MODIFIED INTRA - DERMAL TEST FOR THE DIAGNOSIS OF PARATUBERCULOSIS IN GOATS

By

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THESIS

Submitted in partial fulfilment of the
requirement for the degree

Master of Veterinary Science

Faculty of Veterinary and Animal Sciences
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Mannuthy, Thrissur

1994

DECLARATION

I hereby declare that this thesis entitled **"Modified Intra-dermal Test for the Diagnosis of Paratuberculosis in Goats"** is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title of any other University or Society.

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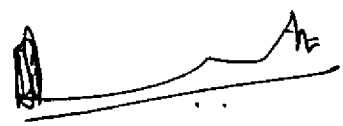
CERTIFICATE

Certified that the thesis entitled **"Modified Intradermal Test for the Diagnosis of Paratuberculosis in Goats"** is a record of research work done independently by **Dr. K. Vinod Kumar** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to him.



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* Dr. J. Abraham for allowing me to utilize the facilities at Kerala Agricultural University meat plant for the slaughter of animals.

* Dr. A.D. Mercy and Dr. M. Nandakumaran, Dept. of Nutrition for their willing help in the estimation of serum magnesium levels.

* Dr. K. Valsala and Dr. N. Divakaran Nair for their expert interpretation of histopathological results.

* Sri. O.J. George and Sri. N. Ravindranathan for the statistical analysis of results.

* Dr. K.R. Harshan for his technical advice and help in histopathology.

* Dr. Mathew Thomas, Dr. P.V. Tresamol and Dr. Devada Menon for helping in the collection of literature.

* Distributed information system, NDRI, Karnal for the technical abstracts.

* Dr. C.P. Gopakumar for the many hours spent on the photography of tissue sections.

* Dr. P.R. Pradeepkumar and other post-graduate students for their cheerful comraderie and practical help.

ACKNOWLEDGEMENTS

I would like to express with pleasure my warm appreciation and sincere gratitude to,

* Dr. M.R. Saseendranath, Chairman, Advisory Committee, for his inspiring guidance, constant encouragement and splendid cooperation.

* Dr. A. Rajan, Dean, for his technical expertise, active interest and invaluable help in both academic and official matters as member of the advisory committee.

* Dr. V.S. Balakrishnan and Dr. P.C. Alex, Department of Clinical medicine, for their helpful suggestions and co-operation as members of the advisory committee.

* Dr. P.T. Georgekutty for his precise instructions and meticulous supervision in both designing and conducting this work.

* Dr. E.P. Paily for his wise suggestions, Dr. P.G. Baby for his encouragement and Dr. K. Vijayakumar for the technical help.

* Dr. Rajagopala Raja, Dr. P. Nandakumar and Dr. Stephen Mathew, for making the goats available for the study.

* Non teaching staff, Dept. of Preventive Medicine for their enthusiasm.

* Director, Kerala State Animal Husbandry Department, for granting me leave to complete the study.

* Kerala Agricultural University for providing financial assistance in the form of junior fellowship.

* To all others who assisted me in one way or other.

* To my father, mother, brother and sister .... for everything.

K. VINOD KUMAR

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INTRODUCTION

Paratuberculosis or Johne's disease occurs among domestic ruminants all over the world. Despite being recognized as one of the most serious diseases affecting livestock industry, the disease has not had the intensive investigation as it demands. It could be because the threat posed by Paratuberculosis was recognized only recently, even though the disease was first noticed almost one hundred and seventy years ago by d'Aroval in 1826 (Chodini et al., 1984).

The name "paratuberculosis" means "besides nodule forming disease". As the name implies, the early workers considered this disease to be an aberrant and infrequent form of tuberculosis. Most of the current knowledge on paratuberculosis was obtained during two periods of intensive research. The first period from 1890 to 1910 was led by Johne, Frothingham, Bang, Twort and Ingram. They established the separate identity of both the disease and the etiological agent M. paratuberculosis, an acid-fast bacilli. The second period of intensive research from 1955 to 1970 had Hole, Gilmour, Sigurdsson and Larson in the fore-front. They used better diagnostic techniques like complement fixation and fluorescent antibody techniques, and brought light to the

difficulties in both the diagnosis and control of paratuberculosis.

The insidious nature of paratuberculosis, the lack of a practical and accurate diagnostic test and the failure of many farmers to recognize the disease among their stock have made epidemiological surveys difficult. Abattoir data and postmortem studies since 1980 recorded eight to fourteen per cent incidence of paratuberculosis among dairy cattle in USA and UK. Substantial economic loss could be attributed to the morbidity and mortality among affected animals (Chodini et al., 1984 and Scott et al., 1988). In addition, the recent reports suggesting a mycobacteria identical in all aspects to M. paratuberculosis as the possible etiological agent for a fatal and chronic ileo-colitis of human beings (Crohn's disease) attaches a possible zoonotic importance to this disease (Spangler, 1992a).

Presence of a large number of healthy carriers among the infected herds complicates the control of paratuberculosis. Routine diagnostic tests fail to detect such carriers and they act as a source of infection to susceptible animals. Early detection and removal of such "sub-clinical carriers" is now recognized as the most important aspect of control of paratuberculosis (Sherman et al., 1989).

Most of the diagnostic tests commonly being employed for other bacterial diseases are inaccurate when used for the diagnosis of paratuberculosis because of very high levels of false positive and false negative reactions. More than 50 years of intensive research has produced only two tests of absolute accuracy. One of them, the lymph node biopsy is a surgical procedure whereas the other, the DNA probe, involves advanced technology. Results of other customary techniques like ELISA and fecal culture do not justify the extra labour and expenses involved in performing them.

Goats play a major role in the agricultural economy of India. This country has the largest goat population of 109 million in the world. Goats contribute to 79.3 per cent of total meat and 12.9 per cent of total hide in India. The total value of products from goats in India during 1990 was calculated to be US $ 1.6 billion (Devendra, 1992). The contribution of goats in terms of the nutrition of rural folk, effective utilization of family labour, generation of family income and utilization of agro-industrial and domestic wastes is, however, several times more than the estimates made on the basis of economic returns.

Paratuberculosis among goats was reported by Pande (1940) for the first time in India among goats of Assam. The presence of the disease among Indian goats on a major scale is

beyond doubt as the disease has been reported from most of the farms engaged in the production and distribution of kids to the field (Lall, 1973). But a comprehensive epidemiological survey, which forms an important part of any control programme, is yet to be undertaken. A dependable accurate diagnostic test practicable under Indian conditions is an essential tool for such a survey.

Diagnosis of the infection in goats is more difficult than in cattle. It is because the goats do not manifest the clinical symptoms such as diarrhoea which are often associated with paratuberculosis in cattle. Post-mortem examinations seldom reveal any typical lesions like corrugated intestinal mucosa in goats. Lastly most of the work concerning diagnosis of paratuberculosis have been conducted in cattle, and they give a different set of results when employed for goats.

Intra-dermal test was first developed by Von priquet in 1907 (Skinner, 1949) for diagnosis of tuberculosis, and the technology was later adopted by Bang (1909) for the diagnosis of paratuberculosis. This test is ideal for Indian conditions as all the field veterinarians are trained for it, and it does not involve expensive chemicals and sophisticated instruments. But this test also suffers from poor levels of sensitivity and specificity, which are below 50 per cent in both instances.

Efforts to improve the accuracy of the test in cattle for the diagnosis of tuberculosis has resulted in the development of the Stormont test (Kerr et al., 1946a)which had very high levels of accuracy. But so far no study has been done to improve the efficacy of intra-dermal test for the diagnosis of paratuberculosis in goats. If such an improvement could be brought upon, it will be a very helpful step in the diagnosis and control of paratuberculosis in goats.

The present study was undertaken to compare the efficacy of the conventional single intra-dermal test against two modified intra-dermal tests for the diagnosis of paratuberculosis in goats.

REVIEW OF LITERATURE

2.1 General history

A form of enteritis with chronic diarrhoea was reported in cattle by d'Aroval in 1826. Hans and Nelsen (1881, cited by Chodini et al., 1984) reported corrugation and thickening of the intestinal mucosa of cattle dying from the disease. Johne and Frothingham (1895) described the disease and demonstrated the presence of acid-fast bacilli in sections of the diseased intestine, and concluded it to be an atypical form of tuberculosis. Bang (1906) differentiated the disease from tuberculosis and called it as 'pseudotuberculosis disorder' or Johne's disease.

Twort and Ingram (1913) isolated the organism and named it as Mycobacterium enteritidis chronicae paratuberculosae bovis johne. McFadyan and Sheather (1916) described the disease for the first time in goats. Thereafter numerous reports of the disease affecting both domestic and wild ruminants have appeared from all parts of the world (Doyle and Spears, 1951; Slotys, 1951; Choudhari et al., 1964; Riemann et al., 1979; Chodini et al., 1984; Benedictus et al., 1987 and Koh et al., 1988).

Incidence in India

Twort and Ingram (1913) reported the disease for the first time in India. The second case was reported by Sheather (1918). A survey in Assam during 1933-37 period detected the disease in 23 organized farms (Pande, 1940).

Rahimuddin et al. (1940) reported the disease among sheep at Hosur and Rajagopalan (1947-48) reported the disease among goats at Mukteswar.

The disease was reported among goats from many parts of the country (Rajan et al., 1976; Sriraman et al., 1982; Kumar and Prasad, 1986; Kumar et al., 1988; Koul et al., 1988 and Krishna et al., 1989).

2.2 Diagnosis of paratuberculosis

A vast amount of work has been done on the various methods used for the diagnosis of paratuberculosis and an extensive amount of literature has been published. Frequent, comprehensive reviews have kept the knowledge upto date (Sigurdsson, 1956; Goudsward and Terporten-Pastoors, 1972; Julian, 1975 and Chodini et al., 1984). But there is a scarcity of literature concerning works done on goats, despite the advantages in utilizing small ruminants over cattle for experiments (Goudsward and Terporten-Pastoors, 1972) and also

the fact that pathogenesis of the disease was similar in cattle and goats (Gilmour, 1976b).

2.2.1 General aspects of diagnosis

The carrier animals may be positive to intradermal allergic test and negative to all other diagnostic tests or vice versa (Nag, 1960).

Pearson (1962) reported that many workers have failed to differentiate between clinical and sub-clinical animals which has resulted in confusion regarding the value of serological and allergic tests for the diagnosis of Johne's disease.

Pearson and McClelland (1962) described the phenomenon of "cross over" between allergic and serologic titres as infection progressed.

The serologic reactions sometimes did not correctly correlate to the histopathological status because the organisms after oral infection multiplies at a faster rate than rate of increase of antibody titre (Merckal et al., 1968a).

Larsen et al. (1969) stated that there was no effective test to accurately diagnose Johne's disease in cattle which harbour the organism. These apparently healthy

animals shed too low numbers of organisms in feces to be detected in culture.

The immunologic tolerance exhibited by carrier animals to M. paratuberculosis was attributed as the reason for the long incubation period and the erratic results of serologic tests (Benedixen, 1977b).

Chodini et al. (1984) in their review inferred that none of the thirteen available diagnostic methods for Johne's disease were performing satisfactorily to identify the infected animals, and many tests produced a number of false positive and false negative results. They correlated such inaccuracy to the poor quality of available antigen, presence of many antigenically similar bacteria in atmosphere and the state of anergy in advanced clinical cases.

Paliwal and Somvarshi (1984) stated that the false negative reactions were due to the generalized T cell impairment in clinically affected animals as evidenced by changes in lymph nodes.

Benedictus and Basma (1985) observed that a combination of complement fixation test, intra-venous Johnin test, fecal culture and biopsy of rectal mucosa detected only 96 per cent of clinical cases.

The disease has a very chronic course and several months or even years may elapse between the time of infection and time of appearance of clinical symptoms (Blood et al., 1989).

Spangler et al. (1991) observed that vaccination interfered with the serological diagnosis of paratuberculosis.

2.2.2 Agar-gel immuno-diffusion tests (AGID)

Merckal et al. (1965) designed an agar-gel immuno diffusion test to be used in lambs for the diagnosis of Johne's disease and the results were obtained much earlier than complement fixation test. The animals even six weeks after infection showed a positive response in agar-gel immuno diffusion test as compared to the six months required by complement fixation test. The gel diffusion test accurately reflected the progression of infection than other serological tests.

Merckal et al. (1968b) observed that the precipitin lines continued to increase as the disease advanced in cattle and decreased whenever the animal was recovering.

Goudswaard and Terporten-Pastoors (1972) reported that the results of agar-gel immuno-diffusion test in goats were

better than the results of complement fixation test in diagnosing sub-clinical infection in goats.

Merckal (1973) observed precipitin lines in paratuberculous cattle to become clearer as the disease advanced.

Sherman and Gezon (1980) observed agar-gel immuno diffusion test and fecal culture to have good diagnostic sensitivity and specificity in Johne's disease. The false positive reactions due to Corynebacterium antigens was avoided by repeated testing in goats.

The agar-gel immuno diffusion test was found to have accuracy of 96.9 per cent in detecting clinical cases of Johne's disease in cattle (Sherman et al., 1984).

Kormendy et al. (1984) found that cross reactivity existed between M. avium and M. paratuberculosis in agar-gel immuno-diffusion.

Sherman et al. (1989) and Spangler et al. (1992a) reported agar-gel immuno diffusion test to be of poor value in sub-clinically infected cattle.

2.2.3 Counter-immuno electrophoresis (CIEP)

Muhammed et al. (1978) has found the counter-immuno

electrophoresis to be at least four times more sensitive and rapid than agar-gel immuno-diffusion test in the diagnosis of Johne's disease in cattle.

2.2.4 Complement fixation test

Twort and Ingram (1913) reported that a suspension of artificially cultured M. paratuberculosis was of little use as an antigen for complement fixation test.

Bang (1914) found that by using M. tuberculosis var hominus culture as an antigen, only 50 per cent of animals answering the intra-dermal test as positive, were detected by complement fixation test.

Hole (1952) recommended modified complement fixation test than intra-dermal test and fecal examination for diagnosis of Johne's disease in cattle.

Sigurdsson (1956) reported less than two per cent of false positive reactions for complement fixation test in "apparently healthy" sheep having Johne's disease.

Chandler (1956) reported that a micro complement fixation test using perspex trays had 84 per cent sensitivity and 77 per cent specificity in clinical cases and 95 per cent sensitivity and 97 per cent specificity in sub-clinical cases, of paratuberculosis in cattle.

Landau et al. (1959) proposed conducting complement fixation test only on cattle which were positive to intradermal tests so as to improve the results.

Larsen et al. (1963b) found the complement fixation test to be unreliable in cattle.

Merckal (1968a) considered the complement fixation test to be of unreliable diagnostic value for the diagnosis of Paratuberculosis in cattle, sheep and goats.

Goudswaard et al. (1976) compared five serological tests under field conditions for diagnosis of Paratuberculosis in cattle and found the complement fixation test to be of poor value.

Rieman et al. (1979) and Lisle et al. (1980) also recommended complement fixation test as effective for diagnosis of natural infections of paratuberculosis in cattle.

Kulshrestha et al. (1984) advised combination of fecal examination with complement fixation test for accurate results.

Ratnamohan et al. (1986) used a heat extracted soluble M. avium antigen for diagnosis of Johne's disease in cattle and reported 74.4 per cent sensitivity and 92.3 per cent specificity.

Norrie and Spencer (1989), by using the micro complement fixation test reported 74.4 per cent sensitivity and 92.3 per cent specificity in clinical cases among cattle.

Saxegaard (1990) reported that both clinical and sub-clinical cases of Johne's disease in cattle answered the complement fixation test.

2.2.5 Direct examination of feces

Acid-fast staining of direct fecal smears by Ziehl-Neelsens technique proved to be a very efficient method of diagnosis in early days. The presence of acid-fast bacilli in characteristic clumps was taken as positive whereas presence of single acid-fast bacilli was treated as suspicious (Doyle and Spears, 1951).

Slotys (1951) found that direct method of fecal sample examination was as equally effective as antiformin method, floatation method and modified methods in diagnosis of Paratuberculosis.

Taylor (1951) and Hole (1952) found the direct examination of feces to be of poor value in diagnosis of pre-clinical Johne's disease.

Rankin (1958a) reported that the sensitivity of fecal

examination in clinical cases of Johne's disease in cattle was as low as 30 per cent.

A modification in preparing the smear by placing two slides perpenticularly upon each other and spreading the fecal material along the edge of upper slide, followed by staining the seepage area under it, was claimed to be of better value (Cunningham and Gilmour, 1959).

Hole and Maclay (1959) st..ted that microscopical examination of feces had a high positive value in detecting characteristic clumps of the organism. But a negative result had no significant value.

Pearson and McClelland (1962) observed that in the early stages of the infection acid-fast organisms were difficult to find. The accuracy of fecal examination in clinical cases was below 30 per cent. Floatation and centrifugation methods were no better than ordinary method.

Gilmour (1965a) found microscopical examination of feces to be of little value in detecting pre-clinical Johne's disease in cattle.

Merckal et al (1968b) reported that fecal culture and direct fecal examination was the method of choice for diagnosis of

Johne's disease in cattle. Many mycobacteriae which was differentiated from M. paratuberculosis only by fecal culture.

A negative result of fecal sample examination had little meaning as for the diagnosis of Johne's disease in cattle, sheep and goats (Lall, 1973).

Kumar et al. (1982) had developed a prior-oxidation acid-fast staining method as an improvement on direct Ziehl-Neelsen's technique in Johne's disease of sheep.

Chodini et al. (1984) opined that rectal pinch examination had many false negative results since the test sampled only the last two to three feet of rectum which is not a preferred site by the Mycobacteria.

Kulshrestha et al. (1984) found the examination of fecal samples to be comparatively simple. But a negative result did not rule out infection.

Paliwal et al. (1984a) found the fecal sample examination to be more reliable than the allergic test.

Paliwal et al. (1985b) found that fecal examination in cattle gave positive results in only 1/19 of pre-clinical, 7/8 of clinical and the single advanced case of Paratuberculosis.

Patel et al. (1987) observed that invasion of rectal mucosa was taking place only in the advanced stage of the disease.

2.2.6 Enzyme linked immunosorbant assay (ELISA)

Thoen and Muscoplat (1979) applied ELISA for the first time to diagnose mycobacterial infections in animals and could obtained positive results from the sera of cattle experimentally infected with homologous species of Mycobacteriae only.

Thoen and Bruner (1982) proposed application of ELISA for the diagnosis of paratuberculosis in goats.

Merckal (1984) recommended ELISA as the test of choice in all animals for the diagnosis of Johne's disease. But the many false positive reactions were still a problem.

Huanz (1989) proposed a biotin-avidin amplified ELISA to increase the level of sensitivity. This test gave more true positive results than ordinary ELISA, complement fixation test and Johne's intra-dermal test in fecal culture positive cattle as well as animals from an infected herd. They obtained more than 89.4 per cent sensitivity for the new test.

Sugden et al., 1989 proposed that use of a lipo-arabinomannan (LAM) in ELISA increased the sensitivity from 23.5 per cent to 70.6 per cent and specificity to 100 per cent.

Milner et al. (1989) and Milner et al. (1990) observed 56 per cent sensitivity and 99 per cent specificity for ELISA in the diagnosis of clinical Johne's disease in cattle.

Molina et al. (1991) reported 87.5 per cent sensitivity and 93.95 per cent specificity for ELISA in diagnosing paratuberculosis among goats.

Valentin et al. (1991) achieved differentiation between titres due to actual infection and vaccination by combining ELISA with western blotting.

Collins et al. (1991a) reported 47.3 per cent specificity and 99 per cent sensitivity for ELISA in cattle whereas Spangler (1992b) reported 71 to 73 per cent sensitivity and 61 to 83 per cent specificity for ELISA in cattle for diagnosis of Paratuberculosis.

Singh et al. (1992b) reported that ELISA test was most reliable test for the detection of sub-clinical infection in goats.

2.2.7 Fluorescent antibody test

Preliminary investigations by Gilmour et al. (1965b) on application of fluorescent antibody test for the diagnosis of paratuberculosis gave good results in sheep with pre-clinical infections of Johne's disease.

Gilmour and Gardiner (1968) reported that fluorescent antibody test was effective in advanced stages of the disease, but could not differentiate between infections due to M. johnei and M. avium, and gave many false positive reactions.

The fluorescent antibody test gave results earlier than complement fixation test and was three times sensitive than complement fixation test for diagnosis of experimental paratuberculosis in cattle. The fluorescent antibody test titres varied from week to week, with no correlation between the results of the test and histopathological findings (Gilmour and Gardiner, 1969).

Goudswaard et al. (1976) reported that fluorescent antibody test was as specific as complement fixation test in naturally infected cattle with subclinical paratuberculosis.

Gilmour and Angus (1976a) observed that fluorescent antibody test was more specific than complement fixation test and allergic test in experimentally infected calves and abattoir cattle (Gilmour and Angus, 1976b).

Paliwal et al. (1983) conducted experiments with sera from 352 goats and concluded that fluorescent antibody test gave better results than fecal examination and histopathology. The positive animals were detected even when they had no visible lesions in internal organs.

Paliwal et al. (1984b) found the accuracy values of six diagnostic tests in goats with paratuberculosis to be in the order of fluorescent antibody test (100 per cent), histopathology (100 per cent), gross necropsy (98 per cent), fecal examination (70 per cent), clinical signs (69 per cent) and double intra-dermal test (25 per cent).

In cattle, fluorescent antibody test detected 18/19 of pre-clinical cases and the single, advanced clinical case correctly (Paliwal et al., 1985b).

Blood et al. (1989) opined that fluorescent antibody test detected paratuberculosis at an early age, but the results were too inaccurate for practical purposes.

2.2.8 Histopathological changes in paratuberculosis

2.2.8.1 Significance in diagnosis

Sigurdsson (1956) noticed that even in advanced clinical cases of paratuberculosis in cattle, with typical lesions, it was difficult to demonstrate the bacilli.

Groth (1964) could not detect any correlation between age and severity of lesions in sheep.

Buergelt et al. (1978b) found intestinal and lymphatic lesions among 31/51 infected cattle and rectal lesions in only five.

Fodstad and Gunnarson (1979) opined that the demonstration of even macroscopical lesions was of little value in the diagnosis of paratuberculosis, since such changes were not seen often, and were non specific. Histopathology could detect only 54 per cent of infected animals as against 92 per cent by bacterial culture and 47 per cent by microscopical examination.

Summers (1981) reported that 22 out of 74 specimens negative for M. paratuberculosis in mucosal scrapings were positive on histopathological examination of the intestine. Twenty one of these had lesions in mesenteric lymph glands also.

Paliwal and Rajya (1982) examined the intestine and mesenteric lymph nodes of 150 goats sub-clinically infected with paratuberculosis and found lesions in very few of them. They concluded that it could be due to the animals being in the very early stages of development of the disease.

Carrigan and Seaman (1990) found that 36 per cent of diseased sheep had only mild gross lesions in the intestine.

Jubb et al. (1993) found gross and microscopical lesions to be so slight as to be easily missed in some goats dying of paratuberculosis.

2.2.8.2 Lesions in small intestine

2.2.8.2.1 Macroscopic lesions - Clinical cases

The typical gross changes in goats affected with paratuberculosis were very few (Hutyra et al., 1949).

Taylor (1951) stated that if the disease was caused by the bovine strain, the intestinal mucosa may be thickened, and if the pigmented strain was involved, very little lesions except an orange colour on the intestinal mucosa was seen.

Terminal portion of the ileum was the commonly affected area of the small intestine in goats (Rankin, 1958a).

Rajya and Singh (1961) observed the lesions in sheep to vary from extremely congested patches involving considerable portions of the intestine to thickened and corrugated mucosa.

Thickening of the mucosa of ileum was observed in sheep with clinical paratuberculosis (Groth, 1964). He also

noticed that in cases were the thickening of the mucosa was absent, animals were negative for the presence of M. paratuberculosis in feces.

Kluge et al. (1968) observed that in sheep, gross lesions were most prominent in the intestine wherein there was a thickened intestinal wall and in some instances corrugations of a hyperplastic mucous membrane. The terminal portion of the ileum, ileo-caecal valve, caecum and the cephalic portion of the colon were most commonly affected.

Lall (1973) found the thickening of intestinal mucosa in a large number of goats to be so slight as to be imperceptible. Some times cracks were observed on the thickened mucous membrane.

The intestinal lesions among goats with paratuberculosis were found to be quite few by Fodstad and Gunnarson (1979). Corrugation of the mucosa was rare. Only 47 per cent of goats had macroscopical lesions.

Ullrich et al. (1982) found the gross lesions in naturally infected goats to be confined to the intestinal tract. Nodular hyperplasia was observed on the mucosa of the jejunem and ileo-caecum and anterior colon.

Chodini et al. (1984) reported that the earliest

changes observed among sheep were a fleshy or velvetty thickening of the mucosa of ileum and ileo-caecal junction.

Kulshrestha et al. (1984) observed slight to moderate thickening and oedema with rough granular mucosa covered with a cream coloured slimy fluid on the small intestine in sheep.

Krishna et al. (1989) found the jejunem and caecum to be consistently oedematous and having roughened mucosa among goats with corrugated appearance of the mucosa in all clinical cases of Johne's disease.

Carrigan and Seaman (1990) observed that 35 per cent of sheep affected with paratuberculosis had only mild granular changes, 48 per cent had mucosa formed into transverse ridges and 10 per cent had segmental lesions, the lesions being confined to certain segments of the intestine.

Singh et al. (1992) found that the gross pathological changes in the small intestine of goats to be very few. Macroscopic changes were not seen, and were non specific, corrugation of the mucosa was rare.

Jubb et al. (1993) reported presence of rows of tubercle like caseation and calcification in the ileum, ileo-caecal junction and caecum of goats affected with paratuberculosis.

Sub-clinical cases

Rajya and Singh (1961) observed congestion and slight granular thickening of the ileal mucosa in paratuberculosis affected sheep. They also reported nodular, worm like appearance of lymphatic vessels and creamy, slime like mucosa of jejunum.

Lenghaus et al. (1977) found the wall of the intestinal mucosa to be thickened in four sub-clinical cases of Johne's disease in goats.

Paliwal and Rajya (1982) observed tough granular mucosa, congestion and also thickening of the intestinal mucosa among goats affected with pre-clinical paratuberculosis.

2.2.3 Microscopical lesions - Clinical cases

Rankin (1958b) recorded aggregation of epithelioid cells just below the mucosa of the terminal portion of the ileum as a typical characteristic of paratuberculosis in sheep and goat.

Rajya and Singh (1961) noticed granulomatous inflammatory changes involving all layers except the muscular layer, of the intestine in diseased sheep. The epithelium of the intestine in these animals was desquamated and coagulative

necrosis affected all parts of the mucosa, which also showed unnatural vascularity and haemorrhage, and degenerative necrosis of villi. Extensive cellular infiltration with macrophages, epithelioid cells and giant cells were also observed. Acid-fast bacilli phagocytosed by macrophages could be seen under Ziehl-Neelsen's staining.

Lenghaus et al. (1977) found acute and subacute peritonitis, fibrin strands and tags of fibrin tissue in the serosa of intestine, particularly in ileum, caecum and colon of goats.

Fodstad and Gunnarson (1979) while reviewing the post-mortem findings of 2997 goats observed that the intestinal lesions in paratuberculosis affected goats were very few. Presence of bacilli in sections was not uncommon.

The infiltration of intestine by inflammatory cells was so extensive that the usual architecture was replaced by lymphocytes and macrophages (Ullrich et al., 1982).

Krishna et al. (1989) observed thickening of the lamina propria with aggregation of mononuclear cells, macrophages and epithelioid cells. The submucosa was also infiltrated with inflammatory cells.

Granulomatous enteritis, typhilitis and colitis were observed in case of sheep, along with focal aggregation of

epithelioid cells at the tip of the intestinal villi and severe villous atrophy in terminal ileium due to infiltration of inflammatory cells. In 30 per cent of animals focal necrosis in the ileum was observed. Similar changes were observed in the cecum and colon. The presence of acid fast bacilli was closely associated with the extent of epithelioid cell infiltration (Carrigan and Seaman, 1990).

Jubb et al. (1993) pointed out that the most characteristic change cf paratuberculosis in the intestine of goats was the infiltration of lymphocytes and plasma cells in the sub-mucosa.

Sub-clinical cases

Rajya and Singh (1961) and Paliwal and Rajya (1982) observed only very mild changes in sub-clinical cases of paratuberculosis in sheep and goat respectively with cellular infiltration of intestinal mucosa as the only change. No comparable lesions were recorded in early stages of infection.

Chodini et al. (1984) opined that it was very difficult to recognize sub-clinical paratuberculosis of goats histologically.

2.2.8.3 Lesions in mesenteric lymph nodes

2.2.8.3.1 Macroscopic lesions - Clinical cases

Necrosis, caseation and calcification were never seen in cattle, but were common in sheep and goat (Rankin, 1958a).

Rajya and Singh (1961) detected lesions in the mesenteric lymph nodes of all goats with clinical paratuberculosis. The lymph nodes were enlarged and a milky fluid oozed from the cut surface. Mesenteric lymph node of one sheep had nodular out growth, and a few mesenteric lymph nodes had caseous and calcified foci. The mesenteric lymph node of one sheep contained greenish matter.

Lal (1973) observed necrosis caseation and calcification in sheep and goats affected with clinical paratuberculosis.

The oedema and the enlargement of the lymph nodes were noticed in goats with clinical Johnes disease by Lenghaus et al. (1977). Many white foci, 1-2 mm in diameter were visible through the capsule.

Fodstad and Gunnarson (1979) considered the gross changes in the mesenteric lymph nodes to be non specific in goats.

Seaman and Gardiner (1981) found the mesenteric, lymph nodes of sheep with clinical paratuberculosis to be hyperplastic. Cortex had focal epithelioid granulomas some times with central calcification. Medulla was oedematous.

Ullrich et al. (1982) found the mesenteric lymph nodes of naturally infected goat to be oedematous and often with caseation and calcification.

Kulshrestha et al. (1984) found the mesenteric lymph nodes to be enlarged, soft and moist or juicy in consistency in sheep.

Chodini et al. (1984) found the mesenteric lymph nodes to be enlarged and oedematous, with little cortico-medullary distinction in sheep and goat. The lymphatics may be corded as a result of focal granulomas.

Krishna et al. (1989) and Carrigan and Seaman (1990) noticed pale prominent areas in the cortical region of mesenteric lymph nodes of goats with advanced paratuberculosis.

Jubb et al. (1993) pointed out instances of tubercles mineralizing and replacing much of the lymph node in sheep and goats.

Sub-clinical cases

Rajya and Singh (1961) found gross changes in mesenteric lymph nodes of sub-clinically affected sheep to be very mild, and many animals having no lesions at all. The mesenteric lymph nodes of some sheep were enlarged moderately and oedematous. The cut surface was moist in appearance. In rare occasions caseation and calcification were also observed.

Paliwal and Rajya (1982) found similar lesions in goats also. The lymph nodes were enlarged in most of the cases. The capsule was thickened in one instance.

2.2.0.3.2 Microscopic changes

Clinical cases

Rankin (1958a) recorded infiltration of epithelioid cells just below the capsule of the mesenteric lymph nodes in paratuberculous goats.

Rajya and Singh (1961) observed infiltration of epithelioid cells into cortical and medullary sinuses and presence of many acid-fast bacilli in sheep with clinical Johne's disease. Giant cells were also present. In the germinal centre there were marked accumulations of macrophages. Some of which had assumed giant size. In many cases the reactions were extremely diffuse with symplasma

stage markedly distinct. The reticular tissues were distended and oedematous. Lymphoid cells were exhausted and caseous and calcified foci were present. Such lesions varied from necrotic foci, surrounded by a zone of phagocytic cells with acid fast rods, to complete encapsulation of caseous and calcified foci which also had acid-fast rods.

Kluge et al. (1968) reported that the acid-fast bacilli transported to the lymph nodes were usually located in or adjacent to sub-capsular sinuses and peri trabecular sinuses of sheep with paratuberculosis. Ultimately upto 75 per cent of the greatly enlarged mesenteric lymph nodes contained phagocytic cells filled with acid-fast bacilli. Portions of many nodes had undergone liquefactive and caseous necrosis.

Lenghaus et al. (1977) observed in goats foci of epithelioid cells in the cortex of many mesenteric lymph nodes with many acid-fast bacili.

Ullrich et al. (1982) reported that the macrophages and varying number of lymphocytes may replace the usual architecture of the mesenteric lymph nodes. Granulomas were seen in lymph nodes draining the affected part of the digestive tract in goats.

Chodini et al. (1984) found caseation and less frequently calcification of granulomas in lymph nodes of sheep and goat.

Carrigan and Seaman (1990) could not find any giant cells or tubercle formations in paratuberculosis affected sheep. The infiltration was diffuse in 70 per cent of animals. Mesenteric lymph nodes were consistently hyperplastic with generalized lymphoid proliferation and infiltration of epithelioid cells into subcapsular sinuses. The appearance of calcified foci in goats were occasional, according to Singh et al. (1992).

Sub-clinical cases

Rajya and Singh (1961) observed the changes in sub-clinically affected sheep to be few. Moderate infiltration of macrophages into the germinal centres of mesenteric lymph nodes were seen. Sinusoids were free of significant changes. Acid-fast bacilli were seen in tissue sections of most of the lymph nodes.

Paliwal and Rajya (1982) found mild inflammatory reactions in mesenteric lymph nodes of goats with sub-clinical paratuberculosis.

2.2.9 Intra-dermal tests

2.2.9.1 History and development

Even before successful artificial cultivation of M. paratuberculosis, Bang (1909) observed that sub-cutaneous injection of 10 millilitre of 20 per cent heat concentrated synthetic medium (HCSM) avian tuberculin elicited a temperature reaction in animals affected with paratuberculosis.

Dunkin (1934) observed that intra-cutaneous injection 0.1 to 0.2 ml HCSM tuberculin produced a local oedema, hyperaemia and even a small necrotic spot at the site of injection by 48 hours. Two injections of 0.1-0.2 millilitre of HCSM into the same site at 24 hour and 48 hour interval were found identical in reactions as the single injection.

Glower (1941) prepared purified protein derivative (PPD) johnin which was found to give better results than the unpurified product.

Konst and McIntosh (1958) observed PPD johnin to be useful in allergic tests.

2.2.9.2 Immunological aspects

Smythe (1951) observed that the response of an animal to the intra-dermal injection had a direct relationship to the

nature of presence of the mycobacteriae in the bowel of the animal. In young, high reading indicated a recent infection, whereas in animals which cannot with stand the infection, the reading dropped down rapidly and the animal succumbed to the disease both in young and old animals.

Sigurdsson (1956) advocated consideration of other parameters such as redness and tenderness of the area of injection in addition to the increase in skin thickness for reading the results of the intra-dermal tests. It was found that heterologous antigens failed to elicit a reaction in the quantities as for homologous antigen, but did so if a sufficiently large dose was given.

Borodenok (1959) found the reactivity to intradermal injection of avian tuberculin development in sheep experimentally infected with M. paratuberculosis to develop 55-113 days after infection.

Meyn (1961) reported that use of a diluted tuberculin containing only 2,500 to 5,000 international units reduced the false positive reactions.

Larson et al. (1963) observed the cell wall fraction of mycobacteriae to produce hypersensitivity reaction in animals sensitized with heterologous antigen whereas the

protoplasmic fraction of the organism elicited reactions in animals sensitized with homologus antigen.

In an attempt to increase the specificity of the reaction a peptido-glycolipid (Pmko) from M. paratuberculosis was used by Ross et al. (1967) as the antigen and it gave different degrees of reaction in tuberculous and paratuberculous animals.

Merckal et al. (1968a) found that there is no correlation between numbers of organisms and degree of hypersensitivity. The intra-dermal test in general became positive as the necrotic foci were appearing in the tissues and became negative when only the debris remained in the tissues.

Lall (1973) pointed out that the failure of many workers to correlate the results of intra-dermal test with post mortem findings may be due to variation in the immune status of animal, tuberculous animal giving positive result, Johnin used being of low quality or lesions being minimal.

Karpinski and Zorawski (1975) found the sensitivity in sheep to develop by 8 weeks and last for 6 months.

Merckal et al. (1977) found the systemic temperature response to tuberculin was more specific than dermal temperature response but difficult to measure. Skin thickness

increase was found to be more specific than dermal temperature response.

Buergelt et al. (1978b) suggested that the reported lack of accuracy of various diagnostic tests may not be indicative of a defect in the test procedure, but a deficiency in encompassing the entire immunologic scope of the disease.

Lisle et al. (1980a) suggested considering sequential intra dermal test for classifying animals as reactors. The large number of false negative reactions may be due to the lack of sensitivity of the test or an absence of delayed type hypersensitivity to Johnin PPD in many animals infected with M. paratuberculosis.

Ketterer et al. (1981) observed that inoculation of calves with M. avium produced sensitivity and cross reactivity to bovine tuberculin by 8 weeks.

Patel et al. (1987) recommended 72 hours after injection as the optimal time for the maximum reaction to be observed in a single intra-dermal Johnin test among sheep.

A cell wall protein and a cell wall protein-peptido glycan complex were compared by Angus et al. (1989) as antigens for an intra-dermal test. But both were less potent and less specific than johnin PPD in cattle.

Blood et al. (1989) reported the maximum intra-dermal reaction to tuberculin to occur between 48-96 hours after the injection, with majority in 48-72 hours. It was also suggested that the intra-dermal test suffered from the minimal sensitivity of skin observed in many animals.

Srivastava and Kumar (1992) reported that ammonium sulphate precipitated protein from culture supernatent of M. bovis could be used with good efficiency for diagnosing tuberculosis.

Pavlas (1990) tried using ultra-sonicated antigens for intra-dermal tests and found them to give better results than Johnin PPD.

2.2.9.3 Single and double intra-dermal tests

Taylor (1951) stated that comparative tuberculin test was worthless as a diagnostic aid in paratuberculosis of cattle, sheep and goats.

Hole (1952) observed that allergic tests were accurate method of diagnosis in certain stages of infection, but they

appeared to be of little value when the disease was established or clinical.

Sigurdsson (1956) concluded that allergic tests was somewhat disappointing as tools of diagnosis in natural infections with M. johnei in cattle. Infected animals some times did not become positive until the disease was rather advanced, and they became negative again in the later stages of infection, and at least in some animal population, false positive reactions were common. He opined that the allergic tests were useful under certain circumstances, but too much reliance should not be placed on them.

Konst and McIntosh (1958) observed the intra-dermal test to be accurate for diagnosis in the early stages of infection.

Hole and Maclay (1959) found that reactions to allergic tests became most marked during the initial stages of infection, and became less regular as the disease became established. This type of test had a greater experimental than practical value.

Larsen and Vardaman (1959) compared intra-dermal tests with haemagglutination tests and a modified haemagglutination test. Fifteen per cent of cattle answered the intra-dermal test as positive, whereas 50 per cent answered the

haemagglutination test and 75 per cent answered the modified test. Intra-dermal test was the method of choice based on post mortem lesions. But many false negative reactions were also observed. They calculated that 85 per cent of animals reacted to the intra-dermal test before they were one year old and they lost the reactivity within 15 months of being positive.

Mukherji (1959) recommended intra-dermal johnin tests as suitable for diagnosis of paratuberculosis in India, under field conditions.

Belechev et al. (1961) found intra-dermal test to give many false positive reaction in unknown herds.

Pearson (1962) found that in clinical cases of paratuberculosis in cattle, skin tests were of not much value due to false positive reactions and non specificity. There was little difference between 48 h readings and 72 h readings.

Carrying out intra-dermal Johnin testing of 96 cattle, Larsen et al. (1963a) found the test to give 40 per cent false negative and 22 per cent false positive reactions. The sensitivity persisted for 6 month to 36 month in these animals. The animals with persisting sensitivity were most likely to develop the clinical disease later.

Gaggino and Carrille (1964) stated that out of 400 cattle tested intra-dermally with avian and bovine tuberculin, 4.5 per cent were positive only to tuberculosis, 17.25 per cent were positive only to avian tuberculin and 7.5 per cent were positive to both. They suggested cross reactivity between avian and bovine tuberculins.

Gilmour et al. (1965a) found that non-specific reactions limited the value of intra-dermal test. Many infected calves failed to react. They considered the test to be unreliable.

Clark (1969) stated that intra-dermal test in cattle was useful only as a herd test.

Merckal (1973) found the test to be non specific in cattle, since homologous reactions cannot be differentiated from heterologous reactions.

Lall (1973) reported a sensitivity of 89.1 per cent for intra-dermal test in cattle.

Julian (1975) recommended intra-dermal tests as the best test for the diagnosis of the disease in cattle, sheep and goat before clinical disease develops.

Lisle et al. (1980a) observed that intra-dermal tests, as they were currently used, were contra indicated as a routine diagnostic test for paratuberculosis. According to them upto 90 per cent false positive results were seen.

Kumar et al. (1982), working in sheep, found that the criterion of 2.0 mm or more increase in thickness was best to be considered as positive reactions to avian tuberculin whereas 2.5 mm increase was best for johnin.

Chodini et al. (1984) stated that the intra-dermal test had the added advantage of being a field test for which the veterinarians were trained. But they found that in any infected herd, about 50 per cent of the non-infected animals responded and 50 per cent of infected animals did not respond.

Paliwal et al. (1984a) conducted intra-dermal johnin test in 423 sheep with both clinical and pre-clinical diseases. They obtained 29 per cent false positive and 5 per cent false negative results.

Paliwal et al. (1984b) observed that intra-dermal tests appeared to be the least reliable in both clinical and pre-clinical paratuberculosis of goats also.

Kulshrestha et al. (1984) found the allergic test to give negative results in majority of infected sheep.

Paliwal et al. (1985b) found that intra-dermal johnin test in cattle failed to detect some of the preclinical and most of the clinical cases of johne's disease.

Nain et al. (1985) found that the single inter-dermal test had 100 per cent specificity in diagnosis of JD in cattle.

Thorel et al. (1985) found the hypensensitivity reactions in cattle affected with paratuberculosis developed earlier than humoral response.

Zhi et al. (1986) found that in preclinical Johne's disease of cattle, allergic Johnin test was more sensitive than complement fixation test, based on confirmation of infection by fecal culture.

Patel et al. (1987) subjected 421 sheep to single intra-dermal test and read the results in 72 hours after injection. They found the test to be reliable in screening the animals. But some false negative reactions also occurred, probably because the animals were asymptomatic carriers.

2.2.9.4 Stormont test

Kerr et al. (1946b) developed the stormont test for the diagnosis of tuberculosis in cattle. The test exploited the increased sensitivity at the site of injection at the

seventh day optimally. The test proved to be very efficient and had an error value of only 1.6 per cent as against 12-15 per cent by the single intra-dermal and comparative tests.

Lembrechts (1957) found the seven day interval to be more significant. The stormont test was very efficient compared to the single intra-dermal test and differentiated reactions between tuberculous and paratuberculous animals.

Pearson et al. (1962) applied the test for the diagnosis of paratuberculosis in cattle and obtained good results. But they found the additional 5 days and 3 visits as cumbersome.

Popluhar and Vritiak (1964) found the test useful to detect more reactors than single intra-dermal test and recommended to take readings 48 hours after second injection.

Kalra et al. (1984) found the Stormont test to be more sensitive than single and double intra-dermal test for diagnosing Johnes disease in cattle.

Blood et al. (1989) commented extensively on the test. The increased sensitivity of the test was, according to them, due to the attraction of antibody to the site after the first injection. It began by the 5th day in cattle, and was at its peak by the 7th day and ended by the 12th day after injection.

2.2.9.5 Multiple intra-dermal injections

Tsellarius (1971) advocated use of triple intra-dermal Johnin PPD injections to further increase the sensitivity of intra dermal tests.

Craciunescu et al. (1982) found that the specificity of intra-dermal test for the diagnosis of paratuberculosis in cattle was enhanced by giving a second injection to all animals positive to the first injection 72 hours later. Then all animals positive to the second injection were given a third injection 48 hours later and only those animals which were positive to the last injection were considered infected.

2.2.10 Intra-venous Johnin test

Taylor (1951) stated that intravenous Johnin test gave results which were as good as any other test in cattle for the diagnosis of paratuberculosis.

Sigurdsson (1956) described a serious drawback of intra-venous test. It could not be predicted exactly at what time the hyperthermia will occur.

Larsen and Kopecky (1965) found the test to give better results than intra-dermal test and complement fixation test in both clinical as well as sub-clinical cases of

paratuberculosis in cattle. The test was found to suffer from poor sensitivity and specificity.

Allen et al. (1967) found out that the intra-venous Johnin test had only poor levels of sensitivity and specificity.

Larsen (1973) recommended use of fecal culture for confirmation of results of intra-venous test in cattle affected with paratuberculosis.

Merckal (1973), Owen and Thoen (1983), Benedictus and Basma (1985) and Kormendy et al. (1990) found the intra-venous Johnin test to be useful for the diagnosis of paratuberculosis in cattle.

2.2.12 Other diagnostic tests

Merckal et al. (1968b) found that the clinically acceptable titres of 1:32 was difficult to achieve by the haemagglutination test for the diagnosis of bovine paratuberculosis.

Aalund (1971) proposed a bovine leukocyte migration inhibition test for the diagnosis of paratuberculosis. It was

possible to diagnose between Johne's disease and tuberculosis in cattle by this test.

Alhaji et al. (1974) found that peripheral lymphocytes were stimulated by as much as 15 fold by homologous PPD whereas heterologus PPD caused little or no stimulation.

Wallace et al. (1971) reported that floculation of bentonite particles sensitized with old tuberculin and a purified carbohydrate of BCG could be used to differentiate between Johne's disease and tuberculosis.

Benedixen (1977a) observed that a leukocyte migration agarose test could be used for diagnosis of Johne's disease in cattle.

Johnson et al. (1977) observed lymphocyte immuno-stimulation detected more animals than those by fecal culture.

Buergelt et al. (1978a) found a stimulation index of $\geq$2.0 for Johnin PPD in lymphocyte transformation test.

Buergelt et al. (1978c) found that Johnin PPD was better than the avian and mammalian PPD for stimulation of peripheral lymphocytes to diagnose cattle with sub-clinical Johne's disease.

Pemberten (1979) described the technique of mesenteric lymph node biopsy for the diagnosis of paratuberculosis in

cattle, and found it to be in complete agreement with later histopathology of gastro intestinal tract and lymphnodes in 100 clinical cases of Johne's disease in cattle.

Williams et al. (1985) found lymphoblastogenesis tests with peripheral blood mononuclear cells to be having 72 per cent sensitivity and 100 per cent specificity for diagnosis of paratuberculosis in domestic sheep.

Benedictus and Hagasa (1986) found that lymphnode biopsy detected infection in 29 cases out of 223 cattle, where the complement fixation test, intra-dermal test and microscopical examination of feces failed.

Yang and Duan (1987) found the single radial haemolytic test, direct haemolytic test and ELISA to be more sensitive than complement fixation test for the diagnosis of paratuberculosis in cattle. They recommended all three tests as suitable for use in a large scale.

Collins et al. (1989) found that a DNA probe detected M. paratuberculosis from all four species of ruminants and had complete lack of cross reactivity against M. avium.

Hurley et al. (1989) used a DNA probe to detect M. paratuberculosis in clinical Johnes disease of cattle. It detected very small numbers of bacteria and so was more efficient than fecal culture. It took 72 hours to complete.

But the test could not differentiate between M. avium and M. paratuberculosis.

Whipple et al. (1989) found that all but one of 30 isolates from clinical paratuberculosis of cattle, sheep and goat, were identical with the reference strain ATCC 19698. They suggested that due to the genetic similarity, it may be possible to develop a specific diagnostic probe for M. paratuberculosis.

Whitehead (1989) found an epitope specific to M. paratuberculosis at 65000 M.W. region.

Wood et al. (1989) found that an in vitro cellular assay for interferon can detect antigen specific cellular response in white blood culture from cattle with Johne's disease. The test was equally, or more sensitive than fecal culture.

Collins et al. (1990) found that use of a commercial radiometric medium, BACTEC 12 B, in which filter concentrated decontaminated fecal specimens were placed, was superior to conventional methods of fecal culture for diagnosis of paratuberculosis.

Smith (1990) proposed lymphnode biopsy as the method

of choice in cattle, sheep and goats for the diagnosis of paratuberculosis.

Giessen et al. (1992) found that tests using Polymerase Chain Reaction primers and a DNA probe (169, RNA test and 15900 test) were more specific than fecal culture but sensitivity varied from 3 to 23 per cent only.

2.2.12 Serum magnesium levels

Negi et al. (1963) estimated the serum magnesium levels of 26 goats which were positive to the double intra-dermal test by heat concentrated synthetic medium (HCSM) Johnin and found that the values (2.6 to 2.9 mg/dl) did not significantly differ from the serum magnesium values (2.2 to 2.75) of test negative goats.

Patterson et al. (1968) opined that biochemical changes in paratuberculosis affected animals were not specific enough to be utilized for diagnosis.

Shobhanan (1981) found the serum magnesium levels of goats from the Kerala Agricultural University Goat Farm to be ranging from 2 to 2.2 mg/dl.

Reddy et al. (1982) reported a significant reduction in calcium, phosphorus and magnesium levels of sheep with Johne's disease. Serum magnesium level was 1.85 mg/dl.

Zahinuddin and Sinha (1984) reported low levels of serum sodium and potassium while the levels of other minerals including magnesium was normal in paratuberculosis affected goats.

Materials and Methods

MATERIALS AND METHODS

The present study was conducted in the department of Preventive Medicine, College of Veterinary and Animal Sciences, Mannuthy, Trichur during 1991-94.

3.1 Materials

3.1.1 Experimental animals

One hundred and fifty Malabari crossbred goats belonging to the age group of one to five years, of either sex, were selected at random from the Kerala Agricultural University Goat Farm, Mannuthy, Trichur Dt., for the present study. The animals were born and reared in the farm and were showing no apparent clinical symptoms of Paratuberculosis. All the animals were subjected to detailed clinical examination before the start of the experiment. Any animal showing signs of diseases, as well as animals in gestation for more than three months were not included for the study. Animals with characteristic clinical symptoms of Paratuberculosis like unthriftiness, emaciation and diarrhoea were also not included.

One month before the start of the experiment, all animals were dewormed using Albendazole, at the dose of

10 mg/kg body weight, and one week later by Niclosamide, at the dose of 75 mg/kg body weight. The feeding and management adopted were as per the schedule of the Kerala Agricultural University Goat Farm.

3.1.2 Stains and reagents

3.1.2.1 Acid-alcohol

Concentrated hydrochloric acid (35.4 per cent)	- 3 ml
Industrial methylated spirit	- 97 ml

Seven ml of industrial methylated spirit was added to three millilitre of acid, and volume made upto 100 ml by using methylated spirit.

3.1.2.2 Concentrated carbol fuchsin

Basic fuchsin (BDH Pharma)	- 1.0 g
Absolute alcohol	- 10 ml
5% phenol in distilled water	- 100 ml

The basic fuchsin was dissolved in 10 ml absolute alcohol in a mortar and pestle. Then five per cent phenol in distilled water was added, mixed and sieved through a muslin cloth. It was kept in a glass stoppered bottle for a week.

3.1.2.3 Eosin

Eosin powder (water soluble) (RIENDEL)	- 250 mg
Distilled water	- 125 ml
Glacial acetic acid (MERCK)	- 0.5 ml

Eosin was dissolved in 10 ml of distilled water by a mortar and pestle. Volume was made upto 125 ml using distilled water. Glacial acetic acid was added drop by drop. The solution was sieved through muslin cloth and used. The stain was prepared as and when required.

3.1.2.4 Harris haematoxylin

Prepared from two solutions

Solution A

Haematoxylin (E MERCK)	- 600 mg
Absolute alcohol	- 6 ml

Haematoxylin was dissolved in absolute alcohol in a mortar and pestle.

Solution B

Ammonium alum (MERCK)	- 12.0 g
Distilled water	- 120 ml

The two ingredients of solution B were mixed and boiled. While solution B was boiling solution A was added to it quickly and brought to boiling temperature and removed from heat. The flask was then put in cold water and three grams of red mercuric oxide was added part by part. When the solution cooled down five millilitre of glacial acetic acid was added and kept for a week before use.

3.1.2.5 Johnin PPD

Purified protein derivative (PPD) of Johnin was obtained from IVRI, Izatnagar, UP, in vials of 10 doses.

3.1.2.6 Malachite green

Malachite green (Glaxo)	- 2 g
Distilled water	- 100 ml

The powder was dissolved in 10 millilitre of distilled water using a mortar and pestle, and volume was made upto 100 ml using distilled water. It was sieved through a muslin cloth and kept.

3.1.2.7 Methylene blue

Methylene blue (Merck)	- 2 g
Distilled water	- 100 ml

The stain was dissolved in 10 millilitre of distilled water using a mortar and pestle. Volume was made upto 100 ml using distilled water and sieved through a muslin cloth.

3.1.2.8 Serum

Five millilitre of venous blood was directly collected by jugular puncture one week after intra-dermal tests into sterile test tubes and serum was separated. Hundred fold dilutions of the serum was made using de-ionized distilled water (0.1 millilitre serum in 10 millilitre of de-ionized distilled water).

3.1.2.9 Standard solutions of magnesium

A 1000 ppm magnesium standard solution was prepared by dissolving 1.13 gram of magnesium heptahydrate ($MgSO_4$ $7H_2O$) in 1 litre of de-ionized distilled water. The working standards of 1 ppm and 0.5 ppm were prepared by dilution of the 1000 ppm standard.

3.1.3 Equipments

3.1.3.1 Atomic Absorption spectrophotometer - USA
(PERKIN-ELMER - 2380)

The instrument was standardized using 1 ppm and 0.5 ppm standards. The setting was as follows.

Wave length	-	285.2μ
Slit	-	0.7
Flame	-	AAc
Linear range	-	0.5

3.1.3.2 Calipers - A vernier "Pincer" type of calipers was used

3.2 Methods

3.2.1 Experimental design

The one hundred and fifty experimental goats were divided into three groups of 50 each in a random manner as Group I, Group II and Group III.

The group I animals were subjected to the single intra-dermal Johnin test. The site was prepared and 0.1 millilitre of Johnin PPD was injected intra-dermally. The thickness of the skin at the site of injection was measured using calipers, immediately before and 24, 48 and 72 hours after the injection.

Group II animals were subjected to the modified intra-dermal Johnin test I (MID-I). The first injection was given as in the group I animals. Three days later a second

injection of 0.1 millilitre PPD Johnin was given intra-dermally exactly on the same site of the first injection. Thickness of the skin at the area was measured immediately before and 24 hours after the second injection. The single intra-dermal test was also conducted along with MID-I among the animals of this group due to the similarity between procedures for the SID and MID-I tests.

Group III animals were subjected to modified intra-dermal Johnin test II (MID-II). Here also the first injection was done as in the case of group I animals. The second injection was done as in the case of group II animals, but five days after the first injection. The thickness of the skin was measured immediately before and 24 h after the second injection. In this group also single intra-dermal test was conducted along with the MID-II due to the similarity in procedures of the two tests.

Fecal samples from all 150 animals were screened for the presence of Mycobacterium paratuberculosis. Rectal pinch smears from all animals were prepared and examined for the presence of mycobacteriae.

Serum magnesium levels of all the positive as well as an equal number of negative animals from each group were estimated.

Six animals which gave positive result to the test and two animals which gave negative result to the test were slaughtered from each group, and subjected to histopathological study to ascertain the infection.

The results of the three tests were statistically compared.

A detailed description of the methodology follows.

3.2.2 Direct microscopical examination of feces

3.2.2.1 Conventional technique

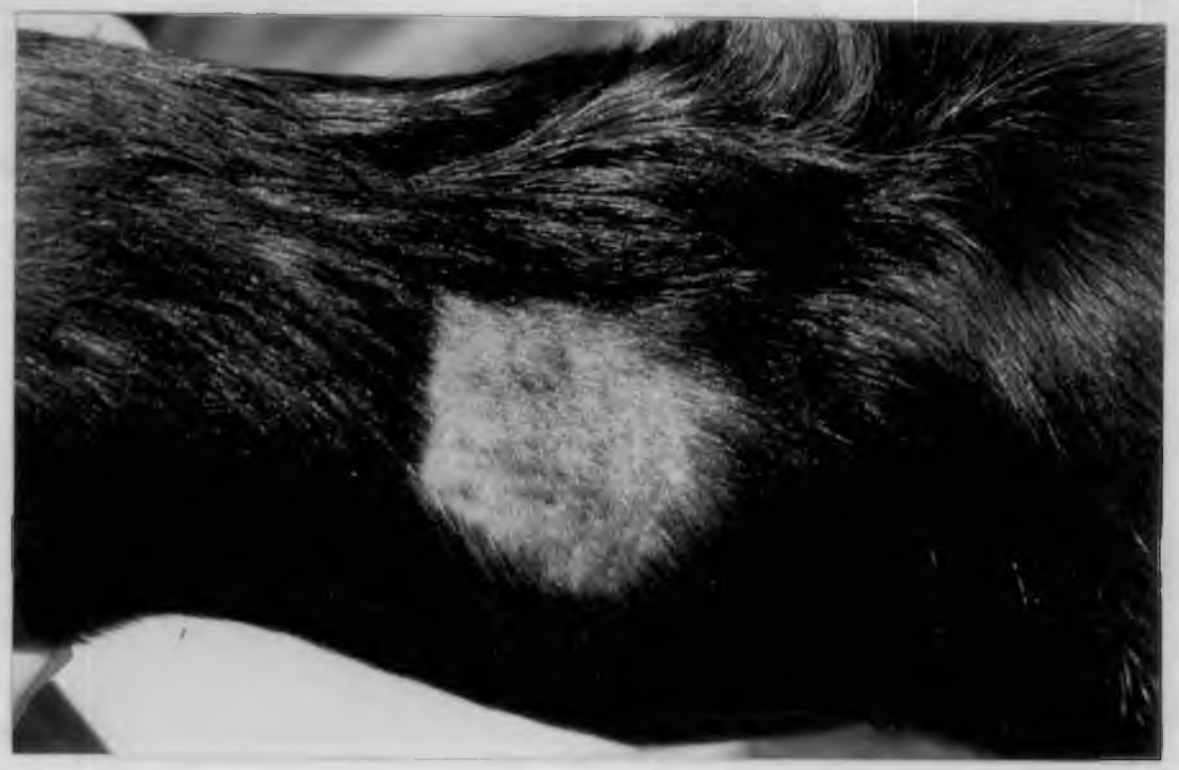
The method of Paliwal et al. (1984a) was followed with slight modifications. Fecal samples from all the one hundred and fifty animals were directly collected from the rectum. Two to three pellets were put in sterile glass vials. Each sample was mixed with 10 ml of normal saline in a mortar and pestle, and the mixture was sieved into test tubes. The fecal suspensions were kept undisturbed overnight and supernatants were collected into sterile test tubes in the morning. These were subjected to centrifugation at 2,000 rpm for five minutes. The sediment of each sample was suspended in one millilitre of normal saline and thick smears of these were made on glass slides. The slides were dried in air, heat fixed and examined for M. paratuberculosis by Ziehl-Neelsen's

Plate I Site immediately after intra-dermal injection

Pea-shaped nodule at the site of entry of Johnin

Plate II Negative reaction at the site 48 h after intra-dermal injection

Absence of inflammatory reaction



clipped, shaved and washed. The next day, the area was examined for the presence of inflammatory reactions. If any reaction was present, the another site was prepared on the other side of the neck. The tests were done three days after preparation of the site.

Measurement of skin thickness was arrived at by taking three consecutive readings for each measurement and taking the average value into account.

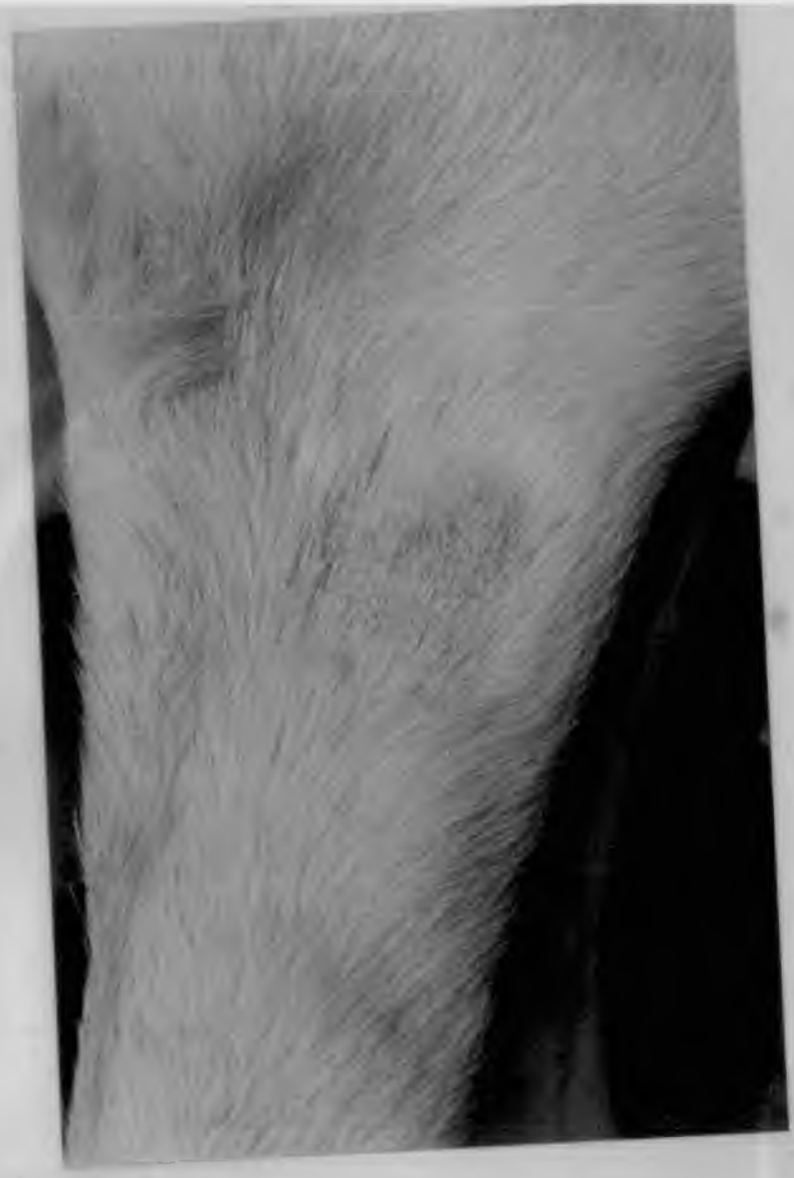
A fold of skin in the middle of the area was held using left fore-finger and thumb. The tuberculin needle was inserted parallel to the skin, with care taken to ensure that it doesnot pierce into deeper layers. The Johnin PPD (0.1 ml) was injected slowly. Accuracy of the technique was ascertained by the necessity of very high pressure for injection and the development of a pealike nodule at the site (Plate I). The nodule was circumscribed by indelible ink. Care was taken to prevent the animal from rubbing the area of injection against any solid objects.

Detection of positive cases was done by the measurement of skin thickness of the area at stipulated periods (Plates III-VI). An increase in thickness of 3 mm or more was considered as a positive result. Inflammatory reaction in the area, like temperature, oedema and hyperaemia

Plate III Measurement of skin thickness immediately before intra-dermal injection

Plate IV Positive reaction at the site 48 h after intra- dermal injection (in the same animal)

Inflammatory oedema and erythema at the site



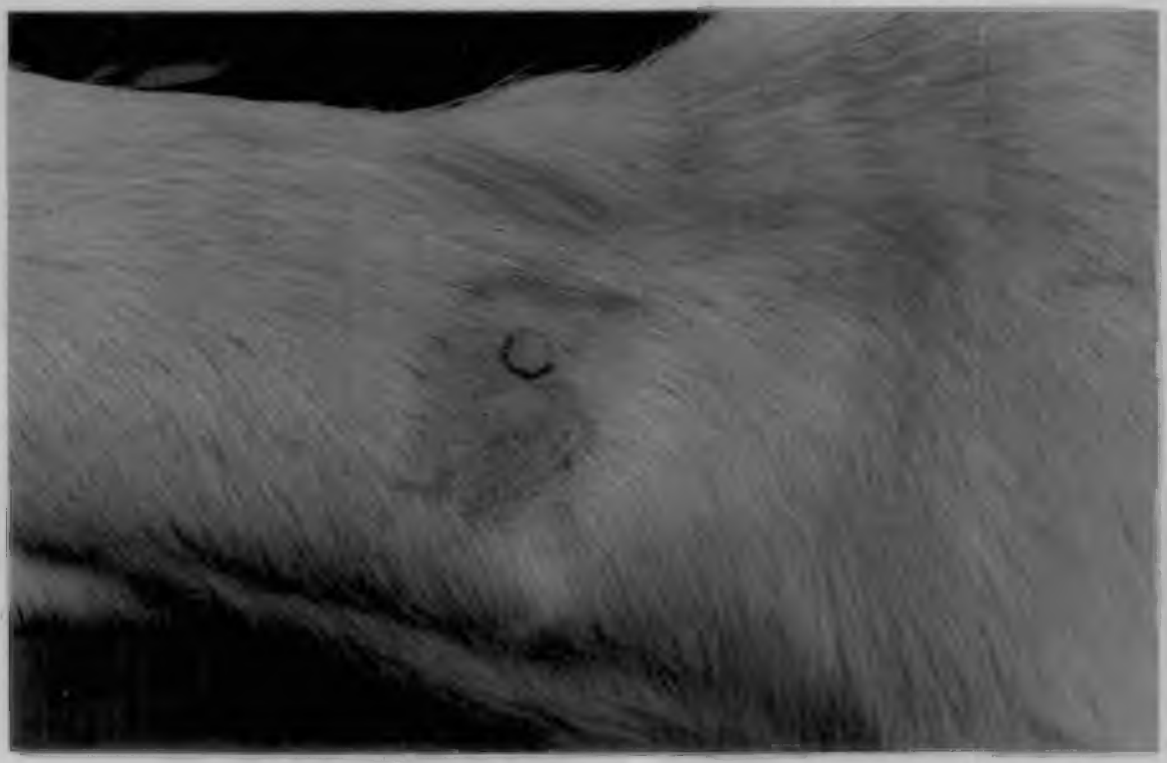


Plate V Site immediately after the second intra-dermal injection (in the same animal)

Plate VI Measurement of positive reaction 24 h after the second intra-dermal injection (in the same animal)

Intense inflammatory oedema and erythema

were also taken into account while classifying the result as positive or negative (Plate II).

3.2.5 Estimation of serum magnesium

Serum magnesium level was estimated as per the method of hollow magnesium-cathode tubers as per the instructions in the users manual of PERKIN-ELMER-2380 atomic absorption spectro-photometer.

3.2.6 Slaughter of animals and collection of materials

Six goats which gave positive results and two goats which gave negative results were slaughtered from each group. The animals were slaughtered within two weeks of intra-dermal tests in all cases. The ileum, jejunum, caecum, colon, rectum and mesenteric lymph nodes were subjected to detailed gross examination. Scrapings of the mucosa of the ileum, ileocaecal junction, caecum, colon and rectum were made into smears and examined for the presence of M. paratuberculosis, by Ziehl-Neelsen's technique (Kelly, 1964). Impression smears of all the mesenteric lymph nodes were subjected to acid-fast staining and examined for the presence of M. paratuberculosis. Small pieces of ileum, ileo-caecal junction, caecum and colon, along with all the mesenteric lymphnodes were collected in 10 per cent formalin for histopathological examination.

3.2.7 Histopathological examination

The tissues collected were fixed in 10 per cent formalin and embedded in paraffin as per the standard techniques (Sheehan and Hrapchak, 1980). Tissue sections were then cut at five microns thickness and subjected to Ziehl-Neelsen's acid-fast staining (Kelly, 1964) and haematoxylin-eosin staining (Sheehan and Hrapchak , 1980).

3.2.8 Analysis of data

The specificity, sensitivity and predictive values of results for each test were calculated as follows (Smith and Easman, 1990).

$$\text{Specificity} = \frac{\text{Number of animals who did not have the infection and gave a negative result}}{\text{Total number of animals not infected}}$$

$$\text{Sensitivity} = \frac{\text{Number of animals who had the infection and gave a positive result}}{\text{Total number of infected animals}}$$

$$\text{Predictive value of positive result} = \frac{\text{Number of animals who had the infection and gave a positive result}}{\text{Total number of animals who gave a positive result}}$$

$$\text{Predictive value of a negative result} = \frac{\text{Number who did not have the infection and gave a negative result}}{\text{Total number of animals who gave a negative result}}$$

For calculating the specificity, sensitivity and predictive values of results for SID test, data from all the 24 slaughtered animals belonging to the three groups were taken into consideration, since SID was done to all animals in all groups. In the case of MID-I and MID-II, data from eight slaughtered animals in the respective groups were taken since modified tests were done to the animals in those groups only.

Statistical analysis of the data was done as per the standard techniques of Snedechor and Cochran (1967).

Results

RESULTS

4.1 Group I Animals

4.1.1 Detection of M. paratuberculosis in fecal sample and rectal pinch

Fecal sample from all the fifty animals were negative for the presence of M. paratuberculosis both by the conventional as well as the modified techniques for fecal sample examination. The smears of rectal pinch were also negative for the presence of M. paratuberculosis in all cases.

4.1.2 Reactions to the single intra-dermal (SID) Johnin injection

The concised results of SID test are given in Table 1. Eleven of the 50 animals tested had positive reactions. The increase in skin thickness of group I animals are given in Fig.1. Measurement of skin thickness for each animal is given in Appendix-I.

Among the positive animals, the proportion of animals having an increase in skin thickness of 3 mm, 4 mm and 5 mm are shown in Fig.2. More than half the number (54.5 per cent) of total positive animals had an increase of 3 mm, followed by animals with 4 mm increase in thickness (27.3 per cent) and

lastly the animals with 5 mm increase in thickness (18.2 per cent). The inflammatory reactions were moderate and manifested by oedema and resentment on palpation of the area.

4.1.3 Post mortem findings

Six animals were randomly selected for slaughter based on the positive results of Johnin test. The results are given in Table 2A. Three of the six animals were infected with M. paratuberculosis whereas the other three were negative for the presence of the organisms, showing a false positive reaction. The results of the histopathological examination of each tissue are given in Table 3.

Two animals which were negative to the test were also slaughtered. One of them was negative for the presence of M. paratuberculosis in it's tissues whereas the other had typical clumps of the organism in mesenteric lymph nodes (Table 2B).

Analysis of the results of the test are given in Table 8. The test had very low level of sensitivity, specificity and predictive values of positive and negative results.

4.1.4 Serum magnesium levels

Serum magnesium values of 22 goats from group I are given in Appendix-II and compared on Fig.3.

The mean values of serum magnesium from 22 animals are shown in Table 4. A very wide variation between the values was noticed. All values except three were towards the lower side of the normal range as evident from Fig.3. No significant variation was noticed between the serum magnesium values of test positive and test negative animals as given in Table 7.

4.2 Group II Animals

4.2.1 Detection of M. paratuberculosis in fecal samples and rectal pinch

M. paratuberculosis was not detected in fecal samples of any of the fifty goats either by conventional or modified techniques for examination of fecal samples. Examination of smears of rectal mucosa also gave negative results.

4.2.2 Reactions to the modified intra-dermal Johnin test No.1 (MID-I)

The results of MID-I and the SID conducted along with it are given in Appendix-III. The concised results are shown

in Table 1. The increase in skin thickness of all animals after SID and MID-I are shown in Fig.4. Eight animals answered the MID-I test as positive. In all eight animals the positive reactions were clearly evident on account of the prominent inflammatory signs at the site of injection like oedema, hyperthermia and pain on palpation. The increase in skin thickness was 3 mm in 62.5 per cent and 4 mm in the rest of positive animals (Fig.5).

In the case of SID test, 12 animals answered the test as positive. Increase in thickness of skin in positive cases were 6 mm for 8.3 per cent, 5 mm for 16.7 per cent, 4 mm for 25 per cent and 3 mm for 50 per cent of positive animals, as evident from Fig.6.

Four animals were positive to both SID and MID-I. The remaining four animals positive to MID-I gave negative results to SID. Seven animals were positive to SID which gave negative results to MID-I. The severity of reactions also varied between the two tests with positive reactions to MID-I being comparatively intensive and severe.

4.2.3 Post mortem findings

Six animals were randomly selected for slaughter based on the positive reaction to the MID-I. The results of M. paratuberculosis infection in the animals are shown in

Table 2A. Results of detailed examination of tissues of the animals are shown in Table 5. Five of the six animals slaughtered, were having M. paratuberculosis infection. Two animals which were negative to MID-I were also slaughtered and examined for the presence of infection. Both animals were negative for the presence of M. paratuberculosis in their tissues (Table 2B).

Analysis of the results of the test are given in Table 8. The test had 100 per cent sensitivity and predictive value for negative results with high values for specificity and predictive value of a positive result.

4.2.4 Serum magnesium levels

Serum magnesium levels of 16 animals of group II are given in Appendix-IV and the results of analysis are given in Table 4. The mean serum magnesium values are shown in Fig.7. The values tended to be on the higher side of the average, but within the normal range in most of the cases. No significant difference was found in serum magnesium values of test-positive and test-negative animals as evident from Table 4.

4.3 Group III Animals

4.3.1 Detection of M. paratuberculosis in fecal sample and rectal pinch

Only one animal had the presence of typical clumps of M. paratuberculosis in feces (Plate VII) as detected by both the conventional and modified techniques for fecal examination. The modified technique did increase the ease of detecting the organism in feces. Examination of rectal pinch revealed only negative results in all the fifty cases.

4.3.2 Reactions to the modified intra-dermal Johnin test No.2 (MID-II)

The results of the MID-II test as well as the SID test conducted along with it are given in Appendix-V. The concised results are shown in Table 1. Seven animals answered the test as positive. The increase in skin thickness after both MID-II and SID are shown in Fig.8.

The comparative increase in thickness of skin in positive animals to both the tests are shown in Fig. 9 and 10. The MID-II had 66.6 per cent of positive results with an increase of 3 mm and 16.7 per cent of positive with increase of 4 mm and 5 mm each. All positive cases of SID were with 3 mm increase.

differences between the serum magnesium levels of test-positive and test-negative animals were not significant, as evident from Table 7.

4.4 Statistical analysis of the data

The specificity, sensitivity and predictive values of results for the three tests are given in Table 8. Statistical analysis by Chi-square technique revealed that no significant difference existed between the three tests if each of the four parameters were taken individually, as evident from Table 9. But if all the four parameters were considered together, there was significant difference between the three tests at 5 per cent level.

4.5 Post mortem findings of *M. paratuberculosis* infected animals

The infected animals presented only mild to moderate inflammatory changes typical of the disease.

4.5.1 Gross changes

Macroscopic changes were absent in the ileum, ileo cecal junction, jejunum, colon, caecum and rectum of nine of the twelve infected animals. The intestinal mucosa was thickened in three animals.

Plate VII *M. paratuberculosis* as typical clumps in fecal sample

Acid-fast bacilli arranged in clumps (Ziehl-Neelsen's staining. 100 x 10)

Plate VIII Gross appearance of mesenteric lymph nodes from goats having paratuberculosis

Oedema and enlargement of the lymph nodes

Plate IX Cross section of mesenteric lymph nodes from goats having paratuberculosis

Dark coloured medulla invading into the cortex

Plate X Mesenteric lymph node with numerous sinusoids from goat having M. paratuberculosis infection

Mesenteric lymphnodes of all twelve animals which harboured the bacilli (Plates XIX and XX) had typical gross changes in varying degrees. The lymph nodes were enlarged to 3-4 times the normal size in some animals (Plate VIII). They were very soft on palpation and upon sectioning of such lymph nodes an excess quantity of water was noticed in them. The medulla in many were dark in colour and encroaching into the cortex (Plate IX). Lymph node from one animal had numerous dilated lymphatic sinusoids (Plate X).

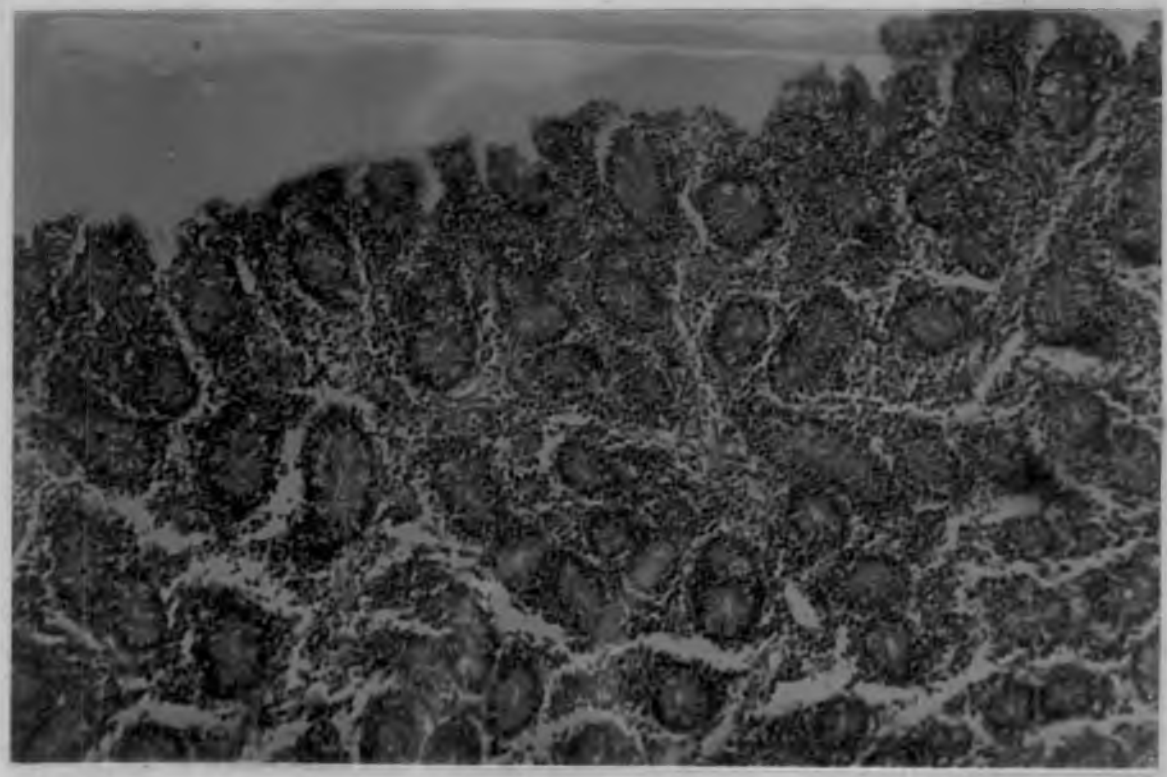
4.5.2 Histopathological changes

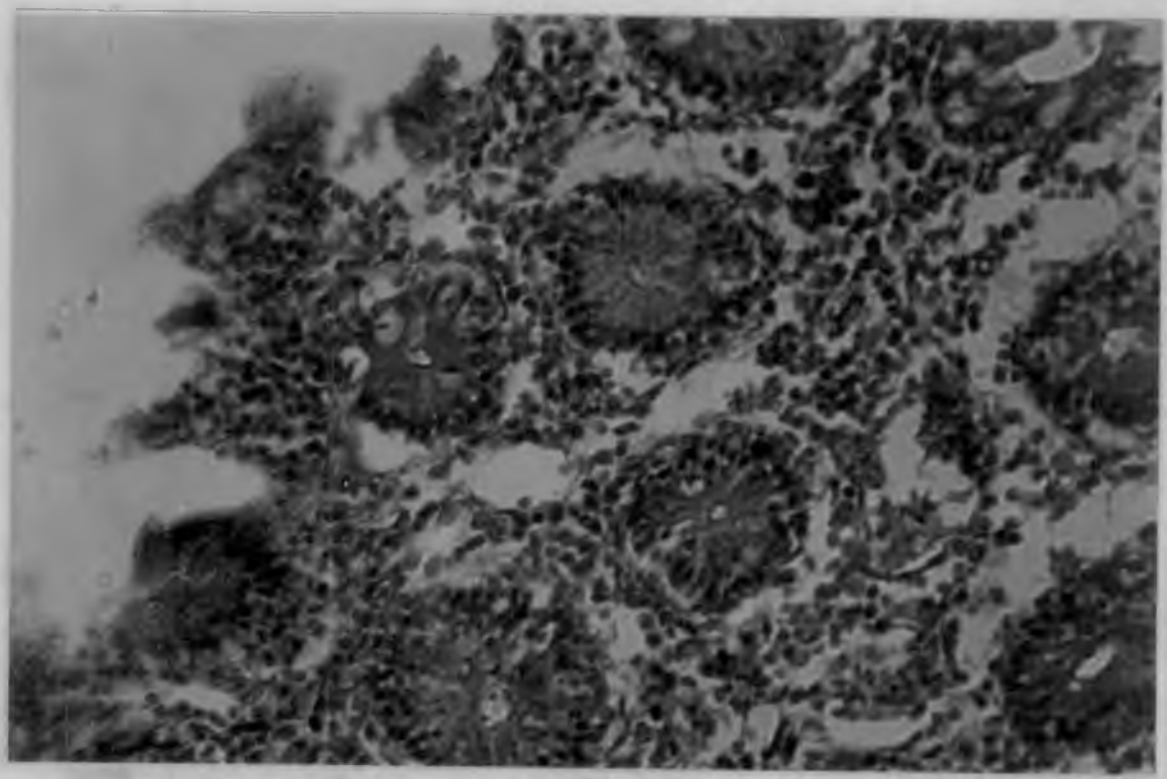
Chronic inflammatory reaction was observed in most of the mesenteric lymphnodes and some of the intestinal tissues. The lymph nodes presented some advanced stages of reaction whereas the intestinal tissue had only moderate changes. The typical lesions noticed are given below.

Ileocaecal junction- Proliferative enteritis characterised by moderate thickening of villi and heavy infiltration of epithelioid cells and macrophages (Plates XI an XII) was seen. There was extensive destruction of glands associated with infiltration of macrophages and epithelioid cells and a few eosinophils. In some cases focal degeneration of glandular epithelium was also noticed.

Plates XI-XII Sections of ileo-caecal junction from a goat with Paratuberculosis

Moderate thickening of villi. Heavy infiltration of epithelioid cells and macrophages. Destruction of glands (H & E staining 10×10 , 45×10).





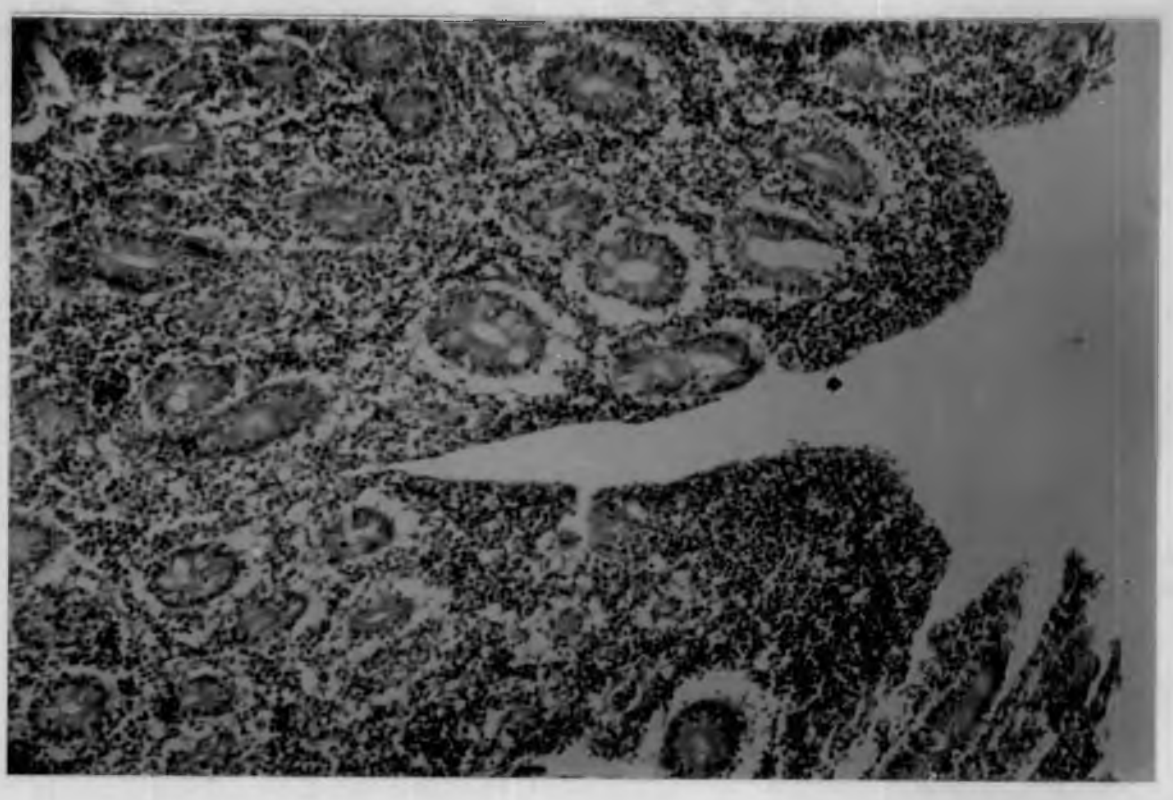
Caecum - Chronic inflammatory changes associated with diffuse glandular necrosis, predominance of lymphocytes and plasma cells were evident (Plate XIII and XIV). The submucosa and muscular layer were also infiltrated with inflammatory cells. Focal glandular atrophy was also noticed.

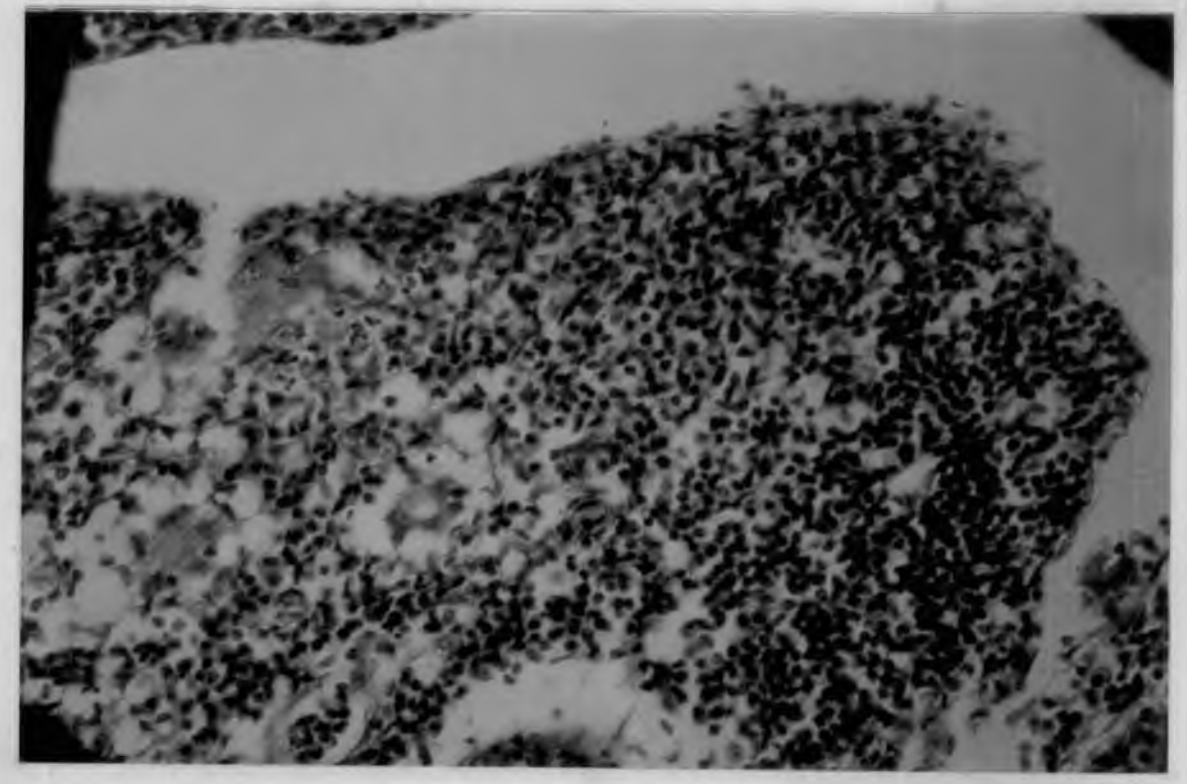
Colon - Proliferative enteritis was evident. Focal necrosis, disappearance of glands, infiltration of lymphocytes and mononuclear cells were evident. In focal areas nodular accumulation of lymphocytes and macrophages were observed in the mucosa. Some of the glands were atrophied (Plates XV and XVI).

Mesenteric lymphnodes - Proliferative lymphadenitis. There was diffuse cortical necrosis. The cortex was densely infiltrated by sheets of epithelioid cells. These cells were seen extending as cords of cells into the medullary area. Numerous plasma cells were noticed in the medullary sinus. Focal areas of necrosis were common. Occasionally bigger homogenous areas of necrosis were seen. Such areas were seen invaded by macrophages and plasma cells (Plates XVII and XVIII).

Plates XIII-XIV Sections of caecum from a goat with paratuberculosis

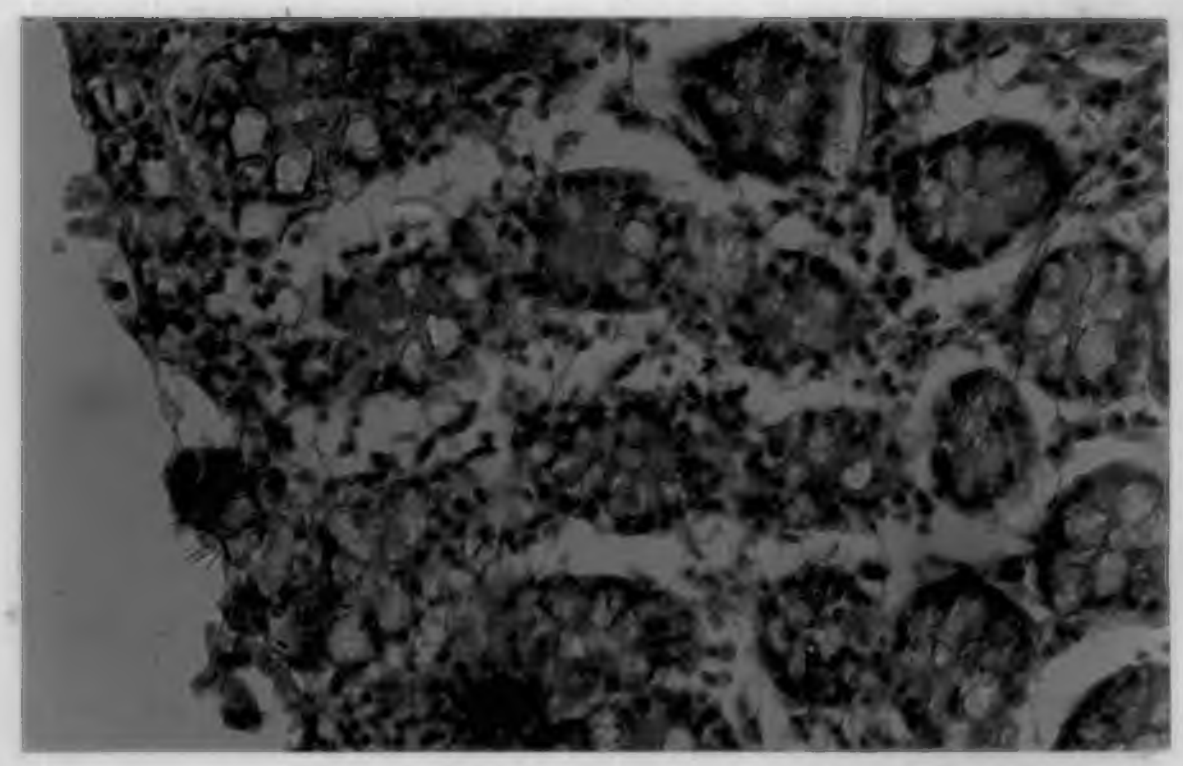
Diffused glandular necrosis. Predominance of lymphocytes and plasma cells (H & E staining 10×10, 25×10).





Plates XV-XVI Sections of colon from a goat with paratuberculosis

Focal necrosis. Infiltration of lymphocytes and mononuclear cells (H & E staining 25×10, 45×10).

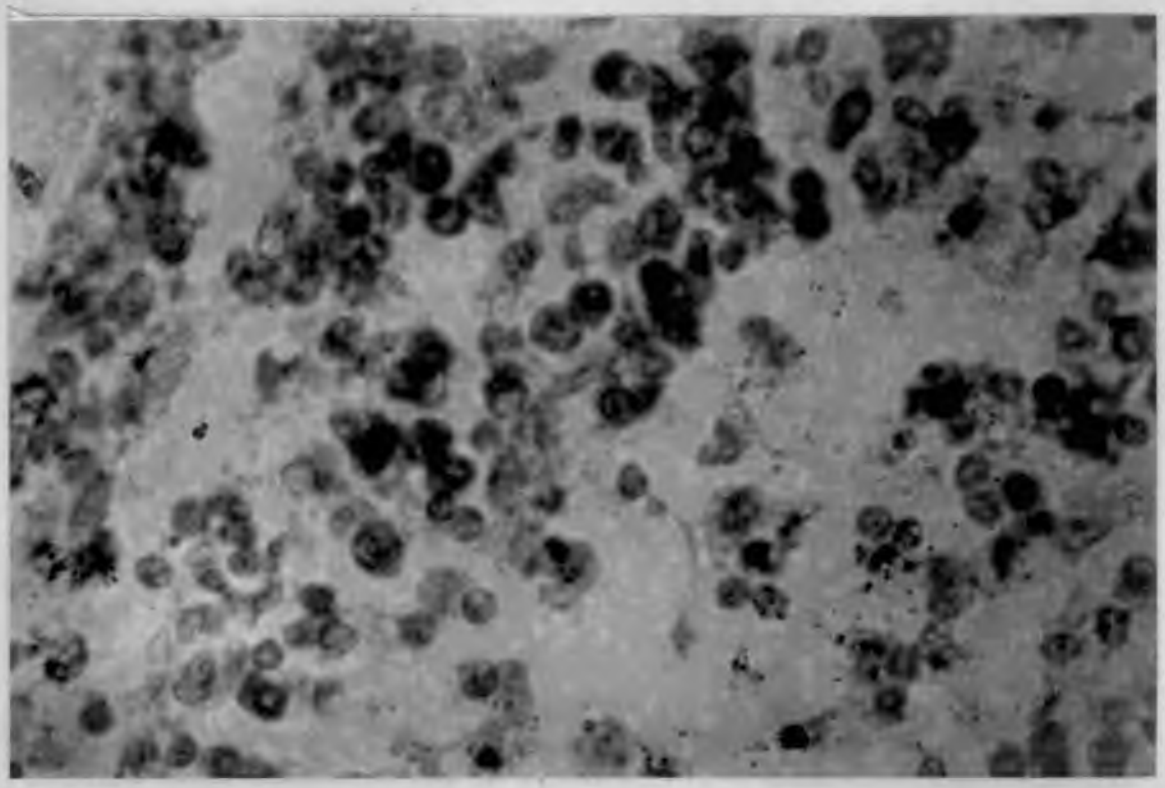


Plates XVII-XVIII Sections of mesenteric lymph nodes from a goat having paratuberculosis

Proliferative lymphadenitis. Diffused cortical necrosis. Infiltration of epithelioid cells into the cortex (H & E staining ×5×10).

Plates XIX-XX *M. paratuberculosis* in lymph node sections

Acid-fast bacilli in characteristic clumps both within and outside the macrophages (Ziehl-Neelsen's staining)



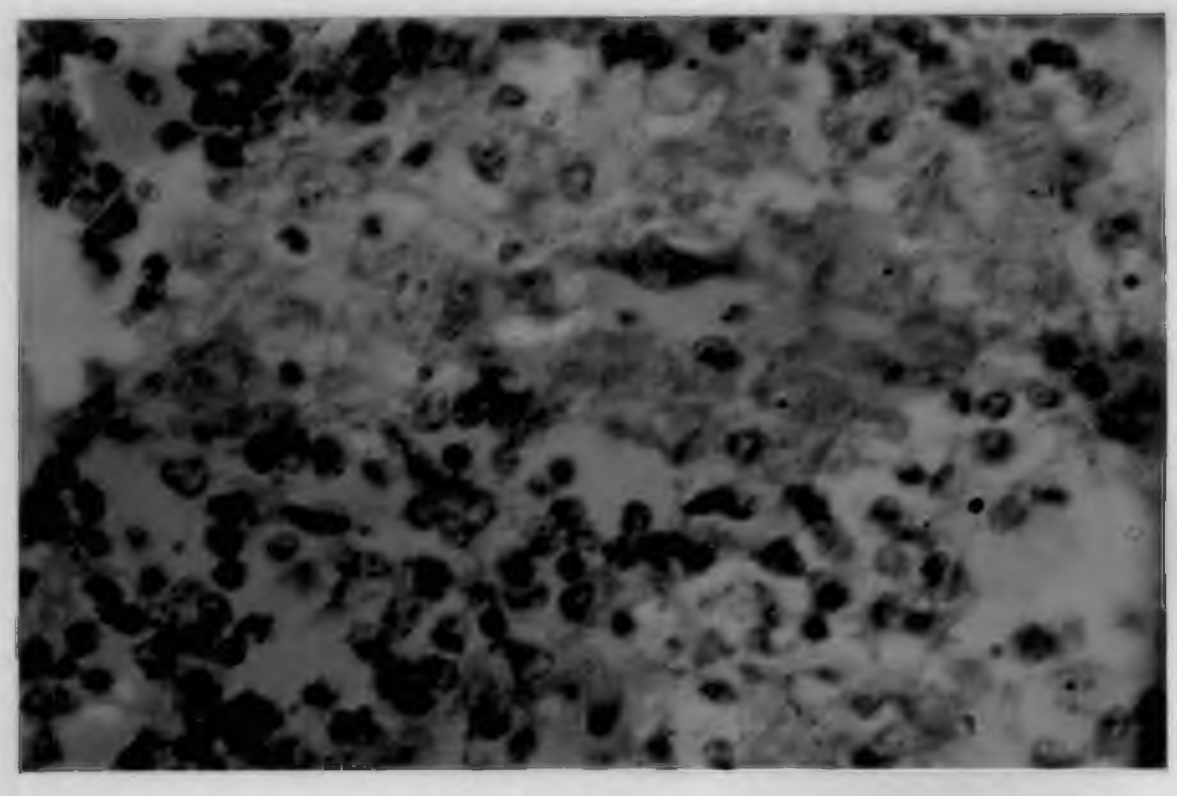


Table 1. Results of intra-dermal tests of animals belonging to Groups I, II and III

Group	Number of animals tested	Number of animals found positive			
		SID	MID-I	MID-II	BOTH
I (SID only)	50	11	--	--	--
II (SID and MID-I)	50	12	8	--	4
III (SID and MID-II)	50	3	--	7	2

Table 2. Results of confirmation of infection in slaughtered animals from Group I, II and III

A. Animals with positive results to intra-dermal tests

Sl. No.	Animal number	SID	MID-I	MID-II	Infection status
Group I Animals					
1.	0174	+	ND	ND	+
2.	0178	+	ND	ND	-
3.	0231	+	ND	ND	+
4.	0307	+	ND	ND	-
5.	0372	+	ND	ND	+
6.	0848	+	ND	ND	-
Group II Animals					
7.	0036	+	+	ND	-
8.	0041	+	+	ND	+
9.	0209	-	+	ND	+
10.	0420	-	+	ND	+
11.	0574	+	+	ND	+
12.	3536	-	+	ND	+
Group III Animals					
13.	0320	-	ND	+	+
14.	0740	+	ND	+	-
15.	0758	+	ND	+	+
16.	0863	-	ND	+	-
17.	0964	-	ND	+	-
18.	0989	-	ND	+	+

ND - Not done

Table 2. Results of confirmation of infection in slaughtered animals from Group I, II and III.

B. Animals with negative results to intra-dermal tests

Sl. No.	Animal number	SID	MID-I	MID-II	Infection status
Group I Animals					
1.	0522	-	ND	ND	+
2.	0261	-	ND	ND	-
Group II Animals					
1.	0550	-	-	ND	-
2.	0564	-	-	ND	-
Group III Animals					
1.	0232	-	ND	-	-
2.	0688	-	ND	-	

ND - Not done

Table 3. Inflammatory reaction and presence of *Mycobacterium paratuberculosis* in the tissues of six slaughtered animals belonging to Group I

Sl. No.	Animal number	Ileum			Ileo-caecal junction			Caecum			Colon			Rectum			Mesenteric lymph nodes		
		Inflammatory reaction	Presence of *M. ptb.*		Inflammatory reaction	Presence of *M. ptb.*		Inflammatory reaction	Presence of *M. ptb.*		Inflammatory reaction	Presence of *M. ptb.*		Inflammatory reaction	Presence of *M. ptb.*		Inflammatory reaction	Presence of *M. ptb.*	
			Scr.	Sect.		Scr.	Sect.		Scr.	Sect.		Scr.	Sect.		Scr.	Sect.		Scr.	Sect.
1.	0174	-	-	-	+	-	+	+	-	-	-	-	-	-	-	-	+	+	+
2.	0176	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3.	0231	-	-	-	+	-	+	+	-	-	+	-	-	-	-	-	-	-	+
4.	0307	+	-	-	+	-	-	+	-	-	+	-	-	-	-	-	-	-	-
5.	0372	+	-	+	+	+	+	+	-	+	+	-	+	+	-	+	-	+	+
6.	0848	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Scr. - Scrapings Sect. - Section *M. ptb.* - *M. paratuberculosis*

Table 4. Mean values of serum magnesium (mg/dl) among test positive and negative animals of groups I,II and III

Group	Number of animals subjected to estimation	Mean values of serum magnesium (mg/dl)	
		Test positive animals	Test negative animals
I	22	1.67	1.42
II	16	3.78	3.71
III	14	4.09	3.94

Table 5. Inflammatory reaction and presence of Mycobacterium paratuberculosis in the tissues of six slaughtered animals belonging to Group II

Sl. No.	Animal number	Ileum			Ileo-caecal junction			Caecum			Colon			Rectum			Mesenteric lymph nodes		
		Inflammatory reaction	Presence of M. ptb.		Inflammatory reaction	Presence of M. ptb.		Inflammatory reaction	Presence of M. ptb.		Inflammatory reaction	Presence of M. ptb.		Inflammatory reaction	Presence of M. ptb.		Inflammatory reaction	Presence of M. ptb.	
			Scr.	Sect.		Scr.	Sect.		Scr.	Sect.		Scr.	Sect.		Scr.	Sect.		Scr.	Sect.
1.	0036	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-
2.	0041	+	-	+	+	+	-	+	+	-	-	-	-	-	-	-	+	+	+
3.	0209	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+
4.	0420	+	-	+	+	+	-	-	+	-	-	-	-	-	-	-	+	+	+
5.	0574	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+
6.	3535	+	-	+	+	+	-	-	+	-	-	-	-	-	-	-	+	+	+

Scr. - Scrapings Sect. - Section M. ptb. - M. paratuberculosis

Table 6. Inflammatory reaction and presence of *Mycobacterium paratuberculosis* in the tissues of six slaughtered animals belonging to Group III

Sl. No.	Animal number	Ileum			Ileo-caecal junction			Caecum			Colon			Rectum			Mesenteric lymph nodes		
		Inflammatory reaction	Presence of *M. ptb.*		Inflammatory reaction	Presence of *M. ptb.*		Inflammatory reaction	Presence of *M. ptb.*		Inflammatory reaction	Presence of *M. ptb.*		Inflammatory reaction	Presence of *M. ptb.*		Inflammatory reaction	Presence of *M. ptb.*	
			Scr.	Sect.		Scr.	Sect.		Scr.	Sect.		Scr.	Sect.		Scr.	Sect.		Scr.	Sect.
1.	0740	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2.	0320	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+
3.	0758	-	-	+	-	-	+	-	-	-	-	-	-	-	-	-	+	+	+
4.	0876	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-
5.	0964	-	-	-	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-
6.	0989	+	-	+	+	-	+	+	-	+	-	-	-	-	-	-	+	+	+

Scr. - Scrapings Sect. - Section *M. ptb.* - *M. paratuberculosis*

Table 7. Statistical analysis of serum magnesium levels (mg/dl) of animals in Group I, II and III

Group	Source	df	Mss	F
I	Between treatments	1	0.3849	0.18
	Error	20	2.0877	
II	Between treatments	1	0.01	0.04
	Error	14	0.2264	
III	Between treatments	1	0.7140	0.62
	Error	12	0.1155	

Table 8. Analyses of the results of SID, MID-I and MID-II

Test	Specificity (Per cent)	Sensitivity (Per cent)	Predictive value of a +ve result (Per cent)	Predictive value of a -ve result (Per cent)
SID	7/12 = 58.33	6/12 = 50	6/11 = 55.5	3/9 = 33.3
MID-I	2/3 = 66.60	5/5 = 100	5/6 = 83.3	2/2 = 100
MID-II	2/5 = 40.00	3/3 = 100	3/6 = 50.0	2/2 = 100

Table 9. Statistical analysis of the efficacy of three intra-dermal tests

Chi-square values

	Sensitivity	Specificity	Predictive value of positive result	Predictive value of negative result	All together
Intra-dermal tests (SID, MID-I and MID-II)	5.2	0.131	1.71	5.6	8.8*

Chi-square value for the three tests together was significant at 5 per cent level

Fig. 1 Increase in skin thickness of group 1 animals after injection.

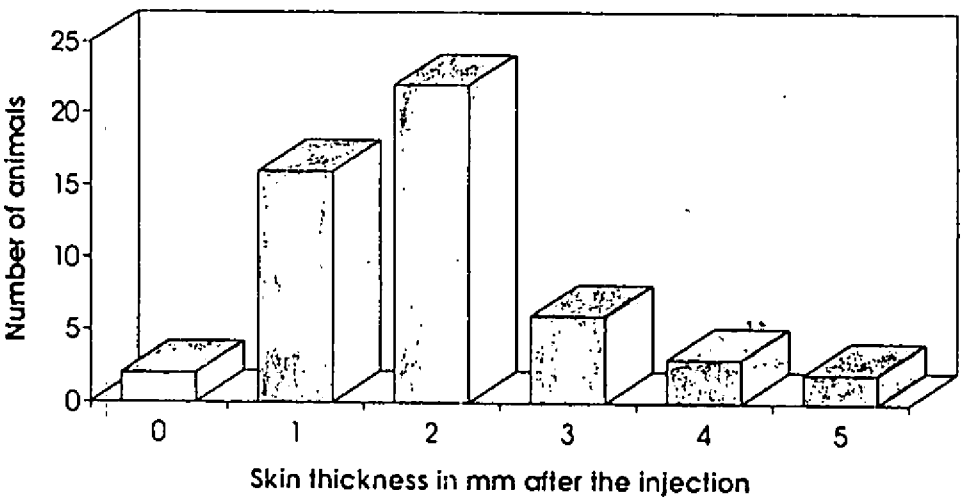


Fig. 2 Proportion of increase in skin thickness of +ve animals in group 1 SID

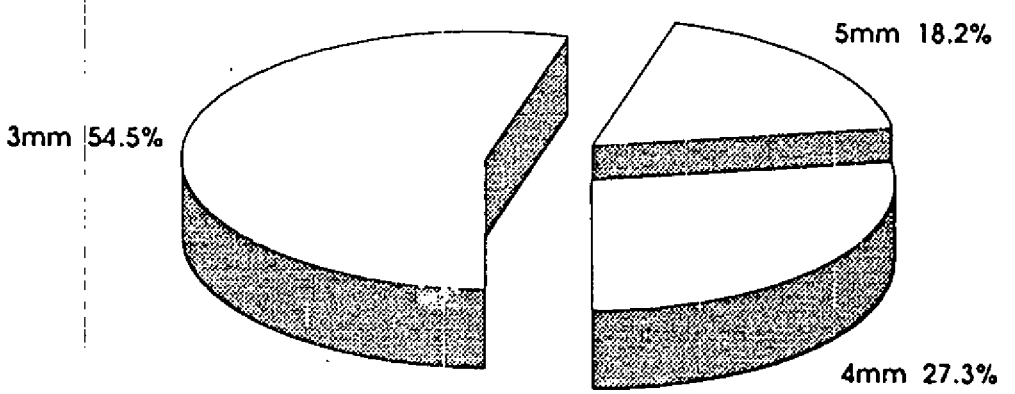


Fig. 3 Serum magnesium levels of group 1 animals.

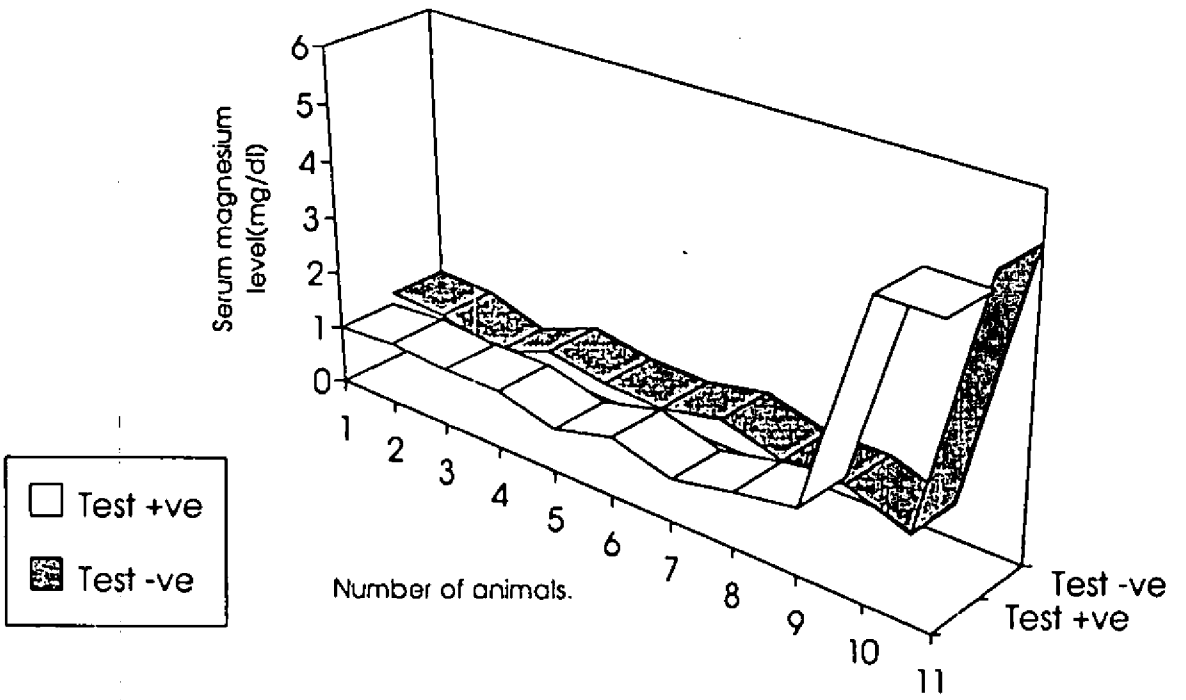


Fig . 4 Increase in skin thickness of group 2 animals after injection.

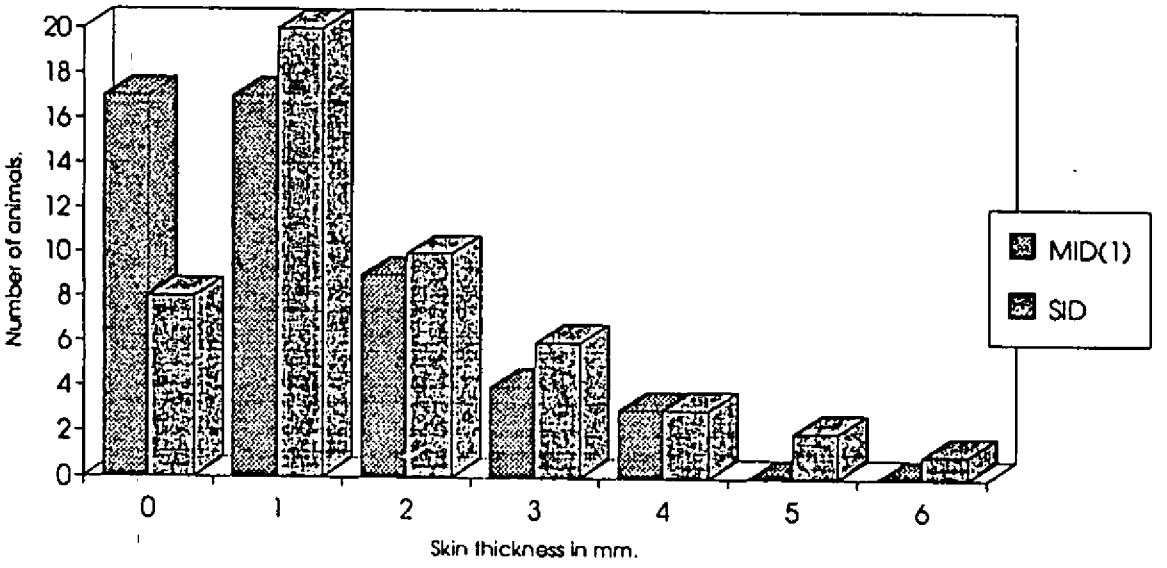


Fig . 5 Proportion of increase in skin thickness of +ve animals in group 2. MID (1).

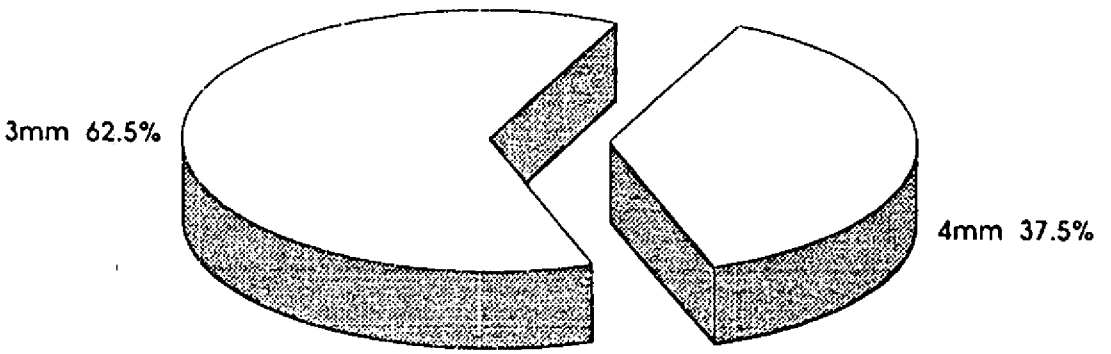


Fig . 6 Proportion of increase in skin thickness of +ve animals in group 2. SID.

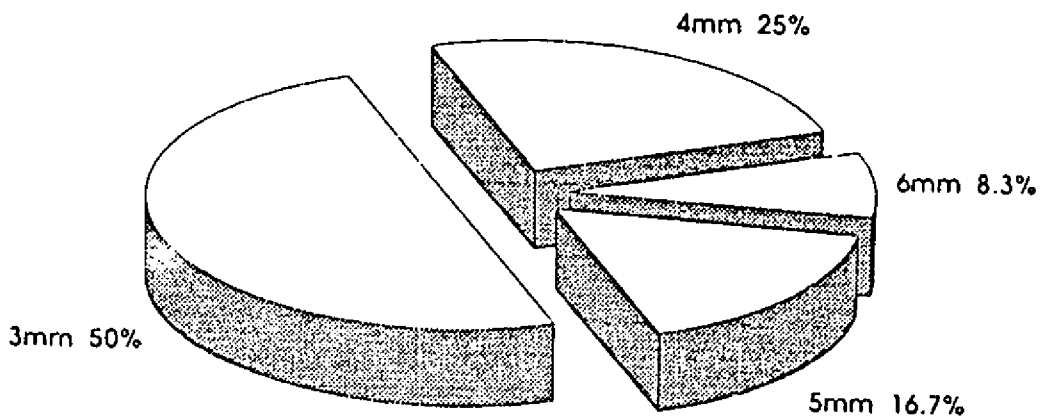


Fig . 7 Serum magnesium levels of group 2 animals (MID.1).

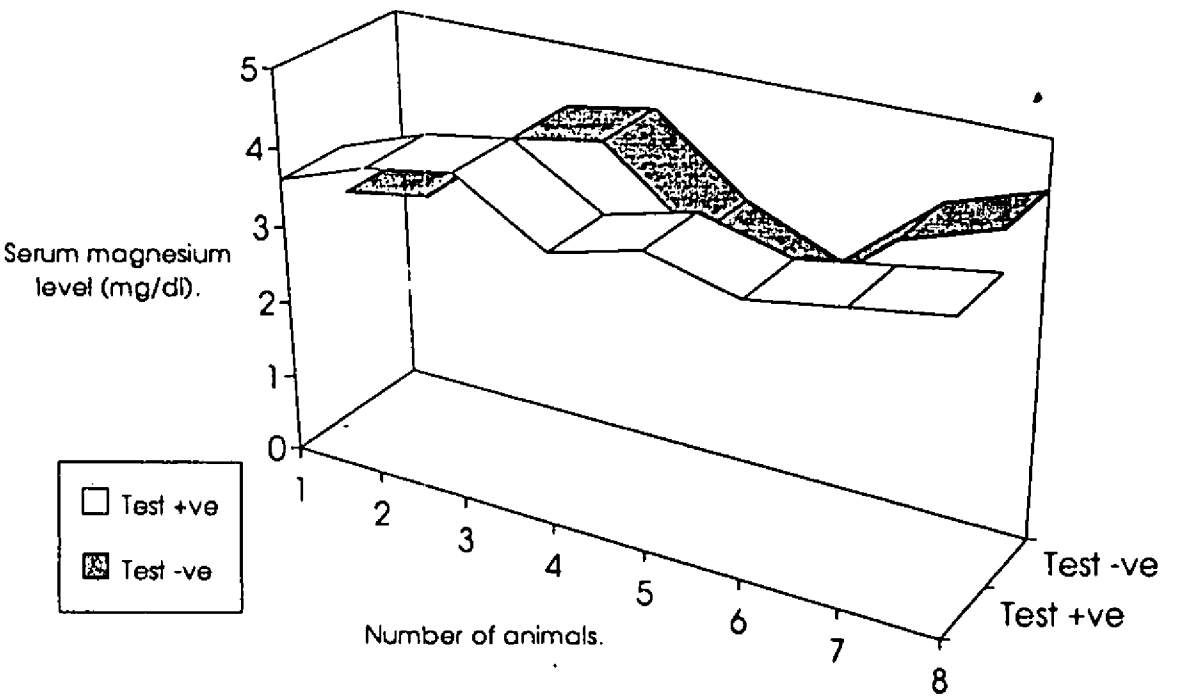


Fig . 8 Increase in skin thickness of group 3 animals after injection.

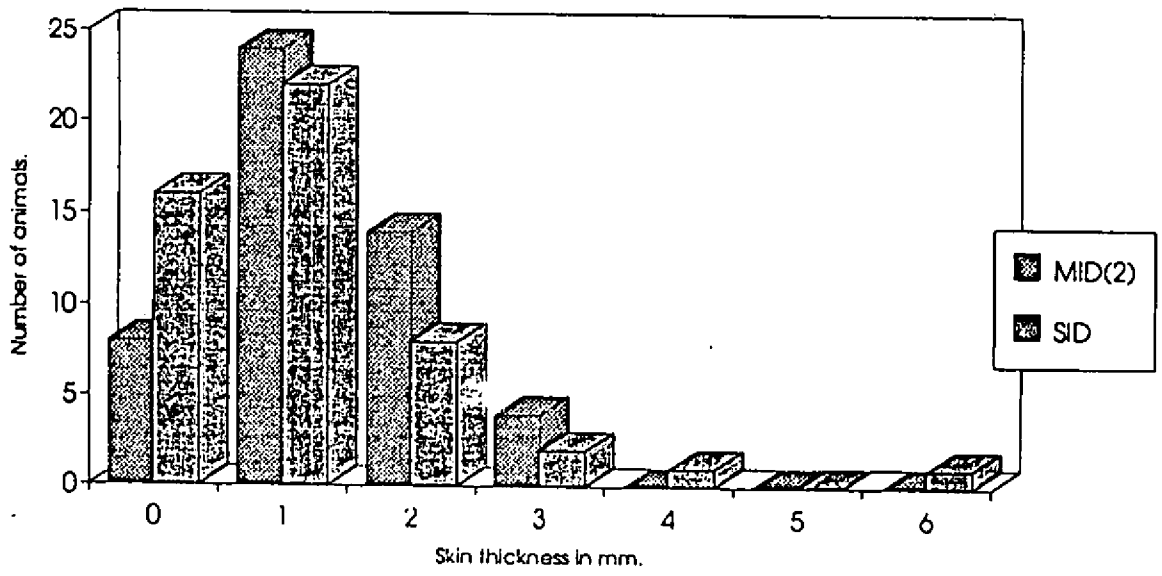


Fig . 9 Proportion of increase in skin thickness of +ve animals in group 3. MID(2).

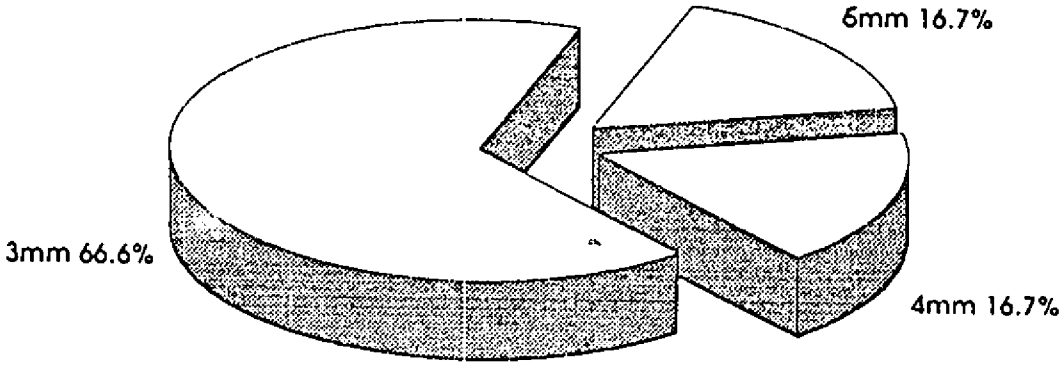


Fig . 10 Proportion of increase in skin thickness of +ve animals in group 3 SID

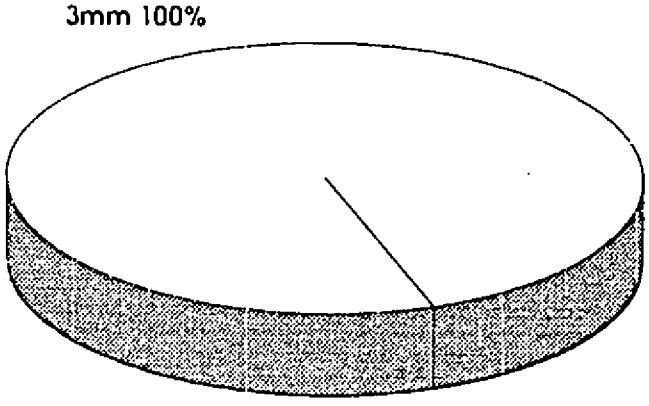


Fig .11 Serum magnesium levels of group 3 animals (MID.2).

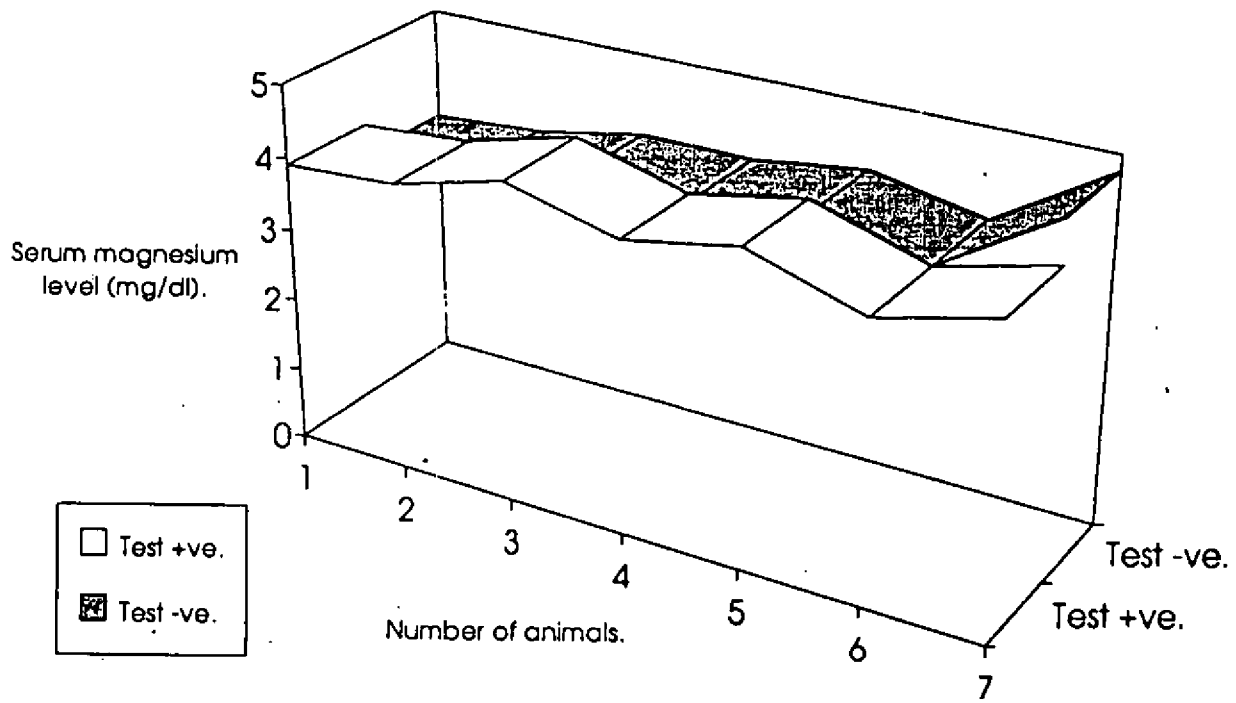
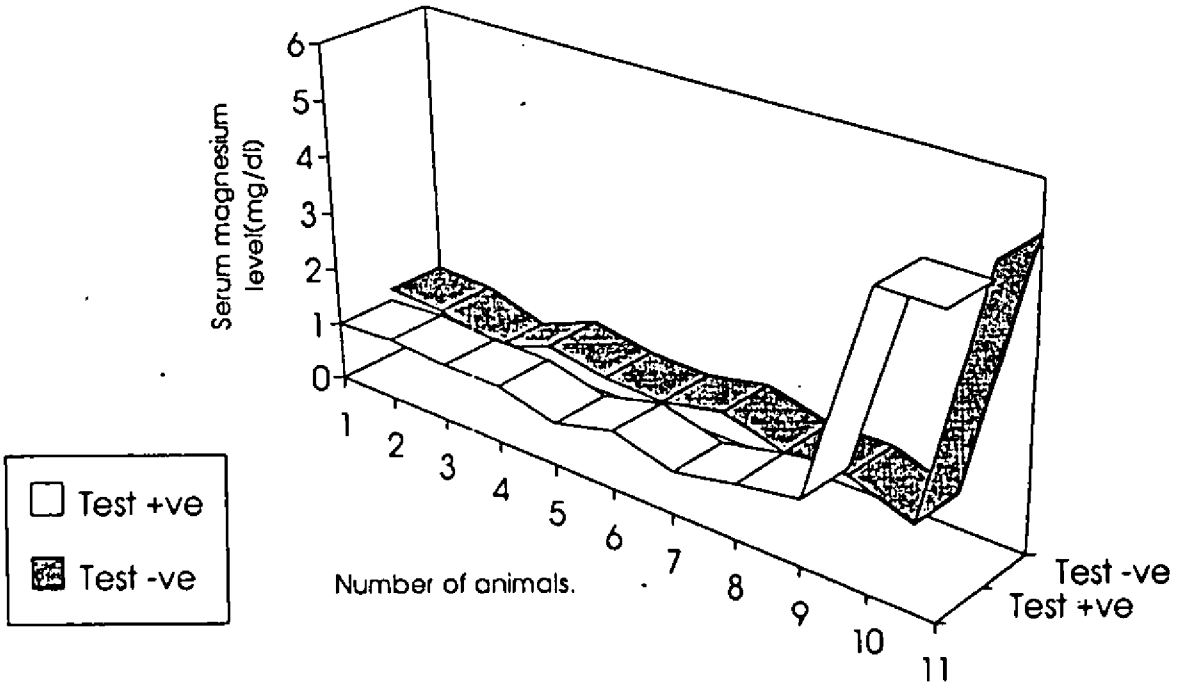


Fig. 3 Serum magnesium levels of group 1 animals.



Discussion

DISCUSSION

The present study was conducted to compare the efficiency of the modified intra-dermal Johnin test and the single intra-dermal Johnin test, for the diagnosis of paratuberculosis in goats.

Direct microscopic examination of fecal sample and rectal pinch

All the animals except one, were negative for the presence of M. paratuberculosis in feces. This particular animal had clumps of acid-fast bacilli in feces. None of the 12 animals which were positive in histopathology were excreting the organism through feces in detectable numbers. The present findings is in agreement with the observations of Gilmour (1985) and Lisle et al. (1980b), who found that affected animals excreted the organism through feces only intermittently and that too in minimum quantities.

Only apparently healthy animals from a known positive herd were selected for the study. The predominantly negative results obtained for the fecal examination could be due to preclinical stage of infection, as Hole (1952), Gilmour (1954), Paliwal et al. (1985b) and Patel et al. (1987) have found the excretion of bacteria by animals in pre-clinical

stage to be very poor. The negative results of the study could be due to factors like time of collection or poor excretion of bacteriae in pre-clinical stage.

Taylor (1951) and Borodenok (1959) reported that animals would be excreting M. paratuberculosis only in the later part of the infection, whether the infection was clinical or sub-clinical. Since the animals in the Kerala Agricultural University goat farm were periodically subjected to intra-dermal tests and positive animals were culled, the chances of finding any animal in the advanced stage of infection was also very little.

The results of the present study also agrees with Merckal et al. (1968b) and Lall (1973). According the them, a negative result during microscopical examination of faces is of little significance, and it is impossible to distinguish the Johne's bacillus from other acid-fast bacilli which are present in feces; whereas Paliwal et al. (1984a) and Kulshrestha et al. (1984) were of the opinion that fecal examination was more reliable than the allergic test to detect clinical shedders. However, the negative fecal test, does not conclude that the animal was free from infection.

Examination of rectal pinch was not giving any positive results in the present study. Doyle and Spears

(1951), Larsen (1972), Chodini et al. (1984), Paliwal et al. (1985b) and Patel et al. (1987) were of the opinion that the rectal pinch examination becomes positive to M. paratuberculosis in advanced stages of the infection rather than pre-clinical stage. In the present investigation all the cases were in pre-clinical stage. This would explain the negative result on rectal pinch examination.

Allergic test

Among the 50 animals belonging to group I, 11 animals (45.5 per cent) gave positive result to SID Johnin test (Appendix I). Among the positive animals 54.5 per cent had 3 mm, 18.2 per cent had 4 mm and 27.3 per cent had 4 mm increase in thickness of skin (Fig.2). Such increase in skin thicknesses is more than those observed (2.5 to 3 mm) by Kumar et al. (1982) in positive cases of paratuberculosis among sheep.

The inflammatory reactions such as erythema, oedema, hyperthermia and pain at the site of injection were very moderate in the case of animals positive to SID.

Reading of skin thickness at 24 h, 48 h and 72 h gave 15 (30 per cent), 13 (26 per cent) and 8 (16 per cent)

positive results respectively. Analysis of the data revealed that three animals were positive only at 24 h and 48 h readings each, and one animals was positive only at 72 h reading. Only four animal out of the total 17 animals (23.5 per cent) answered all three readings as positive (Appendix-I). Therefore, the time of taking the reading was found to have an influence on the outcome of the results of single intra-dermal test. The present findings indicated that it was better to take the readings at 24 h in SID in goats.

These results are in contradiction to the findings of Dunkin (1934) and Lall (1973) who recommended taking readings 48 h after injection, and Pearson and McClelland (1962) who found little difference between taking readings at 48 h and 72 h after injection for cattle. The findings of Kumar et al. (1982) and Blood et al. (1989) who had recommended taking readings at 72 h are also in contradiction to the results obtained in this study.

Various workers have reported SID johnin test as quite satisfactory and reliable (Lall, 1973 and Singh et al., 1992). The present SID results were found to give negative results in majority of the infected animals, and is in contradiction with the observation of these workers. But Paliwal et al. (1984a) observed that allergic test was negative in both clinical and preclinical stages among sheep.

Eight animals (16 per cent) out of 50 tested gave positive results to the MID-I, whereas 11 (24 per cent) of the same group gave positive johnin test results to SID. Among the positive animals to MID-I, 62.5 per cent had 3 mm increase and 37.5 per cent animals had 4 mm increase in skin thickness. These findings agree with Kerr et al. (1946) who found maximum number of reactors to tuberculin among cattle at 3 mm with few 4 mm reactors during stormont test in cattle.

The animals in group II which were subjected to MID-I test had very pronounced and characteristic inflammatory reactions like erythema, hyperthermia and pain at the site of injection. Such reactions made isolation of positive reactors easy.

Eventhough four of these animals gave positive reaction to SID test, the inflammatory reaction of these animals to SID test were not so pronounced. The increased inflammatory reaction revealed that the increased sensitivity due to the first injection of johnin was more at 72 h compared to 120 h in MID-II.

Seven animals (14 per cent) out of 50 animals of group III had positive results for MID-II. Among the positive animals 66 per cent had 3 mm increase in skin thickness

followed by 17 per cent animals with 4 mm increase and 17 per cent animals with above 4 mm increase (Fig.10). These results are comparable to those obtained by Kerr et al. (1946) for stormont test in the diagnosis of tuberculosis among cattle.

Animals in group I and group II had moderate inflammatory reactions at the site of injection. This may lead to the conclusion that increased sensitivity of the skin at the site after first injection was considerably reduced by the time the second injection in MID-II at 120 h was given. This is in contrast to the findings of Lambrecht (1957) and Blood et al. (1989) who recorded maximum sensitivity to tuberculin after the first injection on the 7th day in cattle.

The reduced sensitivity to the Johnin on the 5th day of the injection in the present study is in conformity with the findings of Borodenok (1959) and Karpinski and Zorawski (1975) who observed that goats acquire and lose sensitivity to Johnin earlier than cattle in M. paratuberculosis infection

Pearson and McClelland (1962) and Blood et al. (1989) stated that the Stormont test is difficult to practice since it involved two injections and three visits. The animals which were positive to the SID resented the area being handled and were difficult to control for the injection in MID-I and MID-II.

All three tests had similar percentages of positive levels of positive results ranging between 14.0 to 17.3 per cent. Considering the three groups as homogenous, it can be stated that all three tests had similar levels of positive results. This is in contrast to the findings of Kalra et al. (1984) who found the Stormont test to give more number of positive results than single intra-dermal test.

Sensitivity and specificity

In the present study the SID test had 50 per cent sensitivity and 58.33 per cent specificity (Table 8). These values are too low for an effective diagnostic test. Many workers Sigurdsson (1956), Clark (1969), Muhammed et al. (1978), Paliwal et al. (1984a) and Kulshrestha et al. (1984), have stated that the intra-dermal test is too inaccurate to be used as a dependable diagnostic test. This observation has been substantiated in this study.

The MID-I Johnin test had 100 per cent sensitivity and 66.6 per cent specificity (Table 8). The values are better than both SID and MID-II test. The sensitivity is similar to that observed by Kerr et al. (1946b) for diagnosis of tuberculosis in cattle by stormont test. However specificity is poor.

The test had 100 per cent sensitivity and 40 per cent specificity. Here also the sensitivity is similar to that observed by Kerr et al. (1946) in stormont test in cattle, but specificity is low. Pearson (1962) and Popluhar and Vritiak (1964) have recorded higher levels of specificity and sensitivity for Stormont test.

The specificity was highest for MID-I followed by SID and MID-II (Table 9). Thus among the healthy animals only 66.6 per cent will give a negative result to MID-I, whereas the figure is still lower for SID (58.33 per cent) and MID-II (40 per cent). The others will be giving false positive reaction (33.3 per cent MID-I, 41.67 per cent for SID and 60 per cent for MID-II).

Predictive value of positive and negative results

The MID-I Johnin test had a predictive value of 83.3 per cent for positive results and 100 per cent for negative results, followed by MID-II Johnin test with 50 per cent and 100 per cent and lastly SID Johnin test with 55.5 per cent and 50 per cent respectively.

The results of the SID Johnin test are in agreement with Chodini et al. (1984) who reported 50 per cent false positive and 50 per cent false negative results for single intra-dermal Johnin test in cattle, sheep and goats. But the

results vary from those of Larsen et al. (1963a) (22 per cent and. 40 per cent); Lisle et al. (1980a) (90 per cent false positive); Paliwal et al. (1984a) (29 per cent and 5 per cent) and Paliwal et al. (1984 b)(79.63 per cent and 23.8 per cent in goats).

The false positive reaction to the three tests observed in the present study could also be correlated to the sensitization with Corynebacterium pseudotuberculosis. Jayaprakasan (1986) had recorded this infection at the Kerala Agricultural University Goat Farm. The observation of Chodini et al. (1984) who found the animals infected with Corynebacterium to give false positive reaction to the intra-dermal Johnin test was in conformity with the present observation.

Sensitivity to many other Mycobacteriae present in the soil, like M. avium, could also have caused false positive reactions as proposed by Ketterer et al. (1981) and Paliwal et al. (1984a). The findings of Larsen et al. (1963a) who found the PPD to have antigens capable of producing sensitivity to heterologous antigens is also relevant.

The findings of Smith (1990) point out that some animals may acquire infection and hence sensitivity, to Johnin, but would have overcome it and expelled the bacteriae

from the body. Such animals retain the sensitivity to Johnin which may lead to many false positive results. Jubb et al. (1993) had pointed out the existence of some animals who have no visible lesions, but have M. paratuberculosis infection.

Since vaccination against paratuberculosis is not practiced in the Kerala Agricultural University Goat Farm, the chances of vaccination leading to false positive results as observed by Spangler (1992a) is not a possibility.

False negative results were noticed for single intradermal test. This could have been due to the animals being in the very early stage of infection as recorded by Sigurdsson (1956) or by a temporary stage of unreactivity in animals (Konst and McIntosh, 1958). Lall (1973) reported poor quality of the antigen and advanced stages of the disease or pregnancy as probable causes for false negative reactions. But all these three conditions are not relevant in this study. Diseased or pregnant animals were not utilized for the study. The antigen was of satisfactory quality since it gave good results with other tests.

Estimation of serum magnesium

Group I animals had serum magnesium levels towards the lower range and Group II and III animals had serum magnesium

levels within the normal ranges, but more towards the higher side. The serum magnesium levels observed in this study were lower than the levels (2.2 mg/dl) observed by Shobhanan (1982) in the goats of the Kerala Agricultural University goat farm.

The serum magnesium levels were found to have no effect on the results of the intra dermal test. The serum magnesium levels of test positive and negative animals belonging to group I, II and III were also found to be not significantly different. These observations are supported by the findings of Negi et al. (1963). Reddy et al. (1982) recorded low levels of serum magnesium in sheep with clinical Johne's disease. None of the animals in the present study was having clinical paratuberculosis.

Results of post mortem examination

Post mortem examination of slaughtered animals revealed that 11 out of 18 intra-dermal test positive and one out of six intra-dermal test negative animals had M. paratuberculosis infection.

Gross and microscopic pathologic changes observed in this study is in conformity with the observations of Rajya and Singh (1961).

Only one animal had extensive lesions in all the tissues examined. Five animals had typical inflammatory reactions in most of the tissues examined whereas three animals had chronic inflammatory reactions at the ileo-caecal junction and caecum.

These results agree with the findings of Carrigan and Seaman (1990) who found that 36 per cent of sheep had only mild to moderate inflammatory changes.

Three animals had no visible lesions eventhough they were harbouring the M. paratuberculosis which was detected in histopathology. These observations are in agreement with the findings of Fodstad and Gunnarson (1979). Paliwal and Rajya (1982) had found lesions of paratuberculosis in the intestine and mesenteric lymph nodes of only 18.2 per cent of sub-clinically infected goats.

In the present study three animals which were having M. paratuberculosis in sections of mesenteric lymph nodes were negative for the presence of the organism in ileum, ileocaecal junction, caecum and colon. These findings agree with Summers (1981) who had reported failure to observe M. paratuberculosis in sections of lymph nodes even when sections of the intestine revealed them and vice versa.

One animal had typical, chronic inflammatory changes both in the lymphnode and intestine. But the organism could not be detected from any of these tissues. It could be due to the fact that the infected animals had completely eliminated the infection from the body as stated by Jubb et al. (1993).

Seven animals which were negative for the presence of acid-fast bacilli in the scrapings of the intestine, were identified to have infection on histopathological examination. It would therefore appear that the recommendation of Paliwal and Rajya (1982) to examine the intestinal tissue scrapings for conformation of information has no significance.

Mesenteric lymph nodes were found to be the organ of choice for confirming the result, as all infected animals had the presence of organism in mesenteric lymph nodes. This was followed by ileo-caecal junction (72.7 per cent), and then by caecum (45.5 per cent) in having the organism. The other organs did not contain M. paratuberculosis. These results are in agreement with Chodini et al. (1984), Paliwal et al. (1985a) and Jubb et al. (1993).

Gross changes

The intestine of all the 12 positive animals did not reveal any gross abnormality. The hyperaemia and inflammatory oedema, as described by Groth (1964), Rajya and Singh (1961),

Lenghaus et al. (1977) and Jubb et al. (1993) were absent. Nodular hyperplasia (Ullrich, 1982) could not be observed in any of the goats examined.

The congestion and mild thickening of the mucosa, as suggested by Sigurdsson (1955) and Paliwal and Rajya (1982) were seen in three animals only.

Krishna et al. (1989) observed typical corrugated appearance of the intestinal mucosa and enlargement of the mesenteric lymph nodes in all the four goats died of Johne's disease. But animals in such advanced conditions were not included in this study.

The mesenteric lymph nodes of all positive animals were having typical inflammatory changes. The oedematous nature was in conformation with Lall (1973) and Rajya and Singh (1961). The cortex and the medulla were as described by Kulshrestha et al. (1984) and Krishna et al. (1989). But the caseation and calcification (Rankin, 1958b) and white foci (Lenghaus et al., 1977) were absent, except in one case where the reaction of the mesenteric lymph node revealed degenerative foci in the form of caseous or calcified nodules as reported by Lall (1973).

Histopathological changes

Proliferative enteritis and moderate thickening of the villi of the ileum, ileocaecal junction, caecum and colon were characteristic of the disease, as reported by Rankin (1958b) and Rajya and Singh (1961).

Mononuclear infiltration of the intestinal mucosa was observed in three of the animals and is in agreement with the observations of Summers (1981) and Carrigan and Seaman (1990).

The villous atrophy of the caecum observed in two animals has also been reported by Singh et al. (1992).

The changes characteristic of advanced cases of the disease like loss of architecture of the tissues and presence of fibrin strands, could not be detected in any of the animals. Focal aggregation of epithelioid cells at the tip of the villi has also been observed by Carrigan and Seaman (1990).

The changes in the mesenteric lymphnodes were more of an advanced nature than those in the sections of the intestine. The cortical necrosis, both diffuse and focal, indicate signs of a well-established infection. Such changes have been observed by Lenghaus et al. (1977) and Chodini et al. (1984).

The chronic inflammation is a characteristic feature of paratuberculosis, and was evident in all animals. Rajya and Singh (1961) observed that these changes are the only indication of sub-clinical paratuberculosis in sheep. Paliwal and Rajya (1982) reported only mild inflammatory reactions in the mesenteric lymph nodes of sub-clinically infected goats. The results obtained in this investigation did not agree with these findings. There was severe reactions in the meseteric lymph nodes even in sub-clinical cases.

The correlation between gross and histological changes were variable in the present study. Though the intestine of 18 goats were having mild gross changes, none of those animals were showing severe histological lesion typical of Johne's disease. This finding is in agreement with the observation of Carrigan and Seaman (1990).

The results of this study suggest that MID-I test could be used as an effective test for diagnosis of sub-clinical paratuberculosis in goats. But since this test also does not have absolute accuracy, the chances of combining this test with other serological tests should also be examined.

Summary

SUMMARY

The study was conducted to compare the efficacy of two modified intra-dermal Johnin tests against the single intra-dermal Johnin test for diagnosis of paratuberculosis in goats.

One hundred and fifty adult goats from the Kerala Agricultural University Goat Farm were subjected to the experiment. The animals were divided at random into three groups (Group I, II and III) of 50 animals each.

Group I animals were subjected to the single intra-dermal test (SID). The animals were injected with 0.1 ml Johnin purified protein derivative (PPD) intra-dermally on the neck. Thickness of the skin of the area was measured immediately before and 24, 48 and 72 h after the injection.

Group II and Group III animals were subjected to the modified intra-dermal tests 1 (MID-I) and 2 (MID-II) respectively. Here the first injection was given as for the SID for both MID(I) and MID(II). But a second similar injection was given exactly on the same site on third day for MID(I) and fifth day for MID(II). Thickness of the skin was measured immediately before and 24 hours after the second injection in both cases.

Fecal samples and rectal pinch from all animals were subjected to Zeihl-Neelsen's acid-fast staining to detect presence of M. paratuberculosis. Serum magnesium levels of all the intra-dermal test positive and an equal number of test negative animals were estimated in all three groups.

Six animals giving positive results and two animals giving negative results to the respective intra-dermal tests were slaughtered from each group and presence of infection in them was ascertained by histopathological techniques. The specificity, sensitivity and predictive value of results were calculated for each test and statistically compared.

The MID-I test was found to have highest specificity (66.6 per cent as against 58.33 per cent for SID and 40 per cent for MID-II) and predictive value of a positive result (83.3 per cent as against 55.5 per cent for SID and 50 per cent for MID-II). Both the modified tests (MID-I and MID-II) had 100 per cent sensitivity and predictive value of negative result as against 50 per cent sensitivity and 33.3 per cent predictive value of negative result for SID test.

The presence of M. paratuberculosis could be detected in feces of only one animal and the rectal pinch examinations of all 150 animals could not reveal the presence of M. paratuberculosis.

Serum magnesium levels from all the test positive and test negative animals were within the normal range. The difference between mean values of serum magnesium in test positive and test negative animals of each group was found to be not significant.

Post-mortem examination of slaughtered animals revealed characteristic changes of early paratuberculosis in 10 of the 18 test positive and one of the six test negative animals. The gross changes in intestines were not apparent. Mesenteric lymph nodes from the infected animals were enlarged and oedematous. Histologically the ileo cacecal junction, caecum and colon of infected animals revealed infiltration of inflammatory cells, destruction of glands and focal necrosis. The mesenteric lymph nodes also were having inflammatory cell infiltration and necrosis.

The present study revealed MID-I test to be superior to SID and MID-II test for diagnosis of paratuberculosis in goats. Examination of fecal sample and rectal pinch were found to be of little diagnostic value. Serum magnesium levels were found to be not significantly affected by the disease in goats.

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* Originals not consulted

Appendices

APPENDIX-I

Results of single intra-dermal (SID) Johnin testing in group-I animals

Sl. No.	Animal number	Thickness of skin at the site of injection (in mm)				Difference between thickness of skin before injection and 48 h after injection (mm)	Results of of SID test
		before injection	24 h after injection	48 h after injection	72 h after injection		
1	2	3	4	5	6	7	8
1.	0855	2	4	4	3	2	-
2.	0216	2	5	6	5	4	+
3.	0404	1	3	3	3	2	-
4.	0848	2	4	5	4	3	+
5.	0356	2	4	4	3	2	-
6.	0253	1	3	3	2	2	-
7.	0856	1	5	5	3	4	+
8.	0307	1	6	6	6	5	+
9.	0231	2	6	6	5	3	+
10.	0371	2	4	4	5	2	-
11.	0856	4	7	6	6	2	-

(Contd).

Appendix-I (Contd.)

1	2	3	4	5	6	7	8
12.	0322	1	3	3	3	2	-
13.	0326	6	6	6	6	0	-
14.	0851	2	5	4	5	2	-
15.	0239	2	5	4	5	2	-
16.	0117	1	3	6	4	5	+
17.	0178	3	6	6	5	3	+
18.	0224	1	2	2	3	1	-
19.	0314	1	3	3	2	1	-
20.	0572	2	4	4	4	2	-
21.	0546	2	4	3	3	1	-
22.	0484	1	3	2	3	1	-
23.	0871	2	3	3	2	1	-
24.	0372	2	6	6	6	4	+
25.	0854	2	5	4	3	2	-
26.	0357	2	3	3	3	1	-
27.	0106	4	5	5	5	1	-

(Contd).

Appendix-I (Contd.)

1	2	3	4	5	6	7	8
28.	0875	2	3	3	2	1	-
29.	0462	1	3	3	3	2	-
30.	0575	1	4	3	3	2	-
31.	0313	2	2	3	3	1	-
32.	0047	2	3	3	2	1	-
33.	0522	2	2	3	2	1	-
34.	0261	2	5	4	5	2	-
35.	0015	1	3	3	2	2	-
36.	0326	2	4	5	4	3	+
37.	0486	1	4	4	2	3	+
38.	0113	2	2	2	2	0	-
39.	0174	2	6	5	3	3	+
40.	0208	1	3	3	3	2	-
41.	0294	1	3	2	2	1	-
42.	0300	2	4	4	4	2	-
43.	0477	1	4	3	3	3	+

(Contd).

Appendix-I (Contd.)

1	2	3	4	5	6	7	8
44.	0389	5	5	6	6	1	-
45.	0714	1	2	3	2	1	-
46.	0811	2	3	3	2	1	-
47.	0887	2	3	3	3	1	-
48.	0703	1	2	3	2	2	-
49.	0698	1	3	3	3	2	-
50.	0897	3	5	5	3	2	-

APPENDIX-II

Serum magnesium level (mg/dl) of Group-I animals

SID positive animals			SID negative animals		
Sl. No.	Animal number	Serum Mg (mg/dl)	Sl. No.	Animal number	Serum Mg (mg/dl)
1.	0372	1.00	1.	0474	1.21
2.	0117	1.08	2.	0322	1.16
3.	0856	0.74	3.	0814	0.85
4.	0307	1.00	4.	0253	1.25
5.	0231	1.00	5.	0374	1.06
6.	0486	1.01	6.	0851	1.03
7.	0848	0.72	7.	0106	1.25
8.	0216	0.95	8.	0314	0.97
9.	0477	1.12	9.	0185	0.99
10.	0174	4.90	10.	0356	0.60
11.	0178	4.90	11.	0367	5.20

APPENDIX-III

Results of modified intra-dermal Johnin test-I (MID-I) and single intra-dermal (SID) test of Group II animals

Sl. No.	Animal number	Thickness of skin at the site of injection (mm)		Result of SID test	Thickness of skin at the site of injection (mm)		Difference in thickness before injection and 24 h after II injection (mm)	Result of MID-I test
		Before I injection	48 h after I injection		Before II injection	24 h after II injection		
1	2	3	4	5	6	7	8	9
1.	0260	2	2	-	3	3	0	-
2.	0124	4	4	-	4	4	0	-
3.	0267	6	7	-	7	7	0	-
4.	0272	2	3	-	2	4	2	-
5.	0091	4	8	+	7	8	1	-
6.	0168	4	3	-	4	4	0	-
7.	0110	2	4	-	5	5	0	-
8.	0411	1	5	+	4	5	1	-
9.	0454	3	4	-	4	4	0	-

(Contd).

Appendix-III (Contd.)

1	2	3	4	5	6	7	8	9
10.	0246	4	7	-	5	7	2	-
11.	0268	2	3	-	3	2	-	-
12.	0135	2	4	-	3	3	0	-
13.	0486	3	2	-	3	3	0	-
14.	0132	3	3	-	3	3	0	-
15.	0186	3	3	-	3	4	1	-
16.	0041	2	8	+	6	10	4	+
17.	0249	2	3	-	4	6	2	-
18.	0418	2	3	-	3	4	1	-
19.	0438	2	2	-	2	3	1	-
20.	0413	2	3	-	2	4	2	-
21.	0550	2	3	-	2	2	0	-
22.	0564	2	2	-	2	2	0	-
23.	0574	2	5	+	7	10	3	+
24.	0207	2	2	-	2	3	1	-

Contd.

Appendix-III (Contd.)

1	2	3	4	5	6	7	8	9
25.	3669	3	3	-	2	3	1	-
26.	0429	3	8	+	10	14	4	+
27.	0418	4	3	-	2	5	3	+
28.	3536	1	3	-	2	5	3	+
29.	0420	2	4	-	4	8	4	+
30.	0016	3	4	-	4	6	2	-
31.	0027	3	4	-	3	4	1	-
32.	0291	2	4	-	3	4	1	-
33.	0209	2	3	-	3	6	3	+
34.	0508	2	2	-	2	2	0	-
35.	0314	1	2	-	2	3	1	-
36.	0345	2	6	+	4	5	1	-
37.	0250	3	5	-	3	4	1	-
38.	0245	1	2	-	2	4	2	-
39.	0210	1	3	-	2	3	1	-
40.	0931	1	2	-	2	3	1	-

(Contd).

Appendix-III (Contd.)

1	2	3	4	5	6	7	8	9
41.	0036	2	7	+	4	7	3	+
42.	0161	2	3	-	4	6	2	-
43.	0107	1	3	-	2	1	-	-
44.	0264	1	3	-	2	3	1	-
45.	0569	1	4	+	2	3	1	-
46.	0576	1	4	+	2	3	1	-
47.	0177	2	5	+	4	4	0	-
48.	0360	2	5	+	4	5	1	-
49.	0188	1	3	-	2	2	0	-
50.	0230	2	3	-	2	4	2	-

APPENDIX-IV

Serum magnesium level (mg/dl) of Group-II animals

MID-I positive animals			MID-II negative animals		
Sl. No.	Animal number	Serum Mg (mg/dl)	Sl. No.	Animal number	Serum Mg (mg/dl)
1.	0041	3.6	1.	0250	3.0
2.	0574	4.0	2.	0207	3.2
3.	0429	4.2	3.	0246	4.2
4.	0418	3.5	4.	0508	4.4
5.	3536	3.8	5.	0016	3.5
6.	0420	3.5	6.	0234	3.0
7.	0209	3.7	7.	0272	4.0
8.	0036	3.9	8.	0168	4.4

APPENDIX-V

Results of modified intra-deramal Johnin test-II (MID-II) and single intra-dermal (SID) test of Group III animals

Sl. No.	Animal number	Thickness of skin at the site of injection (mm)		Result of SID test	Thickness of skin at the site of injection (mm)		Difference in thickness before injection and 24 h after II injection (mm)	Result of MID-II test
		Before I injection	48 h after I injection		Before II injection	24 h after II injection		
1	2	3	4	5	6	7	8	9
1.	0878	2	3	-	2	3	1	-
2.	1017	2	2	-	2	3	1	-
3.	0956	1	3	-	3	3	0	-
4.	0696	2	4	-	3	3	0	-
5.	0989	3	4	-	3	6	3	+
6.	0880	3	2	-	1	3	2	-
7.	0982	3	2	-	2	4	2	-
8.	0654	1	2	-	2	3	1	-
9.	0758	2	5	+	2	5	3	+
10.	0964	3	3	-	3	6	3	+

(Contd).

Appendix-V (Contd.)

1	2	3	4	5	6	7	8	9
11.	1002	3	3	-	3	3	0	-
12.	1000	2	2	-	2	4	2	-
13.	0768	2	2	-	3	4	1	-
14.	0974	1	3	-	3	2	1	-
15.	0959	2	4	-	2	4	2	-
16.	0711	2	3	-	3	3	0	-
17.	0733	1	2	-	2	3	1	-
18.	0712	4	4	-	3	5	2	-
19.	0762	2	3	-	2	3	1	-
20.	0835	1	2	-	2	4	2	-
21.	0844	2	3	-	3	4	1	-
22.	0947	2	3	-	2	4	2	-
23.	0752	2	3	-	2	4	2	-
24.	0540	2	2	-	2	4	2	-
25.	0723	1	2	-	2	4	2	-
26.	0867	1	2	-	2	2	0	-

(Contd).

Appendix-V (Contd.)

1	2	3	4	5	6	7	8	9
27.	0738	2	2	-	2	3	1	-
28.	0667	2	2	-	2	3	1	-
29.	0993	2	2	-	3	4	1	-
30.	0688	1	2	-	1	3	2	-
31.	0748	2	3	-	2	3	1	-
32.	0996	2	5	+	3	4	1	-
33.	0740	2	5	+	1	7	6	++
34.	0700	1	2	-	2	5	3	+
35.	0876	3	4	-	3	7	4	++
36.	0680	1	2	-	3	3	0	-
37.	0955	2	3	-	2	3	1	-
38.	B156	4	4	-	4	5	1	-
39.	B561	3	3	-	3	4	1	-
40.	0436	3	3	-	3	4	1	-
41.	0316	1	2	-	4	4	0	-
42.	0351	1	2	-	2	4	2	-

(Contd).

Appendix-V (Contd.)

1	2	3	4	5	6	7	8	9
43.	0022	1	2	-	3	3	1	-
44.	0567	2	4	-	4	5	1	-
45.	0191	1	3	-	3	4	1	-
46.	B367	1	3	-	3	3	0	-
47.	1263	1	3	-	2	3	1	-
48.	0701	3	4	-	4	5	1	-
49.	0320	3	2	-	3	6	3	+
50.	0726	2	2	-	3	4	1	-

APPENDIX-VI

Serum magnesium level (mg/dl) of Group-III animals

MID-II positive animals			MID-II negative animals		
Sl. No.	Animal number	Serum Mg (mg/dl)	Sl. No.	Animal number	Serum Mg (mg/dl)
1.	0989	3.9	1.	0666	3.5
2.	0758	4.0	2.	0880	3.6
3.	0964	4.4	3.	0996	3.9
4.	0740	4.0	4.	0959	3.9
5.	0876	4.3	5.	0974	4.1
6.	0700	3.8	6.	0982	3.8
7.	0320	4.2	7.	0723	4.8

MODIFIED INTRA - DERMAL TESTS FOR THE DIAGNOSIS OF PARATUBERCULOSIS IN GOATS

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ABSTRACT OF A THESIS

Submitted in partial fulfilment of the
requirement for the degree

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1994

ABSTRACT

Efficacy of single intra-dermal Johnin test was mpared against two modified intra-dermal Johnin tests for agnosis of paratuberculosis in goats. One hundred and fifty adult goats were divided at random into three groups and each group was subjected to one of the intra-dermal tests. Group I being the single Johnin intra-dermal (SID) Johnin test whereas in group II (modified intra-dermal test - MID-I) and group III (modified intra-dermal test - MID-II). The second injections were given after third and fifth days of first injection. Six animals giving positive results and two animals giving negative results were slaughtered from each group and confirmation of infection was ascertained by histopathology. Fecal samples and rectal pinch from all the animals were subjected to Zeihl-Neelsen's acid-fast staining for detection for M. paratuberculosis. Serum magnesium levels of all test-positive animals and an equal number of test-negative animals from each group were compared.

After 24 h MID-I was found to be superior to both SID and MID-II tests. The MID-I test had higher specificity (66.6 per cent against 58.33 per cent in SID and 40 per cent in MID-II) and predictive value of positive results (83.3 against

55.5 in SID and 50 per cent in MID-II). The MID-I and MID-II tests had 100 per cent sensitivity and predictive value of negative results as against 50 per cent and 33.3 per cent of SID.

Examination of fecal sample and rectal pinch was found to be of little value in the diagnosis of early paratuberculosis in goats.

Serum magnesium levels between test positive and test negative animals was found to be not significantly different in all three groups.

The results of the present study indicate that MID-I test could be used as an efficient diagnostic test for detection of paratuberculosis among goats.