Vet. Med. J., Giza, 39, No. 1, 57 - 69 (1991).

A LOCALIZED OUTBREAK OF RINDERPEST IN EGYPTIAN BUFFALOES IN 1990

BY

H.M. YOUSSEF*, A.M. SAMI**, A.A.EL-SANOUSI**, M.S. SABER** AND I.M. REDA**

* Department of Veterinary Medicine, Cairo University.

** Department of Microbiology & Immunology, Cairo University.

(Received: 29.1.1991)

INTRODUCTION

Rinderpest is a contagious disease that spreads at a very high rate and causes heavy mortility in buffaloes and other farm animals. The disease is manifested by high fever and watery diarrhea, pustular eruptions and ulcers which develop on the lips, gums, tongue and buccal mucosa.

At present, Europe and most of Asia are free of the disease, except a few countries in south Asia beside Africa.

In general buffaloes are susceptible to rinderpest. The disease has been reported from Egypt, India , Indonesia, Philippines, Malaya, Burma and Thailand (Mohan, 1968). Edward (1927) found that Indian buffaloes were more susceptible than cattle.

Rapid diagnostic methods are available for the diagnosis of rinderpest by demonstration of either the antigen or the antibody, these include agar gel immunodiffusion (AGID) Scott and Broun, (1961) ,

counter immunoelectropnoresis (GIEP) (Ali and less 1979, Uppal et al., 1983). Complement fixation test (C.F.T), Scott et al. (1986) Passive haemogglutination (PHA) Singh et al. (1972), immuno peroxidase stainign (Selvakumar et al., 1981), immunofluoresence (IF) Rossiter and Jessett (1982), virus neutratization (Plovuright and Ferris 1961), and enzyme linked immunosorbet assay (ELISA) test Rossiter et al., (1981).

For controlling rinderpest, tissue culture vaccine (either using calf kidney or lamb kidney) is being extenesively used. Tissue culture vaccine stimulates interferon production before neutralizing antibodies develop in buffaloes (Mohan 1968).

A localized outbreak appeared suddenly among a group of buffaloes in the farm of Faculty of Vet. Med. Giza Egypt. The outbreak ran an acute course with clinical signs, post mortum lesions and high mortalities similar to those recorded in rinderpest of cattle. In the present investigation the clinical findings, P.M. lesions, the epizootological observations Laboratory Investigation and the method of control are discussed.

MATERIALS AND METHODS

1. History of the outbreak:-

On the 19th March 1990 one buffalo out of 25 animal in the farm of Fac. Vet. Med. Giza Egypt showed fever, off food for one day them became recumbant with subnormal temperature and died in the scond day. This picture was repeated in other 4 buffaloes but before their death some of them showed congestion and ulcers on the buccal papillae, inner side of cheeks and severe diarrhea. While other 18 buffaloes showed in appetance, salivation and diarrhea without death.

2. Samples:

Broncheal, prescapular, prefemoral, mesentric lymph nodes and spleen were sampled from dead animals . Whole blood on anti coogulant (heparin) has been collected from febrile animals and transported rapidly on ice to the laboratory. Sera were obtained from acute and convalescent cases.

3. Cell culture:-

Vero cells line has been used for the primary isolation of viral agents from suspected clinical materials. The cell lines were kindly supplied from the virology laboratory, Animal Health Research Institute. Dokki, Giza.

4. Media and hyper immunesera:

M 199 E and Eagle's minimum essential medium (EMEM) were purchased from GIBCO" and used primarily for the cultivation of vero cells.

Rabbit anti RP and BVD virus hyperimmune sera were kindly supplied from the virology laboratory, Animal Health Research Institute, Dokki, Giza. Negative rabbit serum was obtained from normal rabbits. Mycoplasma and virus screened faetal calf serum has been purchased from flow, England and used as 10% dilution as a growth medium or as 2% dilution as maintenance medium.

5. Virus isolation in cell culture:-

For isolation of the virus from the blood, the buffy coat has been separated by centrifugation, washing, resuspension in T.C. medium and inoculated into vero cells. The inoculated vero cells were daily observed for the development of typical cytopathic effect (CPE) up to 7 days post inoculation.

6. detection of virus specific antigen:

Detection of specific antigen (S) in infected T.C. monolayers by using the indirect fluorescent antibody (IFA) technique with specific RP hyper immune rabbit antiseum canjugated with fluorescein isoxthiocynatc according to the method of (Prabhudrock and Sambamurti 1976).

7. Agar gel precipitation test (AGPT):-

A dilution of 1% of Oxoid No.2 agar was prepared in phosphate buffer saline (PBS) with Ph 7.2. The microtechnique on glass slide was performed. The central well of each haxagonol pattern received reference rabbit anti RP, BVD virus hyper immune sera in agar gel precepitation test after White (1958) modyfied by Madboly et al. (1987).

8. Serum neutralization test:-

For detection of neutralizing antibodies against RPV in acute and convalescent sera of diseased animals the test was conducteed according to Rossiter and Jessell (1982).

9. Control measures adopted:-

Notification of the Veterianry authorities, quarantine measures, isolation, disinfection, hygienic disposal of dead animals as well as vaccination of apparently healthy animals with tissue culture rinderpest vaccine which is prepared at the Serum and Vaccine production and Research Institute, Abbasia, Cairo were adopted.

RESULTS

1. Diagnostic Field investigation (clinical signs and P.M. lesions).

The epizootic ran an acute course. The clinical signs began with fever, off food, decrease in milk yield , lacrimation, salivation, nasal discharge, erosions in the mucosa of buccal cavity especially on cheek papillae, inside of the lower lip and adjacent gum, (Fig. 1), severe diarrhea, dehydration, recoumbancy, subnormal temperature and ended by death in 5 cases. The important necropsy findings were erosions on the gums, ventral surface of the tongue, the lymphnodes are oedematous, Peyer's pathches were acutely inflamed.

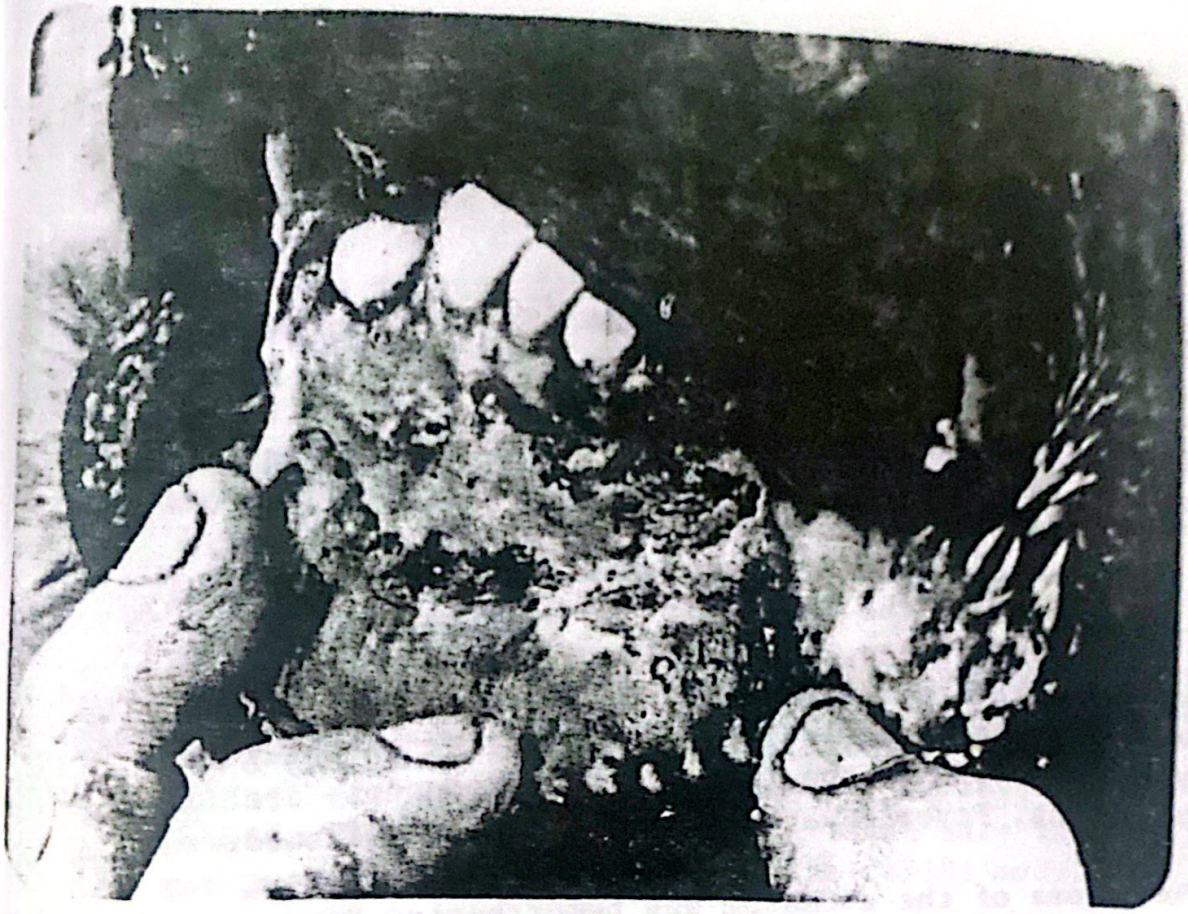
The mucosa of the abomasum was hemorrhagic. Haemorrhage and erosions of the mucosa of the ceacum were observed. The mucosal surface of the last portion of the large intesine showed zebra stripping.

2. Epizootological investigation:-

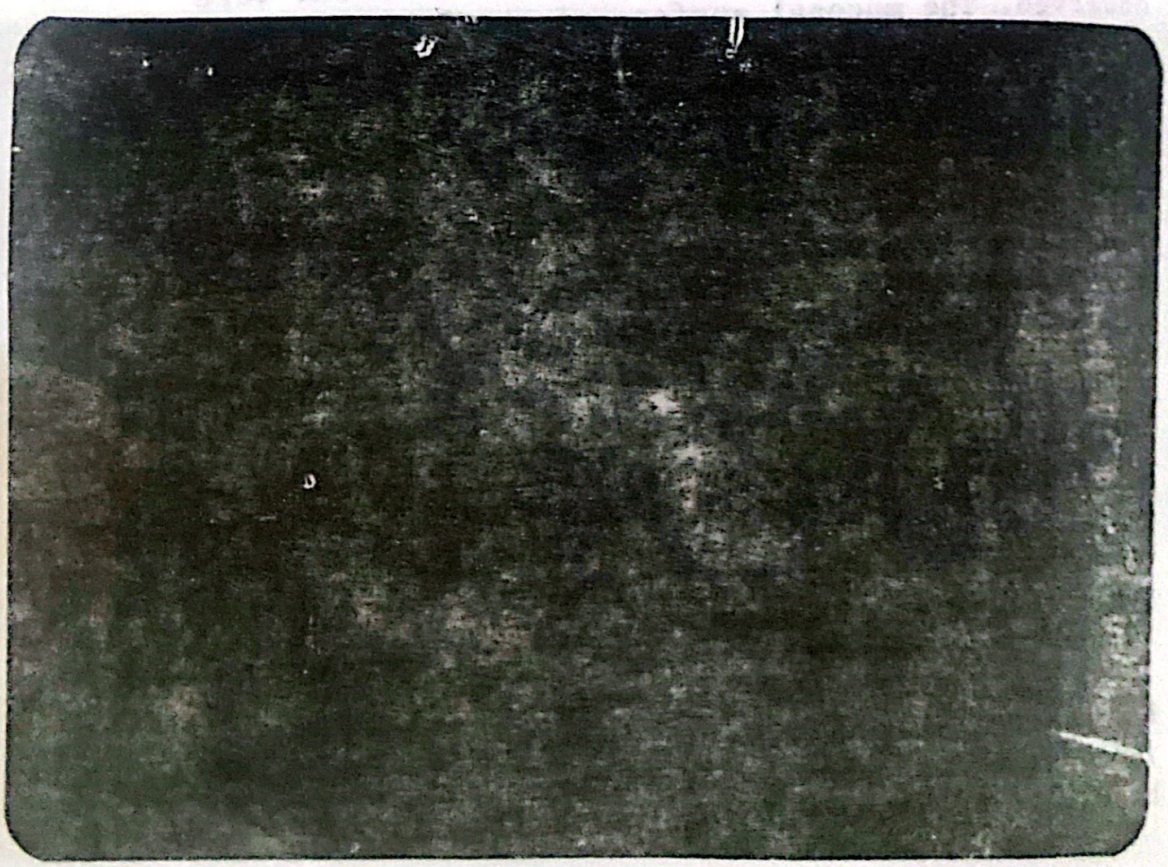
The epizpptological investigation showed that the total exposed animals in the farm were 25 buffaloes. The morbidity rate was 72%, the mortality rate was 20% and case fatality was 27.7 percent.

The first animal in the farm which showed the first clinical signs was observed on 19th of March 1990, it was buffalo of about 2 years old and was considered as the index case of the epizootic. The origin of the outbreak started after addition of a newly purchased group of buffaloes to the original animals in the farm one month before.

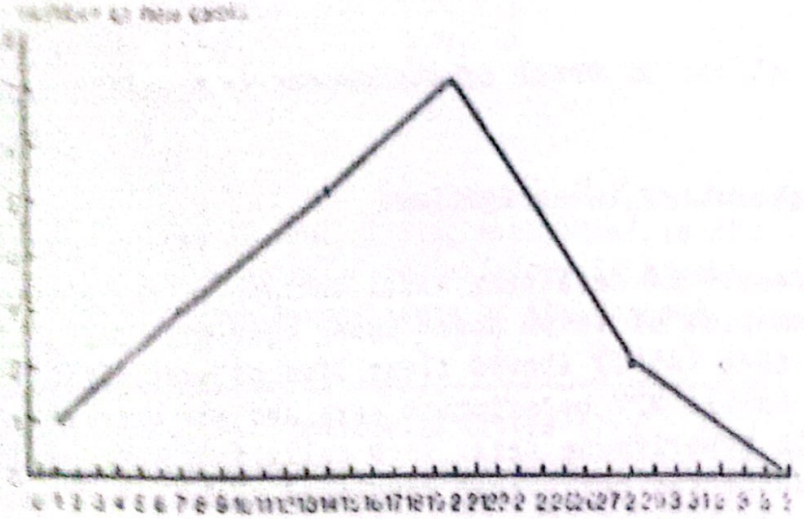
Examination of the epizootic curves Fig. (3,4) revealed that the incubation period was about 7 days with high mortality and morbidity.



(Fig 1) : Erosions on the buccal cavity and gums.



(Fig 2): Infected vero cells showing intracytoplasmic granular fl
orescence.

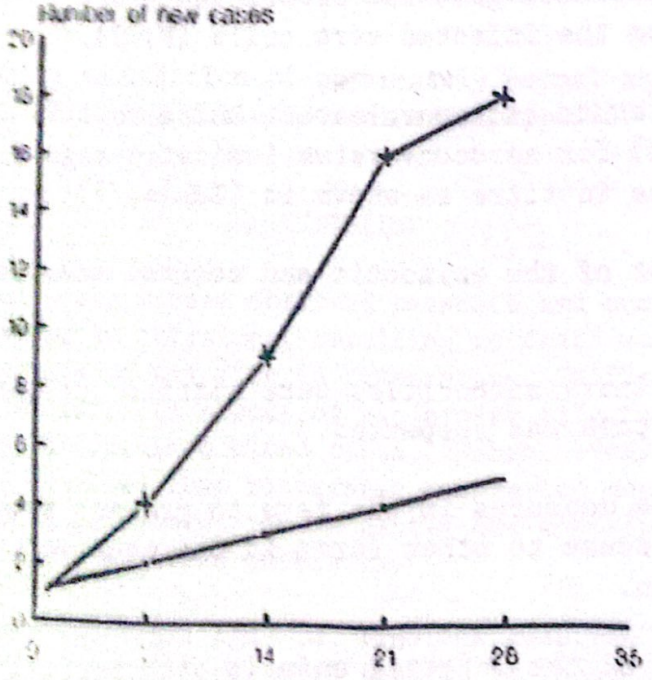


Days

Frequency curve

(Fig. 3)

Frequency curve of the outbreak



Days

Daily cumulat. morb. Daily cumulat. mort.

(Fig. 4)

Daily cummulative morbidity and mortality of the outbreak

3. Laboratory investigation:-

The result of detecting viral antigen in the suspected smaples of lymph nodes using agar gel precipitation test (AGPT) showed clear line of precipitation with rabbit RPV hyperimmune sera and not with BVD rabbit hyperimmune sera. Vero cells inoculated with the suspected buffy coat developed a characterestic cytopathological changes similar to RPV with the formation of giant cells allover the sheet. Reproducible changes were obtained through the 2nd and 3rd passage of the viral isolate in vero cells. The idntification of RP viral isolates was carried out through the detection of RPV specific antigen inside vero cells by the immuno fluorescence technique using RPV. Specific reference rabbit serum (Pirbright, UK.). Granular, diffused greenish fluorescence was emitted from the infected vero cells (F. 2).

Testing of acute and convalescent buffaloe sera (2 weeks apart) for seroconversion indicated significant increase in titre as shown in (Table, 1).

4. Managment of the epizootic and control measures adopted:-

- The veterinary authorities were notified as soon as index case was suspected.
- Quarantine measures in the farm to prevent spread of the disease to other farms in the area were undertaken.
- Isolation of the infected animals with speical attendants.
- Disinfiection precaustion in the farm.
- Hygienic disposal od dead animals by burning.
- Levamesole "citarin Bayer" was used to intiate the reaction of immune system against infection.

Table. 1: Titres of neutralizing antibodies to RPV in sera of infected buffaloes in acute and convalescent stage of the disease.

Serum samples No.	Antibodies titres (log 2)	
	Acute stage	convalescent stage
1	2	6
2	2	6
3	3	6
4	2	6
5	3	6

- Finally vaccination of apparentaly normal animals with RP tissue culture vaccine was applied.

DISCUSSION

Several workers have observed peracute and acute rinderpest in buffaloes, resulting in death even in the absence of any rise in trmperature. Unlike cattle the diarrheic stools of rinderpest affected buffaloes contained blood clots, (Mohan, 1968). This author reported that rinderpest manifested more acute course in buffaloes than in cattle and mortality was much higher in field outbreaks.

The clinical signs and P.M. findings observed in the epizootic in our study were similar to those of rinderpest which were previously described by Mohan (1968).

The epizootological investigation and the epizootological curve revealed that the disease under investigation is a disease of high morbidity, mortality and case fetality with an incubation period of about 7 days.

The laboratory investigation of the epizootic indicated that extracts of suspected lymph nordes of dead animals gave specific line of precipitation in agar gel precipitation test against known rabbit RPV hyper immune sera. This result agree with white (1962) Joshi et al., (1972) and Hajela and Malik (1974). The vero cells inoculated with the suspected a characterestic cytopothological changes similar to RPV these changes were found to be similar to those described by Apple et al. (1981) and Madboly et al. (1978).

The detection of suspected viral antigen in infected T.C. cells with immunofluorescence test using RPV specific reference rabbit serum gave positive test which has been previously achieved by Liess (1963), Ushijima et al. (1969) and Rossiter and Jessett (1982).

Testing of acute and convalescent sera of infected buffaloes showed significant increase in titre which was recorded before by Apple et al. (1981) and Madboly et al. (1987).

The nature of R.P. in Egyptian buffaloes differ completely from cattle in some aspects. First, not all infected buffaloes reacted thermally as in cattle, only 2 animals showed a rise of body temperature ranged from 39-39.5°C, while other animals did not show any rise of temperature and some buffaloes contracted the disease with a very short course and death occurred within 72 hours. The rest of infected animals took a very long course of the disease lasting more than one month (this represent the newly added buffaloes which is considered the source of infection to other susceptible animals). Second , although the buffaloes were vaccinated against R.P. but some of them reacted positively to the disease which indicates that the immunoresponseis not completely protective which may required further investigations to explore the different aspects of immune response in buffaloes.

Most outbreaks take place in quarantine areas from time to time may be due to importation of infected animals from endemic areas on the international scale or due to introduction of newly purchased animals without isolation on the local farm scale which may be the probable cause of transmission of the disease into the farm under investigation.

From the avaliable results it can be concluded that the clinical signs, P.M. findings, epizootological study and laboratory investigation confirm that the causative agent of the epizootic in buffaloes was rinderpest virus.

Finally it is recomended that any newly introduced animals to the original herd must be kept in well isolated stables for a period not less than one month to be sure that the animals are free from the disease.

SUMMARY

Rinderpest virus was isolated from an outbreak affecting group of buffaloes in the farm of Faculty of Vet. Med. Giza Egypt. The clinical signs, the P.M. lesions and epizootological study were recorded.

The specific viral antigen could be detected in lymph nodes of the freshly dead animals by using agar gel precipitation test.

The viral agent was isolated in vero cells and could be demonstrated in infected T.C. cells with the immunofluorscence technique and finally seroconversion of the infected buffaloes was applied to conffirm isolation of the virus.

REFERENCES

1. Ali, B.E.H. and Less, G.E. (1979): The application of immunoelec troprecipitation in the diagnosis of rinderpest. Bull. Anim Hlth. Prod. Africa. 27: 1-6.

2. Apple, M.J.G.; Gibbs, E.P.J.; Martin, S.J.; Termeulen, V. Rima, B.K.; Stephenson, J.R. and Taylor, W.P. (1981): Morbilli virus disease of animals and man. Comperative diagnosis of viral diseases volume, IV, Vertebrate Animal and related viruses, part BRNA viruses, PP. 235-297.

3. Edwards, J.T. (1927): Rinderpest: Some properties of the virus and further indications for its employment in the serum simultaneous method of protective inoculation. Transaction of the Eastern Association of Trapicol Medicine, 7th congress held in India: 3: 699-709 (quoted by Plowright (1968).

4. Hajela, S.K. and Malik, B.S. (1974): Observation on the relative sensitivities of the immunodiffusion, complement fixation and intradermal allergic tests in the detection of rinderpest antigens and antibodies. Ind. J. Anim. Sci. 44 (11): 832-837.

5. Joshi, R.C., Shukla, D.C. and Bansal, R.P. (1972): Rinderpest diagnosis by agar gel double diffusion test. Indd. Vet. J., 49 (5): 449-456.

6. Liess, B. (1963): Fluorescence serological studies cellculture after infection with rinderpest virus. Zbl. Bakt. II., 190: 424-443.

7. Mahboby, H.M.; El-Sanousi, A.; Osman, K.M.; Osman, O.A.; Saber, M.S. and Reda, I.M. (1987): Preliminary studies on the application of some recent technique in the detection of rinderpest viral antigens and antibodies. Assiut Vet. Med. J.; 18. (36): 88-94.

8. Mohan, R.N. (1968): Diseases and parasites of buffaloes. Part I. Viral mycoplosmal and rickettsial diseases. Vet. Bull, 38: 607-576.

9. Plowright, W. and Ferris, R.D. (1961): Studies with rinderpest virus in tissue culture, the anability of cultured virus and its use in virus neutralization test. Arch. Gesamte virus Forsch. 11. 516.

10. Prabhudasok, and Sambamurti, B. (1976): A note on fluorescent antibody technique for rapid diagnosis of rinderpest. Ind. J. Anim. Sci., 46 (8): 454-457.

11. Rossiter, P.B., Jessett, D.M. and Halmes, P. (1981): Micro ELISA test for dtedcting antibodies to rinderpest virus antigens. Trop. Anim. Hlth. Prod. 13: 113-116.

12. Rossiter, P.B. and Jessett, D.M. (1982): Detection of rinderpest virus antigens in vitro and in vivo by direct immunofluorescent. Res. Vet. Sci., 33 (2) : 198-204.

13. Rossiter, P.B. and Jessell, R.M. (1982): Microtibre technique for the assay of rinderpest virus and reutralizing antibody. Res. Vet. Sci. 32, No. 2. 253-256.

14. Scott, G.R. and Broun, R.D. (1961): Rinderpest diognosis with special reference to the agar gel double diffusion test. Bull. Epizoot. Dis. Afr. g: 83-125.

15. Scott, G.R. Toylor, W.P. and Rossiter, P.B. (1986): Manual on the diagnosis of rinderpest. Food and Agrocilture Orgnization of the united Nations.

16. Selvakumar, R., Padmanban, V.D., and Balapraksjam, R.A. (1981): Immunoperoxidose technique in the diagnosis of rinderpest.Gheron. 10 , 137-139.

17. Singh, S.N.; Tanwani S.K. Singh, R.; and Kangvde, C.M. (1972): Detection of rinderpest virus by an indirect haemagglutination test. Indian Vet. J. 49 : 355-359.

18. Uppal, P.K.; Oberoi, M.S. and Bansol, R.P.(1983): A note on the use of winter immunoelectropharetic technique Mycoplasma arignnini, Aspergillus fumigatus and rinderpest virus against antibody system. Indian Vet. J. 60: 767-768.

19. Ushijima, T.; Tajimo, M. and Kishi, S. (1969): Observations on cultured cell infected with pest virus by means of fluorescent antibody technique. Jap. J. Vet. Scic.; 31: 43-49.

20. White, G. (1958): A specific diffusible antigen of rinderpest virus demonstroted by the agar double diffusion precipitation reaction, Nature (Lond.), 1818-1409.

21. White, G. (1962): Gel diffusion test in the diagnosis of rinderpest. Vet. Rec., 74., 1477-1478.