non-laying young stock.....	—	—	
3	4–6 Months.....	12,000	W.L. & B.A.	Adult laying hens.....	50	48	94
		18,000	B.A. & W.L.	Non-laying young stock.....	—	—	
4	4 Months.....	3,000	W.L.	Adult laying hens.....	65	65	90
		4,000	W.L.	Non-laying young stock.....	—	—	
5	6 Months.....	5,000	W.L. × B.A.	Adult laying hens	60	60	90
		7,000	W.L. × B.A.	Non-laying young stock.....	—	—	
6	These fowls received 0·5 c.c. inactivated vaccine* i.m. 1 month previously.	500	W.L.	Young laying hens.....	56	60	98
7	Not previously vaccinated...	38	W.L.	Adult laying hens.....	78	16	96
8	Not previously vaccinated...	183	W.L.	Young laying stock.....	60	36	92

l.v.i. = Live virus immunization.
B.A. = Black Australorp.
W.L. =White Leghorn.
B.A. × W.L. = Cross breed.

* Denotes a locally prepared aluminium hydroxide adsorbed formalinized vaccine of embryo origin using a Cape virulent strain of virus.

" Interval " refers to the interval between the inactivated vaccines and the live virus.

(1949) used a live virulent virus by skin scarification 15–35 days after a formalinized vaccine and a good immunity was produced. Moses *et al.* (1948) came to the conclusion in a series of experiments with fowl plague, that an inactivated virus followed some months later with living virulent virus, was the best method of immunization. Recently Hitchner and Johnson (1948) have described the use intranasally, of the very mild Bl Newcastle strain of virus. They claim that susceptible laying hens were not put off lay and that it could be used effectively by intranasal instillations in day-old chicks, hatched from eggs out of susceptible or immune hens. In spite of the avirulence of the strain, its invasiveness was not blocked by transmitted immunity of the chickens. The H.I. titres with this strain are, on the average, very low and subsequent experience has indicated that the immunity is of short duration, probably not exceeding that of the newer killed vaccines of chicken embryo origin.

In the recent outbreak of Newcastle disease in Hawaii (Adler, Willers and Campbell, 1951), a duck embryo attenuated live virus was used by wing-web application after a killed vaccine had been used. Coronel (1949) reports that in the Philippines the use of virulent live virus after an adsorbed inactivated vaccine, produced a solid immunity.

None of these workers, however, has mentioned the beneficial protection afforded to the ovaries by the residual immunity left from the killed vaccine. As a result of observations made on a large number of birds in the Western Province, it is believed that the residual immunity from the killed vaccine protects the ovaries from the action of the virus.

Newcastle disease is sometimes referred to as the rinderpest of poultry. Its behaviour in poultry, indeed, has many comparable features with rinderpest in cattle. The report by Mitchell and Le Roux (1946) of the combined use of a formol-glycerine rinderpest vaccine followed by the attenuated Kabete goat rinderpest virus is interesting in the light of this work on Newcastle disease. They showed that as the interval between a single injection of inactivated vaccine and the live virus increased, so the severity of the reactions as well as the percentage of reactors decreased. The rapid production of immunity induced by a single injection of formol-glycerine spleen vaccine could be used to control the reaction to the goat virus. An interval of seven days between inactivated vaccine and the live virus appeared to be the optimum, the immunity thereafter being sufficiently strong to completely block the live virus. Immunity produced by a triple vaccination with formol-saline vaccine had diminished considerably after eight months, but it controlled somewhat the severe reactions to the goat virus. The application of an inactivated Newcastle disease vaccine followed shortly afterwards by an avirulent live virus at the commencement of the rise of immunity as was done in the work cited on rinderpest, was not attempted. The optimum interval at which a good, reliable and uniform immunity is produced, while at the same time controlling the severe effects on the egg production, deserves investigation in Newcastle disease. The early use of live virus during the rise of immunity in the face of a virulent outbreak, will certainly narrow down the " blind spot " which develops when the live virus is used during the decline of the transient immunity, as reported here. The use of smaller quantities of inactivated virus and the determination of the optimum interval of the use of the live virus warrants further consideration, since the results obtained in flock 6, Table No. 6, where half a dose of inactivated vaccine was followed by live virus one month later gave a very satisfactory response.

It seems reasonable to believe that the live virus, when applied intra-ocularly, intranasally or intratracheally is superior to the wing-web route in partially immunized birds because (1) the virus is brought into direct contact with the suitable susceptible tissues without the neutralizing effect of the humoral antibodies, (2) the quantity of virus employed is greater.

It is considered that the combination of the use of the live virus after the inactivated vaccine is a very safe method of permanently vaccinating laying stock. No control on the duration of immunity has, however, been attempted. The serum H.I. titre was regarded as an indicator of whether a response to the vaccination had occurred or not. In all cases thus far tested, fowls showing positive reactions, when challenged, proved to be immune. Negative titres, however, do not necessarily mean that the birds are susceptible, for in the cases of vaccination with inactive vaccines, no H.I. titre is present at one month, yet there is a considerable immunity. Studies made have shown that after an inactivated vaccine had been injected, a peak titre was obtained within eight days, after which a rapid decrease occurred (Hanson, Winslow, Brandly, Upton, 1950). At four weeks, the titre was always negligible.

The combination method of vaccination also appears very satisfactory and safe in young stock. It is, however, strongly recommended that a long enough interval after the inactivated vaccine should be allowed, before live virus is administered, as it appears that the invasiveness of the vaccine strain (Beaudette Roakin strain) is much poorer than the highly virulent strains.

The chief objections to the use of live virus immunization are:—

(1) A loss in egg production.

(2) The occasional severity of the reaction.

(3) The possibility of spread to neighbouring unvaccinated stock.

It is felt that the combination method of vaccination eliminates very largely these objections, but it has its own disadvantages, e.g. the extra cost of vaccination, the extra handling of the birds, and the many variable factors in the susceptibility of the birds from flock to flock, the vaccines from batch to batch and the manner of application of the vaccines by different owners.

SUMMARY.

The use is described of Beaudette's Roakin strain live virus intra-ocularly on fowls previously vaccinated with an aluminium hydroxide adsorbed formalinized chick embryo vaccine. Good response to this combination was recorded from three months after using the killed vaccine. When the virus was applied per wing-web, a reliable percentage of reactions only took place when the interval between the two vaccines was five months or more.

Experiments determining (1) the most effective routes of application of the live virus, (2) the optimum dilutions of the live virus, (3) the interval necessary between the use of the inactivated and the live virus for the intra-ocular and wing-web routes of application of the live virus and the effect on the egg production are described.

Allantoic fluid, infected with Roakin strain virus, having an embryo Ld 50 of not less than 10^{-8} was diluted 1/10 in 50 per cent. glycerine-saline containing 1/8,000 crystal violet. The crystal violet was incorporated to colour the vaccine and so to facilitate its application. One drop of this diluted virus was applied intra-ocularly with an eye-dropper.

Experiments conducted indicate that the intra-ocular route of application of the live virus is superior to the intranasal.

The use of live virus after killed vaccine was characterised by absence of the drop in egg production which usually follows live virus immunization in susceptible fowls.

In field tests conducted five large flocks, totalling 25,000 laying hens and 49,000 young stock (one to five months old) were vaccinated satisfactorily with a combination of these two vaccines. The severe effects of the live virus on egg production was controlled. Two small control susceptible flocks of 38 and 183 laying hens showed a total drop in egg production of 50 per cent. and 24 per cent. respectively, when they were treated with live virus in the same manner.

One flock of 500 immunized with 0·5 c.c. of locally prepared adsorbed killed vaccine and vaccinated with live virus per eye, one month later, showed a good H.I. response without a drop in egg production.

Response to the live virus was judged on the production of a serum H.I. titre higher than 1/160. Where good reactions occurred more than 50 per cent. showed a titre of 1/1,280 or higher.

The use of the Roakin strain following an inactivated adsorbed vaccine after an adequate period, is recommended as a safe method of vaccinating permanently laying hens and younger stock. The use of the virus per eye does not set up a local inflammation or an increase in mortality.

Acknowledgments.

Grateful thanks are due to Dr. R. A. Alexander, Director of Veterinary Services, for his permission to publish this work and for his interest and constructive criticism in preparing the article.

We acknowledge with grateful thanks the ready co-operation and assistance of the many poultry farmers in the Western Province who made their flocks available for the field experiments and especially Mr. R. Heimann, of " Dalmore ", Retreat, C.P. who placed his flocks at our disposal. Thanks are also due to Miss P. Kerling and Mr. P. van Rooyen for technical assistance rendered.

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