SEROPREVALENCE OF CHLAMYDIAL INFECTIONS AMONG BUFFALOES OF HIMACHAL PRADESH, INDIA

R.C. Katoch, Mandeep Sharma, Rajesh Chahota, Prasenjit Dhar, B.C. Chauhan and Chaman Singh

ABSTRACT

*Chlamydia psittaci* has public health significance and has been encountered in a variety of clinical syndromes, viz. pneumonia, polyarthritis, keratoconjunctivitis, rhinitis and abortion and nasal tract of normal yaks. Samples (8.7%) were found to harbour *Chlamydia psittaci* in agar gel precipitation test out of a total of 207 serum samples collected from buffaloes from various places of Himachal Pradesh.

INTRODUCTION

The challenge to achieve food security for all is greater now than it has been before as one out of six people around the globe is currently being underfed. India has the world’s largest cattle wealth; it produces more milk than any other country in the world. A lion’s share of milk production is from buffaloes. Livestock husbandry provides employment to about 20 million either directly or indirectly. Prudently, animal health care needs more attention in view of exotic and newly emerging diseases. India alone has more than 73 million buffaloes and these belong to world’s best breeds (FAO, 1989). Buffaloes contribute 33% or milk production and 60% of total meat in India. Despite their economic importance, the productivity of buffaloes is still limited by their slow growth rate, high age maturity, short lactation periods and poor productivity.

The health care coverage for buffaloes is also not up to the mark. Chlamydiosis is a disease of a wide variety of vertebrates, including domestic animals, birds, human beings and wild fauna. The occurrence of chlamydiosis in various species of domestic livestock is of significant public health importance because the organisms are easily communicable to human beings. There is a constant necessity to find carriers/ reservoirs that are harbouring *Chlamydia psittaci* in nature and act as a focus of infection to the susceptible population and multiplies within the cytoplasm of the living cells. The genus *Chlamydia* has recently been reclassified into two genera, viz. *Chlamydia* and *Chlamydophila* and nine species *Chlamydia muridarum*, *Chlamydia suis*, *Chlamydia trachomatis*, *Chlamydia abortus*, *Chlamydia caviae*, *Chlamydomphila felis*, *Chlamydomphila pecorum*, *Chlamydomphila pneumoniae* and *Chlamydomphila psittaci* (Everett et al. 1999). This study was conducted to reveal the prevalence and distribution of chlamydial infections in buffaloes.
MATERIALS AND METHODS

A total of 207 serum samples of buffaloes were collected from various parts of Himachal Pradesh. The serum samples were screened for chlamydirosis by employing agar gel precipitation test (AGPT) using known chlamydial antigen that was prepared from infected chicken embryo yolk sac membranes from positive cases of the disease. The yolk sac membranes were triturated individually in glass/china pestle and mortar using sterile neutral sand and 10 ml phosphate buffer saline (PBS) (0.1 M pH 7.2) containing 0.02 % formalin. Two uninfected yolk sac membranes were used for the preparation of negative antigen control. The triturated yolk sac membranes were centrifuged at 5,000 rpm for 30 minutes to remove fatty layer and heavier particles. The supernatant so obtained was centrifuged at 12,000 rpm for 1 h at 4°C in a cooling centrifuge. The pelleted chlamydiae were resuspended in small quantity of PBS and were used as antigen.

RESULTS AND DISCUSSION

Chlamydirosis has been recorded in cattle by many workers. In this study, a total of 18 samples (8.7%) were found to be positive to C. psittaci using AGPT out of 207 serum samples that were screened (Table 1). In a study by Pisarz (1995) employing enzyme immunoassay on 491 cattle and 476 buffaloes serum samples reported 31.8% cattle and 5.5% of buffaloes were positive for C. psittaci, whereas Sting and Mandi (1995) detected antibodies to C. psittaci by indirect enzyme immunoassay in 90% of 106 cows screened. Our studies corroborate the findings by Pisarz, 1995.

However, Joshi et al., 2001 employed a complement fixation test and recorded seroprevalence of C. psittaci to the extent of 53.57% from the serum collected from 28 buffaloes by a counter immuno electrophoresis test. Storz (1968) reported association of C. psittaci in bovine pneumonia. Several workers, including Kiuchi and Inaba, 1952 and Gupta et al. 1976, also reported

<table>
<thead>
<tr>
<th>Sr. No.</th>
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<th>No. of samples</th>
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<td>5.</td>
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<td>0</td>
<td>-</td>
</tr>
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<td>6.</td>
<td>Chamba</td>
<td>4</td>
<td>0</td>
<td>-</td>
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<td>-</td>
</tr>
<tr>
<td>11.</td>
<td>Kinnaur</td>
<td>0</td>
<td>0</td>
<td>-</td>
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<td></td>
<td>Total</td>
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isolation of C. psittaci from pneumonia in aborted calves. Sharma and Baxi (1983) and Dhingra et al. (1986) on the basis of serological studies on bovine sera commented that chlamydial infections might be of much higher incidence. This work provides further grounds for their apprehension. The CFT has been adjudged to detect the rising antibody titres in bovine sera (Holliman et al., 1994; Nanda et al., 1992).

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REFERENCES


A CASE OF FIBROADENOMA OF MAMMARY GLAND IN A BUFFALO HEIFER

P. Annapurna, V. Rama Devi, Md. Riyazuddin and N. Sasidhar Babu

Mammary gland neoplasms are commonest in dogs whereas they are relatively infrequent in mares, ewes, sows, cows and buffaloes. It is paradoxical that cattle with a highly developed mammary gland have the lowest incidence of mammary neoplasms. Cows rarely develop mammary tumours although many dairy cows are allowed to survive to cancer age.

Perusal of the literature revealed reports of few cases of neoplasms in the mammary glands of buffaloes. Singh et al. (1991) noticed the occurrence of papilloma and fibro adenoma of udder tissue in buffaloes in a survey of tumors in animals. Joshi et al. (1994) recorded a case of fibropapilloma of the mammary gland in a buffalo calf. The present paper deals with a case of fibroadenoma of the mammary gland in a buffalo heifer.

Figure 1. Note the presence of growth on the right side of the udder.

CASE HISTORY

A graded Murrah buffalo aged about two years was brought to the local veterinary dispensary with a history of growth in the mammary gland that had persisted for the previous six months. On examination, the growth was found firm in consistency and occupied the right side of the udder (Figure 1).

RESULTS AND DISCUSSION

After a thorough investigation, the growth was removed surgically. Grossly, it was firm, nodular and encapsulated (Figure 2). The cut surface was dry and grayish white in appearance. Tissue pieces were collected in 10% formalin and sent to the Department of Pathology, N.T.R College of Veterinary Science, Gannavaram - 521 102, Krishna Dt, A.P., India.
Science, Gannavaram, for histopathological examination. The tissue pieces were processed and sections were stained with Haemotoxylin and Eosin.

Microscopically, the sections revealed the presence of numerous tubules lined by cuboidal epithelium with basophilic nuclei. There was retention of some normal lobular pattern with marked proliferation of intra and inter lobular fibrous tissue (Figure 3). The stroma also contained blood vessels that were congested. At places, the tubules were lined by more than one layer of cells that were confined in place by the basement membrane. There was no evidence of malignancy in sections. Based on these findings, the growth was identified as fibroadenoma. Screening of the available literature revealed four cases of fibroadenoma of udder in bovines (Singh et al., 1991). Moulton (1978) mentioned a few cases of primary adenocarcinoma of the mammary gland in bovines which involved the duct system, without arising from the acinar epithelium. The cause of fibroadenoma of mammary gland in the present case may have been hormonal disturbance as opined by Joshi et al. (1994) in case of fibropapilloma of mammary gland in a buffalo calf.

REFERENCES


STUDIES ON ULCERATIVE MAMMILLITIS OF BUFFALOES IN ANDHRA PRADESH (INDIA)

U.V.N. Malleswara Rao, B. Sreedevi and T. Venkata Reddy

ABSTRACT

In the recent years, a hitherto unknown disease of buffaloes characterized by an acute inflammation of one or more teats, ulceration, necrosis and sloughing of the affected teats has been reported in coastal districts of Andhra Pradesh. The mammary gland is apparently free from the lesions, and also milk abnormalities and systemic signs are absent. The onset and chronological progress of the symptoms noticed in the affected buffaloes are very much similar to a well-established disease of cows caused by a herpes virus called “Bovine ulcerative mammillitis” reported in many countries. A total of 101 milk samples were collected from the affected buffaloes and were subjected to physical, chemical and bacteriological tests of mastitis. None of the samples were found positive and no pathogenic bacteria or fungi could be isolated from the suspected samples. The filtered whey separated from the milk of affected teats was tested by intradermal inoculation into rabbits. Dermonecrosis could be reproduced in the rabbits at the site of inoculation suggesting the infectious nature of the etiological agent. Virological investigation of the material showed two light scattering zones in 10-40% sucrose density gradients. Electron microscopic examination of the purified material revealed hexagonal enveloped viral particles suggestive of a herpes virus. Histopathology of the rabbit skin lesions showed marked cellular infiltration, epidermal vesiculation, thinning of stratum corneum and syncytial aggregation of nuclei in the dermis, Cowdry type A intranuclear inclusions in the epidermal cells. Hyperimmune serum was raised against the purified virus in the rabbits. Agar gel precipitation test was performed and a line of identity could be observed between the samples. Based on the above findings and type of lesions observed, the condition was referred to as “Ulcerative mammillitis” of buffaloes.

INTRODUCTION

In India, majority of the population depends on agriculture and animal husbandry for their livelihood. Most of them are small and marginal farmers. Of late there is a steady increase in the buffalo population in states like Andhra Pradesh as there is an increasing demand for buffalo milk. Besides this, upgrading of native buffaloes with the Murrah breed is being practiced for increasing the milk potential of the local animals, with the result there is an increase in the occurrence of different diseases as the stress and strain on the animal is high due to their high productive capacity. Of the different diseases, teat afflictions, constitute a major economic problem encountered in buffaloes in coastal districts of Andhra Pradesh. In the recent years a hitherto unknown condition resulting in ulceration, necrosis and sloughing of the affected teats has been noticed in buffaloes. The condition did not show response to any type of treatment unlike the cases of clinical mastitis.

The symptomatology of the condition include acute enlargement of one or more teats, ulceration, necrosis and sloughing of the affected teats. There was no involvement of mammary tissue and milk was normal at all stages of the disease. This disease with unknown etiology is becoming a major threat to the farmers in the coastal areas of Andhra Pradesh. Clinically the onset and chronological progress of the symptoms noticed in the present condition are very much similar to a well established disease of cows known as “Bovine ulcerative mammillitis” which was
reported in different countries like Great Britain, Australia and United States. Hence, the term “Ulcerative mammillitis” is used to describe the condition noticed in affected buffaloes taken up for investigation. In view of the above observations, an attempt was made to investigate the possible etiological agent of the disease.

**MATERIAL AND METHODS**

**Samples:**

The buffalo herds in coastal districts of Andhra Pradesh (INDIA) provided the basic material for the study. The herds comprised mostly of graded Murrah buffaloes maintained under semi-intensive system by the local farmers. A total of 101 milk samples were collected from buffaloes showing typical symptoms of ulcerative mammillitis, with sudden enlargement of one or more teats that were red, hot and painful.

**Laboratory diagnosis:**

Fore milk from the affected teats was drawn directly on to a strip cup and observed for the presence of any abnormality in physical appearance. The procedure of Schalm and Noorlander (1957) was adopted to carry out California mastitis test, using a reagent modified by Sharma and Rajani (1969). The bromothymol blue indicator card test was performed following the method of Randhawa et al., (1983) and bromocresol purple test was performed as per the method of Merchant and Paker (1952). The whiteside test was carried out according to the method of Schalm et al., (1971) and the chloride test was performed as per the standard procedure. The somatic cell count of milk samples was carried out according to the method of Schalm et al., (1971) and electrical conductivity was measured using a manual meter (Milk Checker, Eisai, Tokyo, Japan).

**Cultural Examination:**

The milk samples were subjected to bacteriological examination to check the possibility of bacterial etiology of the disease. Bacteriological investigation was undertaken by transfer of a loopful of the milk sample to tryptose broth and incubation at 37 °C overnight. Milk agar slants were prepared as per the procedure given by Christie and Koegh (1940). The broth culture was streaked on milk agar slants for primary isolation of pathogens. A tentative identification of bacterial growth was done, based on colony morphology and gram’s staining. The pure cultures were identified up to the genus level using appropriate selective media (Mannitol salt agar, Mac Conkey lactose agar and blood agar) and biochemical tests which were conducted as per the Bergey’s manual of systematic bacteriology (Sneath Peter et al., 1986).

**Testing of whey in laboratory animals:**

Five ml of milk was collected under aseptic conditions from the affected quarters and microbial rennet was added to each sample at a concentration of 0.25 gm/kg of milk. All the major milk proteins were coagulated leaving a clear whey. The separated whey was collected and centrifuged at 6,000 x g for 10 minutes. Then the supernatant was filtered through 45 mm millipore filters. The whey from the milk of healthy mastitis-free lactating Murrah buffaloes was separated and used as negative controls. The test whey samples along with negative controls were injected intradermally in 0.1 ml quantity into healthy adult New Zealand white rabbits and the sequential skin changes were recorded every day for two weeks.

**Evaluation of pathogeneity of bacterial isolates:**

The bacterial isolates obtained from the milk of affected buffaloes were grown in tryptose broth at 37°C for 48 h. The broth was centrifuged at 3,000 x g for 10 minutes and the supernatant was decanted. From the pellet, one per cent cell suspension was prepared and was inoculated intradermally in 0.1 ml quantity into rabbits as per the method of Cameron et al., (1979) and the sites of inoculation were observed for development of lesions in subsequent days.

**Demonstration of the virus:**

About 250 ml of milk was collected from a typical case of ulcerative mammillitis and whey was separated using microbial rennet. The material was purified using sucrose density gradient
purification (Brakke, 1967). About 100 ml of the whey was clarified at 5,000 x g for 15 minutes in a refrigerated centrifuge. Then, the supernatant was subjected to high speed centrifugation at 1,50,000 x g for 2 h at 4°C (Sorvall, Ultracentrifuge OTD-75, rotor AH-629). The pellet obtained was suspended in 6 ml of phosphate buffered saline (pH 7.2) and was again centrifuged at 1,000 x g for 10 minutes at 4°C. The supernatant was collected and layered on 10-40% linear sucrose density gradients prepared in 0.1 M phosphate buffered saline. This was centrifuged at 1,30,000 x g for 2 h at 4°C in a swing out rotor (Sorvall, rotor AH-629). The two light scattering zones observed were collected and suspended in equal volume of phosphate buffered saline. The material was pelleted at 1,50,000 x g for 2 h at 4°C in an angle rotor (Sorvall, rotor, T-865) and was suspended in 500 µl of phosphate buffered saline.

Electron microscopy studies were conducted to demonstrate the viral particles from the sucrose density gradient purified pellet. The method of Gibbs et al., (1970a) was followed with slight modification for particle morphology studies. The material was examined under transmission electron microscope (JEOL-100S Japan, IIHR, Bangalore) and electron micrographs were taken at different magnifications.

**Histopathology:**

A small quantity of purified virus was inoculated intradermally into rabbits, which were observed for the development of lesions. The microscopic changes produced by the virus were studied as per the procedure given by Rweyemamu et al., (1969).

**Agar gel precipitation test:**

Hyperimmune serum was raised against the purified virus in rabbits as per the method described by Rweyemamu and Johnson (1969a). The test was performed as per the standard procedure (Talwar and Gupta 1997). Antiserum was charged in the central well and antigens (whey samples) were charged in the peripheral wells. The whey obtained from the milk samples of healthy mastitis free Murrah buffaloes was used as a negative control.

**Neutralization test:**

A 0.1 ml of each whey sample was injected intradermally into an immunised rabbit and all the samples were inoculated into a control rabbit, which was not immunised. Subsequently, the changes were observed in both the rabbits for and the results compared.

**RESULTS AND DISCUSSION**

**Clinical signs:**

In the present study it was observed that the size of the teat increased to two to three times the normal size and the appearance was glossy and shining with loss of flexibility (Figure 1). Out of 101 cases examined, 28% of the cases showed ulceration on the affected teat (Figure 2). In four percent of the cases there was circumscribed necrosis at the base of the teat resulting in dryness of teat skin (Figure 3). In case in which the necrosis set in a larger area of teat, it resulted in partial sloughing of the teat, which was noticed in 12% of the animals (Figure 4). Even at this stage it was observed that the physical appearance of the milk was normal. Subsequently, there was sloughing of the entire teat leaving only the tissue around the teat canal. When the calves suckled the normal teats, spontaneous flow of milk from the affected teat was observed through the exposed teat canal. Spontaneous recovery was observed in 9% of animals without any treatment.

The affected animals were devoid of febrile symptoms. General metabolic functions of the animal were normal and there was no apparent abnormality with the udder and the milk was also normal. Sharma et al. (1998) also described the same clinical sings in bovine herpes mammillitis of buffaloes in India. Similar clinical signs were described by many workers in ulcerative mammillitis of cows (Gibbs et al., 1970, Turner et al., 1976, Janett et al., 2000).
Figure 1. Ulcerative mammillitis of buffaloes – showing acute inflammation of the affected teat with glossy appearance.

Figure 2. Ulcerative mammillitis of buffaloes – showing the typical sign of ulceration on the affected teat.

Figure 3. Ulcerative mammillitis of buffaloes – showing development of circumscribed necrosis at the base of the affected teat.
Examination of milk samples:
The gross examination of 101 milk samples from ulcerative mammillitis did not reveal any abnormality in physical appearance. The colour, consistency and odour of milk were normal at all stages of the disease. The samples were found to be negative in all the chemical tests of mastitis. The pH of milk from affected teat was found to be within the normal range of 6.5 to 6.7. The average somatic cell count of all the samples was found to be between 100,000 to 500,000 cells/ml of milk. The electrical conductivity values obtained were in the range of 2 to 5 millisiemen’s/cm for all the samples. The work of Sharma et al. (1998) also revealed that the milk of buffaloes from cases of bovine herpes mammillitis was negative for the bromothymol blue test.

Culturing of milk samples:
The suspected milk samples were subjected to cultural examination for the identification of bacteria or fungi involved in the condition. No growth was observed in 32 % of samples. Staphylococci were isolated in 56 % of samples and gram negative bacteria were isolated in 12 % of the samples. None of the samples revealed any fungal growth. None of the Staphylococcal isolates were found to be positive for coagulase and haemolysin production tests. Hence, they were considered as coagulase negative, nonhaemolytic strains of staphylococci which do not have any pathological significance. Based on the growth on selective media and results of the biochemical tests, all the gram negative bacteria isolated were considered as pathologically nonsignificant organisms. These results are in accordance with the findings of Sundaresh and Janakisundaresh (1997) who reported that there were no significant pathogenic bacteria associated with the disease.

Testing of whey in laboratory animals:
The bacterial etiology of the disease was ruled out based on the results of physical, chemical and cultural tests of the suspected milk samples. To further strengthen the above findings, the bacterial isolates from the suspected milk samples were tested for their pathogenicity by inoculation into the skin of rabbits. A total of 25 samples were tested, out of which 12 samples produced severe lesions. Six whey samples produced severe dermonecrosis in the rabbits. The whey obtained from the milk samples of healthy mastitis free Murrah buffaloes did not produce any skin reaction. The sequential changes produced in the rabbit after inoculation are as follows:

One day post inoculation: The lesions observed were light pink coloured and just palpable, approximately 1 cm in diameter.
Two days post inoculation: The lesions were raised, circular and had a well defined erythema at periphery and pale centre on palpation. A varying degree of plaque like thickening was observed.

The whey of normal milk samples was completely absorbed without any visible change at the site of inoculation.

Three days post inoculation: The lesions were erythematous and slightly hardened upon palpation.

Four days post inoculation: Extensive erythema and hardening of the lesions was observed.

Five days post inoculation: Formation of dermonecrotic lesions was observed (Figure 5)

One week post inoculation: The swelling and redness disappeared and formation of brown scab was evident (Figure 6).

Ten days post inoculation: The scab were ready to detach from the lesions.

Two weeks post inoculation: Most of the scabs sloughed off from the lesions leaving an area of hairless skin.

**Evaluation of pathogenicity of bacterial isolates:**

One percent cell suspension of bacterial isolates was inoculated intradermally in 0.1 ml quantity along with negative control into the rabbits. No detectable skin changes could be observed in the rabbits at the site of inoculation. These results clearly
indicated that the dermonecrosis produced in the skin of rabbits by inoculating the whey samples may not have been due to the effect of the bacteria or the bacterial toxins present in the suspected milk. In view of the above findings, the work was continued further to investigate the possible viral etiology of the disease.

**Virus purification and electron microscopy:**

The sucrose density purification of the material showed two light scattering zones. One in between 10 and 20% and the other between 20 and 30 % gradients. Electron microscopic examination of two bands revealed the presence of hexagonal viral particles. Some of the virions had envelope and some were seen as naked particles (Figures 7 and 8). The morphology of the viral particles was suggestive of a herpes virus though further characterization of the virus was required for 100 % confirmation.

Several authors reported that electron microscopy can be used as an important diagnostic tool for the identification of bovine herpes mamillitis cases in cows (Martin et al., 1969, Rweyemamu et al., 1969, Gibbs et al., 1970a, Gibbs et al., 1972). Sharma et al., (1998) also employed the technique of electron microscopy in the diagnosis of bovine herpes mamillitis condition in buffaloes.

Though, in the present study the virus was demonstrated from the milk, scrapings or a piece of tissue from the affected teat serves as the material of choice for conducting electron microscopy.

Figure 7. Electron micrograph of the purified virus showing enveloped hexagonal capsid (X 1,40,000).
However, in the present study thought was not given to the collection or processing of these materials as the etiology was totally unknown at the beginning of the investigation. Hence, further studies using appropriate tissues for isolation and electron microscopic studies to confirm the role of bovine herpes virus in the causation of these specific symptoms noticed in the present study was required.

**Experimental reproduction of the disease in rabbit:**

After isolation of the virus from the affected cases, it was again inoculated into a rabbit, to prove the Koch’s postulates. Following inoculation it was observed that the purified virus experimentally reproduced the lesion in rabbits within 24 h.

**Histopathology:**

The microscopic changes produced by the virus in skin lesions of the rabbit were marked infiltration of inflammatory cells, epidermal vesiculation, thinning of stratum corneum and syncytia formation in the dermis (Figures 9 and 10). Cowdry type A intranuclear inclusions were also noted in the epidermal cells. Several authors recorded similar observations on the histology of natural and experimental cases of bovine herpes mammillitis of cattle (Martin et al., 1969, Rweyemamu et al., 1969, Gibbs et al., 1970, Gibbs and Collings 1972). Similar microscopic changes were also recorded in bovine herpes mammillitis of buffaloes by Sharma et al. (1998). This gives a strong experimental evidence that the present condition might have been caused by a virus. According to the reports of Pepper et al. (1966), Deas and Johnston (1966), Castrucci et al. (1972) rabbits serve as excellent laboratory animals for conducting experiments for bovine herpes mammillitis virus isolated from cows, whereas mice were refractory. To know whether the same property would be exhibited by the virus isolated from ulcerative mammillitis of buffaloes, laboratory animal susceptibility was checked by inoculating the purified virus into both rabbits and mice. The demonecrotic property of the virus could be reproduced in rabbits whereas mice did not show any skin reaction. This suggests that the disease may be due to a strain of bovine herpes mammillitis virus though it has to be proved by sending the material to a reference laboratory.

According to Rweyemamu and Johnson (1969) there are seven strains of bovine herpes mammillitis virus recognised in cows. Until today, there is no information available regarding the bovine herpes mammillitis of buffaloes except for one report by Sharma et al. (1998) from India. In contrast to the disease position in other countries, in India bovine herpes mammillitis is not a major problem in crossbred cows; the majority of similar cases occur in buffaloes. Hence, the possibility of a specific strain of bovine herpes mammillitis virus affecting only buffaloes is to be investigated.

**Agar gel precipitation test:**

Hyperimmune serum was raised against purified virus in the rabbits and the agar gel precipitation test was conducted with different field samples collected from the disease condition. A line of identity could be observed between different samples. This indicates that there is a cross reaction between the different samples or there may be a single strain of virus present in all the cases.

**Neutralisation test:**

A neutralisation test was conducted in immunised rabbits using different whey samples collected from ulcerative mammillitis cases. It was observed that immunised rabbits prevented the development of demonecrosis upon intradermal inoculation of the samples in contrast to unimmunised rabbits. This experiment gave an evidence that there was sufficiently high antibody production against the virus in the rabbits. Similar trials can be undertaken for the development of a vaccine, which protects buffaloes against ulcerative mammillitis under field conditions after the causative factor is fully established.

In conclusion, in the present study an attempt was made to investigate the probable etiological agent of ulcerative mammillitis of buffaloes in Andhra Pradesh. The results of the present investigation indicated that the condition may be due to a virus, probably a herpes virus of buffaloes. However, the findings are to be further confirmed by testing a greater number of samples collected from clinical cases in different areas of Andhra
Figure 8. Electron micrograph of the purified virus – showing the naked hexagonal capsid (X 1,40,000).

Figure 9. Histopathology of the rabbit skin inoculated with the purified virus – showing epidermal vesiculation, thinning of the stratum corneum and marked cellular infiltration H and E x 280.

Figure 10. Histopathology of the rabbit skin inoculated with the purified virus – showing syncytial aggregation of nuclei in the dermis H and E x 280.
Pradesh. Further work is to be undertaken towards the molecular characterisation of the virus and development of a good vaccine which helps in control of the disease in buffaloes.

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REFERENCES


THE ROLE OF SWAMP BUFFALO IN AGRICULTURAL PRODUCTION OF SMALL FARM HOLDERS

Do Kim Tuyen

INTRODUCTION

In Vietnam, the buffalo is very popular animal. These animals are of the Swamp type. Traditionally, buffaloes were raised by small farm holders for multi-purpose in agricultural production. The local swamp buffalo have low meat and milk production and low reproductive efficiency due to long gestation period, long calving interval, silent heat, and low conception rate. However, swamp buffaloes are well suited to poor feeding management as well as adapted to the hot-humid tropical climate conditions. Buffalo raising is closely integrated with paddy rice cultivation, and it plays an important role in total agriculture production of the country. The buffalo is also considered a friend of the farm family not only for draught power and fertilizer but also for taking full advantage of feed resources, free time, and subsidiary labor etc. of small farm holders in rural areas.

From 1990 to the present, the growth rate of the Swamp buffalo population of Vietnam has been affected by various factors. Among these are that cattle are considered more important than buffaloes in livestock development plans that there has been no policy for buffalo development for last 10 years, fast national population grow, local economic development, mechanization and fast urbanization.

Population and distribution

Although buffalo population in the world is increasing about 1.3% annually, the rate of increase of swamp buffalo in Vietnam is low (0.73%). At present, the buffalo population of Vietnam is about 2.9 million head, and the cattle population is 4.16 million. Because the body size of the buffalo is larger than that of cattle so annually, total buffalo meat production has always contributed more than 50% of total ruminant meat production of the country.

There was a reduction in the population of swamp buffalo from 1995 to 1999 due to a decrease in the grazing land as a result of an increase in the area under cultivation (Table 1). This situation happened in the lowland, particularly in the Mekong and Red river delta provinces, where the buffaloes are grazed extensively. However, there has been an increase in number in area where grazing lands are still available; the rate of increase in the buffalo population of Vietnam in last the 10 years has been very low (0.73) when compared with that of the cattle population (2.8%).

Table 1. Buffalo population changes in Vietnam (1990 to 2000).

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<tr>
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<td>2,962800</td>
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<td>1996</td>
<td>2,953700</td>
<td>103.6</td>
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<td>1997</td>
<td>2,943700</td>
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<td>103.3</td>
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<tr>
<td>1999</td>
<td>2,955728</td>
<td>103.5</td>
</tr>
<tr>
<td>2000</td>
<td>2,958355</td>
<td>104.5</td>
</tr>
<tr>
<td>2001</td>
<td>2,807874</td>
<td>98.38</td>
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<tr>
<td>2002</td>
<td>2,814452</td>
<td>98.61</td>
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Source: State Statistic Department, 2002

Ruminant Division, Department of Agricultural and Forestry Extension (DAFE), No. 2 Ngoc Ha, Ba Dinh, Hanoi, Vietnam
The Swamp buffalo is raised in all parts of Vietnam, but it is more concentrated in the Northern Mountain and Upland regions because the ecological conditions of this region are suitable for buffalo production. More than 52% of buffalo population is raised in the Northern Mountain Uplands, where the buffaloes are more valuable to the small-holder farmers not only for farm work and transportation of farm products but also as a source of income of the farmers.

**Body size and growth**

The body size of the swamp buffalo in Vietnam is not as big as the river type buffalo, but it is twice as large as that of the local yellow cattle. The average live-weight of the adult female swamp buffalo is about 420-450 kg and that of adult males is about 480-550 kg. The body weight at birth of the buffalo calf is about 22-25 kg. The live-weights of adult swamp buffaloes differ between regions (Table 3). Buffaloes in the Mekong delta tend to be larger size than in other regions of Vietnam. Thu (1987) reported that swamp buffaloes in Dong Thap province of the Mekong delta were the biggest in Vietnam with an average liveweight of 593 kg for adult females and 700 kg for males.

The small farm holder’s priorities in raising buffaloes were draught power, manure as fertilizer for crop fields, and as a savings bank. So meat production was not the main focus of these small farmers. Traditional farming practices are used extensively and generally include day-time grazing supplemented with rice straw when the animal is confined or at night.

Feed insufficiency during the dry season and cold season is a major factor causing slow to negative body growth in buffaloes. Techniques for improved utilization of rice straw, such as urea treatment or silage and hay making are available but adoption of these technologies has been extremely low in buffalo feeding due to lack of economic incentives.

**Reproduction**

Buffalo attain puberty at a later age than cattle (Table 4). The river type exhibits first estrous earlier (15 to 18 months) than swamp type (21 to 24 months). First conception occurs at an average body weight of 250 to 275 kg, which is usually attained at 24 to 36 months of age.

In Vietnam, female buffaloes attain puberty at around 3 years old. Their first calving is at 4 to 5 years of age, and they continue to have calves to the age 18 years. The average duration of estrous varies from 24 to 72 h. Normal estrous interval or cycle is about 20-22 days. The incidence of silent estrous is high; in general, only 25-30% of buffalo have clear symptoms of estrous (Nguyen, B.X., 1994).

The fertility rate of the swamp buffalo herd, on average, is low, at about 30-40%. The factors contributing to the low reproduction rate are numerous, such as the stress of working conditions, poor nutrition and management, shortage of grazing area and poor knowledge about reproduction on the part of farmers. The “silent estrous” is also a serious problem when attempts are made to implement artificial insemination (AI). Up to the present, availability of good quality breeding bulls at village level is still a limitation for increasing reproductive efficiency in swamp buffaloes. Farmers often mate female buffaloes to good bulls available in the village, regardless of the risk of inbreeding and usually a field-master bull mates all female buffaloes in a given area.

**Feeding and management**

In Vietnam, farmers have a long tradition and experience of buffalo raising but techniques are mostly primitive. Management practices are based on extensive systems and buffaloes are freely grazed on natural grasslands, forests, road sides, canal banks, rice fields after harvesting and dikes, etc. They are also fed with rice straw or other crop residues in the dry season and the working season. Farmers normally do not fatten buffaloes before sale for meat but middlemen occasionally buy and fatten them with good quality grass and some concentrate before sale. Buffaloes are mainly slaughtered when they are not able to work due to old age or as a result of accidents. Housing for buffaloes is simple and often made of local materials such as wood or bamboo with a palm-leaf roof in the mountain areas, but in the plain area the housing for buffalo is better with concrete floors, brick walls and tile roofs.
Table 2. Buffalo population in different regions in 2000.

<table>
<thead>
<tr>
<th>Ecological regions</th>
<th>Population(1,000 heads)</th>
<th>Growth rate(%)</th>
<th>The proportion of Population(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Northern mountain</td>
<td>1,721</td>
<td>0.49</td>
<td>52.00</td>
</tr>
<tr>
<td>2. Red river delta</td>
<td>166</td>
<td>-4.1</td>
<td>8.40</td>
</tr>
<tr>
<td>3. North Central coast</td>
<td>676</td>
<td>1.19</td>
<td>22.00</td>
</tr>
<tr>
<td>4. South Central coast</td>
<td>128</td>
<td>0.78</td>
<td>4.60</td>
</tr>
<tr>
<td>5. Central highlands</td>
<td>51.4</td>
<td>0.78</td>
<td>1.60</td>
</tr>
<tr>
<td>6. North East South</td>
<td>141.8</td>
<td>-5.1</td>
<td>6.40</td>
</tr>
<tr>
<td>7. Mekong river delta</td>
<td>71.4</td>
<td>-5.88</td>
<td>5.00</td>
</tr>
<tr>
<td><strong>Total Vietnam</strong></td>
<td><strong>2,958</strong></td>
<td><strong>0.101</strong></td>
<td><strong>100.00</strong></td>
</tr>
</tbody>
</table>

Source: State Statistic Department, 2000

Table 3: Basic measurements and live-weights of adult swamp buffaloes in Vietnam.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Mekong data(1)</th>
<th>South eastern Provinces(2)</th>
<th>Central area(3)</th>
<th>Northern Provinces(2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Height at withers(cm)</td>
<td>127</td>
<td>126</td>
<td>121</td>
<td>118</td>
</tr>
<tr>
<td>Length of body(cm)</td>
<td>141</td>
<td>132</td>
<td>128</td>
<td>127</td>
</tr>
<tr>
<td>Height girth (cm)</td>
<td>192</td>
<td>193</td>
<td>191</td>
<td>180</td>
</tr>
<tr>
<td>Live-weight (kg)</td>
<td>458</td>
<td>434</td>
<td>417</td>
<td>358</td>
</tr>
<tr>
<td>Male</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Height at withers(cm)</td>
<td>131</td>
<td>129</td>
<td>129</td>
<td>121</td>
</tr>
<tr>
<td>Length of body(cm)</td>
<td>143</td>
<td>135</td>
<td>136</td>
<td>133</td>
</tr>
<tr>
<td>Height girth (cm)</td>
<td>198</td>
<td>197</td>
<td>198</td>
<td>190</td>
</tr>
<tr>
<td>Live-weight (kg)</td>
<td>494</td>
<td>464</td>
<td>494</td>
<td>428</td>
</tr>
</tbody>
</table>

Source: (1) Tu (1987); (2) Hong (1978); (3) Thc (1985)

Table 4: Female reproductive characteristics of cattle and buffaloes

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Cattle mean (range)</th>
<th>Buffalo mean (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sexual season</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age at puberty (months)</td>
<td>15 (10 – 24)</td>
<td>21 (15 – 36)</td>
</tr>
<tr>
<td>Oestrous: Interval(day)</td>
<td>21 (14 – 29)</td>
<td>21 (18 – 22)</td>
</tr>
<tr>
<td>Duration(hours)</td>
<td>18 (12 – 30)</td>
<td>21 (17 – 24)</td>
</tr>
<tr>
<td>Ovulation: Type</td>
<td>Spontaneous</td>
<td>Spontaneous</td>
</tr>
<tr>
<td>Time of onset (hours)</td>
<td>30 (18 – 48)</td>
<td>32 (18 – 45)</td>
</tr>
<tr>
<td>Number of eggs shed</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Life span of corpus lutetium</td>
<td>16</td>
<td>16</td>
</tr>
<tr>
<td>Gestation length (days)</td>
<td>280 (276 – 293)</td>
<td>315 (305 – 330)</td>
</tr>
<tr>
<td>Age at first calving (months)</td>
<td>30 (24 – 36)</td>
<td>42 (36 – 56)</td>
</tr>
<tr>
<td>Calving interval (months)</td>
<td>13 (12 – 14)</td>
<td>18 (15 – 21)</td>
</tr>
</tbody>
</table>
After harvesting, farmers in most areas let their buffalo free to look after themselves in natural grazing areas. But in irrigated areas, farmers have to look after their animal every day. Tending buffalo is usually relegated to children and old family members who cannot work hard.

The function of swamp buffalo in agricultural production

A. Farm integration: In Vietnam, agriculture production system now is mainly that of the small farm holder. Buffaloes are still the main animal for preparing paddy fields and for transportation of farm products. The integration of crops and buffaloes make use of available crop by-products, and transforms them into protein in the formed of meat and milk for human consumption. Manure from buffalo is good fertilizer for paddy fields and also improves soil structure. So buffaloes represent buffer assets which can be realized at any time, adding further stability to the self-sufficient crop and livestock production.

B. Farm cash income: Swamp buffalo can utilize waste and crop by products such as straw, and grasses, which cannot be utilized by humans. It can serve as long term capital reserve by using local natural resources as long as fodder resources are freely accessible at no charge. Rearing buffalo is a means of financial security for the small farm holder. In addition, sale of progeny or unproductive buffaloes and buffalo dung provides cash income to the small farm holders.

C. Food security: Food security can defined by a combination of balance between availability and need, avoidance of food shortage and nutrition deficiencies and ensuring food quality. Buffalo can transform non-edible food inedible for humans into high quality food for human consumption. Increased buffalo production may add to food security: the poor farmer access to more food at low cost and increase local products, and the reduces imports and saves foreign currency.

The role of buffalo in small farm holder

In Vietnam, swamp buffaloes are the main source of draught power in agriculture and crop cultivation. The large feet, slow steady movement, and heavy draught capacity of swamp buffaloes make them particularly well suited for paddy cultivation in swampy, waterlogged rice-fields. In addition, there are various social, cultural and economic conditions which make the use of buffalo for draught a very appropriate and profitable farming technique. However, it is difficult to guaranties the draught power and thus, the buffalo’s contribution as a source of draught power to the nation’s economy has been quite often underestimated.

An important by-product of the swamp buffalo was manure which farmers used as fertilizer in plant cultivation. This made the cost of crop production lower. Moreover, small form buffalo production is highly sustainable from biological, social, economic and ecological stand points.

The draught buffalo would gain weight if a supply a feed was available and a good selling price would be received. Besides draught power from female buffalo, farmers obtained other by-product in form of the offspring when the females were bred. The household’s asset were thereby increased. The buffalo was counted as an asset by the member of the family. This asset will be converted into cash as required.

The estimation of swamp buffalo meat production for the year 2000 is shown in the Table 5. Because of many prejudices about buffalo meat made it has a low rating with consumers. In Vietnam, only old buffaloes are slaughtered without for meat, which is inevitably of poor quality. In fact, buffalo meat can have high quality comparable with beef.

Swamp buffalo production is of less economic importance in term of the GDP, but its economic importance to small farm holders is generally underestimated. Their multipurpose contributions increase incomes of the poor in rural areas.

Problems and Constraints

As in many countries in the world, the swamp buffalo is the most neglected and forgotten animal in Vietnam due to many reasons as follows:

Institutional problems
- More consideration is given to cattle production compared to buffalo production in livestock development plan. There has been no
Table 5: Meat production of buffalo in 2000.

<table>
<thead>
<tr>
<th>Ecological regions</th>
<th>Meat production</th>
<th>Growth rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Northern mountain</td>
<td>18.9</td>
<td>2.78</td>
</tr>
<tr>
<td>2. Red river delta</td>
<td>4.1</td>
<td>5.90</td>
</tr>
<tr>
<td>3. North Central coast</td>
<td>7.2</td>
<td>2.26</td>
</tr>
<tr>
<td>4. South Central coast</td>
<td>1.9</td>
<td>4.11</td>
</tr>
<tr>
<td>5. Central highland</td>
<td>1.5</td>
<td>12.51</td>
</tr>
<tr>
<td>6. North East South</td>
<td>10.3</td>
<td>8.65</td>
</tr>
<tr>
<td>7. Mekong river delta</td>
<td>4.6</td>
<td>-5.22</td>
</tr>
<tr>
<td>Whole country</td>
<td>48.5</td>
<td>1.42</td>
</tr>
</tbody>
</table>

Source: State statistics Department, 2000

extension program and little research budget support for buffalo production, so buffalo production systems received little attention by state management organizations from the central to the grass-root levels.

- From 1990 to 2002, there was no program and no policy for buffalo development and no extension service concerned with buffalo from the Department of Agricultural and Forestry Extension (DAFE) of MARD.
- Lacking of grazing land areas by the result of reforestation program and shortage of forest during the winter season affected to the growth rate and low reproductive efficiency of swamp buffalo.

**Production problems**

- Acceptability of buffalo meat: A major problem of swamp buffalo development is low acceptability of buffalo meat. It is considered to be more fibrous, darker, less tender and less nutritious. The main problem is that buffalo meat is mainly from old animals and those reared under poorly planned nutrition.
- Price distortion of buffalo over cattle. Buffalo contribute more red meat than cattle to the market, but the live-weight price of buffaloes is lower than that of cattle, so the butcher tends to prefer buffaloes as he sells buffalo and beef at the same price. In the slaughtered house, the number of buffalo slaughter per day is greater than the number of cattle but in the market, no body is selling buffalo meat. All of buffalo meat is sold at the same price as beef.
- Low productivity: low growth rate, and especially, low calving rate, the reasons for this including lack of common grazing areas and bulls.
- Lacking of breeding improvement program and policy support for raising or keeping breeding buffalo bulls for natural service.

**SUMMARY AND CONCLUSIONS**

Swamp buffalo production in Vietnam still plays an importance role in total agriculture production of small farm holders, especially farmers in the mountain areas by supplying draught power, fertilizer and taking full advantage of available feed resources, time and subsidiary labor.

The annual rate of increase of the buffalo population during the last 12 year has been very low (0.78%) compared with cattle (2.8%). There is a need to improve the buffalo population in the coming years by establishing a breeding program for swamp buffaloes and focusing on selection of good bulls and cows for producing high quality buffalo beef.

It is necessary to improve feeding and management systems for swamp buffaloes of small farm holders for higher meat production and better reproductive efficiency.

In the coming years, there is a need for a state budgeted breeding program which would include research and extension services for buffalo development in Vietnam, better use of available by-products and transfer of appropriate technology to farmers for improving buffalo production in rural areas.
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