Some Attenuated Rinderpest Virus Strains for Protecting Poultry Against Ranikhet Disease

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Three strains of attenuated rinderpest virus (goat adapted rinderpest virus, lapinized avianized rinderpest virus, and lapinized rinderpest virus) were employed to protect young chicks against lethal challenge with virulent Ranikhet disease virus, given at varying ages. The results were compared, with the Mukteswar strain of Ranikhet disease virus vaccine given via the intranasal route.

Subcutaneous inoculation of 4,000 rabbit infective units of Nakamura strain III of lapinized rinderpest virus protected 17 days old chicks from immune hens against lethal challenge with virulent Ranikhet disease virus given on the 17th, 31st, 45th and 59th post-vaccination days. This protection was even better than the one incited by the intranasal instillation of Mukteswar strain of Ranikhet disease virus vaccine. Other attenuated strains of rinderpest virus, tried simultaneously failed to incite any immunogenic response in 17 days old chicks against virulent Ranikhet disease virus.

INTRODUCTION

Numerous efforts to evolve vaccines for the control of Ranikhet (Newcastle) disease are in progress. The use of live virus vaccines may result in the perpetuation of the disease (Brandly *et al.*, 1946; Robertson, 1964), and inactivated virus vaccines are said to induce variable immunity (Brandly *et al.*, 1946). The results so far achieved are thus very inconsistent and no single vaccine has so far proved successful in all the geographical areas and in all the different populations.

In recent years the use of related pathogens, as immunizing agents, particularly for those virus diseases which are caused by members of the myxovirus group, have given a new method of approach. Adams *et al.* (1959) immunized human beings against measles with canine distemper virus. Warren *et al.* (1960) succeeded in protecting pups against distemper by using measles virus. Polding and Simpson (1957) suggested a possible relationship between the causal viruses of canine distemper and rinderpest and Goret *et al.* (1958) protected ferrets against distemper by using lapinized rinderpest virus and calves against rinderpest by using ferret-adapted distemper virus.

Marxer *et al.* (1958) indicated that injection of an extract of tobacco mosaic virus into chicks engendered immunity to Ranikhet disease.

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Both rinderpest and Ranikhet disease viruses belong to the myxovirus family. The present study was undertaken to exploit their biophysical similarities in protecting young chicks against the Ranikhet disease.

MATERIAL AND METHODS

Experimental chicks:

Four hundred and twenty-five chicks of different breeds from the eggs of hens previously immunized with the Mukteswar strain of Ranikhet disease virus vaccine were divided into four groups: Groups B, C, D and E of 90 chicks each and vaccinated with different viruses/vaccines. The remaining 65 chicks which formed Group A were kept as unvaccinated controls. An outbreak of coccidiosis killed 64 of the experimental chicks. In addition, 32 chicks died due to various reasons not attributable to vaccination of the Ranikhet disease. At the time of challenge, therefore, 73 chicks each were left in Groups B, C, D and E and 37 chicks in Group A (Table 1).

The viruses:

(*i*) Goat-adapted rinderpest virus. Contents of three ampoules of freeze dried virus received from West Pakistan Veterinary Research Institute, Lahore (each said to contain approximately 9,000 goat infective units) were suspended in 100 ml. of normal saline solution. Each 0.5 ml. of the suspension containing approximately 135 goat infective units was given subcutaneously to each chick in Group B.

(*ii*) Nakamura strain III of lapinized rinderpest virus. Contents of four ampoules of freeze dried virus received from Nippon Institute of Biological Sciences, Tokyo, Japan (each containing approximately 200,000 rabbit infective units) were suspended in 100 ml. of normal saline solution. Each 0.5 ml. of this suspension containing approximately 4,000 rabbit infective units was injected subcutaneously to each chick in Group E.

(*iii*) Lapinized avianized rinderpest virus. Contents of four ampoules of freeze dried virus received also from Nippon Institute, Tokyo, (each containing approximately 200,000 egg infective units) were suspended in 100 ml. of normal saline solution so that each 0.5 ml. of this suspension contained approximately 4,000 egg infective units. A dose of 0.5 ml. of this suspension was given subcutaneously to each chick in Group C.

(*iv*) Mukteswar strain of Ranikhet disease virus vaccine. One ampoule containing 1 ml. frozen virus vaccine was procured from the Department of Poultry Husbandry, West Pakistan Agricultural University, Lyallpur. It had a tube haemagglutination titres of 1: 640. One drop of 1 in 10 dilution of this vaccine, suspended in normal saline solution, was instilled intranasally to each chick in Group D.

(v) The virulent Ranikhet disease virus. It was a local isolate from chicks died of Ranikhet disease at the Poultry Experiment Station, West Pakistan Agricultural University, Lyallpur. A 1 in 10 dilution of the chorio-allantoic fluid from the eggs inoculated with the fresh isolate, was used as a challenge dose throughout the present study. Each bird in this project received 0.5 ml. of the virus intramuscularly.

Procedure:

The experimental chicks, when 17 days old, were given different viruses/vaccines as detailed above and were observed for 16 days. Beginning the 17th post-vaccination day a batch of chicks from each group was challenged with virulent Ranikhet disease virus, every 14th day. The schedule of the challenge, age and distribution of chicks in different groups is detailed in Table 1.

TABLE 1. *The age of chicks and schedule of challenges with virulent Ranikhet disease virus.*

Challenge Number	Age (in days)	Post-vaccination days	Distribution of the chicks					
			Group A	Group B	Group C	Group D	Group E	Total
First ..	34	17	10	20	20	20	20	90
Second ..	48	31	10	20	20	20	20	90
Third ..	62	45	10	20	20	20	20	90
Fourth ..	76	59	7	13	13	13	13	59
	Total ..		37	73	73	73	73	329

After each challenge, the chicks were kept under observation until the 14th post-challenge day. The symptoms and mortality were recorded daily. In case of death, the spleens of the dead chicks were immediately removed, pooled group and date-wise and stored at—30°C. Standard spot haemagglutination test was performed on the spleens thus collected after passaging them in 10 day-old embryonated eggs for the confirmation of Ranikhet disease.

RESULTS

The inoculations of goat-adapted rinderpest virus, lapinized avianized rinderpest virus, Mukteswar strain of Ranikhet disease virus vaccine, and lapinized rinderpest virus did not produce any visible reaction in the vaccinated chicks. Their growth remained normal throughout the present study.

On 2nd and 3rd post-challenge days, all chicks in Groups A, B, C and D became depressed and were found huddled together with drooping and ruffled feathers. Some chicks showed occasional nodding of the head which became

more frequent as the time elapsed; eventually involving the whole body in rythmic jerks. Some exhibited locomotor ataxia when made to move. On about the 4th post-challenge day, simple shakings of the head and neck turned into rythmic involuntary muscular spasms of varying intensity. Some chicks were found lying in a recumbant position. A clear mucous discharge which in some cases became copious, was found dripping from beak, as well as, from the nostrils. A few chicks showed partial or complete paralysis. Some of the affected chicks were so moped and so deeply comatosed that they appeared dead except for an occasional gasping.

A foeted greenish diarrhoea was observed in few chicks especially during the 3rd and the 4th challenges. In these cases the droppings became extremely thin and uratic as the disease progressed. The nasal discharge also became copious.

One chick each from Groups C and D during the first challenge and two chicks from Group D during the 2nd challenge displayed symptoms showing serious nervous involvements, and exhibited peculiar postures such as torticollis and emprosthotonus. Besides these symptoms, a variety of abnormal movements, such as posterior propulsions, somersaulting and walking in circles were also observed. These manifestations intensified when the chicks were either approached or handled. With the passage of time, these birds gradually improved and in about six months they became almost normal. Their growth, however, was somewhat impaired.

The deaths reached its peak on the 5th post-challenge day showing decline thereafter. As many as 98.44 per cent of the affected birds died within 10 days following challenge.

The chicks from Group D did not show any symptoms nor death during the 4th challenge. (Table 2).

The chicks from Group E remained healthy and bright in all the challenges throughout the post-challenge period showing no symptoms of Ranikhet disease, except two chicks of the second challenge which died from causes other than those of Ranikhet disease.

The results of all the four challenges (Table 2) indicated that the overall percentage of mortality in the vaccinated groups due to Ranikhet disease was 98.6 per cent in Group B, 97.2 per cent in Group C and 16.4 per cent in Group D. Group E, on the other hand, showed no mortality due to the disease. All the birds in the control group A died following inoculation of virulent virus.

These results suggest that an immunogenic relationship existed between Nakamura strain III of lapinized rinderpest virus and virulent Ranikhet disease virus: 4,000 rabbit infective units of the former when given subcutaneously

protected 17 day old chicks against virulent Ranikhet disease virus up to 76 days of age. Other attenuated strains of rinderpest virus, *viz.*, goat-adapted rinderpest virus and lapinized avianized rinderpest virus, however, did not incite any protection.

This protection was even better than the one induced by the intranasal instillation of Mukteswar strain of Ranikhet disease virus vaccine.

TABLE 2. *Showing overall mortality due to experimental infection with virulent Ranikhet disease virus after confirmation with spot haemagglutination test.*

Group	Challenge Number	Age in days	Number of Chicks		Per cent mortality
			Challenged	Died	
A	First	34	10	10	100
	Second	48	10	10	100
	Third	62	10	10	100
	Fourth	76	7	7	100
	Total		37	37	100
B	First	34	20	20	100
	Second	48	20	20	100
	Third	62	20	20	100
	Fourth	76	13	12	92.3
	Total		73	72	98.6
C	First	34	20	19	95
	Second	48	20	20	100
	Third	62	20	19	95
	Fourth	76	13	13	100
	Total		73	71	97.2
D	First	34	20	9	45
	Second	48	20	2	10
	Third	62	20	1	5
	Fourth	76	13	0	0
	Total		73	12	16.4
E	First	34	20	0	0
	Second	48	20	0	0
	Third	62	20	0	0
	Fourth	76	13	0	0
	Total		73	0	0

DISCUSSION

The results indicate that 4,000 rabbit infective units of Nakamura strain III of lapinized rinderpest virus, when given subcutaneously into chicks of 17 days age protected them from parental infection with virulent Ranikhet disease virus up to the 76th day of age. This relationship apparently does not fall in line with the accepted limits of the antigenically distinct measles-rinderpest-distemper (MRD) sub-group as reported by Chanock and Coates (1964). But the results seem in line with Hashmi and Husnain (1961) who while demonstrating antihaemagglutinating factors against Ranikhet disease virus in convalescent phase sera of rinderpest affected buffaloes hypothesized the existence of a possible functional relationship between Ranikhet disease and the rinderpest viruses.

As many as 73 chicks of Group D, when at 17 days of age were given one drop each of 1: 10 dilution of Mukteswar strain of Ranikhet disease virus vaccine intranasally. No untoward reaction or mortality was observed in any of the chicks following the intranasal instillation of Mukteswar strain of Ranikhet disease virus vaccine, as against the observation of Hashmi and Yaqoob (1959) who recorded 16.3 per cent mortality in day-old chicks vaccinated intranasally. This can perhaps be explained by the difference in the age of the vaccinated chicks in the two studies. Chicks thus vaccinated when challenged with virulent Ranikhet disease virus on the 17th, 31st, and 45th post-inoculation days showed successively diminishing percentage mortality as the gap between the vaccination and challenge increased. In the 4th challenge, given on the 59 post-inoculation day, there were no deaths.

A comparison between the results of the lapinized rinderpest virus and the Mukteswar strain of Ranikhet disease virus vaccine show that the former was relatively more effective. Furthermore, the protection against lethal challenge with virulent Ranikhet disease virus was solid and lasted at least beyond the early growth period (76 days age). Further studies to ascertain the actual age uptil which this protection lasts are, however, indicated.

The nature of this resistance is not clearly understood. It may be a simple antigen-antibody reaction, or a cross-protection similar to the one between vaccinia and smallpox viruses, or it may be due to some cellular blockade of the host cells (Slater and Murdock, 1963). This resistance may as well be an interference as recorded by Hanson and Alberts (1959) and postulated by Hashmi and Husnain (1961). The resistance against Ranikhet disease virus following subcutaneous inoculation of lapinized rinderpest virus is an interesting feature, but the failure to produce any resistance either by goat-adapted or lapinized avianized rinderpest virus in chicks under identical conditions is still more interesting.

It is well known that the attenuation of viruses is an endless process, which, if continued, may not only modify the disease-producing ability of a viral pathogen in its original host but may, at the same time, modify its pathogenicity and antigenicity in some other hosts. A somewhat similar discrepancy was also, observed by some earlier workers. Goret *et al.* (1958) by administering ferret-adapted canine distemper virus could protect two out of four calves against rinderpest but Polding *et al.* (1959) using virulent distemper virus failed to achieve any resistance.

Different strains of the same virus may differ from each other in some of their basic properties. Franklin and Wecker (1959) and Rott (1964) have reported that of all the available strains of Ranikhet disease virus only the 'Italien' strain was not inactivated by hydroxylamine. Rott (1964) stated that although the cause of that was not altogether clear, there was much to suggest that the composition of the envelope limited the activity of hydroxylamine. Rott and Shafer (1962) recorded that whereas the haemaglutinating activity of all the Ranikhet disease virus strains was not affected by the treatment of ether, such an activity was largely destroyed by ether in the case of 'Italien' strain of Ranikhet disease virus.

Taking stock of these observations, it can be hypothesized that either rinderpest virus has undergone attenuation to such an extent in goats and rabbit/chick embryos that it has lost its immunogenic ability against Ranikhet disease virus which remained in tact in lapinized rinderpest virus, or that these two strains differed from lapinized rinderpest virus in their basic structure/properties. It can also be possible that rinderpest virus during its passage and adaptability in rabbits may have acquired certain characters or factors derived from the rabbit cells which may account for its different antigenic behaviour as compared to other attenuated strains of rinderpest virus tested in the present study.

The clinical picture produced by the virulent Ranikhet disease virus in the experimental chicks resembled, in general, the disease picture given by Hagan and Bruner (1961) and Brandly (1962).

This study has shown promise of a safe and effective method of protecting young chicks hatched from immune hens against Ranikhet disease by the subcutaneous use of lapinized rinderpest virus. No side effects were observed following inoculation and chicks were found immune up to the age of 76 days. It appears that lapinized rinderpest virus may be recommended as a substitute for the in-vogue Mukteswar strain of Ranikhet disease virus vaccine for immunizing chicks below the age of two months.

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