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# Research Article
# 'Therapeutic Management' of Incurable Paratuberculosis Using 'Indigenous Vaccine' in Goatherds, Endemically Infected with Johne's Disease

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## Abstract

**Background:** Johne's disease is chronic incurable enteritis mainly responsible for reduced productivity in domestic livestock leading to extensive economic losses to the dairy industry world-wide. Therapeutic efficacy of 'First indigenous vaccine' developed using novel 'Indian bison type' biotype of *Mycobacterium avium* subspecies *paratuberculosis* (MAP) strain 'S 5' was evaluated for the treatment of clinical Johne's disease in the farm herds of Jakhrana breed of goats. Farm herds of this important milch breed of semi-arid region of Rajasthan were endemically infected with Johne's disease. **Materials and Methods:** Response to 'Indigenous vaccine' against Johne's disease has been studied twice in this farm unit, first time in 2006-07 (Vaccine trail-I) and second time in the present study from 2013-14 (Vaccine trial-II). Data on improvement in health, clinical condition, productivity, reproductive performance, milk yield, survivability, morbidity, mortality, culling and shedding of MAP in feces were recorded before and after vaccination. In vaccine trial-II, 225 adult goats and 70 and 39 kids (above 3 months age) born to un-vaccinated and vaccinated goats were vaccinated one time between 2013 and 2014, respectively. **Results:** Reduction in shedding of MAP in this vaccine trial-II of infected goats and 1st generation kids was 45.5 and 100.0%, respectively. Presence of MAP in the blood of vaccinated goats was reduced by 23.0% at 360 DPV. Peak titers were achieved around 90 DPV and all vaccinated goats sero-converted by 360 DPV. High to very high morbidity, mortality and cullings encountered before vaccination in the infected Jakhrana goatherds were mainly due to Johne's disease and were highly reduced after vaccination. Vaccination not only reduced clinical disease but also improved production performance (milk and meat production). Average gain in body weights were distinctly superior in the vaccinated goats and in the 1st generation kids born to vaccinated mothers. After vaccination there was overall improvement in the health of animals, kid survival rate, per animal productivity with respect to milk production and growth rates. **Conclusion:** Study concluded that 'Indigenous JD vaccine' developed using native MAP biotype can be employed both for the 'Therapeutic management' of the disease in the endemically infected goatherds and for the prevention of disease in naive and non-infected goats. The study can serve as model for the utilization of large population of non-productive domestic livestock and for the management and control of incurable Johne's disease in endemically infected herds and flocks in the country.

Key words: Johne's disease, indigenous vaccine, Indian bison type, therapeutic efficacy, sero-conversion, endemic

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Data Availability: All relevant data are within the paper and its supporting information files.

## INTRODUCTION

*Mycobacterium avium* subspecies *paratuberculosis* (MAP), the etiological agent of incurable Johne's Disease (JD) is mainly responsible for heavy economic losses due to reduced production of milk and meat. Country is slowly losing best germplasm of native breeds of domestic livestock being endemically infected with JD[1]. Though India is the highest milk producer in the world but low per animal productivity of the majority of 12.0 million goat heads is the major concern of Indian livestock industry[2]. The JD being production disease is mainly responsible for low per animal productivity and clinically infected domestic livestock suffer from gradual loss in body weights with or without diarrhea (intermittent or continuous), progressive wasting and death, besides other losses (lower fertility, higher morbidity and mortality, increased culling etc.). Live MAP bacilli have been cultured from pasteurized milk and milk products[3] and from soil and water resources[4] of the country. The MAP has also been associated with Crohn's disease in human beings[5,6]. In the absence of national control policy, bio-incidence of MAP is not only high but increasing at fast rate in the domestic livestock both in farm and farmer's animals[1]. Due to low per animal productivity and zero salvage value of cows (ban on cow slaughter), there is major shift towards agri-farming from the animal husbandry, wherein, goat or sheep are now preferred over cows.

Vaccination against JD has shown promise in the control of MAP infection[7]. 'Indigenous vaccine' developed using native 'Indian bison type' strain ('S 5') of goat origin (a new biotype)[8] was both 'Therapeutic' and preventive in goats, sheep and cattle[9-11]. Present study evaluated 'Therapeutic potential' of 'Indigenous vaccine' for the treatment and control of caprine Johne's disease in the farm herds of high milk producing Jakhrana breed, wherein goats have been suffering with sub-clinical, clinical and advanced clinical JD, in the absence of control measures.

## MATERIALS AND METHODS

**History:** Of the 23 recognized goat breeds in the country, Jakhrana is one of the important large size breed, black in colour with ears white and is highly valued for milk production. Breed belongs to Jakhrana village in the neighbouring Bharatpur district of Rajasthan state and is under threatened category since number of pure animals is below 5000 in the native tract. In order to evaluate, conserve, improve and multiply the breed genetically, a farm herd of Jakhrana goats was established at Central Institute for Research on Goats (CIRG), Mathura, (adjacent to Bharatpur district) in 2005, by purchasing animals from the home tract. Breed has good adaptability to semi-arid region and potential to improve milk production of non-descript goats.

**Vaccination history:** Farm herds of Jakhrana breed of goats at CIRG had frequent cases of weakness and diarrhea, which were confirmed as suffering with Johne's disease and were first time vaccinated against Johne's disease in May, 2007 (vaccine trial-I) using 'Indigenous vaccine' developed under the Department of Science and Industrial Research (DSIR) project for the development of 'Indigenous vaccine'. Of the 42 goats screened before vaccination 38.0% were positive for MAP infection, by microscopy. After vaccination, there was marked improvement in the body condition and health (weakness and diarrhea) of goats and was apparent from the distance. Body coat regained luster, shining, pliability, regeneration of hairs and improvements in survivability of the new born kids (100.0%). There was reduction in mortality from 24.6 to >5.0% in subsequent years after first 'One time' vaccination of the herds. After the first vaccination trial-I on 24-5-2007, there was break in further vaccination in trial-I and the effect of vaccine lasted up to 2011. Again there was increase in the health problems in the farm after 2011, which peaked in 2012-13. Second time in the vaccine trial-II, a total of 225 goats above 3 months of age were vaccinated on 3-9-2013. The vaccine trial-II again showed improvement in health and significant reduction in mortality and morbidity at the farm and improvement in production.

**Animals:** In the present vaccine trial II of the Indigenous JD vaccine in the farm herds of Jakhrana goats at CIRG, Mathura, all the 116 adult (>12 months) goats (87 females and 29 males) and 109 kids (3-12 months) were included. Of these 109 kids available in trial II, 70 were vaccinated on 28-3-2014 (naive) and 39 kids (1st generation born to vaccinated goats) were vaccinated on 29-8-2014. Physical condition and body score of goats before vaccination was poor (weak, debilitated and emaciated) and 50-75% goats suffered either sub-clinical or clinical or advance clinical JD. Each of goats had ear tags for further monitoring. Semi-intensive management of the herds at the farm was optimum in terms of nutrition, housing and hygiene.

**Vaccine:** 'Indigenous JD vaccine' used in this study was first developed in 2005 using native 'S 5' strain of 'Indian bison type' (a new biotype of MAP not reported outside India)[8,12].

This novel strain was isolated from Jamunapari breed of goat at CIRG, Makhdoom in 1998. This goat was suffering with advance clinical disease and later died of the disease (JD). 'Indigenous JD vaccine' has been evaluated for the 'Therapeutic potential' in naturally infected spontaneous cases of JD in goats and sheep[10,13] and also as preventive vaccine (vaccination and challenge)[9]. One dose of vaccine contained 2.5 mg dry weight of 'S 5' MAP culture containing $5\times10^9$ bacilli $mL^{-1}$ (Heat inactivated at 72°C) in Gerbu adjuvant (Biotechnik, Germany). All goats above >3 months age were vaccinated with 1 mL (2.5 mg $goat^{-1}$) dose of 'Indigenous JD vaccine' subcutaneously in the middle of neck region behind ear on left side.

**Monitoring parameters:** Goats were monitored for improvement in physical condition, health and production status of the goats before and after vaccination. Improvements in health status were observed by way of mortality, morbidity and reproductive performance. Production parameters included growth rate, survivability of animals naturally infected with MAP and milk production in terms of lactation days and yield. Immunological parameters were monitored by ELISA titer and MAP bacteremia by blood PCR. Status of shedding of MAP in fecal samples by microscopy before and after vaccination was the most critical to evaluated vaccine response.

**Change in physical condition (weakness and diarrhea):** Improvements in physical traits in goats were monitored by appearance, alertness and changes in body coat condition (colour, roughness and shining) and status of diarrhea if any at monthly intervals after the fvaccination. Sick animals as and when encountered were treated symptomatically.

**Collection of data, samples and tests:** Fecal, blood and serum samples of representative goats were collected at the interval of 30 Days Post Vaccination (DPV) up to 360 days.

**Fecal microscopy:** Shedding of MAP in fecal samples was monitored by microscopy as per the method of Singh *et al.*[12]. Shedding was quantified on (0 to + 4 scale) and any goat that was found positive on +1 to +4 scale were taken positive for MAP infection.

**MAP bacteremia and genotyping**

**DNA isolation and IS900 PCR:** The DNA was isolated from the blood samples and subjected to specific IS900 PCR as per Singh *et al.*[14] using P90 and P91 primers[15]. Presence and yield of the specific PCR product (413 bp) was considered as positive for MAP infection.

**IS1311 PCR_REA:** The IS1311 PCR was carried out using M56 and M119 primers as per Singh *et al.*[14]. Restriction digestion (IS1311 PCR_REA) reaction was carried out using endonucleases *Hin*fI and *Mse*I as per Sevilla *et al.*[16] and genotype profiles were interpreted as per Whittington *et al.*[17].

**Indigenous ELISA:** Serum samples collected during the study were screened by 'Indigenous ELISA kit', developed for screening of goats and sheep against JD Infection. Indigenous ELISA kit uses semi-purified protoplasmic antigen (PPA) from the highly virulent native isolate (S 5) of MAP 'Indian bison type' biotype of goat origin. The test was originally developed and standardized for goats and sheep[18] and was later applied for bovines[11]. The OD values were transformed to S/P ratio and goats in positive and strong positive categories in S/P ratio were considered positive for MAP infection or sero-converts[19].

**Body weights:** Average gain in body weights of vaccinated goats at 30 days intervals after vaccination were statistically analyzed using unpaired 't-test' with Welch correction by GraphPad InStat 3.0 software.

## RESULTS

Goats in vaccination trial II were monitored on following parameters and results are described.

**Mortality rate:** Mortality rate which was very high (50.5%) in 2012-13 (before vaccination) was significantly reduced (5.0%) after vaccination in (2013-2014). There was total of 53.8% reduction in mortality in 360 DPV. Nine goats that died in 360 days post vaccination, were due to causes other than JD (Table 1, 2). Age-wise also there was reduction in mortality in each age group and there was 75.0, 75.0 and 20.0% reduction in mortality in 0-6, 6-12 and >12 M age groups, respectively.

**Morbidity and culling:** No significant health problem or illness was noticed in the herd after vaccination trial II. At 180 DPV there was significant reduction in clinical cases of diarrhea, enteritis and anorexia in all age group and this status was maintained upto 360 DPV. There was reduction of 15.1% in the cases of diarrhoea/enteritis at 360 days post vaccination

Table 1: Mortality profile and response to Johne's disease vaccine on the basis of reduction in losses due to death rates in the herd of Jakhrana goats (2005-2015)

| Experimental period in years | Mortality n (%) | Vaccination | | Herd health status (before and after vaccination) |
|---|---|---|---|---|
| | | Date and age | Vaccinated goats (n) | |
| 2005-06 | 8.5 (198)** | - | | Herd suffering from high mortality and morbidity (diarrhoea, weakness, coccidiosis and high culling) |
| December, 2006-May, 2007 | 12.7 (242) | 24-5-2007 (>3 M) | 228 | Increase in mortality due to diarrhoea, weakness, coccidiosis and gid |
| Jun-December, 2007 | 5.7 (270) | - | | Mortality reduced due to effect of vaccine and reduction in cases of diarrhoea and weakness |
| 2008-09 | 24.6 (279)# | - | | Mortality high due to outbreak of coccidiosis in young kids (1-2.6 M) |
| 2009-10 | 6.5 (258) | - | | Mortality continued to reduce due to complete reduction of cases of diarrhoea and weakness in young and adult goats |
| 2010-11 | 3.4 (270) | - | | Do |
| 2011-12 | 5.3 (275) | - | | Do |
| 2012-13 | 50.5 (212) | - | | Immune titers became zero and there is again spurt in cases of diarrhoea, weakness and cullings of goats |
| 2013-14 | 5.0 (255) | 3-9-2013 (>3 M) | 225 | Mortality reduced due to effect of vaccination, the effect was sharp since goats had been vaccinated against JD earlier and could recall residual memory |
| 2014-15 | 2.9 (276) | - | | Mortality continued to reduce due to peak in vaccine response and peak in milk production of goats |

n: Total No. of goats in the herd, **M: Age in months, >3 M is minimum age for vaccination, #Unusually high mortality was due to outbreak of coccidiosis in kids between 1-2.6 months of age, Peak milk production of individual goats never increased from 1.5 L day$^{-1}$ since establishment of farm unit in 2005, but in 2014-15 peak milk production/day reached 3.85 L day$^{-1}$ in 4-5 goats

Table 2: Mortality rates (Age-wise) and causes of deaths in the herd of Jakhrana goats before and after JD vaccination (2013-2014)

| | Mortality/death rates | | | | |
|---|---|---|---|---|---|
| Age groups | Causes | Before vaccination (January-September, 2013), n (%) | After vaccination (October, 2013-May, 2014), n (%) | Reduction in mortality rates (%) | Causes |
| 0-6 M | Pneumonia (9), predation (1), autolysis (1), toxeamia (1), enteritis (1) and acidosis (1) | 14 (87.5) | 2 (12.5) | 75.0 | Septicaemia (1) and peritonitis (1) |
| 6-12 M | Pneumonia (1), hemonchosis (1), tympany (1), anemia (1), enteritis (2), ND (1) | 7 (87.5) | 1 (12.5) | 75.0 | Gid (cerebral coenurosis) |
| >12 M | Pneumonia (1), pregnancy toxemia (1), hemorrhagic enteritis (4), toxemia (3) | 9 (60.0) | 6 (40.0) | 20.0 | Autolysed (4), pregnancy toxemia (1) and septicemia (1) |
| Total n (%) | 39 | 30 (76.9) | 9 (23.1) | 53.8 | |

Mortality or death rate, n: Number, M: Age in months, ND: Nothing-specific detected

Table 3: Morbidity rates (Age-wise) in herd of Jakhrana goats before JD vaccination (April-September, 2013)

| | Morbidity/sickness | | | |
|---|---|---|---|---|
| | Age groups (n) | | | |
| Causes | 0-6 M | 6-12 M | Adults (>12 M) | Total n (%) |
| Diarrhoea/enteritis | 72 | 27 | 256 | 355 (73.8) |
| Dull/off feed/anorexia | 32 | 8 | 9 | 49 (10.2) |
| Pneumonia | 3 | 3 | 1 | 7 (1.4) |
| General weakness | 0 | 0 | 1 | 1 (0.2) |
| Abdominal pain/colic | 1 | 3 | 2 | 6 (1.2) |
| Others** | 10 | 6 | 46 | 62 (12.8) |
| Total | 118 | 47 | 316 | 481 |

Morbidity: Sickness, n: Number, M: Age in months, **Other causes: Mange/dermatitis-1, conjunctivitis-1, mastitis-1, bottle jaw-1, tympany/bloat-2, Lameness/arthritis-5, fracture-21, wound/abscess-29

Table 4: Morbidity rates (Age-wise) in herd of Jakhrana goats after JD vaccination (October, 2013-March, 2014)

| | Morbidity/sickness | | | |
|---|---|---|---|---|
| | Age groups (n) | | | |
| Causes of morbidity | 0-6 M | 6-12 M | Adults (>12 M) | Total n (%) |
| Diarrhoea/enteritis# | 95 | 16 | 131 | 242 (69.9) |
| Dull/off feed/anorexia | 6 | 4 | 12 | 22 (6.3) |
| Pneumonia | 2 | 1 | 0 | 3 (0.9) |
| Others** | 3 | 1 | 43 | 49 (14.1) |
| Total | 108 | 22 | 216 | 346 |

Morbidity or sickness, n: Number, M: Age in months, **Other causes: Hernia-1, mange/dermatitis-1, abortion-2, mastitis/udder oedema-25, dystokia-1, tympany/bloat-4, lameness/arthritis-13, fracture-2, wound/abscess-30, #Symptoms strongly suspected for JD (endemic in Jakhrana goatherds, since establishment of farm)

(Table 3-5). Of the total 162 goats culled in 2 years (before and after vaccination) for various reasons, there was a marked reduction (n-19, 76.5%) in the culling (forced removal) after vaccination (Table 6, 7). Reduction in cases of weakness, emaciation and stunted growth (clinical symptoms strongly suspected due to JD) was 40.9%, before and after vaccination in 360 days time interval. In the goatherds, 6 months post vaccination, animals were treated for repeated parasitic and tick infestation using Ivermectin.

Table 5: Morbidity rates (Age-wise) in the herd of Jakhrana goats after JD vaccination (April, 2014-October, 2014)

| Morbidity/sickness | | | | |
|---|---|---|---|---|
| | Age groups (n) | | | |
| Causes of morbidity | 0-6 M | 6-12 M | Adults (>12 M) | Total |
| Diarrhoea/enteritis# | 42 | 13 | 89 | 144 (58.7) |
| Dull/off feed/anorexia | 9 | 5 | 29 | 43 (17.5) |
| Pneumonia | 1 | 0 | 0 | 1 (0.4) |
| Others** | 4 | 4 | 44 | 57 (23.2) |
| Total | 56 | 27 | 162 | 245 |

Morbidity rate or sickness, n: Number, M: Age in months, **Other causes: Hernia-0, mange/dermatitis-2, abortion-1, mastitis/udder oedema-6, dystokia-0, tympany/bloat-3, lameness/arthritis-15, fracture-0, wound/abscess-30, #Symptoms strongly suspected for JD (endemic in Jakhrana goatherds, since establishment of farm)

Table 6: Culling of goats (Age-wise) in the herd of Jakhrana goats before Johne's disease vaccination (October, 2012-September, 2013)

| | Age groups (n) | | | |
|---|---|---|---|---|
| Causes of culling | 0-6 M | 6-12 M | Adults (>12 M) | Total n (%) |
| Weak and emaciated# | 9 | 6 | 23 | 38 (26.5) |
| Stunted growth# | 24 | 9 | 10 | 43 (30.0) |
| Off color | 20 | 7 | 2 | 29 (20.2) |
| Unfit and suffering with deformities | 0 | 0 | 5 | 5 (3.5) |
| Unfit for breeding | 0 | 3 | 1 | 4 (2.8) |
| Old age | 0 | 0 | 24 | 24 (16.7) |
| Grand total | | | | 143 (88.2) |

M: Age in months, #Symptoms strongly suspected for JD (endemic in Jakhrana goatherds, since establishment of farm)

**Body weights:** Mean gain in of body weights over 1 year (2013) period was 11.0, 2.47 and 18.1%, in vaccinated adult males and females and naive kids, respectively (Fig. 1). Mean gained in body weights up to 90 DPV was 3.35±0.40 kg in 39 kids born to vaccinated goats (vaccinated on 3-9-2013) in March, 2014 (Table 8). The effect of vaccine was evident in kids born to vaccinated goats. The naive kids (vaccinated on 3-9-2013) which were stunted also started growing after the vaccination. However, there was decline in the body weights of few goats which may be due to stress of being in very advance stage of clinical disease. This observation was in agreement to the findings of Singh *et al.*[13], when they used same vaccine in sheep flocks.

Table 7: Culling of goats (Age-wise) in the herd of Jakhrana goats after Johne's disease vaccination (October, 2013-September, 2014)

| | Age groups (n) | | | |
|---|---|---|---|---|
| Causes of culling | 0-6 M | 6-12 M | Adults (>12 M) | Total n (%) |
| Weak and emaciated# | 1 | 0 | 2 | 3 (15.7) |
| Stunted growth# | 0 | 0 | 0 | 0 (0.0) |
| Off color | 2 | 1 | 0 | 3 (15.7) |
| Unfit and suffering with deformities | 3 | 1 | 5 | 9 (47.3) |
| Unfit for breeding | 0 | 0 | 2 | 2 (10.5) |
| Old age | 0 | 0 | 2 | 2 (10.5) |
| Grand total | | | | 19 (11.7) |

M: Age in months, #Symptoms strongly suspected for JD (endemic in Jakhrana goatherds, since establishment of farm)

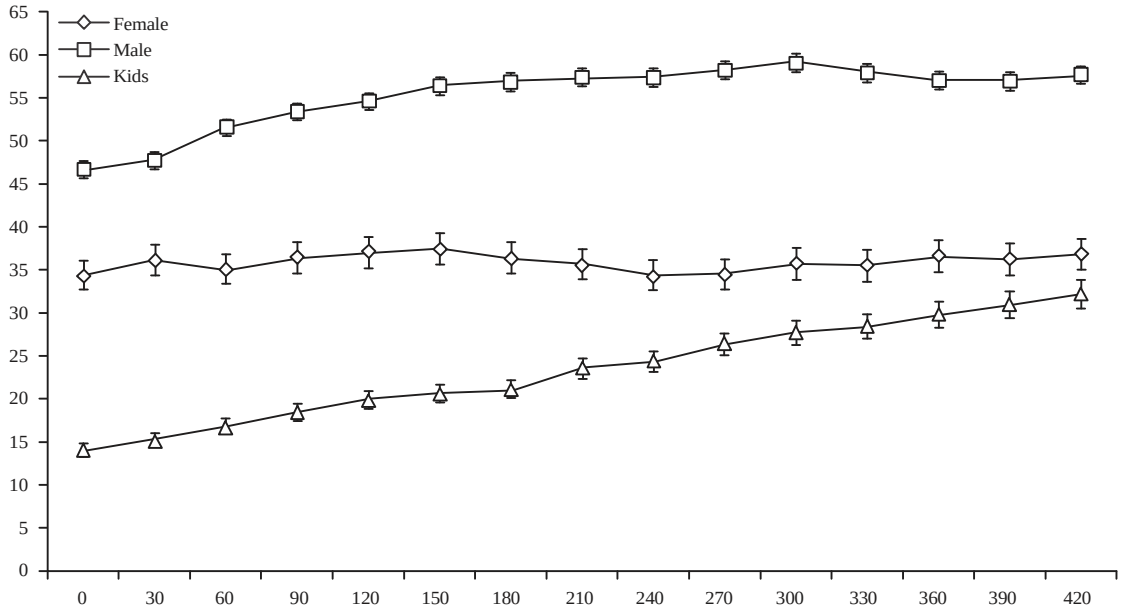


Fig. 1: Average gain in body weights (kg±SE) at monthly intervals in the Jakhrana goats (0-420 days post vaccination)


Fig. 2: Monitoring of physical profile of adult female (3) and male goats (2) from 0-420 Days Post Vaccination (DPV)

Table 8: Average gain in body weights of goat kids (1st generation) from 0-90 DPV after vaccination against Johne's disease

| | | Average gain in body weights (kg±SE) Days Post Vaccination (DPV) | | | | |
|---|---|---|---|---|---|---|
| Age group (0-90 days) | Animals (n) | 0 | 30 | 60 | 90 | 0-90 |
| Kids | 39 | 14.79±0.37 | 16.89±0.48 | 19.95±0.67 | 18.14±0.61 | 3.35±0.40 |

SE: Standard error, DPV: Days post vaccination

Table 9: Lactation profile (month-wise) of the Jakhrana goats before (February-September, 2013) and after (October, 2013-May, 2014) vaccination for the estimation of improvement in milk production due to 'Indigenous JD vaccine'

| Milk yield (kg±SE) | | | |
|---|---|---|---|
| Period | Before vaccination (n) | Period | After vaccination |
| February (2013) | 16.17±6.99 (N = 03) | October (2013) | 28.91±1.81 (N = 13) |
| March (2013) | 36.53±3.08 (N = 24) | November (2013) | 50.93±5.41 (N = 15) |
| April (2013) | 42.91±2.63 (N = 25) | December (2013) | 51.38±3.60 (N = 15) |
| May (2013) | 38.82±2.09 (N = 25) | January (2014) | 42.38±2.60 (N = 15) |
| June (2013) | 32.94±2.26 (N = 25) | February (2014) | 32.10±2.40 (N = 15) |
| July (2013) | 24.67±2.37 (N = 23) | March (2014) | 24.66±4.37 (N = 20) |
| August (2013) | 25.32±2.10 (N = 17) | April (2014) | 40.64±6.08 (N = 11) |
| September (2013) | 12.24±2.07 (N = 15) | May (2014) | 29.88±5.10 (N = 10) |
| Total | 28.7 goat$^{-1}$ month$^{-1}$ | Total | 37.61 goat$^{-1}$ month$^{-1}$ |

Gain of 8.91 kg of milk goat$^{-1}$ month$^{-1}$. N: No. of goats in milk during the period under report

**Milk yield:** Lactating goats showed increase in milk production and there was gain of 8.91 kg of milk per goat per month, after 180 days post vaccination, when compared with pre-vaccination status of these goats in previous lactation (Table 9, 10).

**General body conditions:** There was marked improvement in the body condition of vaccinated goats which was apparent from distance. Body coat regained luster, shining, pliability, regeneration of hairs and eyes were shining bright which could be easily differentiated from distance on the basis of improved body condition after 180 days post vaccination (Fig. 2). Cases of weakness and diarrhoea were reduced significantly in all age groups of vaccinated goats. Most of the vaccinated goats developed 'Take' at the vaccination site.

Table 10: Lactation profile of Jakhrana goats at 180 days post vaccination

| Periods | Milk yield (kg±SE) (After vaccination*) |
|---|---|
| September (2014) | 56.32±2.94 (N = 16) |
| October (2014) | 52.58±2.28 (N = 27) |
| November (2014) | 53.11±2.23 (N = 27) |
| Total | 54.0 goat$^{-1}$ month$^{-1}$ |

*New vaccinated milking goats, N: No. of goats in milk during the period under report, NB: There was gain of 23.3 kg of milk goat$^{-1}$ month$^{-1}$ as compared to same period in previous years

Table 11: Profile of shedding of MAP in feces as marker for status of disease intensity using microscopy

| | | Intensity of MAP shedding at different days post vaccination | | | | |
|---|---|---|---|---|---|---|
| Goat ID | Sex | 0 | 90 | 180 | 240 | 360 |
| 440 | Female | 1+ | 1+ | Neg | Neg | Neg |
| 824 | Female | 2+ | 2+ | 1+ | Neg | 1+ |
| 769 | Female | 1+ | 2+ | 1+ | Neg | Neg |
| 588 | Male | 4+ | 2+ | 2+ | 1+ | 1+ |
| 598 | Male | 2+ | ND | 1+ | Neg | Neg |
| 276 | Male | Neg | Neg | Neg | Neg | Neg |
| 659 | Male | 3+ | 2+ | 1+ | 1+ | 1+ |
| 975 | Kid | 3+ | 1+ | Neg | Neg | Neg |
| 1022 | Kid | Neg | Neg | Neg | Neg | Neg |
| 1024 | Kid | Neg | ND | 1+ | Neg | Neg |
| 1026 | Kid | 2+ | 1+ | Neg | 1+ | Neg |
| Total goats (11), n (%) | | 8 (72.7) | 7 (77.7) | 6 (54.5) | 3 (27.2) | 3 (27.2) |

Reduction in shedding intensity of MAP in representative goats from the herd was 45.5% from 0-360 DPV, classification of shedding intensity, Neg: Negative, +1: Low shedders, +2 to +3: Moderate shedders, +4 and above: High shedders, DPV: Days post vaccination

**Shedding of MAP in feces:** At 0 DPV, 72.7% of goats were shedding MAP in feces, which represented very high rate of MAP infection (Bio-load or prevalence of JD). Shedding of MAP in representative goats was 72.7, 77.7, 54.5, 27.2 and 27.2% at 0, 90, 180, 240 and 360 days post vaccination. There was significant reduction (45.5%) in the shedding of MAP between 0 and 360 DPV both in terms of intensity of MAP (+4 to +1) and number of goats shedding MAP (Table 11). However, few goats that reverted were re-vaccinated and again shedding of MAP declined over period of time after re-vaccination.

**Monitoring of MAP using IS900 blood PCR:** At 0 day blood samples of 41.1% (11/17) goats were positive for MAP exhibiting bacterimia by IS900 blood PCR. Clearing of MAP from the blood circulation was observed in vaccinated goats and only 18.1% goats were positive for MAP infection at 360 DPV (23.0% reduction of MAP bacterimia). Removal of MAP from blood circulation was first and early symptom of the vaccine response.

**Humoral immune response (sero-conversion):** In vaccinated goats, peak titers were achieved around 90 DPV monitored using 'Indigenous ELISA kit'. In majority of goats ELISA titer declined slightly, which was maintained afterwards at 360 DPV and 90.9% goats sero-converted at 360 DPV.

## DISCUSSION

First 'Indigenous vaccine' developed and commercialized by CIRG, Makhdoom against JD using the most prevalent and highly pathogenic 'Indian bison type' bio-type of *Mycobacterium avium* subspecies *paratuberculosis* (strain 'S 5') has proved both 'Therapeutic and preventive' in protecting goats against incurable Johne's disease. Though number of reports exist on the prophylactic properties of the JD vaccine, however there are few reports on the 'Therapeutic potential' of the vaccine[10,11,13]. The vaccine was safe and no un-toward reaction or abscess formation was observed at the site of inoculation using 'Indigenous JD vaccine' in the Jakhrana goat herd. However, some studies reported large and fistulated nodules formation at the site of injection[7].

Progressive losses in milk production, reduction in body weights and diarrhoea or loose feces were the prominent clinical symptoms of Johne's disease that were observed in majority of goats in Jakhrana unit before vaccination. Laboratory findings on the basis of screening of representative goats at 0 Days Post Vaccination (DPV) also showed that goatherds were suffering with MAP infection and 72.7% were positive in fecal microscopy, 41.1% in IS900 blood PCR and 64.7% in indigenous ELISA. Sampling and screening of 116 goats before vaccine trial II showed 77.5% (90/116) goats

were shedding MAP. This fecal shedding was reduced to 45.5% at 360 DPV after vaccination. However, few goats continued shedding of MAP after vaccination were revaccinated. This was mainly due to fact that initial dose of vaccine was not sufficient for the goats in advance clinical JD. However, vaccination of goats with advance clinical JD and already in fragile condition of health, is an additional stress. Therefore, higher dose of vaccine in already fragile condition (initial stage) of goats proved counter-productive in our earlier studies. Therefore, revaccination after time interval of 9-12 months, was better option. Since Jakhrana goats at CIRG were well fed (under optimal nutrition condition) it may look worth taking risk with higher dose in few goats. Another important factor in our vaccine trials (I and II) was the continued re-infection of vaccinated goats, which may lead to persistence re-infection with MAP bacilli daily for long time. Or immune system of the goats in advance clinical stage require more time for repair, since repair is slow and it may need longer time and may not be repaired due to extensive damage to lymph-nodes/lymphoid organs caused by MAP. The time of revaccination of goats in endemic herds will depend on the time of reversion in the status of shedding of MAP or time period when shedding again start increasing. This may not be universal and is likely to change with each farm unit, depending on the extent of damage and load of MAP, nutritional status, hygienic conditions etc. Therefore, monitoring of shedding of MAP is an important criteria which should be carefully followed to record the vaccine response. Increase in shedding of MAP after initial response by exhibiting reduction of shedding is indication of dwindling titer of MAP or titer not sufficient to kill the MAP bacilli. This time interval in reversion will depend on nutritional status, other stress factors and more importantly repeated infection (in endemic farms) of goats. This variation in vaccine response has been reported in our earlier studies on herds and we categorized farms with low and high plane of nutrition[11]. However, one thing is clear that in Indian conditions, the JD vaccine response using 'Inactivated MAP bacilli' cannot be for life as reported by many western studies[9], due to high endemicity of MAP in herds and flocks in India[1]. Stress factors like low plane of nutrition, extreme environment temperatures, pregnancy, lactation, extent of damage to lymphoid tissues and concurrent parasitic infestations played critical role in pathogenesis of MAP and also had role in lowered response to vaccination[11]. Environmental stress factors (excessive winter and summer) other health stresses may have made goats vulnerable to re-infection as they may not have completely recovered after vaccination. Hygienic farm management played important role in decreasing shedding of the bacilli in feces that limits opportunities for repeated transmission of MAP[20] in the population. The 'Take' that developed at the site of vaccination also proved that goats suffered with MAP infection and in some cases 'Take' was retained for 90-360 DPV, while in others reduced at faster pace.

Study also indicated that vaccination using this 'Indigenous JD vaccine' could be safely practiced in sub-clinical to advance clinical cases of MAP by vaccinating all animals above 3 months old to adult stage and in any physiological state (dry, lactating and pregnant) in the farm/herds/flocks. Other studies on vaccination of the adult animals also showed good results in controlling JD[11,13,21]. Corpa *et al.*[22] also showed that immune response was higher in adult animals as compared to few weeks old animals. Similar results were reported by Singh *et al.*[13] in Bharat Merino and Shroff *et al.*[23] in Patanwadi sheep by vaccinating with goat based 'Indigenous vaccine'. Currently, most of the MAP vaccines used mineral oil adjuvants to evoke more active immune responses[24]. Most of the sheep studies used strain 316F, strain 18 and virulent field strains with oil adjuvant as killed vaccines for the control of Johne's disease[25]. Our studies in other livestock species reported the significant reduction in morbidity, mortality and shedding of MAP in feces after vaccination of the goats endemically infected with MAP, which leads to the reduction in the contamination of the animal environment, therefore daily dose of MAP which depends on environmental contamination for the transmission of disease also get reduced. This is why in our vaccine trials, in-contact control (non-vaccinated) also showed improvement in health but it was not to the level in vaccinated goats. Improvement in in-contact control was mainly due to reduction in re-infection due to reduced environmental load and sharp decline in shedding of MAP by vaccinated goats[9]. In Iceland, Sigurdsson and Gunnarson[26] attained successful eradication of JD by vaccinating lambs once in life time using killed vaccine. Using 'Indigenous vaccine' against MAP infection shown reduction in the prevalence of clinical JD in goat herds[27] by 50-90%.

'Indigenous JD vaccine' exhibited 'Therapeutic effect' as there was reduction in shedding of MAP in feces along with visible improvement in physical condition of vaccinated goats as compared to goats before vaccination. Significant improvements were recorded, body coat regained luster, shining, pliability, regeneration of hairs and increase in growth rate was observed in growing kids after vaccination (Fig. 3). Vaccinated and non-vaccinated controls could be easily differentiated from distance on the basis of improved body

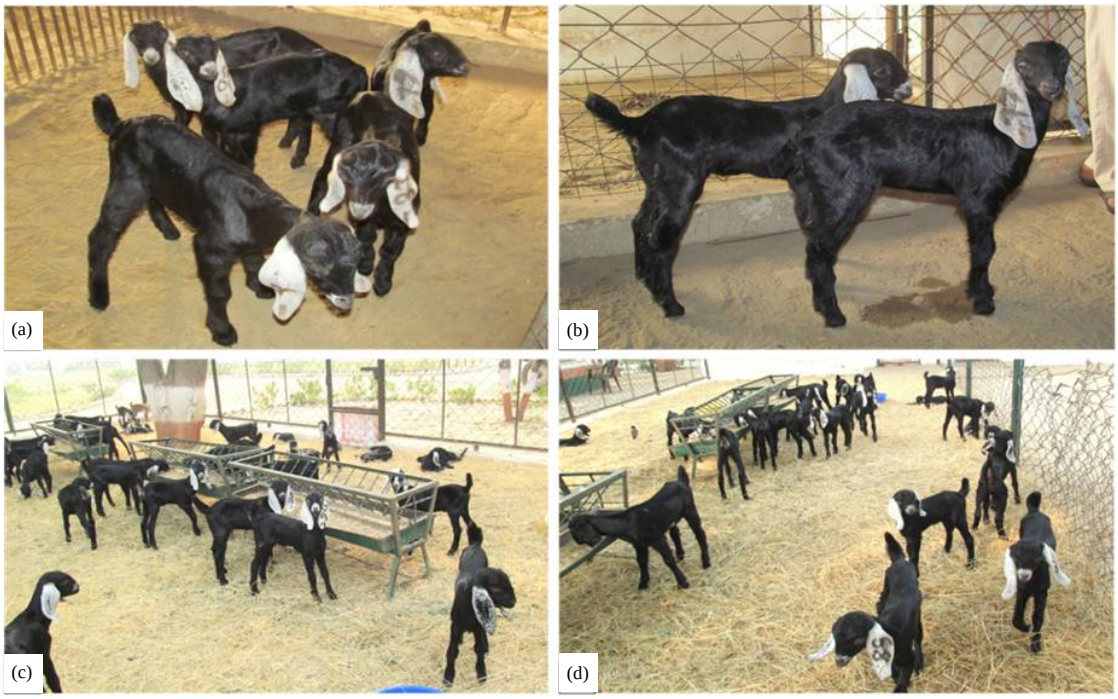


Fig. 3(a-d): Physical profiles of (a-b) Newly born and (c-d) Newly vaccinated kids

condition after 6 month of vaccination. Though reduction in number of MAP shedders was significant after vaccination however it was reduced by 45.5% only. Multi-bacillary shedder turned into pauci-bacillary shedders and was important feature of study. This study suggested that complete recovery (zero shedding) may take more time, since goatherds were highly endemic for JD or MAP infection (77.5% positive by microscopy after screening of 116 goats before vaccination. Similar comparable reduction in number of shedders were reported by Hines *et al.*[28] on vaccination of spheroplastic and cell wall component MAP vaccine in experimentally challenged goat kids.

In the present study, significant reduction in the mortality of goats was observed after vaccination and similar findings were also reported by Gwozdz *et al.*[29]. 'Indigenous vaccine' had strong therapeutic response on the basis of improvements in different parameters (low morbidity, reduced shedding of MAP and high humoral immune response), since most of the parameters were monitored up to the period of 1 year after vaccination therefore, therapeutic effect would be sustained in herd and upto which stage of the infection, vaccine performed better remains to be further investigated. Similar results have been reported by Singh *et al.*[10,11,13] and where goat based vaccine prepared from 'S 5' MAP native strain was equally effective in sheep and reduced the number of fecal shedders.

Antibody titers were monitored in randomly sampled goats at 30 day interval as per Singh *et al.*[9]. Peak titers were achieved around 90 DPV, which declined slightly and were maintained afterwards at 360 DPV. Spangler *et al.*[30] reported rise in the antibody titer at 60-360 DPV in vaccinated calves. However, Corpa *et al.*[22] observed rise in the antibody titer around 60 DPV in goats vaccinated with heat killed vaccine and there was again rise at 180 DPV. But this second rise in the antibody titer was lesser than the primary peak that was observed at 60 DPV. Though this lesser antibody response may not be having the protective effect against MAP infection but it would indicate the degree of activation of immune system against mycobacteria. The Th1 and Th2 responses are not antagonist to each other and IFN-$\gamma$ can play stimulatory effect on B-lymphocytes and antibody production[31].

Study revealed that the 'Indigenous vaccine' had 'Therapeutic effect' in goatherds endemically infected with JD and suffering from sub-clinical, clinical and advance clinical stages of disease. Impact of candidate strain of vaccine has also been observed by Uzonna *et al.*[32], who observed that the 'Native field strain' based vaccine for JD was more effective and efficacious than the commercial vaccines. This study also

report that 'Indigenous vaccine' developed using native biotype, 'Indian bison type' can serve as excellent model for the treatment of infected animals and control of disease in herds. Clinically infected goats on vaccination come back to health and regain their lost productivity levels. Study also underlined need for immediate attention for the management and control of JD in goatherds of Jakhrana breed in India.

## CONCLUSION

'Indigenous vaccine' developed at CIRG, Matura using MAP strain 'S 5' ('Indian bison type') was effective for the therapeutic management of Johne's disease. 'Indigenous JD vaccine' significantly reduced morbidity, mortality and shedding of MAP, reduced bio-load of MAP in blood (bacteremia), improved physical condition, facilitated birth of healthy kids, improvement in body weights gained, enhanced immunity and productivity (milk yield and body weights) in goatherd endemic for JD. Therefore, 'Indigenous JD vaccine' can be employed both in the treatment and for salvaging important goat breeds suffering with sub-clinical, clinical and advance clinical JD from imminent culling and death and before these breeds becoming extinct in near future. Indigenous JD vaccine can be model for the utilization of large population of non-productive and low performing domestic livestock.

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