A Comparative Study on Intra-Nasal, Intra-Ocular and Drinking Water Routes of Vaccination of Young Chicks against Ranikhet Disease

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Chicks from flocks immunized with Mukteswar vaccine strain did not show enough parental immunity at 15 days of age as evidenced by a lethal challenge with virulent Ranikhet disease virus. Mukteswar strain of egg adapted Ranikhet disease virus vaccine, in its present state of attenuation, was found safe for 15 day-old chicks from immunized flock, whether administered by intra-ocular, intra-nasal or drinking water routes.

From the immunogenic point of view, intra-ocular and intra-nasal routes of vaccination were significantly more efficient than the drinking water route. The first mentioned route of vaccination was found to be slightly better than the intra-nasal route, but this difference was non-significant at 5 per cent level. The immunity achieved in the chicks vaccinated by the three routes of vaccination in the present study was far from solid or 100 per cent.

INTRODUCTION

Ranikhet (Newcastle) disease is an acute infectious disease of fowls and turkeys and causes heavy mortality in chickens in every poultry producing area of the world.

In Pakistan and also in India, sub-cutaneous and intra-muscular routes of vaccination have been in vogue for immunizing poultry against Ranikhet disease. However, a variable reaction and some mortality has been reported in recent years due to early vaccination (Hashmi and Yaqoob, 1959; Rafique *et al.*, 1964 ; Qureshi *et al.*, 1965). Different routes have, however, been employed for immunizing young chicks bearing in mind the efficacy, convenience and cost of administration of the vaccine. Hashmi and Yaqoob (1959) concluded that intra-ocular administration of the Mukteswar vaccine strain provided a safe method for protecting day old chicks from immune hens and the immunity lasted for about 2 months.

There is also a difference of opinion on the degree and potency of parental immunity in chicks from immune flocks. A number of reports have indicated absence of a protective immunity in young chicks from vaccinated

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flocks, perhaps due to low level of immunity in the parent flock (Doll *et al.*, 1950, 1951). Others have found that chicks from immune hens either resist artificial infection or develop an asymptomatic infection depending upon the degree of virulence of challenge virus. This immunity was shown to last for varying periods upto 4 weeks of age (Brandly *et al.*, 1946; White *et al.*, 1953). However, field studies have shown that in heavily infected areas passive immunity could not be depended upon for the protection of young chicks under this age (Hashmi and Yaqoob, 1959). It was also demonstrated that normal egg yolk immunity was inadequate to meet the challenge of virulent Ranikhet disease virus enzootic in Pakistan.

The present study was undertaken to determine whether there was any effective parental immunity in chicks from immune flocks and also to make a comparative study on the effects and efficacy of intranasal, intra-ocular and drinking water routes of vaccination in 15 day-old chicks.

MATERIAL AND METHODS

Mukteswar vaccine strain which is now considered milder in reaction (Hashmi and Yaqoob, 1959) due to its continuous passage through egg embryos was used in the present study. It had the haemagglutination titre of 1: 640.

The chicks used for experiment hatched from eggs of immune flocks which had earlier been immunized against Ranikhet disease with Mukteswar vaccine strain. A hatch of 429 chicks of different breeds, when 15 days' old, were vaccinated according to the following schedule: (*i*) Group A of 100 chicks by intra-ocular instillation with one drop of 1: 10 dilution of the vaccine in each chick (*ii*) Group B of 100 chicks by intra-nasal instillation with one drop of 1: 10 dilution of the vaccine in each chick (*iii*) Group C of 100 chicks with 1: 100 dilution of the vaccine in drinking water (3 ml. vaccine mixed with 297 ml. of water) (*iv*) Group D of 129 chicks were kept to serve as unvaccinated control. Sixty four chicks of this group died due to coccidiosis, heat etc., before these could be challenged.

The virulent Ranikhet disease virus used for challenge was a recent isolate from a local outbreak which had been passaged once through 11 days' old embryonated eggs and stored at—30°C. until used. A 0.5 ml. of 1: 10 dilution of this virus given intra-muscularly was used as a challenge dose. The LD50 of this virus in eggs was $10^{-6\cdot6}$.

A lot of 20 vaccinated chicks were selected randomly and challenged with virulent Ranikhet disease virus. The first lot was challenged on the 14th post-vaccination day and was followed later with four more challenges each

10 days apart. A few chicks both from the vaccinated and unvaccinated groups died due to various causes including coccidiosis, heat, etc. Consequently, the number of vaccinated chicks was reduced to ten in the 5th challenge and the number of the chicks in the control groups in 2nd, 3rd, 4th and 5th challenge was thus reduced to half the number of chicks in each of the vaccinated groups.

To assess the level of parental immunity, a group of ten 15 day-old chicks from the same flock were given lethal challenge with virulent Ranikhet disease virus.

Chicks that actually died and also those showing severe nervous symptoms within 10 days after challenge were counted as dead. This mortality was taken as a criterion for breakdown in immunity. For further confirmation of the Ranikhet disease spleens were pooled groupwise, passaged once in chick embryos and subjected to spot haemagglutination test.

RESULTS

All the vaccinated chicks remained healthy, following vaccination and their growth was not interfered with.

Of the ten 15 days old chicks from immune flock which had not been vaccinated, only one survived the challenge with virulent virus. It could, therefore, be presumed that chicks at 15 days age did not have sufficient parental immunity to withstand a lethal challenge.

Those chicks which came down with the disease, after receiving virulent virus, showed general depression, closing of eyes, inappetence and increased thirst within 2 to 3 days of challenge. Some chicks were found lying in recumbent position with their legs flexed and movements like the imaginary pecking on food, shaking of one leg or whole body. Rhythmic involuntary convulsions and gasping were also noticed. Locomotor ataxia leading to staggering gait was not uncommon. Typical Ranikhet disease postures like torticollis, opisthotonus, posterior propulsions and walking in circles was also seen. Prostration and deep coma was usually followed by death.

The post mortem examination of chicks dead after challenge, did not reveal any consistent or definite gross lesions. Some birds showed few petechial haemorrhages in the gizzard and proventriculus. Slight swelling of intestinal mucosa was also noticed. Spleen was sometimes found enlarged and haemorrhagic. In few cases the lungs were hyperaemic.

Mortality within 10 days due to challenge is detailed in Table 1. In group A, vaccinated intra-ocularly, out of a total of 90 birds challenged between 29th and 69th post-vaccination days, only 23 died showing an overall mortality of 25.55 per cent. In group B, vaccinated intra-nasally, 28 of the vaccinated chicks succumbed to the challenge giving a mortality of 31.11 per cent. In

group C, given vaccine in drinking water, a total of 56 chicks died with an overall mortality of 62.22 per cent. All the chicks of control group D, however, died of the challenge.

Mortality for different groups of chicks and in different challenges were analysed statistically. The value of F indicated that there was a significant difference in the vaccination by different routes. The overall mortality in group C vaccinated via the drinking water was higher than the other two groups, A and B. However, the differences between intra-ocular and intra-nasal routes was non-significant at 5 per cent level.

TABLE 1. *Distribution of chicks in different groups, their vaccination, challenge schedules, and mortality due to virulent Ranikhet disease virus.*

Group	Route of vacc.	Age in days when Vaccinated	Age in days when Challenged	Number of chicks Challenged	Number of chicks Died	Per cent mortality
A	Intra-ocular ..	15	29	20	9	45
			39	20	3	15
			49	20	4	20
			59	20	5	25
			69	10	2	20
		Total	..	90	23	25.55
B	Intra-nasal ..	15	29	20	7	35
			39	20	4	20
			49	20	9	45
			59	20	5	25
			69	10	3	30
		Total	..	90	28	31.11
C	Drinking water ..	15	29	20	11	55
			39	20	12	60
			49	20	13	65
			59	20	12	60
			69	10	8	80
		Total	..	90	56	62.22
D	Un-vaccinated control. ..	..	29	20	20	100
			39	12	12	100
			49	10	10	100
			59	10	10	100
			69	10	10	100
		Total	..	52	52	100

DISCUSSION

Mukteswar vaccine strain of Ranikhet disease virus had been shown to cause some reaction and even mortality when administered parenterally in day old and young chicks (Hashmi and Yaqoob, 1959; Rafique *et al.*, 1964 ; Qureshi *et al.*, 1965). In the present study a batch of 300 chicks hatched from eggs of immune hens was administered this vaccine when the chicks were 15 days old through the intra-ocular, intra-nasal and drinking water routes. None of the birds exhibited any untoward symptoms and their growth rate was in no way impaired or slower than their unvaccinated hatch-mates. There were no mortality in any group as a result of vaccination.

This lack or absence of reaction in chicks in the present study as against severe reactions reported by other workers is not easy to explain. This may be due to intra-muscular and sub-cutaneous routes of administration. However, in the present study less potent routes were used, or younger chicks were used for vaccination. It may also be possible that the vaccine in its continuous passage had attenuated further so as to attain non-reacting property even for young chicks. It may also be possible that the chicks were in good condition and had been inoculated in such a season that the vaccine had not caused extra stress on the birds.

The immunity achieved by the Mukteswar vaccine strain given via the three methods mentioned above was far from satisfactory. It will, however, be seen from the results (Table 1) that in the same group more birds were able to withstand the lethal challenge as they grew in age. This seemed to support the feeling that the level of immunity had grown with the growth of the vaccinated chicks. (Brandly *et al.*, 1946; Baldelli, 1957; Gill *et al.*, 1959; Cole and Hutt, 1961).

Comparison between the three methods of vaccination showed that the drinking water route was the poorest. It may be because antigens administered by this route are sometimes lost before ingestion or partly or wholly digested in the alimentary canal or if left intact are absorbed only feebly. The cause of low response may also be explained by the fact that the vaccine might have been placed in relatively unfavourable atmospheric condition while being administered through this route.

The intra-ocular and intra-nasal methods, on the other hand, are more efficient methods of vaccine administration and the results obtained conformed to those of Hashmi and Yaqoob (1959).

The present study indicated that live vaccines administered by the upper respiratory routes gave a greater and more uniform immunogenic response than those administered by other routes. This may be because the respiratory

mucosa was readily and easily penetrable by virus particles (Drinker and Yoffey, 1941). Similar may be the case when virus is administered through the intraocular route. The virus then enters the lymphatics and the lymph nodes and thus causes a stimulation of the antibody forming mechanism of the bird.

There are many factors which may be considered in evaluating the resistance of chicks to a lethal challenge: factors which influence host response and factors which affect the challenge. Two such factors which have been shown to influence the response of the host are parental immunity and age. It has been shown that chicks from immune hens carry yolk acquired immunity for two to four weeks (Brandly *et al.*, 1946; Doll *et al.*, 1951). Secondly, antibodies present in chicks were likely to interfere vaccination with live virus (Doll *et al.*, 1950; Bankowski *et al.*, 1958). It was suggested that very young susceptible chicks were likely to give a poorer response to Ranikhet disease virus vaccine than older chicks (Doll *et al.*, 1950; Hofstad, 1954). This poor immune response following vaccination in young chicks may in part be due to the improper development of the antibody forming mechanism in very young chickens of the present study.

When a programme of vaccination is planned, it is generally assumed that parental antibodies acquired by the chicks from their dams would give sufficient protection against Ranikhet disease during early life. This type of protection, however, is not dependable because the antibody levels of chickens are dependent upon and directly proportional to the serum titres of the parent stock. Out of ten 15-day old chicks from an immune flock challenged with virulent Ranikhet disease virus as many as nine died. This showed that this parental immunity could not be dpended upon especially in areas where the disease was widespread.

The Mukteswar vaccine strain is more potent (Nilakantan *et al.*, 1960) than other strains of lentogenic origin and was probably not neutralized by passively acquired resistance as was evident from 90 per cent mortality in un-vaccinated chicks mentioned above.

From the foregoing experiments, it was also concluded that the normal egg-yolk immunity, if at all present, was inadequate at the age of 15 days to meet the challenge of virulent field strain enzootic in Pakistan and that revaccination at some later age would perhaps be necessary to enable the chicks to withstand any further exposures to virulent virus.

Because of the failure of parental antibody to give chicks adequate protection against field exposure to Ranikhet disease virus, vaccination of baby chicks in poultry producing areas becomes a dire necessity. It is true that vaccination of baby chicks does not stimulate the same immunity levels

or as durable a protection as may be obtained in older birds, yet baby chicks are thus likely to acquire a short term protection which would eliminate the risk of serious losses during their younger age, a time when they are most susceptible to disease. After an appropriate interval, this level of immunity can and should be boosted by revaccination.

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