COMPARISON OF IMMUNE RESPONSE OF THE INDIGENOUS AND CROSS BRED CATTLE OF KERALA

By

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THESIS

Submitted in partial fulfilment of the requirement for the degree

Master of Veterinary Science

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Mannuthy, Thrissur

1994

DECLARATION

I hereby declare that the thesis entitled "Comparison of Immune Response of the Indigenous and Crossbred Cattle of Kerala" is a bonafide record of research work 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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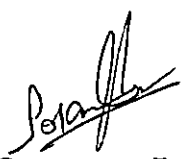
CERTIFICATE

Certified that the thesis entitled **"Comparison of Immune Response of the Indigenous and Crossbred Cattle of Kerala"** is a record of research work done independently by **Shri. P. Francis Bastin**, 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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ACKNOWLEDGEMENTS

I would like to express my sincere thanks and indebtedness to Dr. Sosamma Iype, Professor, Department of Animal Breeding and Genetics, College of Veterinary and Animal Sciences and Chairperson of the Advisory Committee for the proper guidance, encouragement and support extended by her in the development of this research.

I am grateful to Dr. G. Mukundan, Director, Centre for Advanced Studies in Animal Genetics and Breeding and member of Advisory Committee for his valuable guidance and constant help throughout the period of study.

I wish to express my deep sense of gratitude and indebtedness to Dr. P. Nandakumar, Asst. Professor, Department of Animal Breeding and Genetics, and member of Advisory Committee for his constant encouragement, valuable suggestions, proper guidance and useful advices throughout the period of study.

I am also thankful to Dr. P.C. James, Professor, Department of Microbiology, and member of Advisory Committee for his help during the research work and preparation of thesis.

I am indebted to Dr. Girija for her constant support and encouragement throughout the course of this work.

I am grateful to Dr. B. Nandakumaran, Dr. K.V. Raghu Nandanan, Dr. T.V. Aravindhakshan, Dr. Stephen Mathew, Dr. Rajagopala Raja for their valuable help and encouragement throughout the course of this work.

I would like to place on record my heartfelt thanks to Dr. Joju Davis, Dr. Madhavan, Dr. Bindu, K.A., Dr. Mary John, Dr. Raj Menon, Dr. Radhakrishnan, Dr. Gopakumar, Dr. Tirupathi and Dr. Manu Mohan, S., for the valuable time and effort they spent in contributing to this research.

I thankfully acknowledge Dr. Padmakumar, Dr. Vinodkumar and Dr. Raju for donating their blood for conducting this research.

I also acknowledge Dr. Kannan, Dr. G. Ajitkumar, Dr. K.S. Anil and Dr. Prabhakaran, for their valuable support and help during the whole period of study.

I am grateful to Dr. A. Rajan, Dean, Faculty of Veterinary and Animal Sciences for his advices and encouragement.

I am thankful officer in charge of University Livestock Farms at Mannuthy and Thumburmuzhi for the timely help, provided by them.

I am indebted to Kerala Agricultural University, for the financial support rendered.

P. FRANCIS BASTIN

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Introduction

INTRODUCTION

Kerala has a dwarf variety of cattle which is referred to as the indigenous variety of Kerala. These animals are being replaced by their exotic crosses gradually. At present Kerala has about 50 per cent of crossbreds. The organised farms of Kerala which are mostly under the government sector have never been keeping indigenous cattle of Kerala and presently crossbred cattle are kept in these farms. The only farm where local indigenous animals are kept and accurate recording is done in the Kerala Agricultural University farm at Mannuthy for conservation of indigenous cattle of Kerala.

The indigenous cattle of Kerala are disease resistant, heat tolerant and well adapted to the locality. This has been evident during outbreaks of Foot and Mouth disease and other diseases. The local cattle have shown higher disease resistance and lower morbidity and mortality compared to crossbred, though exact records are not available.

Genetic variability, the basic prerequisite for effective selection, has been found in animals and birds exposed to a variety of viral, bacterial and parasitic diseases.

The immune system of an individual consists of three major facets as phagocytosis, cell mediated immunity and humoral immunity which were performed respectively by macrophages, T lymphocytes and B lymphocytes (Cheng and Lamont, 1988) and each of these functions are specialized in the protection against some infections and scarcely effective or ineffective against others. In spite of functional integration of these three cell types basically involved in the three immune functions, they are distinct protagonists under separate polygenic control. Both humoral and cell mediated immunities are under independent genetic control and there exists an inverse relationship between genetic regulations of antibody responsiveness and macrophage activity.

Eventhough immune system is the most important system affecting disease resistance, existence of both non-specific and immunologic specific host defense mechanisms has been established. Non-specific or innate immune mechanisms operate through phagocytic leukocytes (Neutrophills and Macrophages) as well as a variety of soluble mediators as lactoferrins, lactoperoxidases, thiocyanate system, hydrogen peroxide system, lysozyme, cationic proteins and complement proteins. The specific or adaptive immune system consists of leukocytes and antibodies (Brian, 1989).

It was thought necessary to look into the incidence of diseases of the indigenous and crossbred cattle under more or less the same management and also to find out the immune response of two groups of animals. It was possible to use only a harmless antigen for the purpose and hence it was thought appropriate to use human red blood cells to assess the humoral response and 2,4-Dinitro chloro benzene to assess cell mediated immunity. Whether the response to these, would be taken as criteria to the general immune response of animal was to be examined.

The present investigation was undertaken to assess the humoral and cell -mediated immune response status of the indigenous cattle and indigenous exotic crossbred cattle. The following are the main objectives of the present study.

1. To compare the immune response (humoral and cell mediated) of the indigenous and crossbred cattle of Kerala.

2. To study the association between.

   a. Immune response and incidence of common tropical diseases in cattle.

   b. Maternal immune response and neonatal calf diseases and mortality.

Review of Literature

REVIEW OF LITERATURE

Genetic control of disease resistance and immune responsiveness is highly complex involving several systems of body and their interactions. Most important among these systems is the immune system. Immune system comprising of reticulo endothelial cells and a variety of other cellular types and their secretary products. The immune responses can be categorized into three major types namely phagocytosis, humoral responses and cell mediated immune responses performed respectively by macrophages, B cells and T cells (Cheng and Lamont, 1988; Warner et al., 1987). The antibody production of the humoral immune responses is dependent on the interaction of T cells, B cells and macrophages (Unanue et al., 1984). Cellular immune responses involve the action leukocytes including T cells, macrophages, natural killer cells etc. (Nabholz and McDonald, 1983; Adams and Hamilton, 1984; Herberman et al., 1986). The literature pertaining to humoral and cell mediated immune responses and their associations with various viability, fitness and production traits are reviewed.

Humoral immune responses

Burton et al. (1989a) observed that well defined immunological marker traits indicative of disease resistance

may have higher and more accurate heritability estimates than health traits. According to them this may provide an efficient indirect means of genetically modifying disease resistance in domestic animals. The most practical application in the direction would be by challenge test of a harmless test antigen fitting all the required criteria of a marker trait for genetic improvement of disease resistance.

In the words of Biozzi et al. (1979) the mammalian humoral immune response to the complex immunogens as sheep red blood cells (SRBC's) were polygenic in inheritance as evidenced by the studies in mice using sheep red blood cell (SRBC) as a test antigen. Hyldgaard-Jensen, (1979) observed the ability of pigs to produce specific antibody to standardize doses of human and bovine albumin were under genetic control.

Almid et al. (1980) studied the quantitative antibody response to diphtheria toxoid in goats. Buschmann (1980) studied the antibody response in pigs. They could show that this immunological function could be significantly modified by selective breeding for two to three successive generations.

Dominant type of inheritance was noticed in inbred strains of mice selected for high responsiveness to human thyroglobulin when measured by ELISA (Aihara et al., 1983).

Murthy and Ragland (1984) reported significant suppression of antibody response to SRBC in carrgeenan (CGN) treated birds.

According to Santanna et al. (1985), the lines of mice selected for high responsiveness to TNP-LPS had higher response to TI-1 of mice. This finding confirmed that genes accumulated through selective breeding could modify responsiveness to unrelated antigens.

Ferreira et al. (1986) reported that antibody responses of mice to rabbit gama globulin (RGG) and bovine serum albumin (BSA) were controlled by additive effect of several independent loci.

Gyles et al. (1986) concluded significant differences between breeding groups of chickens for antibody response to different classes of antigen as Newcastle virus vaccine, Infectious bronchitis virus vaccine, SRBC etc.

Reynolds and Griffin (1986) concluded that total antibody production was significantly impaired in ewes during late pregnancy.

According to Burton et al. (1989,a) humoral antibody responses of calves to human red blood cells (HRBC) could be used as a marker trait of disease resistance in dairy calves.

Gross and Siegel (1990), concluded that the antibody response of individual chicken to SRBC were influenced by their heterophil-lymphocyte ratios.

According to Loudovaris et al. (1990) the genetic regulation of magnitude of antibody response to SRBC was associated with major histocompatibility complex (MHC) in chickens.

Pinard et al. (1991) reported influence of dam on humoral immune response of fowls to SRBC. They also concluded that major histocompatibility complex (MHC) genotype accounted for only 3.5 - 7.5 per cent of variation in antibody titre. Significant differences in humoral immune response of pigs to salmonella vaccine were reported between groups of half sibs (Zhuchaev et al., 1990).

Primary response

Hyldgaard-Jensen (1979) concluded that peak antibody titre to bovine and human albumin was obtained 2-3 weeks after the primary immunization in pigs and the primary antibody response were influenced by adjuvant and dose of antigen.

In poultry, additive genetic variation was noticed for high and low antibody titres at 5 days after SRBC inoculation (Siegel and Gross, 1980).

Zijpp and Leenstra (1980) and Zijpp et al. (1983) reported that in poultry, the mean total antibody titre to SRBC was highest on day seventh, after the primary inoculation.

Zijpp et al. (1983) concluded that on day three and seven of post primary injection of chickens with SRBC, random size effect was not significantly different from day zero ($P \geq 0.05$). Selection for an improved primary antibody response can be based on total antibody titre level at day five post injection.

Ubosi et al. (1985) reported that by fourth day post-primary injection of chickens with SRBC, differences in response were noticed. But peak values reached by same time in all populations (6 days) after the primary inoculation. According to Burton et al. (1986) the primary humoral immune response in neonatal calves tends to be more highly inherited than the secondary response when assessed by ovalbumin and HRBC as antigens.

Zijpp et al. (1988) concluded that CGN treated chicks had a lower primary antibody response to SRBC than untreated birds.

According to Burton et al. (1989a) peak, primary antibody titres of calves occurred to HRBC by day seven to 14

post primary immunization. The peak antibody titre to sheep red blood cells occurred on day six and seven post primary immunization of chicks (Miller et al., 1992).

Secondary responses

Zijpp et al. (1983) reported that in chicken the mean total highest antibody titre to SRBC occurred on day five after the secondary inoculation. The cockerels had higher total antibody titres than pullets on day zero and three post injection. They also noticed significant sire and dam effect on secondary response.

According to Ubosi et al. (1985), peak antibody titres to SRBC in poultry reached by three days of post secondary immunization.

According to Zijpp et al. (1986), the peak antibody titre to SRBC after post secondary immunization occurred by day seven in chickens.

Davis and Glick (1988) reported that in chickens the anamenestic response obtained at sixth or eighth week post primary injection with SRBC had a higher response than those obtained within two to four weeks after the primary immunization.

Zijpp et al. (1988) stated that treatment of chicks with CGN, suppressed the secondary antibody response to SRBC'S.

Burton et al. (1989a) reported that in calves the secondary antibody response to human erythrocytes peaked by day seven post immunization, whereas the peak secondary antibody titres was reached by day five post immunization with SRBC'S in chickens selected for higher antibody response (Kreukniet and Zijpp, 1990).

But Munns and Lamont (1991) have stated that the peak antibody titres of chickens to SRBC reached on day six post secondary immunization. But Miller et al. (1992a) have concluded that chickens selected for 56 day body weight had peak antibody titre to SRBC'S on day five post secondary immunization.

Cow-calf comparison

According to Hyldgaard-Jensen (1979), age of pigs influenced the total antibody production against bovine and human albumin.

In poultry, peak haemagglutinin values of SRBC occurred between three and six months of age and declined

thereafter (McCorkle and Glick, 1980). Siegel and Gross (1980) stated that in poultry response to SRBC in terms of antibody production was not age specific. In aged mice, the antibody producing ability was higher than young ones (Folch et al., 1982).

According to Nguyen (1983/84) sheep aged three months produced significantly lower antibody levels to chicken RBC than adult sheep. The studies conducted by Lamont and Smith (1984a) demonstrated that effect of age was significant while comparing the antibody production to SRBC among various genetic stocks of poultry.

Zijpp et al. (1986) reported that humoral antibody response of chicks to SRBC was not influenced by age. But Warner (1987) have stated that antibody production in response to immunization with SRBC was greater in young chickens and young dogs.

According to Ayeni (1988), when West African Dwarf goats were immunized with chicken RBC'S and their antibody response assessed by direct haemagglutination, kids of about one month old developed antibody titres which were significantly lower than those of adults. In calves during the first five weeks of life no significant differences were

noticed in antibody responses to immunization with Escherichia coli (Strain J 5) when determined by ELISA (Tyler et al. (1989).

The primary antibody response of chicken to sheep RBC exhibited significant age effect, with chicks of six months age responding at a higher level than four week old chicks (Munns and Lammont, 1991). But old mice of 20-24 months had markedly reduced antibody response than young adult mice of two to three months old when streptococcus pneumoniae vaccine was used as the antigen (Nicoletti and Cerney, 1991).

The secondary antibody responses of Merino sheep to ovalbumin and live Brucella abortus antigen were higher for adult wethers than young lambs and older lambs had a significantly higher antibody response to the above antigen than two week old lambs (Watson and Gill, 1991).

Effect of genetic group

Zijpp (1978) have reported significant breed strain differences in humoral immune response of poultry to SRBC'S.

Banyard and Morris (1980) concluded that when Bos indicus and Bos taurus animals were inoculated with Keyhole Limpet haemocyanin (KLH), a higher antibody response was obtained in Bos indicus animals.

Lie et al. (1983) reported that in cattle, sire families, but not sires differed significantly in their antibody response, when their progeny young males were inoculated with human serum albumin and a synthetic polypeptide.

Differences among genetic groups resulted in significant differences for total antibody titres to SRBC in poultry (Zijpp, 1983a). The ranking of groups of different origin varied with day post injection. Lamont and Smith (1984a) reported significant breed differences in antibody producing capacity of poultry against SRBC and Brucella abortus.

Rothschild et al. (1984) reported significant breed differences in antibody responsiveness of pigs to inactivated B. bromchiseptica. The response was also influenced by dam. The mice selected for high or low antibody responsiveness to Salmonella typhinurium have shown interline differences in their response.

The line differences that exist in antibody responsiveness of chickens to SRBC mere polygenic in inheritance (Okabayashi et al., 1987). Considerable variation in antibody titre to SRBC was found both between and with in populations of poultry and differences were noticed in the

rate of development of immune response to a given antigen between poultry populations (Peleg et al., 1985).

Ubosi et al. (1985) reported significant population differences in response of chickens to SRBC. Regardless of population, peak titres occurred at about same time after primary and secondary immunizations.

Buschmann (1986) found breed differences in the humoral immune response of pigs.

According to Muggli et al. (1987), the levels of infectious bovine rhinotracheitis virus antibodies 60 days post vaccination, did not differ among different breeds of cattle as Angus, Hereford and Red poll calves.

In White Leghorn chickens significant subline differences were noticed in their humoral antibody responses to GAT (glutamic Acid^{60} - alamine^{30} - tyrosine^{10}) (Cheng and Lamont, 1988).

In domestic fowl, antibody response to SRBC have shown differences between white leghorn, Rhode Island Red and Rhode Island White breeds. They have also noticed existence of breed differences in relationship between the antibody titre to two antigens as Brucella abortus and SRBC (Petrovksy et al., 1988). Significant breed differences were noticed in

swine in their humoral immune response to Tetanus toxoid immunizations (Buschman and Meyer, 1990).

Significant line differences were noticed in layer chickens in their humoral responses to SRBC's (Genzel and Weigend, 1990). Significant breed differences were noticed in fowls in their humoral immune responses to SRBC'S (Benda et al., 1990).

Disease association

Gross et al. (1980) reported that chickens selected for ability to produce high antibody titres to SRBC exhibited stronger antibody response to Newcastle disease virus and was found to be more resistant to Mycoplasma galliseptium, Eimeria necatrix, Spleenomegalia virus and feather mites and were less resistant to Escherichia coli and Staphylococus aureus infections. The line of chickens selected for non persistence of antibody titres to SRBC was more susceptible to all infectious agents. Nordskog (1983) concluded that pullets having lower antibody response to certain antigen as ferritins, bovine serum albumin (BSA), and parainfluenza-III virus produced significantly low level of antibodies to Salmonella pullorum.

According to Covelli et al. (1984) mice selected for high antibody responsiveness to SRBC and Salmonella flagellar

antigen had lower incidence of lymphomas but selection for antibody responsiveness did not always appear to influence the incidence of lymphoma. Dunnington et al. (1986) selected chickens for 12 generations for high or low antibody titre, the line with low antibody titre had more susceptibility to Marek's disease than high response line, they were affected at a younger age.

According to Mouton et al. (1988) innate resistance to intracellular pathogens was higher in mice selected for low immune responsiveness in terms of antibody production. This was because of faster antigen catabolism in macrophages of these lines. The mice selected for higher immune responsiveness in terms of antibody production had stronger innate or acquired resistance to all infections that can be cleared by means of antibody production.

In calves diarrhoea prevalence was negatively correlated with high primary antibody responses against HRBC's, but no association was observed for prevalence of pneumonia (Burton et al., 1989a). Chickens selected for four generations for early high antibody response to Escherichia coli and Newcastle disease virus vaccinations. Showed greater resistance to challenge with Escherichia coli (Pitcovski et al., 1989a).

According to Larsgaard (1990) an improved health status was noticed for the goats selected for a higher immune response.

According to Lillehoj (1991), the inbred strains of chickens having higher antibody response and higher T cell responses had a reduced susceptibility to Eimeria tenella infection.

Mortality

According to Zijpp (1983b), the lines of chickens selected for a high antibody response to SRBC had a lower mortality rate. Covelli et al. (1984) reported higher life span for mice selected for high antibody responses to SRBC. But high responsiveness to Salmonella flagellar antigen, did not appear to influence the life span of mice.

Dunnington et al. (1986) selected chickens for 12 generations for high or low antibody titre and the line with low antibody titre had higher cumulative mortality.

The lines of mice selected for high antibody responses to SRCB as measured by ELISA had a positive correlation with life span (Covelli et al., 1989). Similarly chickens selected for four generations for early high antibody response to E. coli and New Castle Disease Virus Vaccinations had a lower

mortality rate (Particularly with respect to Marek's disease) (Pitcovski et al., 1989b).

According to Leitner et al. (1989) broiler chickens having higher antibody titre to E. coli vaccination had lower mortality rate when challenged with pathogenic E. coli.

Leitner et al. (1992) reported birds having higher antibody titre to E. coli vaccination ten days post vaccination had lowest morbidity and mortality rate when challenged with pathogenic E. coli.

Cell mediated immune responses

It has been increasingly common to study delayed type higher sensitivity (DTH) by using various skin sensitivity tests for assessing the cell mediated immunity (CMI) response. These tests help us to assess immune status of the animals. The chemical hapten 2,4 dinitrochlorobenzene (DNCB) had gained much importance now a days and is widely used as a sensitizing agent to assess CMI response.

According to Eliber and Morton (1970), DNCB skin sensitization test could be used as one of the most reliable tests to assess the CMI status of humans by measuring the increased double skin fold thickness. Brummerstedt and Basse

(1973) were the first to assess the CMI response in calves using DNCB.

The sensitized state of animals (calves) by DNCB were indicated by lesions like flare and erythemia from ninth to 12th day of sensitization at the site of application (Jennings, 1979).

Reddi et al. (1981) standardized the dose and number of applications for primary sensitization and challenge dose of 14th day with DNCB in cattle. Subsequently a study was undertaken to assess the efficacy of DNCB sensitization test in evaluating CMI response in goats (Rajan et al., 1981). Valsala et al. (1981) first described DNCB test for assessing CMI response in ducks.

According to Awadhiya et al. (1982) a slight increase in vascular permeability was present at 24 h, but absent at 48 h in poultry sensitized with DNCB.

Rajan et al. (1982) standardised the dose and site of application of DNCB for assessing CMI in pigs.

According to Paliwal et al. (1984), among the in vivo delayed cutaneous hypersensitivity reactions with phytohaemagglutinin (PHA), Johnin and DNCB test, DNCB test was found to be better for assessing CMI response of cattle.

Tiwary and Goel (1985) concluded that DNCB skin test was equally effective in assessing CMI response of chickens as graft virsus host reaction (GVH) reaction and lymphocyte transformation test.

Buschmann (1986) concluded that repeatability was high for T cell dependent cellular parameters in pigs.

According to Burton et al. (1989b) measurements of double skin fold thickness (mm) to DNCB challenge could be used to assess the feasibility of use of DTH response as an index of CMI response in calves and the same could be used as a marker trait to select for genetically improved disease resistance.

Cow-calf comparison

Trindle et al. (1980) reported that in mule deer fawns (Odocoileus hemionus), the CMI was measurable even at the age of one week by using 2,4 DNCB skin sensitization test. Newly hatched turkey poults demonstrated DTH response to PHA and Freund's adjuant containing mycobacterium tuberculin. Two week old poults had a higher DTH than eight week old ones (McCorkle et al., 1984). The DTH reaction of fowls to human gammaglobulin was more intense at six to 12 weeks of age than at three weeks of age (Watable and Glick, 1983).

According to Edelman et al. (1986) difference between T cell reactivity in immunocompetent normal chickens and transplantable fibrosarcoma bearing chickens could be readily detected in vivo at an early age of three to four weeks using PHA wattle test. The wattle swelling indicates the level of CMI response.

According to Warner (1987), the CMI, as measured by DTH to DNCB was greater in young adult beagles than in aged dogs. But he could not detect any age effects in case of chicken using the same test. Similarly when DTH to dinitroflurobenzene (DNFB) was assessed for CMI evaluation in calves, the dermal response was greater in calves aged 11 months than in younger groups (Paulik and Vrzula, 1989).

Corrier and Deloach (1990a) stated that in chickens CMI response could be elicited in young of 10-14 d old using sensitization with Mycobacterium tuberculosis and challenging with tuberculin intradermally. The increase in mean interdigital skin thickness indicate the degree of cell mediated immune response. But, by using PHA, cell mediated immunity could be assessed in young chickens of even three to 14 day old (Corrier and Deloach, 1990b).

Effect of genetic groups

In broiler chickens, CMI response to Diphtheria toxoid

varied between different genetic stocks (Klesius et al., 1977).

Lie et al. (1983) reported significant differences between sire families, but not between sires, in cattle, in their CMI responses.

Significant breed differences were noticed in CMI response of chickens to PHA skin test ($P<0.05$) (Zijpp, 1983a; Lamont and Smith, 1984b). Existence of significant breed differences in CMI response were noticed also in pigs (Buschman, 1986).

Cheng and Lamont (1988) reported significant subline differences in White Leghorn chickens in their CMI responses when assessed by PHA skin test.

According to Benda et al. (1990) also, significant breed differences were noticed in cellular immune response of fowls when assessed by wattle injection with SRBC's.

Disease associations

Brown et al. (1967) studied the efficacy of DNCB skin tests in untreated Hodgkin's diseases human patients and found that DNCB skin sensitization could be used as one of the most reliable skin tests in evaluating the CMI status of patients by measuring the double skin fold thickness.

Lukhis et al. (1984) concluded a little difference in skin reaction to DNCB between healthy and leukotic cattle (lymphoid leukosis). This indicate that there exists a little change in the function of T-lymphocytes in bovine leukosis.

Chickens that were to develop clinical Marek's disease had significantly lower prechallenge levels of CMI, than those that are resistant to Marek's disease. It has also been proved that there exists some correlations between high levels of CMI and resistance to other diseases in addition to Marek's disease (Chauhan et al., 1984).

In Johne's disease infected cattle, the CMI response was significantly suppressed when assessed by DNCB skin sensitization (Paliwal et al., 1984). According to Tiwary and Goel (1985) CMI deficient chickens artificially produced by neonatal thymectomy and inoculation of antithymocyte serum have shown significantly reduced CMI response to DNCB skin test in terms of reduced skin thickness following challenge with DNCB.

Chickens bearing transplantable fibrosarcomas failed to show any CMI responses in form of wattle swelling when assessed by PHA (Edelman et al., 1986).

According to Burton et al. (1989b) prevalence and severely of naturally occurring diarrhoea and pneumonia did

not significantly affect quantity of response to DNCB in form of increased skin thickness.

Desmukh et al. (1990) reported significant increase in CMI responses of kids naturally infected with goat pox, when assessed by DNCB skin test. But significant reduction in CMI responses were seen in fowls having Marek's disease, when assessed by Dinitro fluro benzene (DNFB) contact sensitivity test (Gupta et al., 1990). Similarly the dogs suffering from atopic dermatitis had markedly reduced CMI response to DNCB in terms of increase in skin thickness (Nimmo Wilkie et al., 1991).

Area of reaction (diameter)

The contact sensitivity reaction site was reported to be slightly raised above the normal skin and was found to be extended over an area ranging from 4.2 cm to 5.8 cm (Reddi et al., 1981). They also observed that, the sites of challenge were greatly thickened and endurated in some animals and the diameter of the area measured about 6.5 cm.

Rajan et al. (1982) reported that the mean thickness of the skin and the diameter of area of reaction in healthy pigs by DNCB test at 24 and 48 hours were found to be 6.07 $\pm$ 0.38 mm and 1.25 $\pm$ 0.06 cm and 4.71 $\pm$ 0.66 mm and 1.04 $\pm$ 0.11 cm respectively.

Materials and Methods

MATERIALS AND METHODS

The present experiment was conducted at University Livestock Farms located at Mannuthy and Thumburmuzhi and the farm for conservation of Vechur cattle at Mannuthy of Trichur district.

The animals employed for the present study comprised 30 adult indigenous dwarf cattle and their 36 calves and 40 adult crossbred cows and their 40 calves kept at University Livestock Farms formed the material for study.

Humoral immune response

Test antigen preparation

Human erythrocytes were chosen as test for immunization of the animals since they were complex, foreign and harmless (Burton et al., 1989a). Type A Rh^+ human RBC in anticoagulant were washed three times in sterile phosphate buffered saline (PBS, 0.01 M, pH 7.2) by repetitive centrifugation (1400 rpm for ten minutes) and were gently resuspended in fresh sterile phosphate buffer saline. Twenty per cent (vol/vol) washed packed human red blood cells in Alsevers' solution (a physiological sugar solution) were

prepared for each two week immunization period and stored at 4°C in sterile glass bottles capped tightly with syringe caps.

Administration of antigen

Immunization with the test antigen (20 per cent HRBC suspension) were conducted on day zero (primary immunization) and day 14 (secondary immunization). The test antigen was injected at a rate of 1 ml per 50 kilogram body weight via the juglar vein.

Harvest of serum for monitoring antibody level

Blood samples were collected at the zero, seven and 14 day following primary immunization. Secondary immunization was done at 14th day of primary immunization. Blood samples were again collected on third, seventh and 14th days post secondary immunization. Immunizations with the test antigens was conducted immediately after collecting blood samples on the day of primary immunization and on day 14 of primary immunization. The sample collected on the day of primary immunization was intended as preimmunization serum and that from day 14 as the last sample from the primary response. Serum samples from, third, seventh and 14th day post-secondary immunization was to test the secondary immune response.

The blood samples were left standing upright at 4°C for 24 hours before centrifugation at 1500 rpm for ten minutes. The serum from each sample was stored in tightly capped plastic culture tubes at -20°C.

Serology

The cattle serum antibody to human red cells was titrated by standard direct microhaemagglutination procedure using Type A Rh^+ washed packed human red cells suspended to one per cent (vol/vol) phosphate buffer saline as antigen (Zijpp and Lenestra, 1980). Positive and negative control sera were used on every 96 welled u-bottom microtitration plate (Laxbro, Pune) to ensure that titres were with in ± one doubling dilution from pre-established control sera means. The antibody titres were recorded as the highest dilution of serum giving a visible positive haemagglutination (Plate I).

Cell mediated immunity

Assessment of cell mediated immunity

The animals selected for the humoral immune response study were also used for assessing the cell mediated immunity. The cell mediated immunity was assessed using 2, 4, Dinitro 1 chloro benzene (DNCB) skin sensitization test using the procedure described by Reddi *et al*. (1981) with modifications.

2,4-Dinitro 1 chloro benzene test was chosen for assessing the cell mediated immune status since this was found to be superior to other in vivo delayed cutaneous hypersensitivity reactions using phytohaemaglutinin and johnin in cattle (Paliwal et al., 1984). The DNCB skin test was also found to be equally effective for assessing cell mediated immune status a graft versus host reaction and lymphocyte transformation test (Tiwary and Goel, 1985).

2,4-Dinitro chlorobenze was applied at the neck region 15 cm anterior to the shoulder blade. In this region an area of 2.1 cm diameter were marked with the help of a metallic ring and this area was clipped close to skin with scissors. The metallic ring was placed over the prepared site and two percentage DNCB solution in acetone was dripped slowly, two to four drops at a time using a tuberculin syringe with a hypodermic needle. The solution was allowed to dry immediately by blowing so as to prevent it from running down the neck region. 0.5 ml of two per cent DNCB in acetone was applied for two consecutive days at the same site. All the animals that were sensitized by the above method were challenged at opposite side of neck on the 14th day. The challenge dose was 0.5 ml of two percentage DNCB in acetone. The double fold skin thickness and diameter of area of reaction were measured just before the application of

challenge dose and exactly 24 hours, 48 hours and 72 hours post DNCB challenge.

To assess the various phases of cutaneous delayed type hypersensitivity, in the absence of inherent skin thickness, test site measurements were subtracted from each other as 24 h minus pre-challenge thickness to indicate onset of peak response, 48 h minus 24 h thickness to reflect the persistency of peak response and 72 h minus pre-challenge thickness to indicate duration of total response. Similarly skin diameter of area of reaction were also assessed.

Disease prevalence

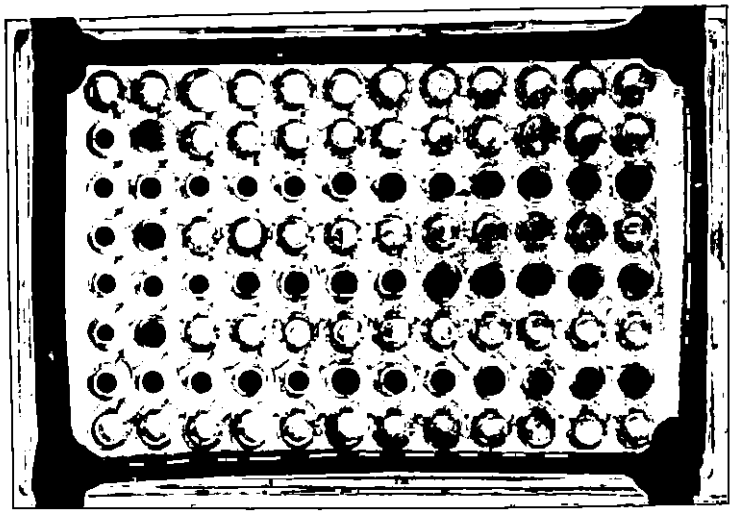
The data on the incidence of respiratory diseases and mastitis was collected from the experimental herds namely indigenous and crossbred cattle at University livestock farm, for a period of about two years. Similarly data on neonatal and adult mortality were also collected. Association between various immune response traits and the incidence of above mentioned disease situations and mortality were assessed.

Statistical analysis

The antibody titres were expressed as $\log_e + 1$ since the observations did not follow a normal distribution. The comparison of means were done using students 't' test. The

degree of relationship between mother and progeny in their immune traits were measured by coefficient of correlation. The incidence of diseases and their association with immune traits were assessed. Chi-square (χ^2) analysis were conducted to assess the disease incidence between crossbred and local indigenous cattle.

Plate I Microhaemagglutination test



Results

RESULTS

In the present investigation the humoral and cell mediated immune response of the indigenous cattle along with the temperate crosses of the local non-descript cattle were compared. The humoral immune response was assessed using HRBC as the test antigen and cell mediated immunity was assessed using DNCB.

Humoral immunity

Primary response: Effect of genetic group

The primary immune response of both the genetic groups viz., crossbred and the indigenous cattle peaked by day 14 of post primary injection of the test antigen. The mean serum antibody profiles on raw and $\log_e$ transformed data are in the Tables 1 and 2. All comparisons of means were done on the basis of transformed data.

The preimmunization antibody titres on $\log_e$ transformed data were 0.300 ± 0.055 and 0.315 ± 0.059 for crossbred and the indigenous calves respectively. The comparison of means using 't' test revealed no significant difference between the indigenous and crossbred calves (Table 3).

The means of preimmunization antibody response of crossbred and the indigenous cows were 0.396 ± 0.069 and 0.399 ± 0.067 respectively. The comparison of means revealed no significant difference.

The means of day seven responses of the crossbred and the indigenous calves were 1.782 ± 0.075 and 1.801 ± 0.471 respectively. No significant difference was noticed between the means. The means of day seven response of crossbred and the indigenous cows were 1.927 ± 0.066 and 1.976 ± 0.067 respectively. Here also means did not reveal any significant difference.

The means of peak primary response on day 14 for crossbred and the indigenous calves were 2.021 ± 0.066 and 2.216 ± 0.071 respectively. No significant difference was observed. The means for peak primary response of cross-bred and the indigenous cows were 2.142 ± 0.063 and 2.136 ± 0.062 respectively. Here also the difference between the means were not significant.

Secondary response: Effect of genetic group

The average secondary antibody response in both the genetic groups peaked by day seven of secondary immunization (Tables 1 and 2).

The mean secondary day three response of crossbred and the indigenous calves were 2.758 ± 0.079 and 2.816 ± 0.069 respectively. The means were not significantly different (Table 3).

The mean secondary day three response of crossbred and the indigenous cows were 2.756 ± 0.072 and 2.888 ± 0.051 respectively. Comparison of means revealed that the difference was not significant (Table 3).

The peak secondary antibody response on day seven of secondary immunization in crossbred calves, the indigenous calves, crossbred and the indigenous cows were 2.951 ± 0.053, 2.969 ± 0.101, 2.950 ± 0.078, 3.030 ± 0.061 respectively. Comparison of means of crossbred with the indigenous calves revealed no significant difference. Similarly comparison of means of cows of the indigenous and crossbred cattle showed no significant difference.

The mean day 14 response (secondary) of crossbred calves, the indigenous calves, crossbred adults and the indigenous adults were 2.114 ± 0.086, 2.137 ± 0.101, 2.099 ± 0.90 and 2.265 ± 0.068 respectively. The difference between the means were not significant.

On an average both the genetic groups attained peak response by seventh day of secondary immunization. But

50 per cent of crossbred adult cattle reached peak by day three post secondary immunization. But 67.5 per cent of the indigenous adult cattle reached the peak response by day three post secondary immunization. Among the calves, 50 per cent of both reached peak by day three of post secondary immunization.

Cow-calf comparison

The correlation between average peak antibody response of crossbred cows with their calves were found to be low ($r = 0.187$). Similarly correlation between peak antibody response of the indigenous adults with their calves were also low ($r = 0.167$).

Eventhough statistically not significant, the peak primary antibody response to HRBC was higher for the adult group than their young ones. The average peak secondary antibody response was higher for the adult crossbred cattle than their young ones. But in case of the indigenous cattle the average secondary antibody response was slightly higher for the calves than their adults. But these differences were not statistically significant (Fig.1 and 2).

Correlation of peak primary antibody response with peak secondary response

The correlation of peak primary antibody response of

an individual with its peak secondary response were found to be highly significant in all the groups except the indigenous cows (Table 4). In case of crossbred calves, crossbred adults and indigenous cows was 0.5991, 0.5927 and 0.7445 respectively.

Incidence of diseases based on average peak antibody response

The incidence of diseases in all the four groups were observed during the period of study. Respiratory diseases, mastitis, along with mortality rates in the groups were recorded (Table 5).

The animals in each groups were classified based on the level of antibody titre on the 21st day. Grouping on the antibody titre was made as <3, 3-3.5 and >3.5 based on the transformed data of peak response. The number of animals in different groups and animals affected with either respiratory diseases or mastitis were looked into (Table 5). It was seen that, in case of crossbred calves out of 40 animals, three animals had an antibody titre greater than 3.5, 21 animals were in 3.0 to 3.5 group and 16 animals under less than 3.0 group. The three animals in the >3.5 group did not contract any disease. But among 20 animals in 3.0-3.5 group, five contracted respiratory diseases and five died. Five animals

in the less than three group had respiratory diseases and three animals died.

There were five the indigenous calves in the >3.5 group and they contracted no diseases. Seventeen out of 36 calves were in 3.0-3.5 group and only one calf contracted respiratory disease. Fourteen calves were in <3.0 group and incidence of respiratory diseases was two during the study period.

Out of 39 crossbred cows, five were in the >3.5 group, 24 in the 3.0 to 3.5 group and 10 were in the <3.0 group. Of the first group one animal contracted mastitis. From the 3.0 to 3.5 group seven animals had respiratory diseases, and 12 animals had mastitis. Three animals had respiratory diseases, six had mastitis and one death was recorded in the less than 3.0 antibody response group.

Among 30 the indigenous cows, five were in the >3.5 response group, 15 in 3.0 to 3.5 response group and 10 in <3.0 response group. In the >3.5 response group, one had respiratory disease. In the 3.0 to 3.5 and <3.0 response group incidence of respiratory diseases was two in both groups. In the less than 3.0 response group, one mortality has also occurred.

Association of maternal humoral immunity with calf diseases and mortality

Based on average peak antibody response, the adult cattle were grouped into three groups namely >3.5, 3.0-3.5 and <3.0 response group. The incidence of diseases among the calves of these adults during the study period are shown in Table 6.

The calves of five indigenous cattle, in the >3.5 response group and they contracted no diseases. Out of the fifteen calves of 3.0 to 3.5 response group, only one contracted respiratory disease. Among the 10 calves in less than 3.0 response group, the incidence of respiratory disease was two. But no significant association could be obtained between maternal humoral immune response and calf diseases and mortality.

In case of crossbred cattle, there were five calves in >3.5 response group, 24 in 3.0-3.5 response group and 10 in <3.0 response group. In the >3.5 response group incidences of respiratory disease was two and mortality of one was noticed. In the 3.0-3.5 response group incidence of respiratory diseases was four and mortality, five. Among the <3.0 response group, the incidences of respiratory diseases was

three and mortality was two. Here also no significant associations could be established.

Cell mediated immunity

Means, standard deviations and coefficients of variation for response of animals to percutaneous application of DNCB are in the Tables 7, 8 and 9. Mean responses in terms of double skin fold thickness peaked by 24 h post challenge in all the experimental groups.

Skin thickness: Effect of genetic group

The mean double fold skin thickness before the DNCB challenge were 5.406 $\pm$ 0.176, 6.00 $\pm$ 0.180, 6.47 $\pm$ 0.138 and 6.066 $\pm$ 0.200 respectively for the crossbred calves, the indigenous calves, crossbred adults and the indigenous adults (Tables 7 and 8). The prechallenge double fold skin thickness of the indigenous calves were found to be significantly higher ($t = -2.358$) (Table 9). But no significant differences were seen between prechallenge thickness of adults.

The mean values of rate of onset of peak response (24 h thickness - 0 h thickness) were 6.6875 $\pm$ 0.386, 6.778 $\pm$ 0.443, 7.21 $\pm$ 0.309 and 7.23 $\pm$ 0.329 for crossbred calves, the indigenous calves, crossbred adults and the indigenous adults

respectively. The rate of onset in peak response did not show any significant differences in both young and the adult group.

The persistency of peak response (24 h to 48 h thickness in mm) were 1.8125 ± 0.341, 1.416 ± 0.405, 1.131 ± 0.349 and 1.43 ± 0.297 respectively for crossbred calves, the indigenous calves, crossbred adults and the indigenous adults. No significant differences were seen between the two genetic groups in the persistency of peak response.

The mean of duration of response (72 h thickness - 0 h thickness in mm) were 2.969 ± 0.376, 3.25 ± 0.447, 4.39 ± 0.400 and 4.03 ± 0.504 respectively for crossbred calves, the indigenous calves, crossbred adults and the indigenous adults. No significant differences were seen between the two genetic groups in their duration of response.

No significant differences were noticed between indigenous and crossbred cattle post DNCB challenge in terms of increased skin thickness (Fig.3 and 4).

Cow-calf comparison

The correlation of average peak cell mediated immune response of crossbred adults with their calves were low (r = 0.016). Similarly correlation between peak cell mediated

immune response of the indigenous adults with their calves were also low (r = 0.078).

Incidence of diseases based on average peak double fold skin thickness

The incidence of diseases in all four groups were observed during the period of study. Respiratory diseases, mastitis and mortality in the groups were studied (Table 10).

The animals in each groups were divided based on the level of peak cell mediated immune response in terms of skin thickness (24 h thickness). Grouping on skin thickness was made as <7 mm response group, 7-9 mm response group and >9 mm response group. The number of animals in the different groups and animals affected with either respiratory diseases or mastitis were observed (Table 10). In all the groups there was no significant association between skin thickness and incidence of diseases. Out of 32 crossbred calves, 22 were in the <7 mm response group and incidence of respiratory diseases was six and had a single mortality. In the 7-9 mm response group, there were five animals and incidence of respiratory disease three and mortality one. In the >9 mm there was one case of respiratory disease.

Among the crossbred cows, in the <7 mm response group, there were 25 animals out of 38, of which five animals

contracted respiratory diseases and nine animals had mastitis. In the 7-9 mm response group, there were seven animals. Out of these, incidence of respiratory diseases was three and of mastitis, seven. This group had one mortality also. In the >9 mm group there were three animals and the incidence of respiratory disease was one.

There were 22 animals in the <7 mm response group of the indigenous adults. Of this, the incidence of respiratory diseases was three. There were five animals in the 7-9 mm response group and incidence of respiratory disease was two. In the >9 mm response group there were three animals and incidence of respiratory disease was one.

There were 26 animals out of 36 animals in the <7 mm response group of the indigenous calves and this group has single incidence of respiratory disease. There were four animals in the 7-9 mm response group and incidence of respiratory disease was two. In >9 mm response group there were six animals and incidence of diseases was nil.

Association of maternal cell mediated immunity with calf diseases and mortality

Based on average peak cell mediated immune response in terms of double fold skin thickness at 24 h, the adult cattle were grouped into <7 mm, 7-9 mm and >9 mm response groups.

The incidence of diseases among their calves were in Table 11. Among all the groups no significant associations could be seen between maternal immunity and calf diseases and mortality.

In the <7 mm response group of the indigenous cows, there were 22 animals and incidence of respiratory diseases among their calves was three. In the 7-9 mm response group there were five animals and incidence of respiratory disease was one among their calves. In >9 mm response group there were three animals and incidence of respiratory disease among their calves was one.

Among the crossbred cattle there were 25 animals out of 38 animals in the <7 mm response group and incidence of respiratory disease was five and mortality one among their calves. In the 7-9 mm response group there were seven animals and incidence of respiratory diseases was four and mortality one among their calves. In >9 mm response group there were six animals and incidence or respiratory disease among their calves was one.

Area of reaction (diameter)

Means, standard deviations and coefficients of variation for response of animals in terms of skin diameter reaction to percutaneous application of DNCB are in Tables 12,

13 and 14. Mean skin diameter in all the experimental groups peaked by 24h post challenge of DNCB.

Effect of genetic groups

No significant differences between the skin diameter reaction of both the indigenous and crossbred cattle were seen. The mean diameter of during peak response was 4.74 ± 0.278, 4.89 ± 0.34, 4.97 ± 0.26 and 4.736 ± 0.303 respectively for crossbred calves, the indigenous calves, crossbred adults and the indigenous adults. No significant differences were seen between the young and adult group during peak response.

The mean skin diameter of 24 h - 48 h diameter were 0.878 ± 0.18, 0.769 ± 0.429, 0.913 ± 0.206 and 0.64 ± 0.202 respectively for crossbred calves, the indigenous calves, crossbred adults and the indigenous adults. No significant differences were noticed for this response also between the two genetic groups.

The mean skin diameter reaction of 48 h - 72 h diameter were 1.43 ± 0.175, 1.075 ± 0.224, 1.36 ± 0.131 and 0.84 ± 0.188 respectively for crossbred calves, the indigenous calves, crossbred adults and the indigenous adults. Here also no significant difference between the two genetic groups could be established.

The mean skin diameter reaction of 24 h-72 h diameter were 2.31 ± 0.299, 1.897 ± 0.248, 2.27 ± 0.246 and 1.48 ± 0.354 respectively for crossbred calves, the indigenous calves, crossbred adults and the indigenous adults. Here also no significant difference between the two genetic groups could be established.

Cow-calf comparison

The correlation between peak skin diameter response of crossbred adults with their calves were found to be of medium (r=0.29). Similarly correlation between peak skin diameter response of the indigenous adults with their calves were also of medium range (r=0.331).

Though no significance, the mean peak skin diameter reaction of crossbred adults were slightly higher than their calves. But in case of the indigenous, the mean peak skin diameter response was slightly higher for their calves than their adults. This was also not significantly different.

Incidence of diseases and mortality in the indigenous and crossbred cattle

Chi-square (α^2) anlysis were conducted to assess the disease incidence between the two genetic groups viz., crossbred and the indigenous cattle. The indigenous cows had

significantly lower incidence of respiratory diseases and mastitis compared to crossbred cows. (α^2 = 3.8 and 19 respectively). The mortality rate of the indigenous cows were significantly lower compared to crossbred calves (α^2 = 8)

Table 1. Mean and $\log_e$ transformed antibody titre to human erythrocytes of calves

Experimental group	Days post-immunization	n	Titre			$\log_e$ plus one		
			Mean	SD	CV	Mean	SD	CV
Crossbred calves	0^{1*}	40	1.8	2.4	1.33	0.300	0.349	1.163
	7	40	104.2	119.81	1.15	1.782	0.473	0.265
	14^{2*}	36	159.56	157.69	0.989	2.021	0.396	0.196
	17	38	998.74	1086.57	1.088	2.758	0.485	0.176
	21	40	1257.60	1402.72	1.115	2.951	0.337	0.114
	28	39	266.77	377.26	1.414	2.114	0.534	0.253
Indigenous calves	0^{1*}	36	2.0	2.83	1.414	0.315	0.359	1.138
	7	36	107.78	120.96	1.122	1.801	0.471	0.261
	14^{2*}	36	166.49	188.23	1.131	2.216	0.432	0.214
	17	36	1079.35	1426.76	1.322	2.816	0.420	0.149
	21	36	1564.44	1567.42	1.002	2.969	0.607	0.204
	28	36	295.11	383.55	1.300	2.137	0.616	0.288

n = number of observations
SD = Standard deviation
CV = Coefficient of variation

1 = Primary immunization
2 = Secondary immunization

Table 2. Mean and $\log_e$ transformed antibody titre to human erythrocytes of cows

Experimental group	Days post-immunization	n	Titre			$\log_e$ titre plus one		
			Mean	SD	CV	Mean	SD	CV
Crossbred cows	0[1]	40	3.43	6.083	1.76	0.396	0.436	1.102
	7	40	133	142.44	1.07	1.927	0.417	0.216
	14[2]	40	208	202.89	0.975	2.142	0.399	0.186
	17	40	1025.6	1421.35	1.386	2.756	0.458	0.166
	21	39	1494.0	1580.75	1.058	2.950	0.488	0.166
	28	40	282	395.76	1.404	2.099	0.572	0.272
Indigenous cows	0[1]	30	2.864	3.43	1.198	0.399	0.407	1.020
	7	30	144.43	144.53	1.00	1.976	0.405	0.205
	14[2]	30	201.51	201.96	1.00	2.136	0.382	0.179
	17	30	1055.14	1288.76	1.221	2.888	0.308	0.107
	21	30	1553.3	1563.48	1.00	3.03	0.376	0.124
	28	30	300.97	376.611	1.251	2.265	0.414	0.183

n = number of observations
SD = Standard deviation
CV = Coefficient of variation

1 = Primary immunization
2 = Secondary immunization

Table 3. Comparison of antibody response of indigenous and crossbred cattle of Kerala to human red blood cells (t test)

Experimental group	Antibody response					
	0	7	14	17	21	288
Between indigenous and crossbred cows	0.034	0.235	-0.026	0.466	0.263	0.683
Between indigenous and crossbred calves	0.184	0.178	-0.049	0.544	0.162	0.169

Table 4. Correlation of peak primary antibody response with peak secondary antibody response

Experimental group	df	r
Crossbred calves	34	0.5991**
Crossbred cows	34	0.5927**
Indigenous calves	34	0.7445**
Indigenous cows	34	0.2760

df - degree of freedom

r - coefficient of correlation

** - Highly significant

Table 5. Incidence of diseases based on average peak antibody response (log_e transformed data)

Experimental group	Antibody response	n	Respiratory diseases	Mastitis	Mortality
Crossbred calves	>3.5	3	0	0	0
	3.0-3.5	21	5	0	5
	<3.0	16	5	0	3
Crossbred cows	>3.5	5	0	1	0
	3.0-3.5	24	7	12	0
	<3.0	10	3	6	1
Indigenous cows	>3.5	5	1	0	0
	3.0-3.5	15	2	0	0
	<3.0	10	2	0	1
Indigenous calves	>3.5	5	0	0	0
	3.0-3.5	17	1	0	0
	<3.0	14	2	0	0

Table 6. Association of peak maternal humoral immunity with calf diseases and mortality (log_e transformed data)

Experimental group	Antibody response of dams	n	Calf disease and mortality	
			Respiratory diseases	Mortality
Indigenous cattle	>3.5	5	0	0
	3.0-3.5	15	1	0
	<3.0	10	2	0
Crossbred cattle	>3.5	5	2	1
	3.0-3.5	24	4	5
	<3.0	10	3	2

Table 7. Mean double fold skin thickness of calves to DNCB challenge

Experimental group	Hours post-immunization	n	Skin thickness (mm)		
			Mean	SD	CV
Crossbred calves	0h	32	5.406	0.9956	0.1842
	24h-0h	32	6.6875	2.186	0.3268
	24h-48h	32	1.8125	1.927	1.063
	24h-72h	32	3.7187	2.035	0.5471
	48h-72h	32	1.9062	1.284	0.6733
	72h-0h	32	2.969	2.128	0.376
Indigenous calves	0h	36	6.00	1.08	1.180
	24h-0h	36	6.778	2.66	0.392
	24h-48h	36	1.416	2.43	1.716
	24h-72h	36	3.528	2.53	0.718
	48h-72h	36	2.11	1.37	0.648
	72h-0h	36	3.25	2.68	0.825

n - number

SD - Standard deviation

CV - Coefficient of variation

Table 8. Mean double skin fold thickness of adult cattle to DNCB challenge

Experimental group	Hours post-immunization	n	Skin thickness (mm)		
			Mean	SD	CV
Crossbred adult cattle	0h	38	6.47	0.85	0.131
	24h-0h	38	7.21	1.90	0.265
	24h-48h	38	1.131	2.15	1.904
	24h-72h	38	2.815	2.34	0.830
	48h-72h	38	1.684	1.17	0.696
	72h-0h	38	4.39	2.47	0.40
Indigenous adults	0h	30	6.066	1.09	0.180
	24h-0h	30	7.23	1.80	0.249
	24h-48h	30	1.43	1.62	1.135
	24h-72h	30	3.2	2.08	0.653
	48h-72h	30	1.77	1.49	0.848
	72h-0h	30	4.03	2.76	0.685

n - number

SD - Standard deviation

CV - Coefficient of variation

Table 9. Comparison of cell mediated immune response of indigenous and crossbred cattle of Kerala to DNCB challenge (t test)

Experimental group	Skin thickness (mm)					
	0h	24-0h	24-48h	24-72h	48-72h	72-0h
Between crossbred and indigenous calves	-2.358*	-0.153	0.747	0.344	-0.636	-0.481
Between crossbred and indigenous cows	1.677	-0.0504	-0.658	-0.714	-0.274	0.561

* Significant at 5 per cent level

Table 10. Incidence of diseases based on average peak double skin fold thickness after DNCB challenge

Experimental group	Cell mediated immune response (skin thickness mm)	n	Respiratory diseases	Mastitis	Mortality
Crossbred calves	< 7	22	6	0	1
	7-9	5	3	0	1
	> 9	5	1	0	0
Crossbred cows	< 7	25	5	9	0
	7-9	7	3	7	1
	> 9	6	2	3	0
Indigenous cows	< 7	22	3	0	0
	7-9	5	2	0	0
	> 9	3	1	0	0
Indigenous calves	< 7	26	1	0	0
	7-9	4	2	0	0
	> 9	6	0	0	0

Table 11. Association of peak maternal cell mediated immunity with calf diseases and mortality

Experimental group	Double skin fold thickness of adult cattle (mm)	n	Calf disease and mortality	
			Respiratory diseases	Mortality
Indigenous cows	< 7	22	3	0
	7-9	5	1	0
	> 9	3	1	0
Crossbred cows	< 7	25	5	1
	7-9	7	4	1
	> 9	6	1	0

Table 12. Mean skin area of reaction (diameter) of calves to DNCB challenge

Experimental group	Hours post-immunization	n	Skin diameter		
			Mean (mm)	SD	CV
Crossbred calves	24h-0h	32	4.74	1.56	0.332
	24h-48h	32	0.878	1.02	1.162
	48h-72h	32	1.43	0.99	0.69
	24h-72h	32	2.31	1.69	0.732
Indigenous calves	24h-0h	36	4.89	2.037	0.416
	24h-48h	36	0.769	2.576	3.34
	48h-72h	36	1.075	1.346	0.776
	24h-72h	36	1.897	1.472	1.25

Table 13. Mean skin area of reaction (diameter) of adult cattle to DNCB challenge

Experimental group	Hours post-immunization	n	Skin diameter		
			Mean (mm)	SD	CV
Crossbred cows	24h-0h	38	4.97	1.60	0.321
	24h-48h	38	0.913	1.27	1.39
	48h-72h	38	1.36	0.805	0.591
	24h-72h	38	2.27	1.519	0.668
Indigenous cows	24h-0h	30	4.736	1.662	0.351
	24h-48h	30	0.64	1.11	1.73
	48h-72h	30	0.84	1.03	1.227
	24h-72h	30	1.48	1.939	1.31

Table 14. Comparison of cell mediated immune response of indigenous and crossbred cattle of Kerala to DNCB challenge in terms of area of reaction (t test)

Experimental group	Skin diameter (cm)			
	24-0h	24-48h	48-72h	24-72h
Between crossbred and indigenous calves	-0.357	0.427	1.58	1.03
Between crossbred and indigenous cows	0.606	0.406	0.612	0.528

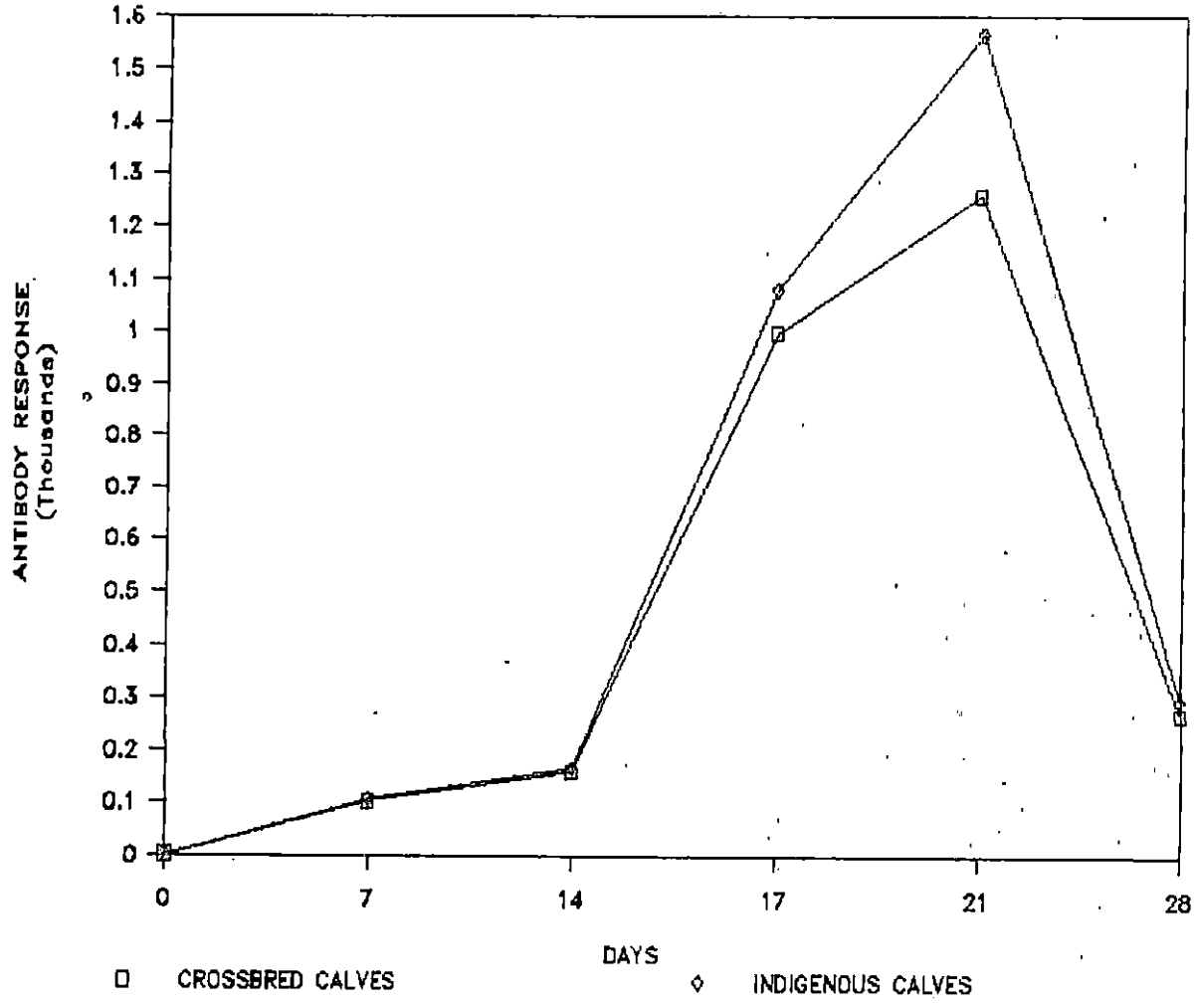


FIG.1 HUMORAL IMMUNE RESPONSE OF INDIGENOUS AND CROSSBRED CALVES

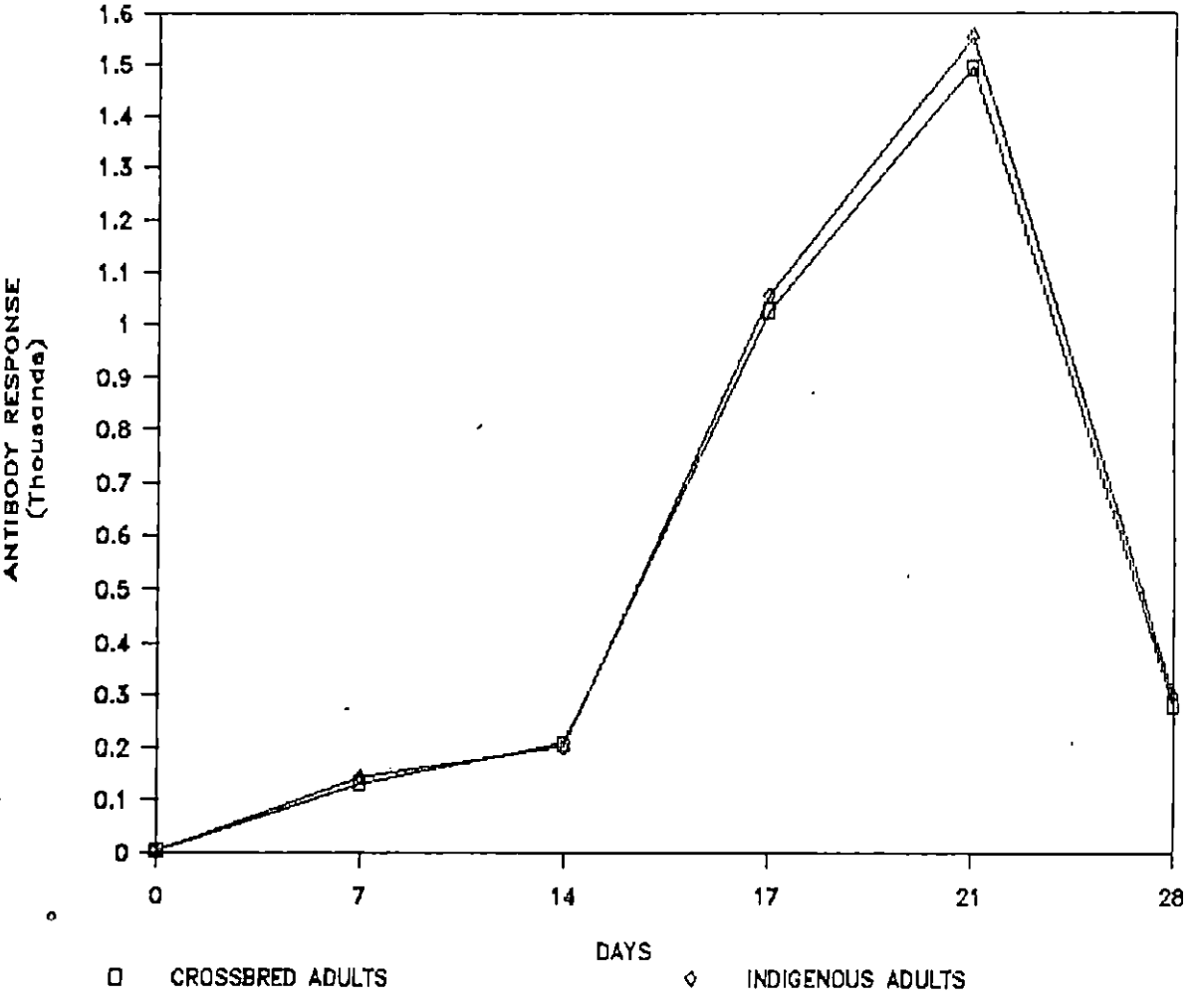


FIG.2 HUMORAL IMMUNE RESPONSE OF INDIGENOUS AND CROSSBRED ADULTS

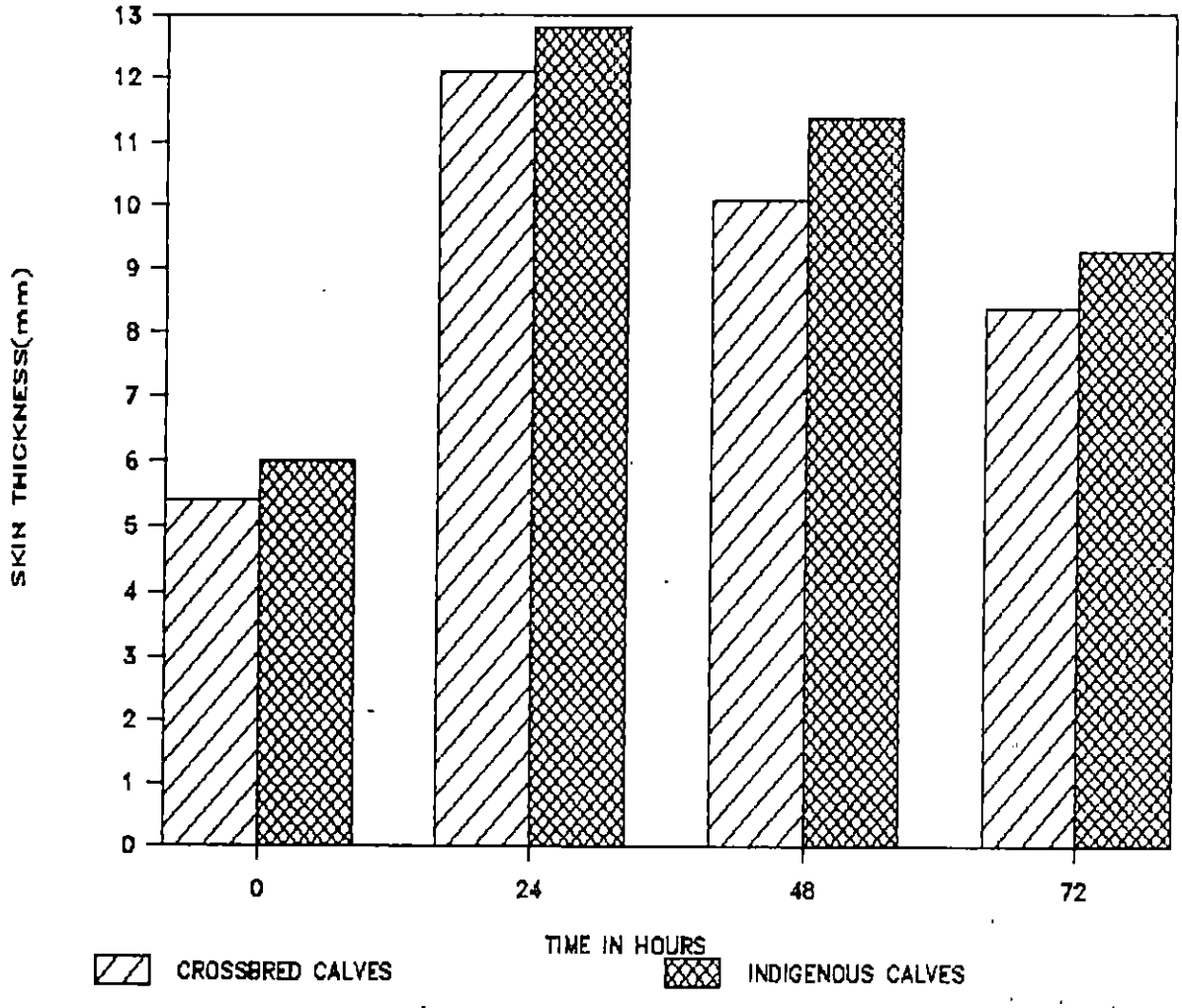


FIG.3 CELL MEDIATED IMMUNE RESPONSE OF INDIGENOUS AND CROSSBRED CALVES

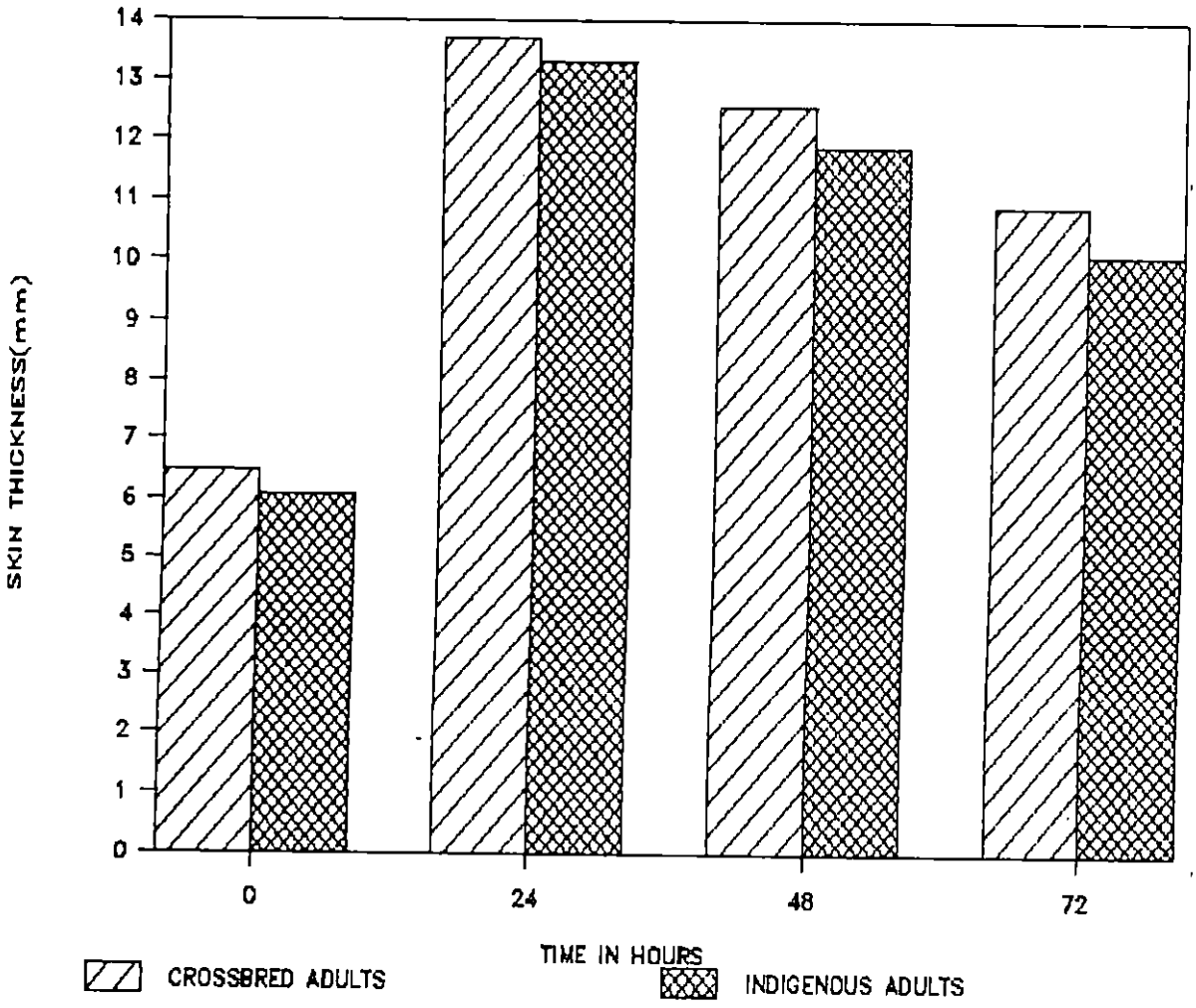


FIG.4 CELL MEDIATED IMMUNE RESPONSE OF INDIGENOUS AND CROSSBRED ADULTS

Discussion

DISCUSSION

Humoral immune response

Primary response

Results are indicative of wide variation between individuals in immune response to human red blood cells (HRBC). The preimmunization titre averaged 0.350 on $\log_e$ transformed data as against the expected value of zero. Burton et al. (1989a) could also observe a similar phenomenon using HRBC as test antigen in calves. This might be due to the cross reactivity between the test antigen and a variety of antigens to which the animal would have been exposed during its life time. This may be also due to some low false positives in the serological test.

Primary response peaked by 14th day and the value averaged around 2.05 in $\log_e$ transformed data, corresponding to observations made by Burton et al. (1989a).

In case of pigs, peak primary response to human and bovine albumin were reached by two to three weeks (Hyldgaard Jensen, 1979). While in case of poultry, the peak primary response to SRBCs was obtained by seventh day of immunization (Zijpp and Leenstra, 1980; Zijpp et al., 1983; Ubosi et al.,

1985 and Miller et al., 1992). These observations clearly indicate the effect of species on the peak primary response is considerable.

Secondary response

The secondary antibody response peaked by seventh day of secondary immunization. This agrees with finding of Burton et al. (1989a). (Secondary seventh day response averaged 2.98 on $\log_e$ transformed data). This may suggest that once the appropriate lymphoid cell clones have been stimulated to differentiate in the primary response, individual animal differences in future anamnestic response diminish perhaps as a function of the generation of the memory T and B cells.

In poultry also, peak secondary antibody response to SRBC was obtained some where between fifth to seventh day of secondary immunization (Zijpp et al., 1983; Zijpp et al., 1986; Kreukniet and Zijpp, 1990 and Miller et al., 1992). This indicates that the secondary immune response is not markedly influenced by species differences and is quick peaking by seven days.

Effect of genetic groups

The results did not yield any significant difference in the primary or secondary immune response of two genetic

groups, viz., crossbred cattle and the indigenous cattle to the test antigen HRBC.

Muggli et al. (1987) also could not find any significant difference between several breeds of cattle and their immune response to bovine infectious rhinotracheitis virus vaccine. In contrast to this Zijpp (1983a); Lamont and Smith (1984a), Petrovsky et al. (1988) and Benda et al. (1990) observed that humoral immune response in poultry was significantly influenced by the genetic group. Here also the influence of species on the immune response appears to be relevant. The absence of any significant difference between the indigenous and crossbred cattle could probably be due to the fact that the crossbred group is derived from indigenous cattle and its exotic crosses and are genetically close. Further the tropical stress may also influence the immune response considerably which requires further elucidation.

The average peak secondary response of both the indigenous and crossbred cattle, was on seventh day of secondary immunization. Fifty percentage of adult crossbred cattle reached the peak titre by third day of secondary immunization. But 67.5 per cent of adult the indigenous cattle reached the peak titre of by third day of secondary immunization.

Zijpp et al. (1983) and Ubosi et al. (1985b) observed that poultry attained peak response by third day of secondary immunization with SRBC.

Cow-calf comparison

The results did not show any statistically significant difference between the primary and secondary antibody responses of adults and their calves.

The peak primary response of both the indigenous and crossbred cows were found to be slightly higher than their calves. The peak secondary response of crossbred cows were slightly higher than their calves. Among the indigenous, peak secondary antibody titre of calves were found to be slightly higher than their dams. But none of these differences were statistically significant.

Watson and Gill (1991) reported that adult wethers had a better response in terms of antibody production. Warner (1987) have observed higher antibody response in young chickens and young dogs. But Nicoletti and Cerney (1991) observed that older mice (22-24 m) had a reduced antibody response than young mice.

The correlation between average peak antibody response of cows with their calves were found to be very low in both

the indigenous and crossbred cattle. This suggests that antibody response is influenced by a variety of environmental factors other than genetic similarity.

Correlation of peak primary antibody response of an individual with its peak secondary response

The correlation of peak primary antibody response of an individual with its peak secondary response were found to be highly significant in all the groups except in case of the indigenous adults.

Burton et al. (1989a) also observed a high correlation between primary antibody responses in an individual with its peak secondary response.

This is indicative of better immune response of an animal to an antigen, that is immune response is individual specific under a set of common environmental factors. This also indicates that primary immune response is a good marker of the animal's secondary immune response as well as total antibody response to an antigen. This indicates that primary immune response generally could be used as a marker trait in assessing the antibody immune response of an individual to an antigen.

Association with diseases and mortality

No significant association could be established between the incidence of diseases and mortality with peak antibody response.

Eventhough no significant difference in humoral response of crossbred cattle and the indigenous cattle, the incidence of diseases in the the indigenous cattle was comparatively lower. Out of 66 the indigenous cattle, the incidence of respiratory diseases was eight and had only one mortality. The incidence of mastitis in the indigenous cattle was zero.

But out of 79 animals in the crossbred cattle, incidence of respiratory diseases and mastitis were 20 and 19 respectively. This group had nine mortalities also.

In case of the indigenous and crossbred cattle there were ten and eight animals in the high response group respectively (>3.5 antibody titre). In this group, the incidence of respiratory disease was one in the indigenous cattle and of mastitis was one in crossbred cattle.

In the medium response group (3.0-3.5 antibody titre) there were 32 animals for the indigenous and 45 animals for the crossbred cattle. The incidence of respiratory diseases

in crossbred cattle was 12 and of the indigenous was three. Crossbred cattle had 12 incidences of mastitis and had five mortalities also. But no mortality occurred for the indigenous cattle during the study period.

In the low responding group (<3.0 antibody titre) there were 24 the indigenous cattle and 26 crossbred cattle. The respiratory disease incidence was four and eight respectively for each group. Crossbreds had six incidences of mastitis also. The indigenous cattle had one mortality, whereas crossbred had four mortalities during the study period in this group.

Chickens selected for ability to produce high antibody titres to SRBC had more resistance to Eimeria neccetrix, splenomegalia virus, but was less resistant to Escherichia coli and Staphylococcus aureus infection (Gross et al., 1980).

Covelli et al. (1984) stated that selection of mice for high antibody responsiveness did not always appear to influence the incidence of lymphomas. Mouton et al. (1988) concluded that mice having low antibody responsiveness had a higher inmate resistance to intracellular pathogens. This was because of faster antigen catabolism in macrophages of these mice.

In calves the diarrhoea prevalence was negatively correlated with high antibody response against HRBC's, but no association was observed for pneumonia prevalence (Burton et al., 1989a). Larsgaard (1990) concluded that goats selected for high immune responsiveness had an improved health status.

Covelli et al. (1984) stated that selection for high antibody responsiveness did not always appear to influence the life span of mice. But according to Zijpp (1983b), Pitcovski et al. (1989a and b), chickens selected for high antibody responsiveness for several generations had a lower mortality rate.

The results of present study agree with general trend that high responder group are less prone to diseases. It should be emphasized that in addition to cell mediated immunity and humoral immune response, there are a variety of factors which influence disease resistance, antibody response and disease susceptibility. The immune response to different antigens ought to vary among animals. The animals of different levels of immune response (both humoral and cell mediated) did not show any remarkable difference in the incidence of diseases and mortality.

Association of maternal humoral immunity with calf diseases and mortality

No significant association could be established between maternal humoral immunity and diseases and mortality of their calves.

The calves of high antibody response group (>3.5) of the indigenous cattle had no incidence of disease, whereas for crossbred cattle respiratory disease incidence was two and mortality one. Each group had five animals each in this group.

The calves of 24 crossbred and 15 indigenous cattle of medium responders (3.0-3.5 antibody response) had four incidences of respiratory diseases and five mortalities among crossbreds and had only a single incidence of respiratory disease among indigenous cattle. Similarly among ten animals each in low responder group (<3.0 antibody titre) had three incidences of respiratory diseases and two mortalities among crossbreds and had two incidences of respiratory diseases among indigenous cattle. Thus, the incidence of diseases and mortality were comparatively lower among indigenous cattle.

Cell mediated immunity

Skin thickness

Mean responses in double fold skin thickness to DNCB challenge were substantial with peak response occurring at 24 h post challenge in all the experimental groups. This is in perfect agreement with finding of Burton et al. (1989b). The average mean skin thickness of skin by 24 h post DNCB challenge was found to be 6.98 cm.

Effect of genetic groups

Mean responses in double fold skin thickness to DNCB challenge did not show any significant difference between the two genetic groups viz., crossbred and the indigenous cattle.

But significant difference was noticed in the prechallenge thickness of crossbred calves with the indigenous calves, with indigenous calves having thicker skin (0.59 mm higher). But double fold skin thickness of the indigenous adults was slightly lower than that crossbred adults eventhough not significant (0.404 mm).

The 24 h double fold skin thickness in indigenous calves had a slightly higher response in terms of increased skin thickness (0.091 mm) than crossbred calves. Indigenous

cows had a higher response of 0.02 mm than crossbred cows. But these differences were not statistically significant.

The average reduction in skin fold thickness between 24 h and 48 h were 20.9 per cent in case of the indigenous calves whereas this was 27.1 per cent in crossbred calves. The average reduction in skin fold thickness between 48 h and 72 h were 39 per cent both in the indigenous and crossbred calves. The average reduction in skin fold thickness between 24 h and 72 h were 52 per cent in the indigenous calves and 55.5 per cent in crossbred calves.

Similarly the average reduction in skin fold thickness between 24 h and 48 h were 19.7 per cent in the indigenous adults and 15.68 per cent in crossbred adults. The average reduction in skin fold thickness between 48 h and 72 h were 30.5 per cent in the indigenous adults and 27.7 per cent in crossbred adults. The average reduction in skin fold thickness between 24 h and 72 h were 44.38 per cent in the indigenous adults and 39 per cent in crossbred cows.

Lie et al. (1983) reported significant differences between sire families, but not between sires in cattle in their cell mediated immune responses. In pigs, significant breed differences were noticed in cell mediated immune response (Buschman, 1986). Benda et al. (1990) also stated

significant breed differences in cellular immune response of fowls.

From the observation of cell mediated immunity in experimental animals, it is apparent that the response of the indigenous calves to the contact sensitivity of DNCB was greater compared to the crossbred, though not a significant level. It is also evident that the indigenous calves had a better persistency of cell mediated immunity compared to crossbred calves.

Cow-calf comparison

Mean responses in double fold skin thickness to DNCB challenge were substantial, with peak response occurring at 24 h post challenge in both the young and adult group of cattle. The mean peak response were found to be higher for the older group when compared with their calves, eventhough no significant difference could be established.

The average reduction in skin fold thickness between 24 h and 48 h were 19.7 per cent in the indigenous adults and 20.9 per cent in their calves. The average reduction in skin fold thickness between 48 h and 72 h and 24 h and 72 h were 30.5 per cent, 44.38 per cent for the indigenous cows and 39 per cent, 52 per cent for the indigenous calves respectively.

The average reduction in skin fold thickness between 24 h and 48 h were 15.68 per cent in crossbred adult and 27.1 per cent in their calves. Similarly the average reduction in skin fold thickness between 48 h and 72 h and 24 h and 72 h were 27.7 per cent for crossbred cows and 39 per cent and 55.5 per cent for crossbred calves respectively.

This finding agrees with Watabe and Glick (1983), the delayed type hypersensitivity (DTH) reaction of fowls to human gamaglobulin was more intense at six to 12 weeks of age than at three weeks of age. But Warner (1987), DTH to DNCB in chickens could not show any effect of age.

The results of the present study agrees with the observation made by Paulik and Vrzula (1989) wherein DTH to dinitroflurobenzene was assessed and the dermal response was greater in older calves than in young calves.

The correlation between average double fold skin thickness at 24 h between the adult cattle and their calves were found to be very low in both the indigenous and crossbred cattle.

Association with diseases and mortality

No significant association could be established between the incidence of diseases and mortality with cell

mediated immune response in terms of double fold skin thickness.

Eventhough no significant difference in CMI response of crossbred and the indigenous cattle could be established, the incidence of diseases in the indigenous cattle was comparatively lower.

Out of 66 the indigenous cattle, the incidence of respiratory diseases was nine. The incidence of mastitis in the indigenous cattle was zero. But out of 70 crossbred cattle, the incidence of respiratory diseases was 19, and of mastitis was 19 and had three mortalities.

In case of the indigenous and crossbred cattle, there were 22 animals each in the <7 mm response (24 h skin thickness) group. In this group the incidence of respiratory diseases was 11 in crossbreds and four in the indigenous cattle and had registered one mortality in the crossbreds and incidence of mastitis was 9 in crossbreds.

In case of 7-9 mm response (24 h skin thickness) group, there were 12 animals for crossbreds and nine for the indigenous. The incidence of respiratory disease was six for crossbred cattle and four for the the indigenous cattle. The crossbreds of this group have registered one mortality also.

In case of >9 mm response (24 h skin thickness) group, there were 11 animals in crossbreds and nine animals in the indigenous cattle. The incidence of respiratory diseases was three for crossbreds and one for the indigenous cattle. The crossbred cattle registered three incidences of mastitis also.

No significant relationship between cell mediated immune response and incidence of diseases as well as mortality could be established from the present study.

According to Chauhan et al. (1984) significant correlations exists between high levels of CMI and resistance to marek's disease and certain other diseases in poultry. Burton et al. (1989b), observed prevalence and severity of naturally occurring diarrhoea and pneumonia of calves did not significantly affect the response to DNCB in form of increased skin thickness.

The lack of association between cell mediated immune response and disease associations in calves appears to arise from a single factor. The major neonatal diseases in calves are alimentary and respiratory infections for which cell mediated immunity appears to offer little protection.

From this study, no recommendation can be made about the use of induced immune response as a criterion for measuring disease resistance or disease susceptibility as well as mortality.

Association of maternal cell mediated immunity with calf diseases and mortality

No significant association could be established between maternal cell mediated immunity with diseases and mortality of their calves.

The calves of low response group (<7 mm) in terms of low values of increased skin thickness at 24 h had three incidences of respiratory diseases in the indigenous cattle and five for crossbred. The crossbreds had one mortality also. There were 22 animals of indigenous cattle and 25 animals of crossbred cattle in this group.

The calves of medium responders to DNCB challenge had a single incidence of respiratory disease and four for crossbreds. The crossbred of this group had registered one mortality also. There were five and seven animals each in indigenous and crossbred cattle of this group.

The calves of high CMI response group had a single incidence of respiratory disease in both the indigenous and crossbred cattle. There were three calves of indigenous cattle and six calves of crossbred cattle in this group.

Summary

SUMMARY

The study was undertaken to compare immune response of the indigenous and crossbred cattle of Kerala as well as the association between immune response and incidence of diseases. The animals employed for the present study comprised 30 adult indigenous cows and their 36 calves and 40 crossbred cows and their 40 calves kept at the University Livestock Farms. The humoral immune response was assessed using human red blood cells (HRBC) as the antigen. The cell mediated immune response was assessed after percutaneous application of 2,4-Dinitrochlorobenzene (DNCB).

Twenty per cent Type A Rh^{+ve} HRBC washed in PBS and suspended in Alsver's solution were given intravenously to all the animals at a rate of one ml per 50 kilogram body weight as the antigen. Primary and secondary immunisations were conducted with the first antigen on day zero and 14 respectively. Serum samples collected on day zero, seven and 14 were subjected to direct microhaemagglutination test for assessing the antibody titres of primary response. Similarly serum samples collected on day three, seven and 14 after secondary immunization were assessed for secondary antibody

response. Since the antibody titres did not follow normal distribution, they were expressed as $\log_e+1$.

The means of peak primary response on 14th day were 2.021 $\pm$ 0.066, 2.216 $\pm$ 0.071, 2.142 $\pm$ 0.063 and 2.136 $\pm$ 0.062 respectively for crossbred calves, local indigenous calves, crossbred cows and local indigenous cows. The means of average peak secondary antibody response on seventh day of secondary immunization were 2.951 $\pm$ 0.053, 2.969 $\pm$ 0.101, 2.950 $\pm$ 0.078 and 3.030 $\pm$ 0.061 for crossbred calves, local indigenous calves, crossbred cows and indigenous cows respectively. No significant differences were noticed between local indigenous and crossbred cattle of Kerala in their serum antibody levels for 60th the primary and secondary humoral immune responses.

The average peak secondary response of both local indigenous and crossbred cattle was on seventh day of secondary immunization. But 50 per cent of crossbred cows and 67.5 per cent of local indigenous cows reached the peak titre by third day of secondary immunization.

The correlation of peak primary antibody response of an individual with its peak secondary response were found to be highly significant for local indigenous calves, crossbred

calves and crossbred cows. But in local indigenous cows, the correlation was not statistically significant.

The cell mediated immunity was assessed using DNCB. Two per cent DNCB solution in acetone was applied percutaneously at neck region for two consecutive days. Two per cent 0.5 ml DNCB applied as the challenge dose at the opposite side of neck on 14th day of sensitization. The double fold skin thickness and diameter of area of reaction were measured just before application of challenge dose and exactly 24 h, 48 h and 72 h post DNCB challenge. The prechallenge double fold skin thickness of local indigenous calves were significantly higher than crossbred calves.

The mean values of rate of onset of peak response were (24 h-0 h skin thickness in mm) 6.6875 $\pm$ 0.386, 6.778 $\pm$ 0.443, 7.21 $\pm$ 0.309 and 7.23 $\pm$ 0.329 for crossbred calves, indigenous calves, crossbred cows and indigenous cows respectively. No significant differences were seen between local indigenous and crossbred cattle in their cell mediated immune responses to DNCB in terms of skin thickness reaction and diameter of area of reaction.

The data on incidence of respiratory diseases, mastitis and mortality of the experimental subjects were collected for a period of two years. No significant

association could be noticed between incidence of diseases and mortality with humoral and cell mediated immune traits. Similarly no significant association could be noticed between dam's immunity and neonatal diseases and mortality.

The Chi-square analysis revealed significantly lower incidence of respiratory diseases and mastitis in local indigenous cows compared to crossbred cows (χ^2 = 3.8 and 19 respectively). The mortality rate in indigenous calves were significantly lower compared to crossbred calves (χ^2 = 8). The salient observations of present study are:

1. No significant differences could be established in the immune response to HRBC, between indigenous and crossbred cattle in adults as well as calves.

2. There was a strong association between primary and secondary humoral immune response.

3. Cell mediated immune response was also not significantly different in indigenous and crossbred cattle both in adults and calves.

4. Indigenous cows had significantly lower incidence of respiratory diseases and mastitis compared to crossbred cows. Similarly the mortality rate in indigenous calves was significantly lower compared to crossbred calves.

5. No association could be established between the incidence of diseases and immune response (humoral and cell mediated) in both local indigenous and crossbred cattle as could be concluded from the similarity of immune response and the simultaneous difference in disease incidence and mortality between the genetic groups.

6. The same group of animals also, the classification based on the level of immune response (both humoral and cell mediated) did not show any remarkable difference in incidence of diseases and mortality. This was true for both the genetic groups.

7. No recommendation can be made about the use of induced immune response as a criterion for measuring disease resistance or disease susceptibility as well as mortality.

From this study, it is to be thought that in addition to cell mediated and humoral immune response, there are a variety of factors which influence disease resistance, disease susceptibility and mortality.

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ABSTRACT

This research work aimed at comparing the immune responses of indigenous and crossbred cattle of Kerala and finding out association if any, with common diseases and also maternal immune response and the neonatal calf diseases and mortality. Thirty adult local indigenous cattle, their 36 calves, 40 adult crossbred cows and their 40 calves formed the material for the study. Primary humoral response to the test antigen, human red blood cells was assessed at zero, seven and 14th day. Secondary immune response to the test antigen was assessed on day three, seven and 14 after booster injection at 14th day. The antibody titre was assessed by direct microhaemagglutination technique. Cell mediated immune response to contact sensitizer 2,4-Dinitrochlorobenzene (DNCB) was assessed by application of two per cent solution on zero and first day followed by percutaneous challenge at the 14th day. Double fold skin thickness and area of reaction were recorded. Humoral and cell mediated immune response, the influence of genetic group, association with diseases and mortality, influence of maternal immune response on the calf immune response and association with calf diseases and mortality were assessed.

The antibody titres were expressed as $\log_e + 1$ to make the distribution normal. Peak primary immune response was

reached by day 14 (2.13) and peak secondary response was on seventh day of secondary immunization (2.98). No significant differences were observed between primary and secondary immune response in different genetic groups namely indigenous and crossbred cattle and also in different age groups viz. dam and calf. The correlation between primary and secondary immune response except in indigenous cows, were highly significant. The cell mediated immune response peaked by 24 h post 2,4-Dinitro chloro benzene challenge both in indigenous and crossbred cattle (7.0 mm). No significant association could be detected between the incidence of diseases and mortality with humoral as well as cell mediated immunity. Similarly association between maternal and calf immune response was also not significant. Indigenous cows had lower incidence of respiratory diseases and mastitis compared to crossbred cattle (α^2 = 3.8 and 19 respectively). Similarly local indigenous calves had significantly lower mortality (α^2 = 8).

The results of the study suggests that primary immune response could be used as an index of secondary immune response. The study also suggests that immune response to a single antigen might not be indicative of general disease resistance. Further, apart from the immune response traits in the present study, there might be several factors which influence the immune response, disease resistance and disease susceptibility.