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*Full Length Research Paper*

# Therapeutic potential of Johne's disease vaccine: A follow up post vaccination study in a goatherd of endangered Jamunapari breed, naturally infected with *Mycobacterium avium* subspecies *paratuberculosis*

**S. V. Singh*, P. K. Singh, M. K. Singh, A. V. Singh and J. S. Sohal**

Microbiology Laboratory, Animal Health Division, Central Institute for Research on Goats, Makhdoom, PO - Farah, District – Mathura (UP), India.

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**The study evaluated 'therapeutic potential' of 'Indigenous Johne's Disease Vaccine' in a goat herd (important endangered Jamunapari breed) endemically infected with *Mycobacterium avium* subspecies *paratuberculosis* (MAP). A total of 526 goats from this herd were vaccinated subcutaneously. Therapeutic potential' was evaluated on the basis of physical improvement, change in body weights, shedding of MAP in feces, sero-conversion rates, mortality rates, growth performance, reproductive performance, milk production, population growth rate, feed and fodder consumption, body weight profile of kids born to vaccinated goats after vaccination and compared with preceding year. Following vaccination, rapid and remarkable reduction in production losses along with improvement in health was recorded. Goats exhibited recovery from symptoms and lesions of sub-clinical, clinical and advanced clinical Johne's disease and improvement in productivity (growth rate, milk production, reproductive efficiency etc.). By decreasing the severity of clinical symptoms, herd incidence, inter-herd transmission of MAP 'indigenous vaccine' helped to salvage majority of 526 vaccinated Jamunapari goats from imminent culling due to weakness, debility and loss of productivity caused by Johne's disease and contributed to the conservation of this endangered breed (Jamunapari) of native goats.**

**Key words:** Goats, therapeutic vaccine, *Mycobacterium avium* subspecies *paratuberculosis*, Johne's disease.

## INTRODUCTION

Goats contribute greatly to the agrarian economy of the country, especially in deserts and hilly tracts by providing livelihood security to large number of small, marginal and landless farmers and labourers. At 120 million (FAO, 2005), India has 16.2% of world's goat population despite 60% removal rate (43.0% slaughter and 17.0% mortality). Of 23 goat breeds, Jamunapari is the heaviest and largest breed valued for milk (Figure 4a and b). Breed in pure form is found in Chakarnagar block of Etawah district of Uttar Pradesh state. Population of Jamunapari goats declined rapidly and at present less than 5000 goat are left in the native tract (FAO, 2008), therefore breed is classified as 'highly threatened'. Population of Jamunapari declined due to susceptibility to Johne's disease (JD), changes in the demography (ravines) of the native tract, farming practices, socio-economic structure and diminishing returns. Breed face extinction and it is feared that genetic resource evolved after years of selection by traditional goat keepers may be lost for ever. Goats are susceptible to *Mycobacterium avium* subsp. *paratuberculosis* (MAP) (Singh et al., 1998 and 2009) and endemic in farm herds (Kumar et al., 2007a, b; Singh et al., 2007a). Clinically, infected goats suffer from loss in body weights with (intermittent or continuous) or without diarrhea, decreased productivity, wasting and death. MAP has also been associated with Crohn's disease in human beings (Hermon-Taylor et al., 2000; Chamberlin

*Corresponding author. E-mail: shoorvir.singh@gmail.com, shoorvir_singh@rediffmail.com.

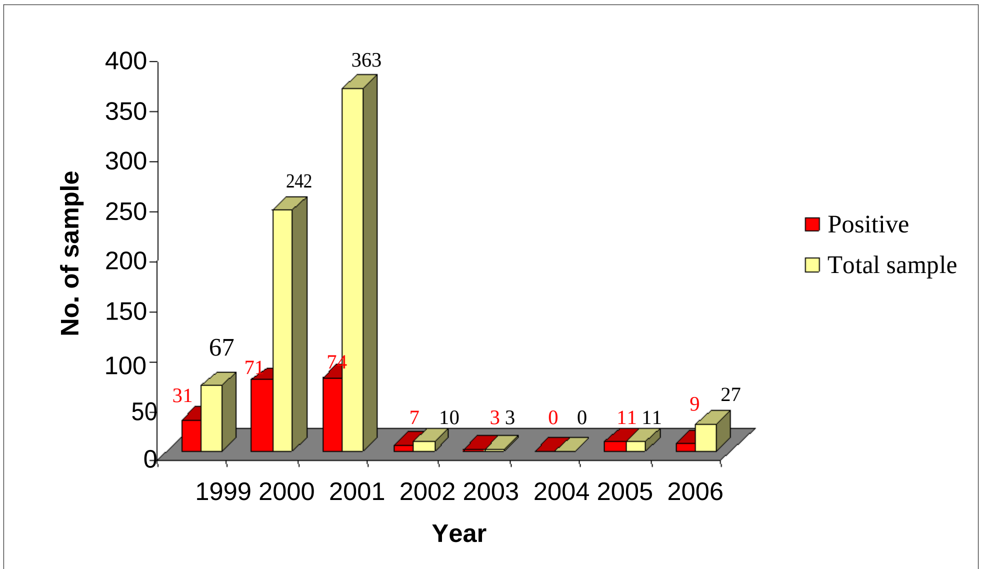



**Figure 1.** Status of Johne's disease in Jamunapari goat herd.

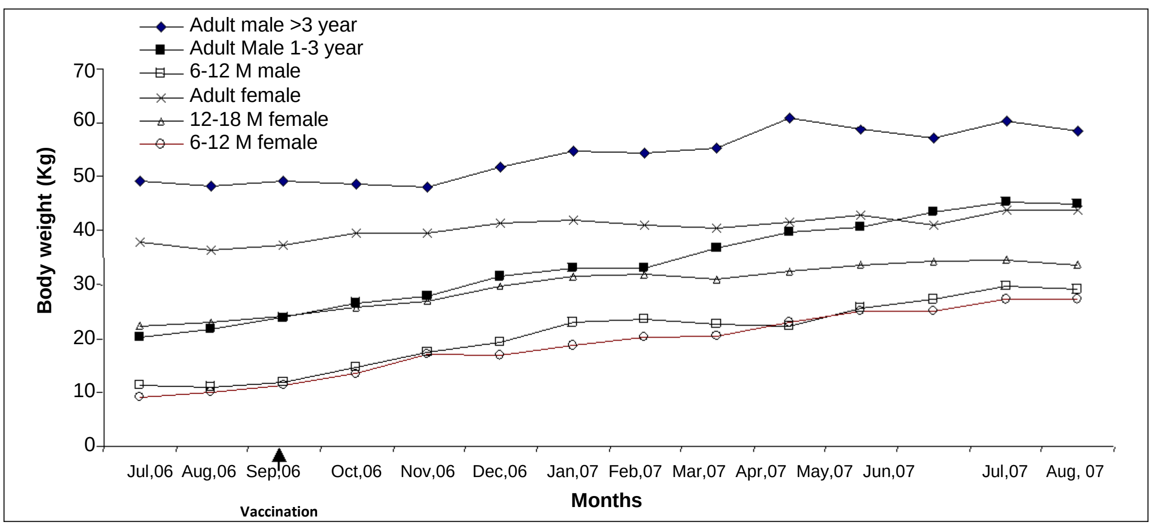



**Figure 2.** Monthly body weight profiles (before and after vaccination) in different age groups (physiological states) of Jamunapari goats.

and Naser, 2006; Singh et al., 2008). MAP has been recovered from pasteurized milk and milk products (Shankar et al., 2010). Diagnosis and control of JD is difficult due to dormancy, long incubation and uneconomical treatment (Harris and Barletta, 2001). Presently, country lacks indigenous diagnostic kits and vaccines for the control of the disease.

JD is endemic in the farm herd of Jamunapari goats located at Central Institute for Research on Goats (CIRG) Mathura (Singh et al., 2009). Significant losses occurred through morbidity, mortality and early culling due to JD (Singh et al., 2007a and b).Test and cull' method practiced for the last 30 years failed to control the disease at this farm. Instead, JD increased in intensity and prevalence (Figure 1, 4c and d and 5a and b). However, vaccination against JD has shown promise to control the infection (Perez et al., 1995). 'Indigenous Vaccine' was developed (Singh et al., 2007b) using native 'Indian Bison type' strain (S 5) of MAP, a new genotype not reported outside India (Sohal et al., 2009). Vaccine trials showed that 'Indigenous Vaccine', was both 'therapeutic' (Singh et al., 2010) and preventive (Singh et al., 2007b). In this study 'Therapeutic potential' of 'Indigenous vaccine' was used to treat goats with sub-clinical, clinical and advanced clinical JD. Study also evaluated the effect of 'Indigenous Vaccine' on health and productivity of endemically infected herd of Jamunapari located at CIRG, Makhdoom. Study aimed to conserve the precious germplasm of this important dairy breed of goats along with revalidation of therapeutic potential of vaccine in terms of reduction in production losses attributed to JD and incidence of disease in the herd.

## MATERIALS AND METHODS

### Genetic stock at Jamunapari farm (CIRG, Makhdoom)

Pure germplasm of Jamunapari breed of goats (best Asian dairy goat breed) existed only with traditional goat breeders located in Chakarnagar block of Etawah district in Uttar Pradesh State. For the conservation and genetic improvement of Jamunapari breed, Government of India established a goat farm at Central Institute for Research on Goats (Makhdoom, Mathura, UP) in 1976, by purchasing goats from Chakarnagar. Minimum of 250 adult of this breed females were maintained for the conservation and breed improvement program, through selection and progeny testing. Number of goats at farm varied between 500 and 650 from 1976 to 2010. Goats were selected on 90 days milk yield and 9 months body weights by avoiding inbreeding. Shifting of purchased goats to farm led to changes in habitat and management (extensive to semi-intensive) leading to confinement and nutritional stress, which over the years resulted in serious health problems especially due to chronic infections like Johne's disease (JD). Jamunapari breed of goat were susceptible for JD (Singh et al., 2009). Endemic JD resulted in higher losses leading to reduction in number of adult goats and since 1976, 3 times new goats (50 to 70 each time) were added by purchasing goats from the native tract to maintain 250 breedable goats for selective breeding.

### Management

Goats (except kids of feedlot experiments and sick) were maintained under 'semi-intensive management' wherein goats were given concentrate ration in the form of pellets (as per National Research Council standards) along with green fodder, dry straw, tree lopping and at least 6 h of grazing. Goats were bred twice a year to have kids in February/March and September/October. Many goats did not kid twice a year and twinning was around 10%. Despite intensive feeding, goats were lean and carcass of goats dying at the farm was always lean and emaciated at necropsy. Similar management, feeding, breeding and health schedule has been practiced for the last 30 years at the farm. Surplus males and few females were sold to farmers and commercial goat keepers (private and government). Therefore, this farm is nucleus herd for the supply of pure Jamunapari goats.

### Herd health management

Goats are vaccinated for foot and mouth disease, entero-toxaemia and Peste des Petits Ruminants besides deworming, drenching against coccidiosis and dipping against ticks, lice and mange. Sick goats were treated symptomatically.

### History of Johne's disease at farm

Farm herd of Jamunapari goats at CIRG experienced losses in health and production due to endemic JD, since establishment in 1976 by way of early culling, morbidity, mortality etc. (Singh et al., 1998).

There was marked decrease in body condition of goats from 1984 to 2006 (Figure 4c and d and 5a and b). Goats in all age groups suffered from clinical JD. Goats were dull, depressed, poor in body condition, weak with (intermittent or continuous) and without diarrhea, stunted, growth rate was reduced etc. Goats were shedding MAP throughout their life. Development of clinical JD hastened due to various stress factors (pregnancy, nutrition, lactation, parturition, concurrent parasitic infections, improper supply of ration, and no additional compensation of energy and protein for JD in diet). Clinical JD resulted in low birth weights of kids, low growth rates, loss in body weights, reduced milk yield, delayed maturity, increased kidding interval, reduced fertility, frequent and prolonged bouts of diarrhea, reduced twining, increased morbidity and mortality, low per goat productivity etc.

### Screening and control of Johne's disease

Farm herd had been screened (yearly/six monthly herd screening' and/or 'suspected goats screening' round the year) for JD since 1984 till vaccinated in September, 2006. Fecal samples were tested by microscopic examination and culture. Goats suspected for JD (suffering from un-treatable diarrhea / weakness / stunting) were sampled and screened for JD. General screening of herd was carried out from 1999 to 2001 and significant numbers of goats were positive for JD by microscopy (Figure 3). However, from 2002 to 2006, only suspected goats were screened.

Goats positive for acid fast bacilli indistinguishable to *M. avium paratuberculosis* (MAP) were culled from herd. Despite removing significant number of positive goats in last 30 years (test and cull) prevalence of JD in the farm did not reduce. Instead, prevalence of JD increased in intensity and severity over the years. Goats continued to suffer from weakness, diarrhea, emaciation and large number of affected goats were culled and died of JD between 1976 and 2006.

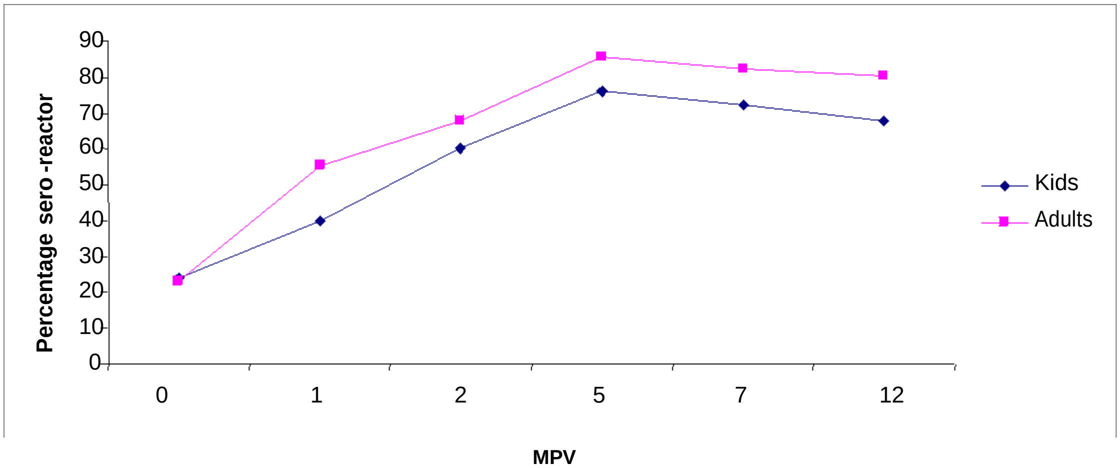
Besides mortality due to JD and other diseases, culling of goats on health (Johne's disease, weakness, loss in body weights, emaciation, debility, hydatidosis etc.) and production (unthriftiness, stunting, stray mating, off-colour etc.) grounds were in regular practice. Endemic JD was responsible for negative energy balance in goats (lean carcasses). Energy losses due to JD were not compensated in ration. Other major health problems in the herd were haemonchosis, lice infestation, pneumonia (Mycoplasmosis), neonatal diarrhea (*E.coli* and Coccidiosis), hydatidosis, mastitis, still-births, abortions, HCN poisoning, weakness and stunting etc.

### Vaccine

Indigenous vaccine was developed using native 'S 5' strain characterized as 'Indian Bison type', a new genotype of MAP, not reported outside India (Sohal et al., 2009). The 'S 5' strain of MAP was recovered from a goat terminally sick with Johne's disease at this Jamunapari farm goat (CIRG, Mathura) in 1998. 'Therapeutic potential' of 'Indigenous Vaccine' has been evaluated in naturally infected/spontaneous cases of JD in goats (Singh et al., 2010) and as preventive in a classical trial (vaccination and challenge) in young goats (Singh et al., 2007a). One dose of vaccine contained 2.5 mg dry weight of S 5' MAP culture containing $5 \times 10^9$ bacilli /ml, in aluminium hydro-oxide gel as adjuvant (Singh et al., 2007a). Inactivation was carried-out at 72°C for 2 h. Attempt was made to save the Jamunapari herd from culling due to JD by treating the spontaneous cases of sub-clinical, clinical and advanced clinical JD with 'Indigenous Vaccine'.

### Vaccination

At the time of vaccination, goats were extremely poor in condition (Figure 4c and d and 5a and b). A total of 526 goats (Males: 161; 3




**Duration (months post vaccination)**

**Figure 3.** Percent sero-conversion (adult goats and kids) in different months post vaccination.

to 6 months, 32; 6 to 12 months, 60; adults, 69; Females, 365; 3 to 6 months, 31; 6 to 12 months, 116; adults, 218) were vaccinated. Prevalence of JD in farm at vaccination was high (Singh et al., 1998); practically all goats were infected with MAP and >60% had sub-clinical to advanced clinical symptoms of JD. Goats above 3 months of age were vaccinated (between 16 and 24, September, 2006), with 1 ml of 'indigenous vaccine' subcutaneously in the neck region.

### Monitoring parameters

Since Jamunapari breed is under highly threatened category, each goat was precious and needed to be saved, therefore, all goats above 3 months of age were vaccinated. Goats were monitored on health (mortality, morbidity, reproductive performance etc.) and production (birth weights, growth rate, birth weight, 90 days, 140 days and total milk yield, kidding interval, age at first kidding etc.) parameters. Status of goats on these parameters before vaccination was used as control and was compared with data after vaccination. Goats were monitored on following parameters to record improvement in the herd.

### Change in physical condition

Overall change in physical condition, health and appearance (colour of body coat and roughness of skin, skin thickness, pliability, alertness/ dullness/depressed etc.) of goats were compared before and after vaccination.

### Shedding of MAP

Shedding of MAP was monitored in fecal samples of serially sampled cohort by microscopic examination of each of 87 samples collected on the day of vaccination and 12 months post vaccination) and by culture on HEY medium on each of 38 samples collected on the day of vaccination and 6 months post vaccination). Feces were finely grounded, concentrated by centrifugation and decontaminated by hexadecyl pyridinium chloride (HPC). For culture, inoculum from 1.0 mlof sediment after decontamination was streaked on HEYM slants with and without mycobactin J (as per Merkal et al., 1982). From the concentrated fecal samples, microscopy was done.

### Sero-conversion

Sero-conversion was monitored in representative goats serologically using indigenous ELISA kit (Singh et al., 2007b). Serum samples were collected from 81 of total 526 vaccinated goats (~15%) from all age groups (kids and adults) at 0, 1, 2, 5, 7 and 12 months post vaccination. Antibody titers were converted to S/P ratio as per Collins (2002). Results were presented as percent sero-reactors at different sampling intervals. Goats in the strong positive category in S/P ratio were considered positive.

### Changes in body weights of goats of different age groups

Changes in average monthly body weights of different age groups of goats were compared before and after vaccination at monthly intervals for 10 months.

### Morbidity and mortality rate

Herd was monitored for morbidity due to various diseases including JD and other causes**.** Herd mortality, before vaccination (2005-2006) was compared with mortality after vaccination (2006-2007 and 2007-2008).

### Milk yield

Changes in milk production before (2005 to 2006) and after vaccination (2006 to 2007 and 2007 to 2008) were compared. Least square means for 90 days milk yield (90 DMY), 140 days milk yield and total milk yield (TMY) were calculated for respective year and seasons.

### Reproductive efficiency

Changes in reproductive efficiency [Age at first kidding (AFK), decontamination for 18 to 24 h at room temperature (RT) with 0.9%

**Table 1.** Change in average body weights.

| Sex | Age group | Goats / observation (number of goats) | Average weight change (kg) | |
|---|---|---|---|---|
| | | | 3 months before vaccination | 4 months after vaccination |
| Male | 6-12 months | 60 | 2.2±0.2 | 9.7±0.6 |
| | 1-7 years | 46 | 3.2±0.4 | 7.8±0.5 |
| Females | 6-12 months | 80 | 1.9±0.1 | 7.4±0.3 |
| | 12-18 months | 97 | 1.7±0.2 | 7.2±0.4 |
| | 1-7 years | 152 | -0.3±0.3* | 4.1±0.6 |

* Adult goats showing negative growth rate.

**Table 2.** MAP culture from feces: Serially sampled cohort.

| Goats sampled | Positives | |
|---|---|---|
| | Pre vaccination | 6 months post vaccination |
| 87 | 28 (32%) | 22* (25%) |

*26.8% reduction in shedding of MAP bacilli in fecal samples.

weight at first kidding (WFK), kidding interval (KI), kidding rate, litter size etc.] were compared before (2005-2006) and after (2006-2007 and 2007-2008) vaccination.

#### Population growth rate and feed and fodder consumption

Population growth rate was also recorded for pre and post vaccination periods. To overcome biasness in quantities of ration consumed by the same herd, total feed and fodder consumption at the farm before and after vaccination was compared.

#### Feed lot experiment

Performance of male kids (body weights gained) under feedlot experiments at 3, 4 and 5 months of age were evaluated before (2005 to 2006) and after (2006 to 2007) vaccination.

#### Body weight profile of kids born to vaccinated goats

Data on body weights of kids born to vaccinated goats were monitored and weights at birth, 3, 6, 9 and 12 months of age were recorded and subjected to least squares analysis.

## RESULTS

### Johne's disease in Jamunapari goat farm

Comparison of least square means for 90 days, 140 days**.**Goats in all age groups exhibited marked improvement in physical condition and clinical symptoms of JD, within 30 days of vaccination, Goats became active, alert, snow white in colour, regained hair loss and body condition. None of the goats showed diarrhea, growth rate increased and goats started recovering from wasting. There was all round improvement (Figure 5b and c) in all of the production parameters (milk yield, body weights, etc.) without any changes in nutrition regimen and management.

### Growth rates and body weights

Comparison of pre and post-vaccination body weights showed most significant improvements in the body weight profile of all the goats in different age groups (Table 1). Average monthly body weight of goats 3 months before vaccination was either steady or declining (Figure 2). However, after vaccination, this rate increased 4 to 5 times within short period of 4 months (Table 1). Body weights of all the goats in different physiological conditions recorded gradual increase in all age groups during experiment (September 2006 to August, 2007) after vaccination (Table 1 and Figure 2). Even very old goats (>5 years) affected with Johne's disease recorded improvements in body weights.

### Shedding of MAP

Screening of fecal samples at 0 day, 6 and 12 months post vaccination showed that there was marked reduction in number of goats shedding MAP bacilli in feces. By culture method, it was observed that 32.1% goats were shedding MAP bacilli (most of the goats had multi-bacillary culture status) and after 6 months post vaccination, number of shedders decreased significantly to 26.8% (Table 2). Similarly, reduction of MAP shedders was also observed In microscopic examination. There was transition of low shedders to non shedders and heavy shedders to low shedders (Table 3).

**Table 3.** Ziehl–Neelsen positive: Serially sampled cohort.

| Goats sampled | Positives | | | |
|---|---|---|---|---|
| | Pre vaccination | | 12 Month post vaccination | |
| | Level of shedder | No. of goats | Level of shedder | No. of goats |
| n= 38 | - | 5 | - | 11 |
| | ++ | 18 | ++ | 21 |
| | +++ | 8 | +++ | 4 |
| | ++++ | 7 | ++++ | 2 |

**Table 4.** Causes of deaths (mortality).

| Year | Causes of deaths (%) | |
|---|---|---|
| | Johne's disease | Others |
| Oct., 2005 - Sept., 2006 (Pre-vaccination) | 28.6(22 / 77) | 71.4 (55 / 77) |
| Oct., 2006 - Sept., 2007 (Post-vaccination) | 4.0(2 / 50) | 96.0 (48 / 50) |

Vaccination done on 20 September, 2006 (number of goats that died due to JD / total deaths).

**Table 5.** Growth performance (Average monthly body weights in kg) of kids under intensive feeding regimen (feedlot).

| Year | Age of kids (n=12) | | |
|---|---|---|---|
| | 3rd Month | 4th Month | 5th Month |
| Kids born in Mar. 2006 (Before vaccination) | 10.8±0.39 | 12.6±0.52 | 13.1±1.15 |
| Kids born in Mar. 2007 (After vaccinated) | 12.85± 0.46 | 14.38±0.37 | 16.15±0.57 |

* n- Number of kids / observations.

**Morbidity and mortality rates**

Diarrhea and weakness, the main symptoms of JD in adult goats, affected large number of goats over the years before vaccination. Morbidity in terms of weakness and diarrhea in preceding year of vaccination was very high in comparison to post vaccination year. Morbidity due to other diseases also reduced just after vaccination, though it increased again after five months post vaccination but in reduced manner.

Comparison of pre-vaccinated (2006) and post-vaccinated (2007) mortality showed that average mortality and weakness due to JD in 1 year (2006) was 28.6%, which indicated high level of MAP infection in herds. There was no mortality due to JD for 3 months (October to December, 2006) immediately after vaccination (Table 4). Mortality due to JD was significantly reduced (4.0%) in the next 10 months (January to October, 2007), after vaccination. There was significant reduction in overall mortality in the herd including all other causes of mortality.

**Sero-conversion / humoral immune response**

Humoral immune response in vaccinated goats was measured by indigenous ELISA at monthly interval for 1 year after vaccination. Sero-conversion rates were higher in each of the next screening of serum samples up to 5 months post vaccination (MPV) and after that the humoral immune titer was maintained by the vaccinated goats (Figure 5) for the next 10 months.

**Improvements in physical traits of Jamunapari herd (feed lot experiments)**

Performance of male kids (body weights gained) under feedlot experiments at 3, 4 and 5 months of age before and after vaccination showed that vaccinated male kids attained higher body weights in 2006 to 2007 at 3, 4, and 5 months of age as compared to body weights gained by un-vaccinated male kids in the preceding year (2005-06) of feed lot experiment (Table 5).

***Reproduction performance of vaccinated goats***

There was reduction of 28 days in 'age at first kidding' in the primiparous goats after vaccinated in 2006-2007 as compared to before vaccination in 2005-2006 (Table 6). Similarly, improvements were also recorded in body weights of goats at first kidding. Kidding interval was reduced and kidding rate increased in vaccinated goats

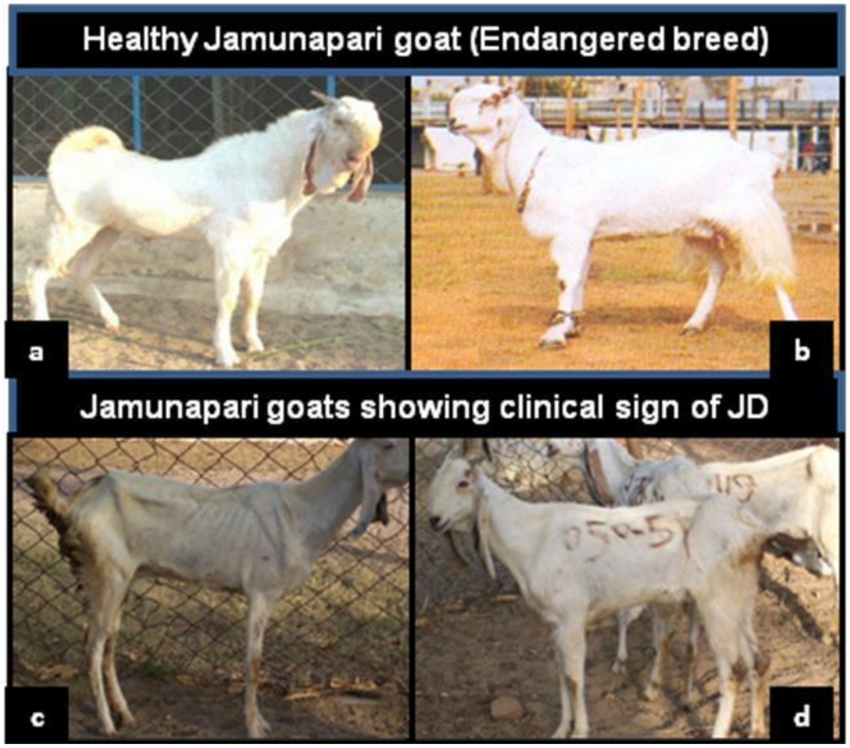



**Figure 4.** Healthy (a and b) and clinical Johne's disease affected goats (c and d) of Jamunapari breed.

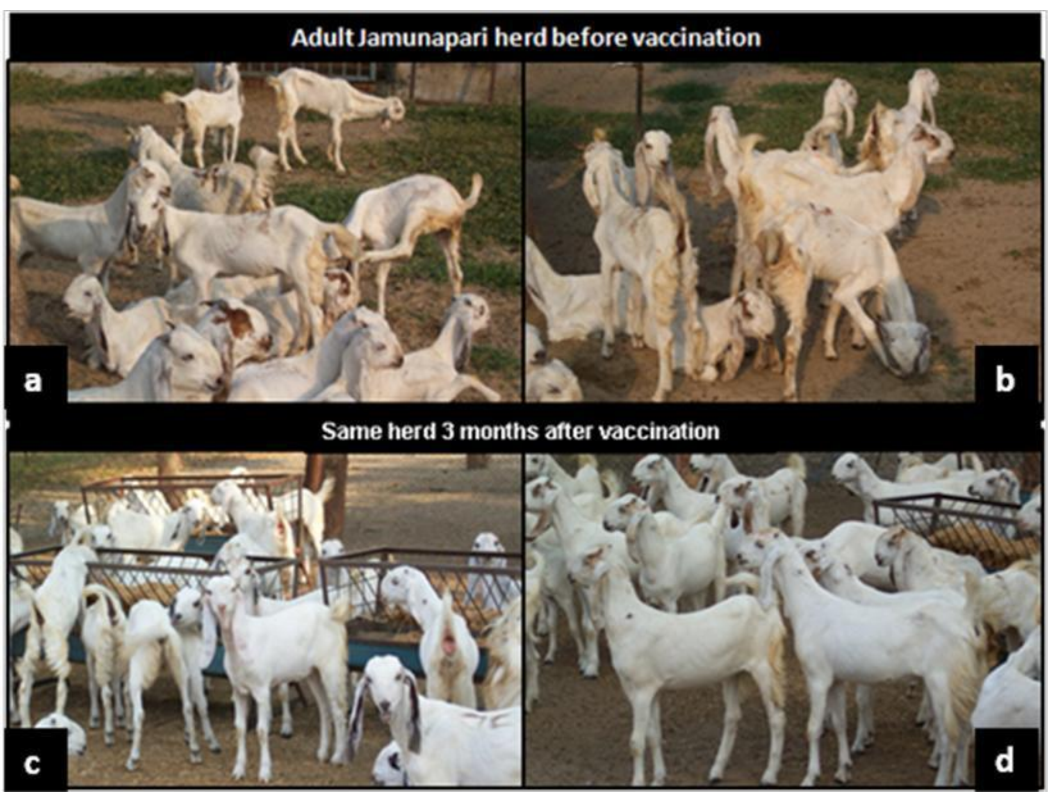



**Figure 5.** Physical condition of goats before (a and b) and after (c and d) vaccination with 'Indigenous Vaccine'.

**Table 6.** Reproductive performance.

| Reproductive trait | Pre-vaccination 2005 –2006 (n) | Post-vaccination 2006 – 2007 (n) |
|---|---|---|
| AFK (days) | 787 ± 24 (58) | 740 ± 24 (55) |
| WFK (kg) | 37.8 ± 0.35 (55) | 34.2 ± 0.32 (58) |
| KI (days) | 324 ± 11 (127) | 309 ± 13 (96) |
| Kidding rate (%) | 126 | 137 |
| Litter size(%) | 138 | 142 |

AFK: Age at first kidding; WFK: Weight at first kidding; KI: Kidding Interval; n: Number of goat / observations.

**Table 7.** Milk production* performance (Least squares means).

| Period | Milk yield (Liters) | | | | Lactation length |
|---|---|---|---|---|---|
| | 90 day | 140 day | Total | Milk yield/ lactation length | (day) |
| 2005-2006 (Pre-vaccination) | 87.5±1.8 (170) | 132.1±3.9 (163) | 141.8±4.1 (171) | 0.797±0.015 (171) | 201.5±3.3 (171) |
| 2006-2007 (Post-vaccination) | 107.6±2.7 (90) | 150.1±5.5 (87) | 172.6±5.8 (94) | 0.932±0.02 (94) | 180.3±4.7 (94) |
| Percent improvement | 22.3 | 13.6 | 21.8 | 16.9 | 10.5 |

Figures in parentheses are number of observations / goats (n); * Production trait.

**Table 8.** Population growth.

| Period | Adult goats (A*) | Kids born (B*) | Total goats (A+B) = (C*) | Kids died (Birth-12 Month) (D*) | Population growth (%) (B–D)×100 / (A*) |
|---|---|---|---|---|---|
| 2005–06 (Pre-vaccination) | 237 | 281 | 518 | 33 | 104.64 |
| 2006-07 (Post-vaccination) | 223 | 301 | 524 | 54 | 110.76 |

* Number of goats

(2006-07) as compared to un-vaccinated goats in 2005-2006 (Table 6). Kidding percentages on the basis of breedable does available and does tupped were 110.6 and 136.8%, respectively. Percent litter size was also high in post-vaccination year.

## Improvements in milk yield

Comparison of least square means for 90, 140 days and total milk yield, during two years after vaccination showed significant increase by 22.3, 13.6 and 21.8%, respectively (Table 7). Marked improvement of 10.5 and 16.9%, was also observed in lactation length and milk yield per day lactation length, respectively in 2006 to 2007 when compared with 2005 to 2006 (Table 7).

## Population growth rate

Population growth rate also recorded increase from 104.6 to 110.7% in Jamunapari herd after vaccination (Table 8).

## Feed and fodder consumption

Consumption pattern of feed fodder in pre and post-vaccination year showed saving of the ration with improvement in overall productivity of Jamunapari herd located at CIRG, Mathura (Table 9). True economic gains were recorded by vaccination of the endemically infected goats against Johne’s disease.

## Comparison of body weight of kids (born from vaccinated goats) with body weights in same season of preceding year

Marked improvement in birth and 3 months weight was recorded in kids born to vaccinated goats in comparison to preceding years. After 3 months of age, new kids were vaccinated and these vaccinated kids continued their improvement in body weights up to six months of age (Table 10).

**Table 9.** Feed and fodder consumption rate.

| Type of feed | Pre-vaccinaton 2005 to 2006 quantity (kg) | Post-vaccination 2006 to 2007 quantity (kg) |
|---|---|---|
| Total concentrates | 123.45 | 78.06 |
| Adult feed | 62.39 | 48.86 |
| Kid pellet | 0 | 17.66 |
| Mesh feed | 29.06 | 11.54 |
| Barley grain | 32.0 | 0 |
| Bhoosa | 1091.6 | 871 |
| Green fodder | 836 | 364 |

**Table 10.** Change in 'age-wise' body weights (least squares means).

| | Body weights (kg) | | | | |
|---|---|---|---|---|---|
| Year of birth | At birth | 3 months | 6 months | 9 months | 12 months |
| 2005-2006* | 3.12±0.03 (300) | 11.07±0.12 (291) | 14.29±0.19 (284) | 19.84±0.26 (244) | 25.31±0.29 (209) |
| 2006-2007* | 3.09±0.03 (283) | 10.89±0.12 (266) | 14.56±0.33 (240) | 21.76±0.26 (201) | 27.86±0.36 (120) |
| 2007-2008** | 3.21±0.04 (133) | 11.39±0.17 (117) | 15.31±0.30 (92) | NA | NA |

* Pre-vaccination; ** post-vaccination; NA, not available.

## DISCUSSION

The concept of therapeutic vaccination is not new as it has been used to treat an ongoing infection (in humans), emerged in the mid-1800s as a therapy for syphilis (Sherwood, 1999). In the early 1900s, the BCG vaccine was used to treat an established infection around the mid-1900s (Plotkin, 1999). It has been a clinical practice since the 1950s to treat asthma and allergies by a therapeutic vaccination (Bergquist, 1955). Beneficial effect of immunotherapy using mycobacterial antigen is also documented (Katoch, 1996). Result of therapeutic vaccine is not limited to humans only because similar therapeutic effect was observed in case of JD also. It is being advocated from many years back that vaccine should be used as part of a control programme as it confer good protection against disease caused by *M. paratuberculosis* and also usually good control of clinical disease, but sub-clinical infection may persist in vaccinated herds, albeit at a reduced level. It has been reported that JD vaccination in infected flock reduce mortality and faecal shedding of the organism in cattle, sheep, deer and goats (Sigurdsson, 1960; Doyle, 1964; Wilesmith, 1982; Saxgaard and Fodstad, 1985; Kormendy, 1994; Fawcett et al., 1995; Gwozdz et al., 2000) but most reports are incomplete and it is frequently difficult to determine if the beneficial effects are due to vaccination or to changes of management. Therefore, in present comprehensive study, effect of changes in management system was avoided and goat herd naturally infected with JD were vaccinated in a farm herd located at CIRG, where similar system of management and feeding schedule was practiced for last 3 decades.

Major concern with Jamunapari breed was conservation in the home tract (Chakarnagar block of Etawah district). The number goats available in this block has significantly reduced in last 10-15 years mainly due to change in farmer's preferences (shifting from animal husbandry to agricultural crops (more stability in returns and less risk due to diseases) and disliking for goat husbandry by younger generation. For the conservation of this precious breed, two herds of Jamunapari goats were established, one each at Central Institute for Research on Goats, Makhdoom and Animal Husbandry Department, Etawah). In Etawah farm also this breed has problems in adaptability due to shortage of browsing material and endemicity of the Johne's disease. At CIRG also, since the establishment of the Jamunapari herd in 1976, goats were under stress due to deficient browsing material in grazing area (breed is essentially a browser) and endemicity of Johne's disease. Since establishment of the farm goats have been routinely screened at six monthly intervals and as and when a goat was suspected against JD. Goats positive in microscopy or culture were culled as regular practice. .Despite culling of large number of goats, JD could not be controlled. Instead prevalence of disease continued to increase in intensity and number of infected goats, since MAP was transmitted through milk, colostrum, in-utero, semen, etc. Therefore, after the success of initial trial of the 'Indigenous vaccine', on infected goats (Singh et al., 2010), present full scale trial was conducted to evaluate 'therapeutic potential' of the vaccine in the Jamunapari goatherd endemic for JD and located at CIRG, Makhdoom. In India due to ban on 'cow slaughter' it is not possible to control Johne's disease by culling of infected animals, therefore, control of JD is possible only by treating the infected animals by vaccination (preventive or therapeutic).

Vaccination of Jamunapari herd against JD dramatically reversed the disease process and reduced the severity of infection as determined by number of health and production parameters monitored in this study. Similarly, Gwodz et al. (2000) in a vaccination trial of lambs already infected experimentally with MAP, observed reduction of mycobacterial burden with high magnitude of systemic cell mediated immune response that confers the animal ability to control infection. However, these observations are in contrast to a previous study where vaccination of sheep already infected with MAP did not modify infection (Gilmour et al., 1965). There were several differences with Gilmour studies e.g., vaccine strain type, infection in animals was experimentally induced by bovine and ovine isolates etc. While in the present study naturally infected goats were selected and Bison type genotype of MAP was dominant strain. Further, the study by Gilmour et al. (1965) was not a comprehensive study in view of little emphasis on production parameters and due to low occurrence of intestinal infection in the infected but un-vaccinated group. The transmission of infection reported by them was not particularly successful and the results were inconclusive.

Similar to Gwozdz et al. (2000) findings, 'indigenous Johne's disease vaccine' had strong and quick therapeutic response on the basis of improvements shown in different production parameters (lowered shedding of MAP, high immune response, low morbidity and mortality), Since most of the parameters were monitored up to the period of one year after vaccination and when vaccinated most of the goats in the herd were in different stages of development of disease therefore, how long (after one year post vaccination) this therapeutic effect would be sustained in herd and in which stage of the infection, vaccine performed better remains to be further investigated.

Progressive weight loss, the main feature of this disease was also observed in majority of goats in the present study before vaccination. Significant improvements were recorded in monthly average gain in body weights of goats of all the age groups. Improvement in growth rate was not limited in goats in growing age but also observed in goats aged between 4 to 7 years. Females (Hines et al., 2007) and Jamunapari breed (Singh et al., 1990) were more susceptible to MAP and was also reflected in the present study. However remarkable improvement in body weight gain was more visible in female goats. This observation is in Contradiction with the field study of a killed *M. a. paratuberculosis* vaccine, Gudair TM, for the control of ovine Johne's disease (OJD) in infected merino sheep flock in Australia (from 1999 to 2004) in which live weight of controls sheep was more in comparison to vaccinated ones (Reddacliff et al., 2006).

Though reduction in number of MAP shedders was not statistically significant after 6 months of vaccination however it got reduced by 26.8%. Colony count at 0 and 12 months post vaccination showed significant reduction in fecal shedding after vaccination. Most of multi-bacillary shedder turned in to pauci-bacillary shedder. Similar reduction was also observed in number of shedders by microscopic examination of serially sampled cohort. Comparable and similar results were reported by Hines et al. (2007) on vaccination of spheroplastic and cell wall component MAP vaccine in experimentally challenged baby goats. Decrease in shedding rates of MAP in herds that had been vaccinated with live and killed vaccine was also observed by Jorgensen (1984) and Kalis et al. (2001), respectively. Recently, Keeble and Walker (2009) developed HSP70 based therapeutic recombinant vaccine and reported that when this vaccine was administrated in conjunction with adjuvant, resulted in significant reduction in bacterial shedding in cattle infected with MAP along with advantage of no mask with diagnostic assay. However, in contrast few studies (Koets et al., 2001 and 2006; Wentink et al., 1994) did not get evidence of the relationship of JD vaccination and reduced fecal shedding.after vaccination. Most of multi-bacillary shedder turned in to pauci-bacillary shedder. Similar reduction was also observed in number of shedders by microscopic examination of serially sampled cohort. Comparable and similar results were recorded by Hines et al. (2007) on vaccination of spheroplastic and cell wall component MAP vaccine in experimentally challenged baby goats. Decease in shedding rates of MAP in herds that had been vaccinated with live and killed vaccine was also observed by Jorgensen (1984) and Kalis et al. (2001), respectively. Recently, Keeble and Walker (2009) has developed HSP70 based therapeutic recombinant vaccine and reported that when this vaccine is administrated in conjunction with adjuvant, resulted in to significant reduction in bacterial shedding in cattle infected with MAP along with advantage of no mask with diagnosis assay. However, in contrast few studies (Koets et al., 2000, 2006; Wentink et al., 1994) did not get evidence of the relationship of JD vaccination and reduced fecal shedding.

Indigenous vaccine reduced the overall annual herd mortality as well morbidity after vaccination. Significant reduction in number of animals died due to JD (on the basis of post mortem lesions) was recorded in post vaccination year in comparison to pre vaccination year. In the present study, protective value of indigenous vaccine is in accordance with our earlier controlled challenge based vaccine trial (Singh et al., 2007b). Interestingly, just few days after vaccination, there was drastically reduction in morbidity due to diarrhea, weakness and some other diseases. That may be due to boosted herd and individual immunity and cross protection to many of other diseases. Considering the importance of vaccination in view of reduction in mortality, morbidity and associated economic importance in sheep and goats, killed vaccine is being tried for use in massively infected sheep flocks suffering with high mortality (Kennedy and Benedictus, 2001) in Spain, New Zealand and in an endemic region in Australia. In Norway, vaccination of kids at the age of 2 to 4 weeks with an attenuated live vaccine has improved the paratuberculosis control in goats, which was failed after several years of unsuccessful efforts by husbandry measures and the isolation and slaughter of clinically affected animals. The prevalence of infection was reduced from 53 to 1%, based on post-mortem examination (Saxegaard and Fodstad, 1985). Similarly, vaccination reduced mortality due to paratuberculosis by 93% (Sigurdsson, 1960).

Cranwell (1993) used vaccination and management changes to control paratuberculosis in a flock with a 9% premature culling rate due to clinical infection and observed substantial reduction in clinical cases after three years. However on the contrary, mortality rate was not affected in a long Australian JD control program using a commercial vaccine. Windsor (2006) despiteobservations of a decline from a high to a moderate prevalence of shedders following vaccinating of the 2-year-old animals, mortalities due to OJD and shedding of *M. avium* ssp. *paratuberculosis* did continue, particularly in wethers.

Protective immunity against mycobacterial diseases, especially paratuberculosis in ruminants, is poorly understood. It is assumed that vaccination stimulates a protective CMI response and strong CMI response to *M. paratuberculosis* infections is associated with the formation of granulomatous tuberculoid lesions and containment of the disease by the host whereas a weak CMI response is associated with diffuse lepromatous lesions and disease progression. Though in present study, CMI response has not been monitored however, in our earlier controlled goat challenge trial with same indigenous vaccine, it was evident that strong and long lasting CMI was induced by this vaccine in goats (Singh et al., 2007b). It was observed that vaccinated animals had higher serum antibody responses than the naturally infected animals (Gwozdz et al., 2000; Juste et al., 1994) also the rate of sero-conversion was enhanced after vaccination (Singh et al., 2007b). Similarly, in the present study sero-conversion rate in naturally infected goats was significantly increased and after 5 months of vaccination, most of the sampled goats had substantially higher levels (peak titer) of anti-MAP antibodies (Figure 3). Feed lot experiment is regularly practiced at CIRG to select high performing goats (on the basis of live weight gain at different time intervals) under intensive feeding to be used for breeding as bucks and does (females). Vaccinated kids undergone in feed lot experiment gained substantial higher body weights after vaccinated in comparison to non-vaccinated kids in previous year. Similarly overall reproduction performance (age at first kidding, kidding Interval and kidding rate etc.) of the female goats also improved after vaccination than non-vaccinated goats in the previous year.

Johne’s disease associated depressive effect on milk yield is the critical factor in the dairy herds. Jamunapari breed of goats are well known for their high milk producing ability, however this important Indian goat breed is now under highly threatened category and needs conservation and JD is one of the main cause for this endangered status (Singh et al., 1990 and 2009). Milk yield of the Jamunapari herd (CIRG, Makhdoom) has decreased gradually over the years and simultaneously there is gradual increase in the incidence of JD in this herd. However, in comparison to pre-vaccination years there was quick and substantial increase in the 90 and 120 days and total milk yield (milk trait) after vaccination. To the best of our knowledge comparable information with respect to increase in milk yield after vaccination of goatherd is not available in the literature so far. In continuation of the improvement in productivity and performance of goats, overall consumption of the feed and fodder by the same herd was very less despite increase in population growth rate in post vaccination year. Milk yield is an important criterion in selection of elite females and males for breeding purpose and for ranking of the sires on the basis of their dam’s 90 days and some times 140 days milk yield (Acharya and Bhattacharyya, 1992). Therefore, with improvement in health and reproductive performance more number of sires of this important breed will be available for selection in coming years, which will facilitate conservation of the breed by distributing surplus sires for breeding in the farmer’s herds.

Vaccination of successive annual crops of kids is likely to have an impact on the level of environmental contamination with *M. avium* ssp. *paratuberculosis,* increase in herd immunity and reduction of horizontal and vertical transmission of MAP. Average gain in body weights (at 3 and 6 M) of new crops of vaccinated kids (2007 and 2008) was higher in comparison to that of kids born to non-vaccinated goats (2006 to 2007). Also in regular herd screening of new crops of kids, very few kids were positive in microscopic examination. After vaccination, kids also responded with high level of anti-MAP antibodies at 60 days after vaccination. All the aforementioned encouraging results of vaccinated goats and their kids strengthened the hopes for potential recovery and cure (conservation) of large number of Jamunapari goats suffering from incurable JD (clinical to advance clinical) and eradication of JD by a long and effective vaccination program using ‘indigenous vaccine’ developed from ‘S 5’ strain of MAP.

## Conclusions

Results of present study confirmed the 'Therapeutic potential' of 'indigenous vaccine' against Johne's disease in Jamunapari goatherd endemic for MAP infection and was valuable and effective for treating goats incubating disease (sub-clinical, clinical and advance clinical). Vaccination with 'Indigenous vaccine' substantially reduced the incidence of JD, transmission of MAP, mortality, fecal shedding and reversed the trend of production losses. Therefore, 'Indigenous vaccine' can be used to save the goats of this highly threatened Jamunapari breed. Since JD is endemic in domestic livestock in India, 'Indigenous vaccine' can help to salvage high productive animals from slaughter and also to control JD at National level, especially in cows which cannot be slaughtered in the country due to ban on 'cow slaughter' for religious reasons.

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