CHAPTER V

DISCUSSION
DISCUSSION

SECTION I

The crossbreeding of cattle in India is rapidly expanding. The objective is to develop cows with increased milk production. Brucellosis is an important disease which threatens efficient animal production. The zoonotic and economic effects of the disease suggested further investigations.

International organizations such as the Food and Agriculture Organization (FAO) and World Health Organization (WHO) have stressed the importance of brucellosis in reducing animal and human production. The FAO/WHO Expert Committee on Brucellosis has met five times (Reports: 1951, 1953, 1958, 1964 and 1971) to review efforts to control the disease on global basis and to recommend diagnostic, control and eradication methods. Even though concerned scientists have added much to the knowledge about the disease and its control, it is considered to be one of the major animal disease problems in most parts of the world. There is further need for development and evaluation of diagnostic tests to detect infection and identify immunization responses. Some of the aspects of brucellosis included in these studies were the antigenic responses of the bovine foetus/neonatal
calves, influence of maternal antibodies on responses to strain 19, antibody titres in a vaccinated herd, susceptibility of selected and assessment of the effects on reproduction.

The present studies were conducted on a cattle development programme by crossbreed: progress which used Hariana-type foundation; brucellosis status was unknown in this found; A brucellosis control programme was undertaken farm by elimination of reactors and vaccination.

HOUSING

The Project consisted of 4 separate housing separate management though procedures were standardised: unit consisted of 16 sheds separated from cattle boxes (Figure 2).

Cows and heifers were housed in the "free stalls". Usually 30-40 milking cows or dry cows included were kept in each loose box. Dry cows include stages of pregnancy were usually kept in a separate "loose-box". Hariana-type cows were mostly kept in rows (facing in system). As a routine, all cows were transferred to calving pens a few days before expected parturition. Young calves were reared...
in groups of 2-3 in each calf-pen. Breeding bulls were separated from the herd.

FEEDING

Milk: Newborn calves from Hariana-type cows, i.e. F₁ calves, were allowed to suckle the dam for full lactation period. Successive generations were weaned at the 4th day and fed pooled milk from a pail.

Concentrate: Concentrate feeds from the Central feed mixing unit of the farm were used.

Fodder: Green fodder from the fodder section of the farm was fed while dry fodder (paddy straw) was procured from outside dealers.

CONTROL PROGRAMME

The foundation Hariana-type cows were initially tested for brucella antibodies by the rapid plate agglutination test during the period from November, 1969 to February, 1970 (Romvary, 1972). The positive cows were segregated in Herd No. 4. The negative cows were vaccinated with Brucella abortus Strain 45/20 adjuvant vaccine (Duphavac N.A.) twice at 6-8 weeks intervals during September-October, 1970 followed by a third dose after one year. The milk ring test (MRT) was continued at tri-monthly
intervals and all MRT positive cows were segregated. All calves were vaccinated with Brucella abortus Strain 19 vaccine between 4-8 months of age.

Since late 1972, milking cows were screened by the MRT at quarterly intervals. All MRT reactors were further tested by the serum tube agglutination test (SAT) and mercaptoethanol test (MET) for confirmation. Cultural isolations were attempted from SAT and/or MET reactors and the proven infected cases were segregated in Herd No. 4. Calfhood vaccination with S 19 vaccine has been practiced and adult vaccination with Duphavac N.A. was discontinued.

No earlier report from India is available in the literature where several aspects of brucella agglutinin have been studied under natural herd conditions on hundreds of indigenous and crossbred cattle.

The serum tube agglutination test is the most widely used method for the diagnosis of bovine brucellosis (Report, 1971). Standard Brucella abortus antigen was readily available in this country (IVRI, Izatnagar).

The tube agglutination test using 2-mercaptoethanol was included in the studies to assist in characterising the IgM (19 S) sensitive antibodies and IgG (7 S)
resistant antibodies. The presence of IgG is often correlated with infection. The test as described by Morgan (1967) is easy to perform and is of assistance in differentiating antibodies resulting from vaccination and infection. It was used in cattle of several ages: viz. neonatal calves, young calves (vaccinated or unvaccinated) and adult cows.

**BRUCELLA ANTIBODIES IN NEWBORN (0-3 DAYS) CALVES**

Brucella agglutinins were present in the blood of 67 of 153 (43.7%) neonatal calves at 20 IU/ml or more (Fig. 3). The prevalence was higher in female (48.8%) than in male calves (36.9%). No studies were conducted earlier to note the relative prevalence of brucella reactors (≥20 IU) between calves of different crossbreeds in India and between sexes. Absence of any statistically significant difference of prevalence between the groups indicate that all the groups behave similarly regarding brucella agglutinins under similar managerial conditions.

There were 12 of 153 (7.8%) of the newborn calves with agglutinins of ≥80 IU/ml. Following sources may provide antibodies in neonates:
A reactors (i.e., >20 IU/ml Brütting's antibody) among newborn calves.
(i) Transplacental } Passively acquired
(ii) Colostral
(iii) Foetal production } Actively acquired
(iv) Neonatal production

In bovines, transplacental transfer of maternal antibodies to the foetus does not take place. Morgan (1967) stated that immunoglobulins do not cross the placental barrier in cattle and the calves are born a-gammaglobulinaemic. However, it is certain that calves in-utero can develop immunoglobulins (Horner, et al., 1973; Gibson and Zemjanis, 1973) and therefore are not always born a-gammaglobulinaemic. Kaniazeft, et al. (1967) tested blood samples collected from the heart blood of bovine foetuses of the abattoir and observed that almost 40% of foetal bovine sera contained appreciable amounts of globulin against infectious bovine rhinotracheitis and bovine virus diarrhea viruses. They concluded that the findings indicated either transplacental transfer or possible "foetal synthesis". Morgan (1971) reported that calves born from infected dams did not have detectable brucella agglutinins before colostral feeding.

Colostrum is a rich source of antibodies and in the calf brucella agglutinins are absorbed through the gut-wall within the first 36 hours of a calf's life after which the globulins are denatured in the gut (Morgan, 1967).
The colostral S 19 antibodies in calves were studied by Baroni (1966). They were followed by a booster part of gestation, calf colostrum as well as found absorption of male of 12 post-partum calves. Merriman (1971) observed immunoglobulins in calf globulins within two days neonatal calves (Stoner, 1971). Maslyanko (1975) reported calf serum samples we detected globulins (IgG) after ingestion of colostrum.

From these studies that agglutinins were derived either passively. We were 117 newborn calves and 54 (46.1%) had agglutinins of ≥ 80 IU (Figs. 3 and 4) in all cases whether at birth before feeding.
BRUCELLA AGGLUTININS
IN NEWBORN CALVES

[Diagram showing agglutinins in newborn calves with various markers indicating total observations, before feeding cedrrom, and after feeding cedrrom.]
high agglutinin titres would suggest that colostrum from such dams could contribute for the agglutinin levels in the calves since colostral antibodies were absorbed from the gut (Morgan, 1967; Merriman, 1971; Stone and Deyoe, 1974).

Morgan (1971) reported that passively acquired colostral antibodies to the SAT had a half-life of about two weeks. The present observation of persistence of brucella agglutinins in calves up to one month in five cases and up to 4 months in one case may suggest the possibility that such antibodies were actively produced by the calves either (i) in neonatal life in response to infection from the infected dam/environment or (ii) in intra-uterine life in response to antigenic (infection) stimulus received from infected dam.

Smith and Ingraß (1965) reported that immunological competence of calf's age depends on the antigen. They observed high antibody production against human serum albumin plus adjuvant in the first week of birth, but *Klebsiella pneumoniae* polysaccharide induced immune responses after one month of age. They also reported that the feeding of colostrum may supress the immune response to certain antigens. Pilet and Mallick (1968) observed
that brucella antigens are capable of inducing immune responses in suckling mice. Steinbach and Krentzer (1973) reported that calves can be actively immunized against 'H' antigen of S. dublin before birth, but the degree of immunological reaction increased after birth with age. These studies suggest the possibility that neonatal calves respond to brucella antigens.

It usually takes about 3 to 5 days time to develop detectable brucella agglutinins in response to infection or vaccination. Since the calves were tested within 3 days after birth, active formation of antibodies following antigenic stimulus from the mother or environment after their birth (i.e. in neonatal life) is a remote possibility.

It was observed that 13 of 36 calves (bled before colostrum feeding) were born with brucella agglutinins. There were two possible sources of these antibodies, i.e. (i) actively produced in utero and (ii) from the dam in transplacental transfer. Since the latter is not known in the bovines, it is concluded that the bovine foetuses were active in antibody production in utero. This conclusion is supported by observations of other workers. Schimmel (1967) detected agglutinins against E. coli in the serum samples of calves prior to ingestion of colostrum. Horner, et al. (1973) observed the presence of specific
immunoglobulins against foetuses. The presence suggested that the development of independent immune response in the bovine foetus to Brucella antigenic agglutinins from the dams, in 143 pairs of dams, confirmed the observation of Emelyaner that the capability of bovine foetal brucella agglutinins.

The antigenic comes from the dams. There was a difference in titres in dams and in the titre in dams, more veterinary agglutinins (Fig. 5).
BRUCELLA AGGLUTININS IN NEWBORN AS COMPARED TO DAM

0—0 Dams agglutinin less than 20 I.U./ml
X—X Dams agglutinin 20 to 39 I.U./ml
A—A Dams agglutinin 40 to 79 I.U./ml
• Dams agglutinin 80 I.U./ml or above

% CALVES
this relationship between the three genetic groups, viz. Jersey cross, Holstein cross and Brown Swiss cross. However, the presence of agglutinins in the dam did not indicate the calf would also have antibodies since in only 53 cases were brucella agglutinins detected both in dam and its calf.

There were no agglutinins in 47.5% of the newborn calves from the dams having antibodies. The reasons could be (1) low agglutinin level in dam or (2) inability of antibodies to cross the placental barrier as mentioned by Morgan (1967) or (3) inability of antibodies to be absorbed from the colostrum by the gut wall. In this study, the quantity of agglutinins in the colostrum was not determined nor was the absorption in the gut of calves evaluated. Vaccination against brucellosis was routinely performed in the herds. It is possible that the cows in these cases were having residual vaccinal antibodies. One would not expect calves born to cows with vaccinal antibodies to also have blood serum titres. There were eleven calves with agglutinins (> 20 IU) whose dams were negative. The only possible source of this agglutinin appears to be its production by the foetus. Thus, this observation coupled with the persistence of agglutinins at one month and four months of age confirm the views of
Gibson and Zemjanis (1973) about the immunological competency of bovine foetus against *Brucella abortus* antigen.

Furthermore, the presence of brucella agglutinins in 20% (2 of 10) of pre-colostral newborn calves (tested within few hours of birth) from non-reactor dams strengthens the hypothesis of production of the antibodies *in utero* by the foetus. It is possible that the foetus may receive the antigenic stimuli from an undetectable (latent) infections of the dam. However, it would be useful to examine the serology of the dams for several months following parturition to help to test this hypothesis. Since the levels of brucella agglutinins were often observed to fluctuate at different times in reactor animals, it is possible that these sero-negative cows were the source of antigenic stimuli to the calves *in utero*. Thus, the detection of presence of agglutinins, in the newborn calves might be useful in detecting latent infection of the sero-negative dams.

Although agglutination, complement fixation and Rose Bengal plate tests have been performed on neonatal calves (Morgan, 1971) there are no previous reports on the MET. In these studies, among 14 calves which were MET positive, only 3 dams were also MET positive. Nevertheless,
it is concluded that the presence of ME-resistant antibodies in the calves was an indication that they were infected and the test can be useful procedure in calves and their dams. Since calves produce the antibodies in utero, the antigenic stimulus must come from the dams. But the dams do not always show antibodies concurrently with their calves as was observed in this study, though they have stimulated the foetus to produce the antibodies. Thus, in a brucella positive herd, it is concluded that ME-test conducted on newborn calves can serve as a method for detecting chronic or latent infection in dams where the serological tests are not conclusive.
BRUCELLA ANTIBODIES IN VACCINATED CALVES

The smooth vaccine strain 19 in agglutinins which interfere with the diagnosis of brucellosis. No previous studies have been conducted in India on the use of S 19 in crossbred calves. The study involved 213 calves of three crossbreeds, viz. Jersey Holstein cross and Brown Swiss cross with S 19 vaccine at 3-8 months of age. The immune responses 3 weeks post-vaccination (Ei breed differences. The largest number of calves showed immune responses ranging from 1280 to 2559 IU/ml. Mukhopadhyay (1968) observed that the individual immune responses following strain 19 vaccination were highest, ranging from 1280 to 2560 IU/ml at 15 days. The mean peak titre was on the 30th day, which was followed by a stationary period up to the 45th day. Agglutinin titres remained high even at 105 days following vaccination. Chekishev (1969) reported that 5 vaccinated calves had peak agglutinin titres during the 6-9 weeks post-vaccination period.
Distribution of agglutinin in calves of different groups at 3 weeks post-vaccination
fast sedimenting (high mol. wt.) antibodies
week and slow sedimenting (low mol. wt.) ant
the second week following S 19 vaccination w
by Rose and Roepke (1964). The peak rise of
wt. and low mol. wt. antibodies was by 13 da
42 days respectively. In these studies (Fig
ME-resistant antibody responses in vaccinate
similar to SAT titres. The largest number c
samples from all three breeds had ME-resista
titres (reciprocal) between 640-1279. There
Jersey cross calves and one Holstein cross c
not have ME-resistant antibodies.

Morgan (1967) reported that ME-resist
(IgG) appeared between 15-21 days post-vacci
were highest by 28-42 days. He also reporte
ME-sensitive (IgM) antibodies appeared (5-7
than those which were ME-resistant. The ant
in the crossbred cattle in this study were h
3 weeks after vaccination which agrees with t
of Morgan.

The persistence of agglutinins follow
of calves was examined. Tests were conducte
and 1 year. Only 3 of 167 (1.8%) calves had
of 160-319 IU/ml and 21 of 167 (12.9%) had a
Fig. 7

Distribution of ME-resistant agglutinins in calves of different genetic groups at 3 weeks post-vacc
titres of 80-159 IU/ml. There were 143 of 167 (85.6%) calves whose titre was less than 80 IU at 4 months post-vaccination. Mukhopadhyay (1968) studied agglutinin responses following vaccination in two calves for 150 days and found they had 80 IU/ml and 320 IU/ml. Siqueria, et al. (1975) observed a rapid decline in titres from one month post-vaccination and all calves were negative at 5 months.

The present studies found that 1 year following vaccination 90% (117 of 130) of the calves had agglutinins less than 80 IU/ml. There were 12 of 130 (9.2%) with titre of 80-159 and only 1 of 130 (0.8%) with \( \geq 160 \) IU. Nuru (1973) reported that 95% animals became negative (titre of 1:50 or less) at 13 months post-vaccination. No breed differences were observed in these studies and no previous work on this subject could be found.

There were only 2 of 165 calves (1.2%) which had agglutinins resistant to ME treatment at 4 months and none at one year after vaccination. These findings agree with the conclusion of Morgan (1967) that ME-resistant agglutinins disappear earlier than other agglutinins in vaccinated calves. There was good correlation in the SAT and MET among high agglutinin titres but among low SAT titres the MET was negative in a high percent of the
serum samples. This observation by Glawischnig and Cortes (15) that MET is superior to the SAT in titres to those caused by infection with neonatal agglutinins on persisting titres were studied (Fig. 8), indicating that agglutinins after one year of vaccination at 0-3 days. Of the four calves which have agglutinins, persistent titres following vaccination with S 19 may be titrated without serum. Additional studies may assist in the identification of neonatal antibodies.

The results of these experiments, vaccinated with S 19 may be titrated without serum, would assist in the identification of neonatal antibodies and brucellosis control efforts.
ANTIBODY AT DIFFERENT STAGES OF CALVES LIFE
(VACCINATED WITH STRAIN 19 AT 3-8 MONTHS)
No previous studies in India on crossbred cattle have been conducted on agglutinins during the first lactation. The SAT and MET were compared. A total of 226 cows of 3 crossbred types viz. Jersey cross, Holstein cross and Brown Swiss cross vaccinated with Brucella S 19 vaccine at their calfhood (3-8 months) were tested during first lactation. With the SAT, 93.6% of the cows had less than 80 IU/ml and were considered negative according to recommendations of the FAO/WHO Expert Committee on Brucellosis (Report, 1964, 1971). The presence of agglutinins at a level of 80-159 IU/ml (doubtful) in 12 cows of 226 (5.3%) and of more than 160 IU/ml in two (0.8%) cows (positive) suggested the possibility of persistent S 19 antibodies or infection. To assist in a diagnosis and characterise the type of agglutinins present MET was performed. Serum of cows with less than 80 IU/ml were negative to the MET and only one serum with a doubtful agglutinin level (80-159 IU/ml) was positive. Two cows having ≥ 160 IU/ml agglutinins by the SAT were also positive to the MET. The MET and cultural isolation had been in agreement in 96% to 97% cases (Nagy and Sorheim, 1969; Nicoletti, 1969). Though the MET positive cases
in the present studies were not proven culturally, it would be suggestive that the MET positive cases were infected. These observations confirmed earlier reports that agglutinin levels of calves vaccinated at calfhood disappeared prior to the first lactation and that cattle showing 160 IU/ml or above on the SAT at this stage should be considered as reactors if the reaction is confirmed by the presence of ME-resistant antibodies. The observations further lead to the conclusion that the SAT is more sensitive but less specific than MET. Rossi and Cantini (1968) observed that the mercaptoethanol test was negative in vaccinated, and uninfected cattle. Nagy and Sorheim (1969) reported that 4% of cows with a low titre (25 IU/ml) were infected on the basis of MET and CFT results. The MET and CFT had a 96% agreement.

Cattle with a divergent vaccination history (S 19 calfhood, Duphavac N.A. 45/20, Duphavac N.A. following calfhood strain 19 vaccine) were studied. The milk ring test on a composite sample from individual cows at tri-monthly intervals was performed and positive cows examined by the SAT and MET. An evaluation of these tests by Nicoletti (1969) indicated that the individual MRT correctly classified 89% of the culture positive cows while the SAT and MET were positive in 52% and 97%,
respectively. Katz, et al. (1976) also concluded that no single milk or blood test was capable of correctly diagnosing all cases of brucellosis because of the complexities in natural brucellosis and complications from calfhood or adult vaccination. He also suggested the use of a combination of tests for the most effective diagnosis of brucellosis.

The MRT with pooled (10-15 cows) milk samples detected 46.7% as reactor (+ or above). A composite milk sample from individual cows was positive in 513 of 4553 (11.2%) of the specimen. A comparison was made with these results and when the composite milk was diluted 1:10 with pooled negative milk. The use of MRT with composite milk samples (dilated) is helpful in detecting reactors.

Individual milk ring tests on composite milk may result in non-specific positive result (Alton and Jones, 1964). In these studies pooled milk from 10 negative cows was used for dilutions. After the milk was diluted, 71.5% were still positive. The results of California Mastitis Test conducted on the individual MRT positive milk samples suggest that subclinical mastitis was not uncommon in these cows. Since 28.5% of the positive individual milk samples were negative after dilution, it is probable that the effects of mastitis were decreased. Hajdu (1964)
reported that there was a correlation between increase in protein constituents in milk and non-specific positive MRT reactions. The results of these studies suggest that the specificity of MRT with individual (composite) milk is increased if the test is performed after dilution with pooled MRT negative milk.

Panda (1969) observed that the MRT with individual samples was equally effective as the SAT in the diagnosis of brucellosis. Pat and Panigrahi (1965) also observed that the results of the SAT and MRT were comparable. In this study, all positive MRT (composite) cattle were tested for agglutinins by the SAT and MRT. There were 96 of 482 cows (19.9%) without agglutinins and 384 of 482 (79.6%) which were MRT negative. The MRT was positive in 20.4% of the MRT positive cows. This suggests a high percentage of MRT positive and SAT positive and doubtful reactions were false positive. Morgan (1967) reported predominant or occasionally only MB-resistant antibody (IgG) in proven cases of brucellosis. Nicoletti (1969) found the MRT to be far superior than the SAT in diagnosing culture positive cows.

In the present studies there was a linear correlation of the degree of milk ring reaction on the MRT (+, ++, +++), and serum agglutinins to the SAT,
although about 20% of the cows with a + MRT reaction had no serum agglutinins. Brus and Jaarstveld (1963) observed that the results of the MRT and blood tests correspond only in about 60% of the cases. In these studies the corresponding figure was about 80%. Sinha and Pathak (1975) also reported marked variations in relative levels of agglutinins in blood and milk.

The variations in the MRT and SAT may be because milk and serum agglutinins develop independently and brucella agglutinins in milk result primarily from udder infection. Since the MRT correlates more closely with excretion of organisms in milk than the SAT (Nicoletti, 1969), the higher sensitivity of the MRT can be advantageous in early identification of probable Brucella excretors. The MRT results can be confirmed by other tests. However, if the cows are considered infected on the basis of MRT reaction only, in that case only a ++++ reaction can be considered positive as lesser reactions were not confirmed by the SAT and MRT in many cases.

Further evidence that the dilution of composite milk on the MRT increased the specificity is that 8.5% of the samples from cows with < 40 IU SAT titres were MRT negative after dilution. However, 5.6% of the cows
with $\geq 160$ IU on the SAT were also suggested that a high level of antibodies can often be correlated with the presence of the organisms in the milk.

The MRT with composite milk dilution technique, is useful in the diagnosis of brucellosis, if the degree of reactivity is taken into consideration.

The presence of weak MRT reactions (40-80 IU/ml) to S 19, Duphavac N.A in multiple doses of S 19) of cattle in this study. The milk samples could be useful to detect antibodies in milk due to vaccination, not included in this study.

There were 482 (composite of which 88 and 88 of these were positive by $\geq 60$ IU/ml. In addition, 100 positive by the MET (++) and above) dilution. It is suggested that in infected cattle the MET and MRT are useful for the SAT. The MET can be of assistance.
Fig. 9
of tests on milk (composite pc and blood)
condemnation of SAT positive cows which are not infected and in identification of chronic infections in cattle with less than positive SAT titres.

The milk ring test, serum agglutination test and mercaptoethanol test were conducted on 11 cows with premature termination of pregnancy.

All these cows were having high agglutinin level but one cow was negative to MRT dilution test and eight were positive to MET. The MRT with undiluted composite milk having +++ and ++++ reactions were correlated (90.9%) with high SAT reaction. Thus, the conclusions about the necessity of conducting several serological tests for detecting brucella reactors and about the correlation of +++ or above MRT reaction with the SAT are confirmed.

Results of studies on agglutinins at 3 month intervals for one gestation/lactation period showed wide fluctuation in titres at different intervals. This suggests that in case the SAT is only diagnostic test used in control programme in a vaccinated herd, as in India, in that event the test is to be conducted repeatedly, definite (3 month) intervals on the same cow so that the cow can be eliminated as and when it would show $\geq 160$ IU/ml.
There was a period of the year when the number of calves was in spring (June) and autumn (September, December). The results of the studies indicate that exposure of brucella-infected personnel to brucellosis during the spring. This is an epidemiological pattern where brucellosis is a seasonal reaction and season of occurrence. The correlation between the seasons of birth of calves and the exposure to brucella indicates the caution that personnel should take when handling these organisms when strictly warranted.
SEASONAL OCCURRENCE OF BRUCELLOSIS

- - - MYE REACTION
- - SAT REACTION

% POSITIVE

MARCH JUNE SEPT. DEC.
SIGNIFICANCE OF BRUCELLOSIS TO REPRODUCTION

Brucellosis is a major cause of bovine reproductive problems in India. Previous workers (Polling, 1947a; Prabhu and Chatterjee, 1970; Khera, 1973) have suggested that crossbreeding programmes result in a higher incidence of brucellosis. These studies were initiated to determine the effects of crossbreeding on the incidence of brucellosis and its effect on reproductive performance under identical managerial, climatic and nutritional conditions.

The results of a control programme undertaken on the farm can be evaluated from the annual incidence of brucellosis shown in Fig.1. The incidence decreased from 14% in 1970-71 to 1.5% in 1971-72 due to the test and elimination methods.

The increase in the incidence (sero-reactors) in 1972-73 may have been influenced by tests performed on vaccinated cattle which were less than 30 months of age. Some of the reactors had been vaccinated as adults with Duphavac N.A. and some were vaccinated as calves with S 19 followed by Duphavac N.A. in their adult life. The reactors were retained until 1973-74 (10.1% incidence) for tests to
hopefully differentiate the agglutinins resulting from vaccination and infection through the SAT and ME tests. The elimination of reactors considered to be infected resulted in a decline in the incidence during 1974-75 and 1975-76. The results indicate the necessity for (i) a fixed policy for vaccination against brucellosis with regard to the type of vaccine and the age of vaccination of animals and (ii) use of supplemental tests for diagnosing infection in organised herds where vaccination is regularly practiced (Pathak, 1969).

The possible differences in the susceptibility of different breeds to brucellosis was part of these studies. A study conducted by Kapur and Grewal (1974) revealed higher incidence of brucellosis in Tharparkar cows (9%) in comparison to Hariana (4%) and Sahiwal cows (1.4%).

In the present study there was no apparent difference among the 3 crossbred groups (viz. Jersey, Holstein and Brown Swiss).

The Hariana-type cattle had a statistically significant higher prevalence of brucellosis when compared to the first and second generation crosses with different breeds maintained under similar conditions. The introduction of exotic breeds on a limited extent into the Indian
cattle did not result in an increased prevalence of brucellosis and when a control programme was applied the prevalence was reduced equally in crossbred and Indian cattle.

In second generation crosses, where the exotic genotype was 75%, there was an increased prevalence. Further studies are indicated to arrive at a conclusion about the increased prevalence (susceptibility) of 75% exotic genotype (B_1) cows.

The effect of brucellosis on reproductive performance in 1929 cattle pregnancies was investigated in the present study. Of the 1929 pregnancies, 191 (9.9%) were terminated prematurely. The rate of foetal absorption was higher among indigenous (Hariana) than crossbred cattle. The abortion rates, were similar among Indian Zebu and European Zebu crosses. Polding (1947 a) reported a relatively higher incidence of abortion (20.4%) among European Zebu crosses as well as Indian Zebu (7.3%) cattle which cohabitated with European Zebu crosses. Prabhu and Chatterjee (1970), however, observed 6.8% abortions among crossbred and 3.7% abortions among indigenous cattle. The relatively low incidence of abortions in the crossbred cattle in this study may have been due to the improved
husbandry practices on the farm. The occurrence of foetal absorptions in different crossbred cattle were similar with the abortion rates. In the Hariana-type cows the foetal absorption rate (10.1%) was much higher than the abortion (3.9%) rate. No explanation is offered for these findings.

Though the incidences of abortion and foetal absorption were similar in crossbred cattle, the percentage of brucella reactors among the abortion and foetal absorption cases varied considerably (Fig. 11). Of 112 foetal absorption cases, only 1.7% were brucella reactors whereas 11.3% of the aborted cows were found to be reactors. Abortions due to brucellosis in the Jersey cross (16.6%) and Brown Swiss cross (14.2%) were approximately double those of the Holstein cross (6%) and Hariana-type cattle (7.7%). These results suggest that though the incidence of brucellosis in the Jersey and Brown Swiss crossbred cattle was lower than that in Hariana-type cattle, these exotic crossbred cattle have more clinical symptoms than indigenous Hariana-type cattle. This may be important in animal breeding programmes.

When the prevalence and clinical effects of brucellosis are considered, the Holstein-Hariana cross cattle appear to be preferential for improving the
productivity of cattle in India. Leech, et al. (1964) reported 6% of the cows in England calved prematurely of which 7% was caused by brucellosis. In the present study, 9.9% of the pregnancies terminated prematurely of which 5.7% (11 of 191) cases were probably caused by brucellosis.

The role of brucellosis (sero-reactors) to reproduction was assessed by studying the reproductive behaviour in 49 brucella reactor cases. This study being the first of its kind, no contemporary findings are available for comparison of these observations.

Average number of services required per conception, 1st service conception rate, average intercalving period were analysed in reactor animals considering the data of the project report (1974-75) as normal value. In Hariana and crossbred cattle the number of services required per conception was greater in reactor cows than in normal cows. The first service conception rate was also less in Hariana and crossbred reactor cows than normal cows. This suggests a negative effect on fertility due to brucellosis. Hignett, et al. (1966) observed a similar decrease in 1st service conception rates in brucella infected cows. In the present study, the interval between parturitions was greater in Hariana and crossbred brucella reactors when compared to normal cows. It was concluded that brucellosis
caused adverse effects on fertility rates when all the parameters were considered. The termination of pregnancy was studied in 47 cases (Fig. 12) of brucella reactors. There were abortions (8.5%), foetal absorpti (4.2%), stillbirths (4.2%) and sterility (10.6%). Among culture positive cows only 3 had a normal calf. The hypothesis on losses due to brucellosis in India propounded by Schawbe (1971) gets the support through the present studies on large number of cattle maintained under uncontrolled conditions. It is suggested that the present observations reflect the losses in productivity that occur among naturally infected animals in dairy farms in India.
Incidence of brucellosis in 2 types of pregnancy termination among 4 groups of cattle

Reproductive performance among brucella reactors