

Evaluation of the efficacy of the crude extracts of *Capsicum frutescens*, *Citrus limon* and *Opuntia vulgaris* against Newcastle disease in domestic fowl in Tanzania

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Received 20 November 1998; received in revised form 12 February 1999; accepted 23 February 1999

Abstract

Prophylactic and therapeutic efficacy of a combination of *Capsicum frutescens* (red pepper), *Citrus limon* (lemon) and *Opuntia vulgaris* (prickly pear) against Newcastle disease (ND) in domestic fowl were evaluated. Eighty-eight broiler chickens were divided into five groups. Birds from three groups were inoculated with velogenic ND virus strain, whereas birds from two groups were left as controls. Two groups received a mixture of the plant extract three days prior to inoculation and birds from one group were given the plant extract for two days following development of clinical signs. Blood samples were collected for haemagglutination inhibition tests (HI) for detection of ND virus antibodies. Body weights were monitored during the experiment. Three birds died from the group that was inoculated with ND virus and treated with the plant extract; two died from the group that received the plant extract as a prophylaxis and inoculated with ND virus; and one bird died from the group that was inoculated with ND virus but not given the plant extract. No death was observed in any of the birds in the control groups. Antibody titers for ND virus rose four-fold in the inoculated birds but remained low in the un-inoculated groups. Mean body weights of birds in group B declined markedly compared to the other groups. The results indicated that there was no prophylactic or therapeutic value of the plant extract against ND. The plant extract showed a negative effect on body weights in birds with ND. © 1999 Elsevier Science Ireland Ltd. All rights reserved.

Keywords: Newcastle disease; *Capsicum frutescens*; *Citrus limon*; *Opuntia vulgaris*

1. Introduction

Newcastle disease (ND) is a viral disease of domestic fowl and is of great economic impor-

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tance in Tanzania (Minga and Nkini, 1986; Kapaga et al., 1989; Melewas, 1989; Gundidza et al., 1993). As with other viral diseases, there is no known specific treatment for ND (Jordan, 1990). Limited therapeutic efficacy of most drugs to viral diseases has led to a dependence on preventive measures, using vaccines which are expensive (Macreadie et al., 1990). Several antiviral agents have been reported to be effective against mammalian viruses, but their uses in poultry disease are limited (Huber, 1988). The main constraint limiting the utility of the antiviral drugs is the toxicity of the drugs to the host cells (Huber, 1988).

Empirical evidence of certain plant preparations being able to cure a number of diseases have been known for a long time but documentation is limited (Minja, 1989). In poultry, stem parts of a plant locally known as 'Mrangari' (*Euphorbia candelabrum*) has been used against ND (Minja, 1989). Fruits of *Capsicum annuum*, in combination with *Iboza multiflora* leaves have been used in the treatment of ND (Minja, 1989). Recently, in Northern Tanzania, a local preparation comprising three plants, namely, *Opuntia vulgaris*, *Citrus limon* and *Capsicum frutescens* has been reported to have therapeutic efficacy against ND in commercial chickens (Mushi 1994, personal communication). The objective of the present study was to evaluate the prophylactic and therapeutic efficacy of extracts of the three plants, namely, *Capsicum frutescens*, *Citrus limon* and *Opuntia vulgaris* against ND using controlled trials.

2. Materials and methods

2.1. Experimental birds

One hundred and ten day-old broiler chickens were purchased from a commercial poultry company, Interchick, (Dar es Salaam, Tanzania), and raised in isolation until 6 weeks of age. Blood samples were collected in 2 ml syringes from the wing vein when the birds were 4 weeks old. Antibody titres against ND were determined by haemagglutination inhibition tests (HI), as described by Allan and Gough (1974). Body weights were recorded on days 0, 3, 7, 14 and 28.

At 6 weeks of age the birds were wing-tagged with assorted numbers. Body weights were determined using a standard balance and blood was collected for serology. The birds were randomly divided into five groups, namely A, B, C, D and E. Chickens in groups A, B and C were kept in separate rooms in one location, whereas those in groups D and E were kept in two separate rooms about 1 km away from the other groups, in order to serve as controls. Groups A, B, C and E comprised 18 birds each, whereas group D had 16 birds.

2.2. Preparation of plant extract

Fruits of lemon (*C. limon*) and red pepper (*C. frutescens*) and stems of prickly pear (*O. vulgaris*) were collected and identified by a plant taxonomist in the Department of Forest Biology, Sokoine University of Agriculture, (Morogoro, Tanzania). The voucher specimens are: Temu 3028 (*C. limon*); Temu 3136 (*C. frutescens*); and Temu 3138 (*O. vulgaris*). All these collections are deposited in the Herbarium of the Department of Forest Biology, Sokoine University of Agriculture.

Two hundred grams each of fresh red pepper fruits (*C. frutescens*) and stems of prickly pear (*O. vulgaris*) were crushed and mixed thoroughly with 100 ml of fresh juice of lemon (*C. limon*) to produce a slimy suspension. The suspension was then made up to 2 l with distilled water, mixed and sieved to obtain a clear slimy juice.

2.3. Administration of plant extract

For every 20 birds, about 500 ml of the plant extract were dispensed in drinking water bowls. Prior to the administration of the plant extract the chickens were deprived of water for 12 h. The plant extract was provided to the birds in groups A and E for three days in the drinkers as done in the field. The chickens in group B were given the plant extract for two days following development of clinical signs in order to evaluate the therapeutic efficacy of the preparation.

2.4. Challenge of the experimental birds

A local velogenic ND virus strain MG/01/01/C isolated from the Morogoro region (Yongolo, 1996) was used in challenging the birds. The virus strain was diluted in normal saline to give embryo infective dose EID_{50}/ml of $9 \log_{10}$. Chickens in groups A, B and C were inoculated with 0.5 ml of the ND virus at day 3 following commencement of administration of the preparation. Following inoculation, the birds were placed in their respective groups and blood samples were taken during days 0, 3, 7, 14 and 28. HI tests were done on sera from all the chickens as described by Allan and Gough (1974). The ND antigen batch 001/93 used was kindly supplied by PANVAC, (Debrezeit, Ethiopia). The birds were also monitored for the presence of diarrhoea, anorexia, respiratory and ocular signs during the course of the experiment. Clinical signs were observed over a period of 21 days. Gross and histopathological examinations were performed on all the dead birds.

2.5. Statistical analysis

The data were analysed using the SAS (1992) general linear model (GLM) procedure.

3. Results

3.1. Clinical signs

All birds looked healthy before and at the time of inoculation. Scant whitish diarrhoeic droppings were observed in groups A, B and C two days postinoculation (PI). Obvious whitish–greenish diarrhoea was seen in all pens of the inoculated birds and some birds had pasted vent feathers. Most of the birds had ruffled feathers and were seen standing in a crouched posture. Signs of difficult breathing were also observed and some of the birds had periorbital oedema and hyperaemia of the cloacal mucous membrane. Many severe signs were observed in birds of group B, which were firstly inoculated and then treated with the plant extract.

One bird which belonged to group B died during day 5 PI, and a day later, three birds from each group (A, B, and C) were found dead. At day 8 PI, one bird in group B died and another one showed nervous signs, characterised by torticollis and moving in circles. One bird from group A died, whereas other birds showed signs of recovery towards the end of monitoring. The birds were monitored until 21 days postinfection. No clinical signs or deaths were observed in groups D and E.

3.2. Gross and histopathological findings

Gross lesions in the dead birds included periorbital oedema, soiled vents with greenish–whitish diarrhoea and hyperaemia of cloacal mucous membranes. Haemorrhages and necrosis of the small intestines were also observed in small segments of about 1–2 cm. The proventriculi of all dead birds were hyperaemic.

On histopathological examination, there were heavy lymphocytic infiltration in the mucosa and submucosa of the small intestines. Extensive necrosis and haemorrhage was also observed in the intestinal mucosa. Lymphocytic infiltration of the intermuscular and subperitoneal connective tissues was observed in two birds from group B. Very reactive spleen with prominent periarteriolar lymphoid tissue and eccentric arterioles was also observed.

3.3. Haemagglutination inhibition test

Prior to inoculation, 22 birds had no antibodies against ND virus, 22 had titres of $1 \log_2$, 37 had titres of $2 \log_2$ and only nine had titres of $3 \log_2$. Mean group antibody titres against ND showed an increasing trend following inoculation in groups A, B and C. On the other hand, ND virus antibody titres remained low throughout the course of the experiment in groups D and E. There was a significant difference in mean group antibody titres between the infected Groups A, B and C, and the noninfected groups D and E (Fig. 1).

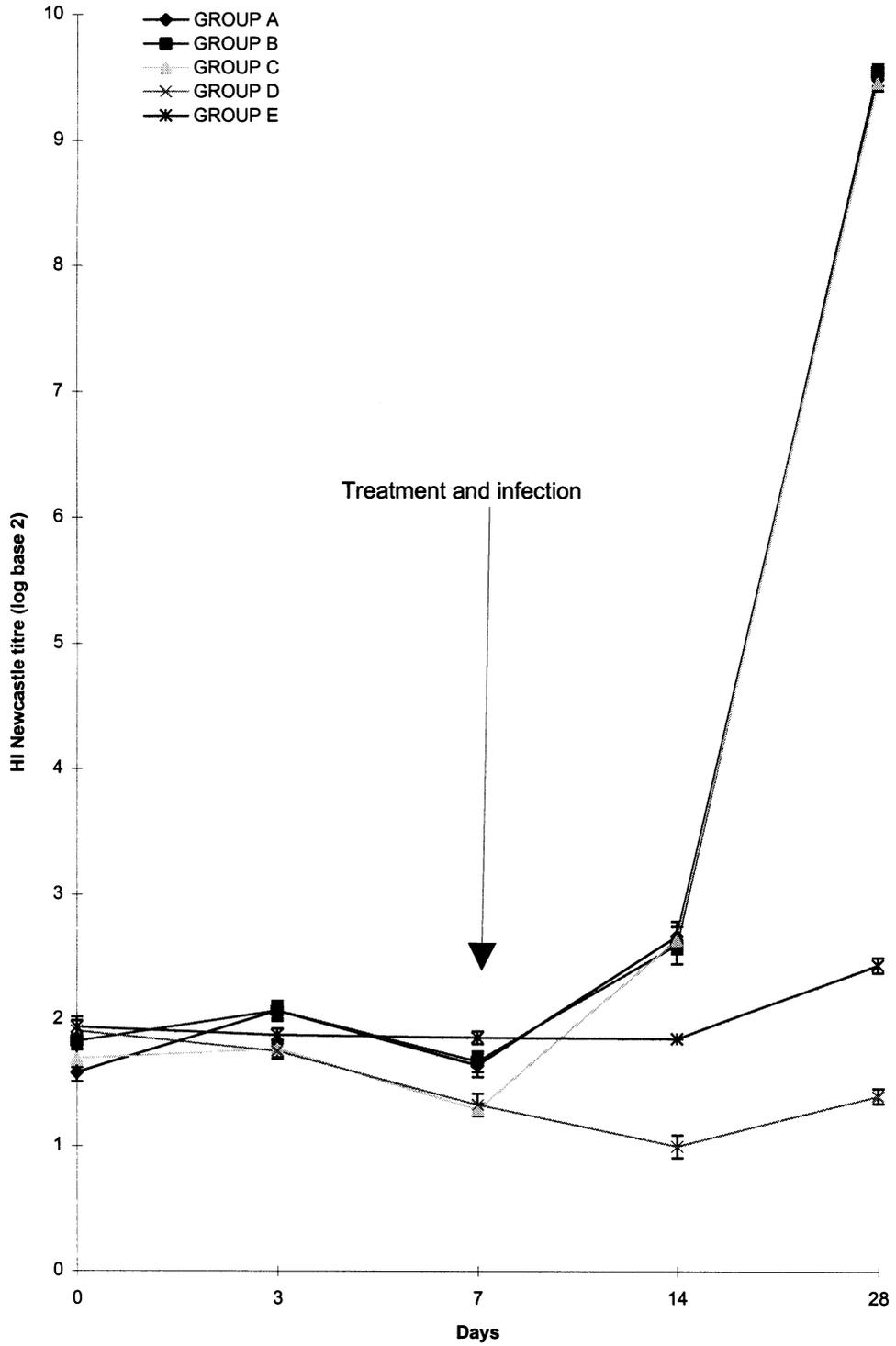


Fig. 1. Haemagglutination inhibition (HI) titres for Newcastle disease in the experimental birds.

3.4. Effects of the plant extract on body weights

Mean group weights of the experimental birds increased gradually, as seen in Fig. 2. However, the differences in weight gain between groups after inoculation with the ND virus was significant ($P < 0.05$). There was no significant difference of mean weights between the groups prior to inoculation ($P > 0.05$).

Following inoculation, the mean group body weight of birds in group B showed a comparatively slower rate of body weight gain. There was a significant difference of mean body weight between group B and the rest of the groups. Group B comprised birds which were inoculated with ND virus and treated with the plant extract for 3 days.

At the end of the experiment, birds of group D had the highest mean weights compared to birds in groups A, B, C, and E (Fig. 2). Group D comprised birds that were neither inoculated with ND virus nor given the plant extract. Groups A and C had similar mean group weights at the end of the experiment, whereas group E had less mean group weights compared to D, A and C. Group E birds received the plant extract as a prophylactic but not inoculated with the ND virus.

4. Discussion and conclusion

In this study, mean antibody titres to ND increased significantly in all birds which were inoculated with ND virus. There were no significant changes in antibody titres in the noninfected birds ($P > 0.05$). This is an indication that there was an active infection in the inoculated groups compared to the noninoculated groups. The finding of birds with no antibody against ND virus prior to inoculation and others with low titres is an important observation. This indicates that chicks from the same batch might have different levels of yolk sac antibodies, which is an important factor that determines severity of the clinical signs in case of a disease outbreak. In the present study, various clinical signs were observed. Some of the experimentally-infected birds showed severe clinical signs while others had mild form of the disease.

It was observed that only six birds died with typical clinical and pathological features of ND virus infection following inoculation. These birds had no antibodies to ND virus prior to inoculation. The lack of antibodies to ND virus might be the major factor that contributed to the mortalities of the infected birds. The observed mortality in group B which was inoculated and treated with the plant extract could be attributed to the low antibody titres against ND. However, there was no significant difference in terms of deaths between the inoculated experimental groups ($P > 0.05$).

Lack of protection of the naive inoculated birds against ND in groups A (prophylactic protocol) and B (therapeutic protocol) suggests that the plant extract has no prophylactic or therapeutic activity. The recovery of the rest of the birds in the groups that had been inoculated with ND virus therefore appears to be due to the presence of the antibodies against ND virus, as shown in the HI test. Similar situations might be occurring in the field, whereby commercial flocks which have varying degrees of antibody titres to ND are treated with the plant preparation during disease outbreak. This might easily be mistaken for a therapeutic activity of the plant extract.

Lack of both prophylactic and therapeutic activity of the plant extract as seen in the present study may not be surprising if the key constituents of the three plants are considered. The key constituents of *C. frutescens* (Solanaceae) are: capsaicin, carotenoids, flavonoids, volatile oil and steroidal saponins; those of *C. limon* (Rutaceae) are: limonene, alpha-terpinene, alpha-pinene, beta-pinene, citral, coumarins, bioflavonoids, vitamins (A1, B1, B2, B3, and C), mucilage and volatile oil; and those of *Opuntia* (Cactaceae) are: mucilage, sugars, vitamin C and fruit acids (Chevallier, 1996). None of these constituents have been reported to exhibit direct antiviral activity.

Birds in group B had significantly low mean group weights at the end of the experiment compared to the rest of the groups ($P < 0.05$). On the other hand, it was observed that birds of group D which were neither inoculated nor given the plant preparation had the highest mean group body

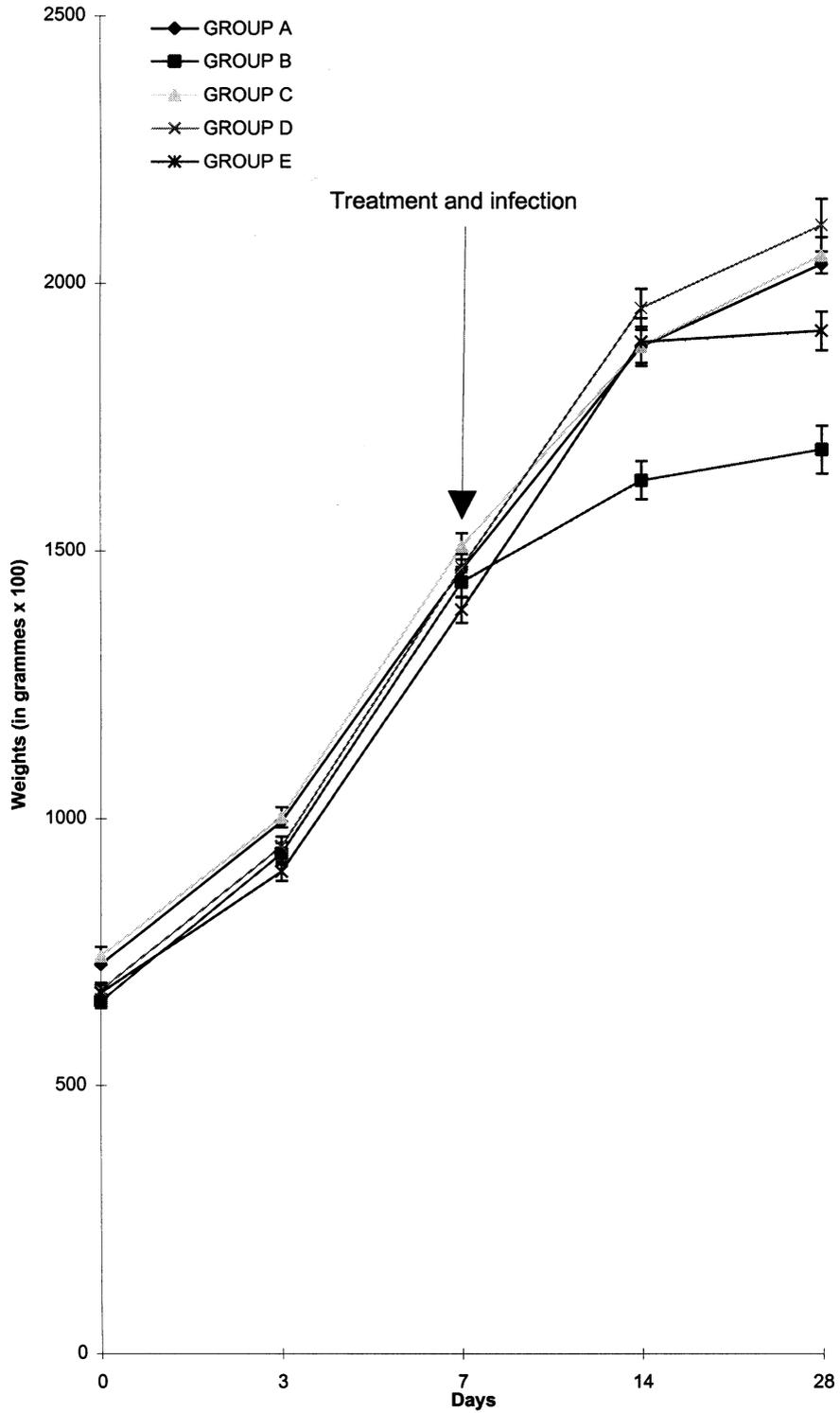


Fig. 2. Trends of mean group body weights of the experimental chickens.

weights. This also shows that apart from the loss due to mortality caused by ND, there is also a loss in terms of reduced weight gain. These findings strongly suggest that the plant preparation has a negative effect on body weights of chickens which are under disease stress. Other plants traditionally used to treat ND include *Capsicum* spp., *Euphorbia* spp. and *I. multiflora*, but the efficacy of the preparation of these plants against ND is not known (Chavunduka, 1976; Minja, 1989).

The present study has revealed that antibody response rather than therapeutic intervention with the plant extract is the key factor in the recovery from ND in chickens. It is therefore concluded that the local plant extract has neither prophylactic nor therapeutic efficacy against ND virus strain MG/01/01/C from Morogoro. In addition, the plant extract has a profound negative effect on body weights of birds with ND virus infection. It is suggested that, currently, routine vaccination in the field should continue as the most appropriate method of controlling ND in poultry.

Acknowledgements

The authors are grateful to the Sokoine University of Agriculture for supporting this work. The assistance by the Faculty of Veterinary Medicine staff, Professor Chamshama of the Faculty of Forestry and that of Dr J. Nyange of Animal Disease Research Institute (ADRI), Dar es Salaam, Tanzania, is acknowledged.

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