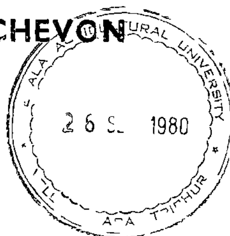
DIFFERENTIATION OF BEEF FROM CHEVON BY SEROLOGICAL METHODS



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BY

N MOHAN DAS

THESIS

Submitted in partial fulfilment of the requirement for the degree

MASTER OF VETERINARY SCIENCE

Faculty of Veterinary and Animal Sciences
Kerala Agricultural University

Department of Veterinary Public Health
COLLEGE OF VETERINARY AND ANIMAL SCIENCES
MANNUTHY - TRICHUR

1980

DECLARATION

I hereby declare that this thesis entitled DIFFERENTIATION OF BEEF FROM CHEVON BY SEROLOGICAL METHODS is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship, or other similar title, of any other University or Society.

Mannuthy

26-7-1980

Signature of the candidate

Name of the candidate : N. Mohan Das

CERTIFICATE

Certified that this thesis entitled DIFFERENTIATION OF BEEF FROM CHEVON BY SEROLOGICAL METHODS is a record of research work done independently by Sri.N.Mohan Das 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.

Name of the Guide: Dr.P.Prabhakaran
(Chairman, Advisory Board)
Designation: Associate Professor
Department of Veterinary
Public Health

Mannuthy
26-7-1980

ACKNOWLEDGEMENT

I wish to record my deep sense of gratitude and indebtedness to Dr.P.Prabhakaran; Associate Professor, Department of Veterinary Public Health, for his advice and guidance during the course of my work.

I am grateful to Dr.R.Padmanabha Iyer, Professor, Dr.M.Soman, Associate Professor and Dr.A.Rajan, Professor, members of the Advisory Committee for their valuable help and suggestions.

Dr.S.Sulochana, Associate Professor has given me invaluable technical help which I cannot forget. I wish to record my gratitude towards her.

Dr.R.Kalyanasundaram, Professor of Parasitology has helped me in taking the photographs and I am greatly indebted to him.

Dr.J.Abraham, Assistant Professor, Dr.V.L.Somanathan, Junior Assistant Professor, Dr.George T. Oommen and staff of the Department of Veterinary Public Health have helped me in various manner during the period of my study.

My gratitude towards Dr.C.K.Ouseph, Veterinary Surgeon, Trichur Municipality for help in collection of

meat samples and to Dr.K.E.Ramakrishnan, Dr.K.R.Johny, Veterinary Surgeons, Veterinary Biological Institute, Palode for preparation of freeze-dried antigen is recorded.

I am grateful to the Government of Kerala and the Director of Animal Husbandry for granting me study leave, and to the Kerala Agricultural University for providing financial assistance during the period of my work.

My thanks are due to the Dean, College of Veterinary and Animal Sciences, Mannuthy for providing facilities for this study.

I thank Sri.T.K.Prabhakaran for typing the manuscript.

N. MOHAN DAS

DEDICATED TO MY WIFE DAYAMANY

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INTRODUCTION

Adulteration and misrepresentation of meat are common maladies affecting the meat trade and industry in India. On an average 25 to 30 percentage of the edible meat sold in various parts of India is adulterated (Jacob, 1976). The probable cause of such malpractices can be attributed to various factors like preferential demand of meat, cost, religious taboos and the innate tendency of certain unscrupulous traders to amass wealth through illegal practices. Accurate identification of adulteration is not easy for the consumer as it involves techniques to be practiced only in the laboratory.

In India adulteration is mostly done in chevon (Goat meat) and mutton (Sheep meat) with beef having a low price. As the price of chevon and mutton is growing day by day, there is proportionate increase in their adulteration as well.

Various methods of identification of meat of different species based on anatomical, physical, chemical and serological differences have been described by several workers (Kaplan and Buck, 1951; Helm et al. 1971; and Sherikar et al. 1979). However, the result has been variable and often specific conclusions have not been obtained. Of the different tests employed

serological tests have been found to give more accurate results. For identification of meat, various tests like Electrophoresis, Passive haemagglutination inhibition, Precipitation, Gel-diffusion and Immuno adsorbant chromatography have been employed. But gel-diffusion and precipitation tests have advantage over others as they could be conducted in the field laboratories with simple equipments and the results of gel-diffusion test could be kept as a record of evidence.

Voluminous data have accumulated on the results of investigation on the identification of beef as a seperate entity, over the past two decades, but the information from the available literature on the differentiation of mutton from beef is very little (Shunmugam and Ranganathan, 1972). So far no work seems to have been done in Kerala with the object of identifying the meat of any species.

More than eighty per cent of Keralites are meat eaters and perforce the demand for meat is high (Report of the study group appointed by the Government of Kerala, 1978). Among them a sizable group have preferential demand for chevon/mutton over beef for sentimental reasons. Under such conditions, adulteration of chevon

with beef is rampant in Kerala. The administrators therefore, have the responsibility of ensuring the supply of unadulterated meat and to safe guard the interest of the consumers. This can be done only by methods which will help to identify the meat of different species of animals.

Hence the present study was undertaken with the objective to evolve an easy and suitable method for identification of adulteration of chevon with beef following serological method and to decide the minimum detectable proportion of adulterants.

REVIEW OF LITERATURE

Adulteration of meat has been a problem for centuries. The Equine Meat Inspection Act of 1920, enacted by the United States Department of Agriculture was intented for prevention of adulteration of meat (Edelmann *et al.* 1943). Laws were enacted to prevent fraudulant practices in meat trade in the City of Florence as early as 13th and 14th centuries (Brandly, *et al.* 1966). Various techniques have been adopted for identification of the species of animal from which meat was obtained.

According to Kaplan and Buck (1951) the identification based on physical characteristics and chemical tests are often inconclusive. Helm *et al.* (1971) stated that the chemical tests employed for the identification of meat have serious disadvantages in that they are too time consuming, inconclusive and not sensitive enough to detect level of adulteration that might reasonably be expected to occur. Sherikar *et al.* (1979) have also observed that differentiation of meat based on anatomical and chemical characteristics were inclusive.

Edelmann *et al.* (1943) recorded that biological method for identification of meat deserves greatest consideration. Many workers have observed that more reliable results were obtained by using techniques based

on immunological reaction (Oswald, 1952; Weinstock, 1953; Penso, 1955; Merchant and Packer, 1967).

The precipitation test has been regarded as a precise and suitable test by Kaplan and Duck (1951). The test is based on an immunological reaction where in the combination of an antigen and antibody causes visible aggregation of precipitate. Konnad and Thiory, (1955) also recommended the precipitation test as a means of identification of meat in respect of the species. Brandly et al. (1966) stated that the precipitation test was ideal for the detection of adulteration of meat. The precipitation test was found to give easier and more reliable results in differentiation of meat (Merchant and Packer, 1967).

While pointing out the efficiency of precipitation test in distinguishing minced meat, many workers have stated that the test was specific except for closely related species (Proom, (1943); Homann, (1947); Weitz, (1952); Sing and Yadav, (1962); Nelson, (1977) and Kamiyama et al. (1978)). Katsubi and Imaizumi (1968) reported that the precipitation test could be employed successfully only when the proportion of adulterant meat in the mixed sample exceeded 25 per cent.

Among the serological tests, gel-diffusion test has been found to be of great value in the identification of meat samples (Pike and Edward, 1957; Scholot foldt and Hanni Simon, 1960). In comparison with tube test, agar gel-diffusion method is advantageous since it could be preserved as a permanent record for evidence (Pike and Edward, 1957). According to Heever, (1962) gel-diffusion test was found to be useful for identification in "Biltong" (Air dried strips of meat). Thornton and Gracey (1974) observed that gel-diffusion test could be employed successfully for identification of meat samples heated to 80°C for not more than 10 minutes. According to Ponti et al.(1978) electro immunodiffusion and cross over electrophoresis do permit identification of the type of meat used in products that have been heated to 80°C or 90°C for two hours.

According to Garvey et al. (1979) certain varieties of rabbits like Newzeland and Dutch breeds are considered to be the best for the purpose of antibody production and biological variations can be minimised by proper management during the experimental work. Katsubi and Imaizumi (1968) have established that the species specificity of antisera produced is dependent not only on mode of

immunisation but also on the individual variations among the rabbits for their capacity to produce species specific sera which is not uniform.

DePagondo and Bordner, (1943) used animal serum as antigen. According to P oom, (1943) alum precipitated serum as antigen gave high titre and more specificity for the antiserum. Weitz, (1952) confirmed the superiority of alum precipitated serum as antigen. Brandly et al. (1966) recommended alum precipitated serum for the production of high titered specific antisera in rabbits. Kaplan and Buck (1951) used horse serum without treatment as antigen. Pinto, (1961) used alcohol precipitated serum protein as antigen. Katsubi and Imaizumi (1968) found that heat inactivated sera and autoclaved sera possess antigenicity.

Bolin, (1931) reported that a meat extract was better than serum as antigen for the production of hyperimmune serum. Oswald, (1953) demonstrated that antibodies could be produced against the desired species using alum precipitated muscle extract as antigen. Moran and Melton, (1961) observed that both muscle extract and serum of the same species could be used for obtaining antiserum with high titre. Shunmugam and

Ranganathan (1972) used equal quantities of aqueous extract of muscle and serum of respective animals as antigen to produce antisera to differentiate between mutton and beef by tube precipitation test. Merchant and Packer(1967) reported that the saline extract of minced meat was better antigen for the production of antisera. Actomyosin from frozen meat of different species were used as antigen by Warnecke and Saffle (1968). They have also recommended freeze-dried skeletal muscle extract with adjuvant as antigen. Salt soluble muscle proteins were used as antigen in case of spoiled meat by Margitic and Jay (1970). Sing and Yadav (1962) used plasma as antigen.

Ostertag (1934) observed that subcutaneous injection of antigen was badly tolerated by rabbits. DeFagondo and Bordner (1943) reported that antiserum could be produced by innoculating healthy rabbits either by subcutaneous route or by intra venous route. Proom (1943) advocated the intra muscular route for the injection of antigen for the production of a high titred serum in rabbits. Specific anti horse serum was produced in rabbits by intra peritonial route of injection of antigen by Giensberg(1948). He preferred this route of injection because of the rapid

absorption of the substance from the peritoneum and due to the fact that the tissue saturation was only slightly lower than intra venous route. The method of choice for production of higher titred serum in rabbits was by repeated innoculations of alum precipitated serum by intra muscular route (Temagi, 1954).

According to Proom (1943) as the course of immunisation was repeated there was a corresponding increase in the titre of the homologous antibody and a decrease in the specificity of the antiserum and a highly sensitive antiserum could only be obtained at the expense of specificity. Gradual increase in the quantity of antigen per injection is safer for immunisation (Cohn, 1952). Kushner and Kaplan (1961) reported that intense immunisation tends to provoke development of strong antisera with low specificity because it elicited the production of large quantities and varieties of precipitins to a maximum number of both antigens in the immunisation mixture and antigenic determinant group on each population of antigen. Soetarjo (1964) observed that Kaplan and Buck method of multiple injection produced antisera with less specificity while Proom's method proved to be superior as it produced antisera with high

specificity. Hatsubi and Imaizumi (1968) observed that alum precipitated raw beef extract failed to produce antibodies in rabbits, whereas similar treatment with horse meat was successful. He recommended inactivated bovine serum as a good antigen to produce a high titred antiserum in rabbits. Warnecke and Saffle (1968) reported that the rabbits when repeatedly injected intra peritonially with two milli litre of saline extract of muscle containing 10 to 15 mg of protein per ml, they failed to produce hyperimmune serum capable of reacting with homologous antigen in gel-diffusion test. He recommended intra muscular injection of freeze-dried skeletal muscle extract with adjuvant for the production of specific sera against saline extract of the muscle. Christian (1970) pointed out that when a rabbit was injected with more than 10 mg of protein as antigen initially in Freund's adjuvant or saline, the level of precipitins produced was nil or low due to over immunisation. When small quantities of protein (one micro gram) in saline was administered it resulted in under immunisation, failing to produce the desired precipitins with interference of non-precipitating antibodies produced.

According to Furminger (1964) a high degree of

cross-reaction occur between myosin of species within the class. Sherikar *et al.* (1979) observed that inspite of cross-reaction, the serological method could be employed for differentiating meats of various species, by calculating the ratio of diffusion coefficient.

Pinto (1961) stated that the cross-reacting antibodies could be removed by heterologous sera by addition of 1 in 200 diluted heterologous serum to three parts of hyperimmune serum, mixing it, allowing to stand for 15 minutes and centrifuging at 4000 rotations per minute (r.p.m.). This technique is efficient to render the serum species specific. The method advocated by Hafeeze *et al.* (1961) was the dilution of the antisera to 1 in 1000 for the removal of cross-reacting antibodies. According to Zwaan (1963) dried antigen could be used for absorption in order to increase the efficiency with an added advantage that it will not result in undue dilution of the antiserum. Hochwald and Thorbecke (1962) preferred the use of absorbing antigens in small increments in contrast to adding large amounts all atonce, since the former method enhanced the performance efficiency of the serum. Warnecke and Saffle, (1968) recorded that the cross-reaction could be removed by

absorption with small amount of freeze-dried antigen of the cross reacting species, by adding approximately .8 mg of freeze-dried antigen per ml of the antiserum. The mixture was shaken thoroughly at room temperature and kept for four hours and placed at 4°C for 14 hours. The antiserum was centrifuged at 2000 r.p.m. to remove the precipitate containing cross-reacting antibody antigen complex. Prasad and Misra (1978) made an attempt to remove cross-reaction by treating the sera with "rivanol". But they failed to achieve the target, resulting in loss of specificity even to homologous antigens.

Preparation of species specific sera was attempted by certain workers. Katsubi and Imaizumi (1968) prepared species specific antisera by using heat treated serum as antigen in rabbits. Their opinion is that precipitation test could be employed successfully only when the proportion of adulterant meat in the mixed sample exceeded 25 per cent. Helm et al. (1971) used gamma globulin isolated from rabbit antiserum for rapid detection of homologous antigen after subjecting it to heat treatment (56°C) and found a satisfactory method to avoid cross-reactions.

Chase (1967) stressed the necessity of preventing haemolysis during collection of blood for serum production.

Strong haemolysis results in quick enzyme degradation of immunoglobulin on storage. According to him sterile immune serum with good titre can be kept at 4°C for more than 35 years without loss of potency of precipitin. Van Orden and Trifers (1968) observed an increase in antigen precipitating capacity of chicken sera during storage at 4°C. Chase (1967) found chicken sera should not be frozen as the precipitins will be lost. Crowle (1973) has pointed out that none of the common means of preservation such as refrigeration at 4°C, freezing and lyophilisation could be relied upon to prevent changes in precipitin titre. When stored aseptically or with preservatives, some antisera maintain activity for months at 4°C, while some rabbit precipitins lose their activity within 12 days. He also observed that precipitins in human antisera against mycobacterium can lose activity overnight. Weitz (1952) reported freeze-dried sera can be kept for two years at room temperature without deterioration.

Crowle (1973) recommended the use of either Merthiolate 0.0001 per cent, Sodium azide 0.10 per cent, phenol 0.25 per cent, Benzethonium chloride two per cent or a combination of 0.05 per cent sodium azide and acriflavine 0.01

per cent or 0.2 per cent Methyl paraben and 0.02 per cent Ethyl paraben as preservaties for antiserum. He has cautioned against the use of merthiolate for preservation of antisera, as merthiolate contains aromatic group which will act against antibodies produced using haptens containing phenol group. Since many of these preservaties contain appreciable amount of nitrogen, estimation of protein by determination of nitrogen content becomes unreliable.

According to Libbey (1975) a good precipitating antiserum should show a very definite ring formation when layered at the bottom of a 1 : 800 dilution of the corresponding antigen and no reaction with a 1 in 50 dilution of the sera of the unrelated species. According to Garvey *et al.* (1979) a positive result is indicated by the development of a sharp ring at the interface of the antigen and antibody. Giensberg (1948) pointed out that a high salt content will upset the precipitation reaction and more than three per cent salt concentration was not advisable for the conduct of the test.

Formation of precipitation lines in any immunodiffusion system was highly dependent on relative concentration of antigen and antibody (Fudenberg et al. 1976). Gel-diffusion test involves precipitation reactions between antigen and antibody in a semisolid rather than a fluid medium. Both

antigen and antibody are present as solutions in seperate wells in an agar coated plate (Garvey et al. 1979). Immuno diffusion media include agar, agarose, cellulose acetate, polyacrylamide and gelatin in the decreasing order of popularity (Crowle, 1973). Some of the advantages of agar reported are that it forms transparent gelly, it is nearly inert, it is compatiable with many different buffer systems, can be prepared at several concentrations and can be easily washed free of most non-reacting constituents of antigen and antibody solution. The major disadvantages of agar are that basic dyes cannot be used with basic antigens, it forms complexes with lipoproteins and cause strong electro-smosis. According to him agar at concentration of one to two per cent is employed usually for immunodiffusion. Though agar can be used immediately after it has gelled, ripening of gel for several days is recommended in order to increase the sensitivity of the test (DeCarvalho, 1960). Jordan and White (1965) have recommended the use of agar in concentration between 1 to 1.5 per cent in cases where gel need not be manipulated after it has been cast. At two per cent agar, diffusion rate was slower and sensitivity began to drop. According to Crowle (1973)

the common solvent for immunodiffusion test is normal saline usually buffered to near or slightly alkaline pH. It has also been stated that test conditions like low salt concentration, high salt concentration, presence of ions like barbital which tend to insolubilise antigen/antibody increases the sensitivity of the test but increases non-specific precipitations. Halpern et al. (1961) have recommended saline, weaker than the physiological saline when rabbit antiserum is used, for stronger and quicker result. Ozerol and Silverman (1969) have reported that bovine, ovine and equine antisera function better in high salt concentration than in the case of normal saline. The influence of the concentration of sodium chloride on agar gel-precipitation test was determined by Jordan and Chubb (1962). They were of the opinion that eight per cent salt concentration was necessary for the detection of threshold amount of either antigen or antibody when chicken sera is used.

MATERIALS AND METHODS

In the present study for the production of hyper-immune sera in rabbits, the method adopted by Proom (1943) was followed. The method advocated by Warnecke and Saffle (1968) was followed for absorption of the hyperimmune sera with modification.

Rabbits.

Antisera was raised in rabbits (<u>Oryctolagus cuniculus</u>) maintained in the laboratory. Sixteen adult healthy rabbits, weighing approximately 1.5 kg were randomly selected and kept under observation for one week. The rabbits were divided into two groups of eight each (Group A and Group B). Rabbits in group A were immunised with beef and rabbits in group B with chevon. Each rabbit was marked with different coloured dyes for individual identification. The rabbits were maintained on a balanced diet. Initially ascorbic acid (One Celin tab: 500 mg for 16 rabbits) was administered in drinking water for three days in order to boost the antibody production power as suggested by Mucillo (1940).

Preparation of Antigens.

Fresh samples of beef and chevon were collected from Municipal slaughter house Trichur. Beef and Chevon were

minced separately and triturated with normal saline in the ratio 3 : 5. The triturates were kept overnight in the refregerator (5°C) with occassional stirring to facilitate extraction of muscle proteins. The following day the triturates were filtered through two layers of sterile muslin cloth and the filtrates were transferred to screw capped vials of 10 ml capacity and stored in deep freeze (-20°C). This was used later as antigen to immunise the rabbits. Through out the procedure measures were taken to avoid contamination of the antigens with micro organisms. Soluble protein contents of the extract were estimated by Biuret method (Gornall et al. 1949). Portion of the antigens were freeze-dried, ampuled and stored in a refrigerator to use as absorbant to eliminate cross-reacting antibodies in the sera.

Antisera.

Immunisation: Each rabbit was administered initially three millilitre of the freshly prepared antigens contain ing 0.38 mg and 0.30 mg of protein per ml of beef and chevon extract respectively by intra peritoneal injection using a 22 gauge needle and syringe. Subsequently four injections were given at regular intervals of four days with 4 ml 5 ml 5 ml and 5 ml of frozen antigen respectively after thawing and maintaining at room temperature. The

site of injection was changed alternately to minimise tissue damage and strain. After the third injection, test bleeding was done to assess the development of antisera by collecting small quantity of blood in a test tube from the ear vein by scarification with a sharp blade. The blood was allowed to clot and the serum was separated. The sera were tested against corresponding known antigens for the development of visible reaction in the form of precipitate both by tube and gel tests.

Harvesting of sera.

On tenth day after the last injection terminal bleeding was done by severing the carotid artery after mechanical stunning. The animals were bled after fasting for twelve hours. The blood was collected in clean sterile 100 ml conical flasks. Precautions were taken to prevent haemolysis. The collected blood was kept at room temperature for three to four hours. Afterwards the clot was rimmed with a sterile glass rod and the conical flasks were kept in the refrigerator (5°C) overnight for shrinkage of clot. The supernatant serum was decanted pooled species wise, centrifuged and collected in sterile tubes. Merthiolate was added to obtain a final concentration of 1 in 10,000 to prevent fungal growth. The

serum thus collected was tested with known antigens (Chevon and Beef) to know its specificity.

Two sets of both goat and beef hyper immune sera were taken in tubes and preserved at 5°C and -20°C to evaluate the effect of storage under the above temperatures on the potency of the sera.

Absorption of sera.

The cross reacting antibodies were removed by the method adopted by Warnecke and Saffle (1968). Small amount of freeze-dried antigen of the cross-reacting species was added. (Approximately .0 mg of the freeze-dried antigen per ml of the antisera). The mixture was shaken thoroughly at room temperature and kept for four hours and placed at 4°C for 14 hours. The antiserum was centrifuged at 2000 r.p.m. for 5 minutes to remove the precipitate containing cross-reacting antibody antigen complex. The absorbed serum was tested with homologous and heterologous antigens (Chevon and Beef) to ascertain the specificity.

Meat samples.

Beef and chevon samples were collected from the Municipal slaughter house, Trichur. In order to mask the identify of the samples, they were false numbered by the

departmental staff. Such meat was used for preparation of antigens for the tests. Saline extract of meat were prepared as mentioned earlier. Besides beef and chevon, a few samples of mutton and buffalo meat were also collected to find as to wheather the presence of these could also be detected by the test employed.

Extract of mixed meat samples consisting of beef and chevon in proportion 50 : 50, 25 : 75, 20 : 80 and 15 : 85 as well as chevon and beef in the same proportion as mentioned above were also used as antigens. Antigens from buffalo meat and chevon in the proportion 20 : 80 was also used for conducting the tests. Saline extract of buffalo meat and mutton were used against beef and chevon antisera to study cross-reactions if any. Tube and gel-diffusion tests were conducted using various antigens and absorbed goat and beef antisera.

Tube test.

Clean agglutination tubes were used for this test. Prepared antigens were diluted to 1 in 10, 1 in 50 and 1 in 100 with normal saline. Approximately 0.05 ml of the beef and chevon sera, were taken in separate tubes. Equal quantity of the antigen was overlayed carefully over the sera avoiding mixing. Simultaneously saline and

serum controls were set. Observation was made at frequent intervals for 30 minutes.

Gel-diffusion test.

Noble agar (DIFCO) one gram and normal saline solution 100 ml were mixed and boiled in a conical flask for the agar to melt and dissolve. Melted agar was poured into Petri dishes to get a thickness of 3 mm for the agar. The Petri dishes were previously coated with 1 per cent agar in distilled water, to prevent seepage of the reactants. The agar was allowed to solidify. A template was prepared to mark wells in the solidified agar in such a way that there will be one central and six peripheral wells equidistant and five milli metre apart, having a diameter of four milli metre. The wells were made by placing the petridish over the template, using a sharp glass tube with four milli metre outer diameter and by aspirating the gel inside the well. For the sake of convenience two sets of wells were prepared in one Petri dish sufficiently apart. The central wells were charged with 0.05 ml antiserum and peripheral wells with same quantity of antigens.

As the trials conducted showed that dilution of

antigen 1 in 10 in the case of single meat and undiluted in the case of mixed meat gives better result in gel-diffusion test, this method was followed. Serum and saline controls were also set. A piece of filter paper moistened with distilled water was placed underneath the cover of the Petri dish to prevent drying of the gel. The Petri dishes after charging, were placed in a refrigerator (5°C) and observed at intervals of 12, 18 and 24 hours for the development of lines of precipitation.

RESULTS

Rabbits:

During the course of experiment five rabbits from group A and two rabbits from group B died at different periods on account of peritonitis/stress.

After the third injection and by 15th day after initial injection all rabbits developed antibodies at detectable levels when test bleeding was conducted. The sera obtained from test bleeding was tested against known antigen in gel-diffusion test. The line of precipitate was appreciable (Fig. 1).

Tests to detect cross-reaction:

Cross-reactions were noticed in both tube precipitation and gel-diffusion tests, in all trials. Positive results were observed when the test was conducted using beef antigen and hyper immune goat antisera. Similar results were obtained when goat tissue antigen and hyper-immune beef tissue antisera were used. Buffalo antigen gave a positive reaction with both antigoat and antibeef hyper immune sera. Similarly mutton tissue antigen reacted with antibeef and antigoat hyperimmune sera (Plate I: - Fig. a and b).

Tests with antisera absorbed for removal of cross-reacting antibodies:

It was observed that antibeef serum absorbed with goat antigen gave positive result with beef antigen and negative result with goat antigen in tube precipitation and gel-diffusion tests. Similarly anti-goat serum absorbed with beef antigen gave positive results with goat antigen but not with beef antigen (Fig. II). Buffalo antigen reacted with the absorbed sera in the same manner as beef antigen. Mutton antigen elicited a positive reaction with antigoat serum absorbed with beef antigen and negative result with antibeef serum absorbed with goat antigen.

Identification of Meat Samples:

Tube test: Among the diluted antigens used for the tube test 1 in 50 appeared to give clear ring of precipitate. Of the 80 samples of meat tested using 1 in 10 and 1 in 50 all were correctly identified as 42 beef and 38 chevon (Table I a and I b). The dilution of antigen 1 in 50 produced more sharp and clear ring of precipitate in the interface than with 1 in 10 dilution. All positive reactions were visible within 15 minutes.

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Gel-diffusion test: The results of the gel-diffusion test conducted on 80 samples of meat (antigen) using undiluted and 1 in 10 dilution against antibeef and antigoat sera have been tabulated in table II a. All samples which were false numbered to mask the identity of the meat sample, were identified correctly as per records. A line of precipitate was observed in the plate in between the homologous antigen and antibody in all positive cases (Fig.III). Forty-two samples were identified as beef and 38 samples as chevon (Table II a and II b). In the case of chevon line of precipitate developed within 12 hours in three samples. Thirty-five samples produced visible line of precipitate between 12 and 18 hours. None of the samples of beef produced a line of precipitate within 12 hours. Between 12 and 18 hours, 19 samples of beef showed visible line of precipitate and the remaining 23 samples developed line of precipitate between 18 and 24 hours.

Tests on known meat.

Mutton

Tube test: Out of the five known samples of mutton tested with unabsorbed antibeef serum, ring of precipitate was observed at the interface within 15 minutes in all

cases. But the result in tests with antibeef serum absorbed with goat antigen was negative even after 30 minutes. Tests conducted with absorbed and unabsorbed antigoat serum against mutton antigen revealed formation of ring of precipitate in the interface.

Gel-diffusion test: All the five samples of mutton tested with unabsorbed antibeef serum produced line of precipitate within 18 hours. While the test with absorbed antibeef serum did not give any line of precipitate even after 48 hours. But gel-diffusion test with absorbed and unabsorbed antigoat serum and mutton antigen indicated positive reaction by formation of line of precipitate within 12 hours. With unabsorbed serum the lines were thicker and clearer than that in absorbed serum (Plate II - Fig. a).

Buffalo meat

Tube test: The five samples of known buffalo meat were tested against unabsorbed antigoat serum. A ring of precipitate formed in the interface within 15 minutes. But when the test was repeated using absorbed antigoat serum with beef antigen in place of unabsorbed serum; no ring of precipitate formed even after 30 minutes.

Gel-diffusion test: Gel diffusion test was conducted using

known buffalo meat antigen against unabsorbed antigoat serum. The line of precipitate developed within 12 hours. But no line developed when the same antigen was tested against antigoat serum absorbed with beef antigen even after 48 hours (Plate II - Fig.b).

Adulterated meat.

Chevon and beef

Tube test: The presence of beef at 50, 25 and 20 per cent level in a mixture with chevon could be detected, but not at 15 per cent level. Similarly varying proportion of chevon in the mixture with beef could be detected using absorbed antigoat serum upto 20 per cent level. The results obtained in the case of mixed meat consisting of beef and chevon at different proportions using 1 in 10, and 1 in 50 dilutions of the antigen have been shown in table III.

Gel-diffusion test: In gel-diffusion test with antibeef serum absorbed with goat antigen, the presence of beef at 50 per cent, 25 per cent and 20 per cent levels in a mixture of beef and chevon showed development of lines of precipitate within 18 hours. Chevon also could be detected in the same manner using antigoat serum absorbed with beef antigen, upto 20 per cent level. But no lines developed at 15 per cent level even after 48 hours (Plate III - Fig. a, b, & c).

These results have been shown in table IV.

Chevon and Buffalo meat.

Five samples of mixture of buffalo meat at 20 per cent and chevon at 80 per cent level reacted in similar manner as that of beef and chevon mixture in tube precipitation test as well as gel-diffusion test.

Generally the intensity of production of precipitate by sera was found to be reduced by absorption technique. In the present study it was observed that this phenomenon was prominant in antibeef serum than antigoat serum. Unabsorbed antibeef and antigoat sera preserved at 5°C and -20°C for six months did not show appreciable difference in the ability to react with homologous and heterologous antigens.

Table I a. Results of tube precipitation test

Meat sample (antigen)	Dilution of antigen		Antisera		Visible Reactions				Conclusion
					Within 15 mts.		Within 15-30 mts.		
	1 in 10	1 in 50	Anti-beef	Anti-goat	Sharpness	Clarity	Sharpness	Clarity	
1	+	++	-	+	++	++	-	-	Chevon
2	+	++	-	+	++	++	-	-	Chevon
3	+	+	+	-	+	+	-	-	Beef
4	+	++	+	-	+	+	-	-	Beef
5	+	++	+	-	+	+	-	-	Beef
6	+	++	+	-	+	+	-	-	Beef
7	+	++	-	+	++	++	-	-	Chevon
8	+	++	+	-	+	+	-	-	Beef
9	+	++	-	+	++	++	-	-	Chevon
10	+	++	-	+	++	++	-	-	Chevon
11	+	++	-	+	++	++	-	-	Chevon

(Contd.)

Meat sample (antigen)	Dilution of antigen		Antisera		Visible Reactions				Conclusion
					Within 15 mts.		Within 15-30 mts.		
	1 in 10	1 in 50	Anti-beef	Anti-goat	Sharpness	Clarity	Sharpness	Clarity	
12	+	++	+	-	+	+	-	-	Beef
13	+	++	+	-	+	+	-	-	Beef
14	+	++	-	+	++	++	-	-	Chevon
15	+	++	+	-	+	+	-	-	Beef
16	+	++	+	-	+	+	-	-	Beef
17	+	++	+	-	+	+	-	-	Beef
18	+	++	+	-	+	+	-	-	Beef
19	+	++	+	-	+	+	-	-	Beef
20	+	++	-	+	++	++	-	-	Chevon
21	+	++	-	+	++	++	-	-	Chevon
22	+	++	+	-	+	+	-	-	Beef

(Contd.)

Meat sample (antigen)	Dilution of antigen		Antisera		Visible Reactions				Conclusion
					Within 15 mts.		Within 15-30 mts.		
	1 in 10	1 in 50	Anti-beef	Anti-goat	Sharpness	Clarity	Sharpness	Clarity	
23	+	++	-	+	++	++	-	-	Chevon
24	+	++	+	-	+	+	-	-	Beef
25	+	++	-	+	++	++	-	-	Chevon
26	+	++	+	-	+	+	-	-	Beef
27	+	++	+	-	+	+	-	-	Beef
28	+	++	-	+	++	++	-	-	Chevon
29	+	++	-	+	++	++	-	-	Chevon
30	+	++	+	-	+	+	-	-	Beef
31	+	++	+	-	+	+	-	-	Beef
32	+	++	+	-	+	+	-	-	Beef
33	+	++	+	-	+	+	-	-	Beef

(Contd.)

Meat sample (antigen)	Dilution of antigen		Antisera		Visible Reactions				Conclusion
					Within 15 mts.		Within 15-30 mts.		
	1 in 10	1 in 50	Anti-beef	Anti-goat	Sharpness	Clarity	Sharpness	Clarity	
34	+	++	+	-	+	+	-	-	Beef
35	+	++	+	-	+	+	-	-	Beef
36	+	++	+	-	+	+	-	-	Beef
37	+	++	+	-	+	+	-	-	Beef
38	+	++	-	+	++	++	-	-	Chevon
39	+	++	-	+	++	++	-	-	Chevon
40	+	++	-	+	++	++	-	-	Chevon
41	+	++	-	+	++	++	-	-	Chevon
42	+	++	-	+	++	++	-	-	Chevon
43	+	++	-	+	++	++	-	-	Chevon
44	+	++	+	-	+	+	-	-	Beef

(Contd.)

Meat sample (antigen)	Dilution of antigen		Antisera		Visible Reactions				Conclusion
					Within 15 mts.		Within 15-30 mts.		
	1 in 10	1 in 50	Anti-beef	Anti-goat	Sharpness	Clarity	Sharpness	Clarity	
45	+	++	−	+	++	++	−	−	Chevon
46	+	++	−	+	++	++	−	−	Chevon
47	+	++	−	+	++	++	−	−	Chevon
48	+	++	+	−	+	+	−	−	Beef
49	+	++	−	+	++	++	−	−	Chevon
50	+	++	−	+	++	++	−	−	Chevon
51	+	++	+	−	+	+	−	−	Beef
52	+	++	−	+	++	++	−	−	Chevon
53	+	++	+	−	+	+	−	−	Beef
54	+	++	−	+	++	++	−	−	Chevon
55	+	++	+	−	+	+	−	−	Beef

(Contd.)

Meat sample (antigen)	Dilution of antigen		Antisera		Visible Reactions				Conclusion
					Within 15 mts.		Within 15-30 mts.		
	1 in 10	1 in 50	Anti-beef	Anti-goat	Sharpness	Clarity	Sharpness	Clarity	
56	+	++	+	-	+	+	-	-	Beef
57	+	++	+	-	+	+	-	-	Beef
58	+	++	-	+	++	++	-	-	Chevon
59	+	++	+	-	+	+	-	-	Beef
60	+	++	-	+	++	++	-	-	Chevon
61	+	++	+	-	+	+	-	-	Beef
62	+	++	-	+	++	++	-	-	Chevon
63	+	++	+	-	+	+	-	-	Beef
64	+	++	-	+	++	++	-	-	Chevon
65	+	++	+	-	+	+	-	-	Beef
66	+	++	-	+	++	++	-	-	Chevon

(Contd.)

Meat sample (antigen)	Dilution of antigen		Antisera		Visible Reactions Within 15 mts.		Within 15-30 mts.		Conclusion
	1 in 10	1 in 50	Anti-beef	Anti-goat	Sharpness	Clarity	Sharpness	Clarity	
67	+	++	+	-	+	+	-	-	Beef
68	+	++	-	+	++	++	-	-	Chevon
69	+	++	-	+	++	++	-	-	Chevon
70	+	++	-	+	++	++	-	-	Chevon
71	+	++	-	+	++	++	-	-	Chevon
72	+	++	+	-	+	+	-	-	Beef
73	+	++	+	-	+	+	-	-	Beef
74	+	++	+	-	+	+	-	-	Beef
75	+	++	+	-	+	+	-	-	Beef
76	+	++	-	+	++	++	-	-	Chevon
77	+	++	+	-	+	+	-	-	Beef

(Contd.)

Meat sample (antigen)	Dilution of antigen		Antisera		Visible Reactions				Conclusion
					Within 15 mts.		Within 15-30 mts.		
	1 in 10	1 in 50	Anti-beef	Anti-goat	Sharpness	Clarity	Sharpness	Clarity	
78	+	++	-	+	++	++	-	-	Chevon
79	+	++	+	-	+	+	-	-	Beef
80	+	++	-	+	++	++	-	-	Chevon

Table I b. Classification of visible reactions observed in the tube precipitation test

No. of meat samples tested	Species		Visible Reactions				Remarks
			Within 15 mts.		Within 15-30 mts		
			Clarity	Sharpness	Clarity	Sharpness	
42	Beef	1 in 10 dilution	++	++	+	+	
		1 in 50 dilution	++++	++++	+++	+++	1. After 15-20 minutes it was observed that sharpness of the narrow bands of precipitate formed spread out.
38	Chevon	1 in 10 dilution	++	++	+	+	
		1 in 50 dilution	++++	++++	+++	+++	2. All positive reactions were visible within 15 minutes.

Table II a. Results of gel-diffusion test

Meat samples (antigen)	Dilution of antigen and charity of line of precipitate		Antisera		Time of appearance of line of precipitate			Conclusion
	Undiluted	1 in 10	Antibeef	Antigoat	Within 12 hrs	12-18 hrs.	18-24 hrs.	
1	+	++	-	+	-	+	-	Chevon
2	+	++	-	+	-	+	-	Chevon
3	+	++	+	-	-	-	+	Beef
4	+	++	+	-	-	-	+	Beef
5	+	++	+	-	-	+	-	Beef
6	+	++	+	-	-	+	-	Beef
7	+	++	-	+	-	+	-	Chevon
8	+	++	+	-	-	+	-	Beef
9	+	++	-	+	-	+	-	Chevon
10	+	++	-	+	-	+	-	Chevon
11	+	++	-	+	-	+	-	Chevon

(Contd.)

Test samples (antigen)	Dilution of antigen and clarity of line of precipitate		Antisera		Time of appearance of line of precipitate			Conclusion
	Undiluted	1 in 10	Antibeef	Antigoat	Within 12 hrs	12-18 hrs.	18-24 hrs	
12	+	++	+	-	-	-	+	Beef
13	+	++	+	-	-	-	+	Beef
14	+	++	-	+	-	+	-	Chevon
15	+	++	+	-	-	-	+	Beef
16	+	++	+	-	-	+	-	Beef
17	+	++	+	-	-	+	-	Beef
18	+	++	+	-	-	+	-	Beef
19	+	++	+	-	-	+	-	Beef
20	+	++	-	+	-	+	-	Chevon
21	+	++	-	+	+	-	-	Chevon
22	+	++	+	-	-	+	-	Beef

(Contd..)

Meat samples (antigen)	Dilution of antigen and clarity of line of precipitate		Antisera		Time of appearance of line of precipitate			Conclusion
	Undiluted	1 in 10	Antibeef	Antigoat	Within 12 hrs	12-18 hrs.	18-24 hrs	
23	+	++	-	+	+	-	-	Chevon
24	+	++	+	-	-	+	-	Beef
25	+	++	-	+	+	-	-	Chevon
26	+	++	+	-	-	+	-	Beef
27	+	++	+	-	-	-	+	Beef
28	+	++	-	+	-	+	-	Chevon
29	+	++	-	+	-	+	-	Chevon
30	+	++	+	-	-	+	-	Beef
31	+	++	+	-	-	-	+	Beef
32	+	++	+	-	-	-	+	Beef
33	+	++	+	-	-	-	+	Beef

(Contd.)

Meat samples (antigen)	Dilution of antigen and clarity of line of precipitate		Antisera		Time of appearance of line of precipitate			Conclusion
	Undiluted	1 in 10	Antibeef	Antigoat	Within 12 hrs	12-16 hrs.	18-24 hrs	
34	+	++	+	-	-	+	-	Beef
35	+	++	+	-	-	+	-	Beef
36	+	++	+	-	-	+	-	Beef
37	+	++	+	-	-	+	-	Beef
38	+	++	-	+	-	+	-	Chevon
39	+	++	-	+	-	+	-	Chevon
40	+	++	-	+	-	+	-	Chevon
41	+	++	-	+	-	+	-	Chevon
42	+	++	-	+	-	+	-	Chevon
43	+	++	-	+	-	+	-	Chevon
44	+	++	+	-	-	+	-	Beef

(Contd.)

Meat samples (antigen)	Dilution of antigen and clarity of line of precipitate		Antisera		Time of appearance of line of precipitate			Conclusion
	Undiluted	1 in 10	Antibeef	Antigoat	Within 12 hrs.	12-18 hrs	18-24 hrs	
45	+	++	-	+	-	+	-	Chevon
46	+	++	-	+	-	+	-	Chevon
47	+	++	-	+	-	+	-	Chevon
48	+	++	+	-	-	+	-	Beef
49	+	++	-	+	-	+	-	Chevon
50	+	++	-	+	-	+	-	Chevon
51	+	++	+	-	-	+	-	Beef
52	+	++	-	+	-	+	-	Chevon
53	+	++	+	-	-	-	+	Beef
54	+	++	-	+	-	+	-	Chevon
55	+	++	+	-	-	+	-	Beef

(Contd.)

Meat samples (antigen)	Dilution of antigen and clarity of line of precipitate		Antisera		Time of appearance of line of precipitate			Conclusion
	Undiluted	1 in 10	Antibeef	Antigoat	Within 12 hrs	12-18 hrs	18-24 hrs	
56	+	++	+	-	-	-	+	Beef
57	+	++	+	-	-	-	+	Beef
58	+	++	-	+	-	+	-	Chevon
59	+	++	+	-	-	-	+	Beef
60	+	++	-	+	-	+	-	Chevon
61	+	++	+	-	-	-	+	Beef
62	+	++	-	+	-	+	-	Chevon
63	+	++	+	-	-	-	+	Beef
64	+	++	-	+	-	+	-	Chevon
65	+	++	+	-	-	-	+	Beef
66	+	++	-	+	-	+	-	Chevon

(Contd.)

Meat samples (antigen)	Dilution of antigen and clarity of line of precipitate		Antisera		Time of appearance of line of precipitate			Conclusion
	Undiluted	1 in 10	Antibeef	Antigoat	Within 12 hrs.	12-18 hrs	18-24 hrs	
67	+	++	+	−	−	−	+	Beef
68	+	++	−	+	−	+	−	Chevon
69	+	++	−	+	−	+	−	Chevon
70	+	++	−	+	−	+	−	Chevon
71	+	++	−	+	−	+	−	Chevon
72	+	++	+	−	−	−	+	Beef
73	+	++	+	−	−	−	+	Beef
74	+	++	+	−	−	−	+	Beef
75	+	++	+	−	−	−	+	Beef
76	+	++	−	+	−	+	−	Chevon
77	+	++	+	−	−	−	+	Beef

(Contd.)

Meat samples (antigen)	Dilution of antigen and clarity of line of precipitate		Antisera		Time of appearance of line of precipitate			Conclusion
	Undiluted	1 in 10	Antibeef	Antigoat	Within 12 hrs	12-18 hrs	18-24 hrs	
78	+	++	-	+	-	+	-	Chevon
79	+	++	+	-	-	-	+	Beef
80	+	++	-	+	-	+	-	Chevon

Table II b. Details of precipitate line formation in Gel-diffusion test

Total No. of meat samples tested	Time of appearance of line of precipitate	Beef		Chevon	
		Undiluted	1 in 10 dilution	Undiluted	1 in 10 dilution
	Within 12 hrs.	0	0	3 (7.9)	3 (7.9)
80	Between 12 and 18 hrs.	19 (45.2)	19 (45.2)	35 (92.1)	35 (92.1)
	Between 18 and 24 hrs.	23 (54.8)	23 (54.8)	0	0

Figures in parenthesis indicate percentage.

Table III. Detection of adulteration of meat at different levels by tube precipitation method.

Number of meat samples tested	Percentage of meat		Reactions			
			Antigen dilution			
			1 in 10		1 in 50	
	Beef	Chevon	Absorbed antigoat serum	Absorbed antibeef serum	Absorbed antigoat serum	Absorbed antibeef serum.
12	15	85	++++	-	+++++	-
6	20	80	++++	+	+++++	++
12	25	75	+++	++	++++	+++
12	50	50	+++	++	++++	+++
12	75	25	+	++	++	+++
6	80	20	+	++	++	+++
12	85	15	-	+++	-	++++

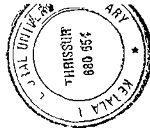
Table IV. Detection of adulteration of meat at different levels by gel-diffusion method

Number of meat samples tested	Percentage of meat		Reactions			
			Antigen dilution			
			Undiluted		1 in 10	
	Beef	Chevon	Absorbed antigoat serum	Absorbed antibeef serum	Absorbed antigoat serum	Absorbed antibeef serum
12	15	85	+++++	-	++++	-
6	20	80	+++++	++	++++	+
12	25	75	++++	+++	+++	++
12	50	50	++++	+++	+++	++
12	75	25	++	+++	+	++
6	80	20	++	+++	+	++
12	85	15	-	++++	-	+++

DISCUSSION

Hyperimmune sera, containing adequate quantities of antibodies were raised in rabbits against beef and goat tissue antigens. The sera were found to be of value in conducting both tube precipitation and gel-diffusion tests. Though there are reports that some rabbits fail to develop antibodies, in the present study all rabbits developed detectable level of antibodies after the third injection. Garvey et al. (1979) reported that certain varieties of rabbits like Newzeland and Dutch breeds are best for the purpose of antisera production. The local strain of rabbit used in this study was found capable of producing hyperimmune sera of satisfactory potency.

Saline extracts of beef and chevon were used as antigen in the present work for immunication of rabbits by intra peritonial injection, following the methods prescribed by Merchant and Packer (1967). The saline extract of whole meat was found to be a competant antigen to elicit sufficient antibody response. Although, the same method was tried by Warnecke and Saffle (1968), they failed to obtain antibodies in serum used for gel-diffusion test. This may be due to over immunisation, because the saline extract of muscle



used for initial injection contained more than 30 mg of protein whereas only about one mg of protein was present in the antigen used for primary injection in the present work. In this context it is pertinent to point out the observation of Christian (1970) that rabbits failed to produce precipitin in the sera if the level of protein in the initial injection of antigen is more than 10 mg.

The cross-reactions observed in the tests using unabsorbed hyperimmune sera against beef and chevon and heterologous antigens such as beef, chevon, mutton and buffalo meat indicated that it contained overlapping antibodies as observed by Tafeze et al (1961), Pinto (1961) and Sherikar et al. (1979). But Shanmugam and Ranganathan (1972) did not observe any cross-reaction between beef and mutton in tube precipitation test. This may be due to the differences in the antigens, used for production of antisera, since they used serum and aqueous extract of muscle together as antigen.

The technique followed for absorption of antisera, for removal of cross-reacting antibodies, using freeze-dried antigen, helps in maintaining the volume and

concentration of the sera more or less in the same level, without affecting its efficacy. Any method of absorption, in general, will reduce the intensity of precipitate formation, as observed by Pinto (1961). Now also the intensity of precipitation was found reduced in absorbed sera.

Of the two dilutions of antigen employed 1 in 50 was found to give better result. Though 1 in 10 dilution produced sufficiently thick precipitate, the margins of the precipitate were difficult to identify from the column of antigen which had slight brown colour and this difficulty was not present in case of 1 in 50 dilution. Observation for the formation of ring of precipitate should be continous for the first 15 to 30 minutes, as the rings of precipitate formed may settle down by gravity after some time of its formation. More over according to Giensberg (1948) in positive cases the whitish ring develops in the interface almost immediately and certainly between five and twenty minutes. The precipitate formed later than this may be due to non-specific reactions. But Kaplan and Buck (1951) considered the reaction within 30 minutes as significant. The major advantage of tube

precipitation test is that the result could be read in a short period of less than 30 minutes.

In gel-diffusion tests the results could be obtained in case of both undiluted and diluted antigens. For single meat antigen, dilution 1 in 10 was adopted to avoid the colour of the undiluted antigen affecting the line of white precipitate formed in positive reactions. But in the case of mixed meat the undiluted antigen was preferred because of their low proportion in the total antigen. Moreover the dilution will further weaken it. The added advantage of gel-diffusion test over tube precipitation test is that the border line cases in tube test, will be distinct in gel-diffusion test. Another advantage that can be cited is that the result obtained could be preserved as visible proof for a longer duration in gel-diffusion test.

All the 80 false numbered meat samples could be identified correctly by both tube precipitation and gel-diffusion tests. Hence the tests could be considered reliable. Chevon gave positive reaction earlier than that by beef. In gel-diffusion test 7.9 per cent of the chevon samples developed precipitate within 12 hours

and 92.1 per cent within eighteen hours. In case of beef none of the samples produced precipitate within 12 hours. Between 12 and 18 hours 45.2 per cent produced precipitate and 54.8 per cent between 18 and 24 hours. The lines of precipitate are clearer and sharper in case of chevon than that in beef. This is probably due to the higher potency of the antigoat serum than antibeef serum. Fudenberg et al. (1976) observed that the formation of precipitation lines in any immunodiffusion system is highly dependent on relative concentration of antigen and antibody. In all cases, positive results were available within 24 hours. This indicates the efficiency of the tests in producing the result in a reasonably short time.

Detection of adulteration of meat is of practical importance. Physical characteristics alone cannot be relied upon, in their identification. When known mutton and buffalo meat were used in teste, they reacted similar to chevon and beef respectively. As reported by Singh and Yadav (1962) the above reaction may be du to the close serological relationship between the species.

According to Katsubi and Imaizumi (1968) the

lowest detectable level of adulteration is 25 per cent in meat. The tests in the present study have revealed that the detectable proportion of one type of meat in a mixture could be as low as 20 per cent. But below that they could not be detected. The probable reason is that the proportion of meat of the particular species in the bulk of the mixture will be so low, that their part in the antigen will be weak to show any visible reaction in the precipitation test. Another reason that could be attributed is that the antiserum might not have had a very high titre to produce a reaction with a weak antigen.

Efficacy of prepared antisera, by preservation at 5°C and -20°C for six months, were found unaltered in precipitation test. According to Crowle (1973) some rabbit precipitins lose their activity within 12 days when preserved at 4°C. But it is not known whether this is applicable to the anti meat sera.

During the course of the study there were occasional break-down in the electricity supply which would have altered the temperature in the refrigerator. But the probable temperature fluctuation, inside the refrigerator did not affect the quality of the antisera. Therefore,

it may be concluded that variations in preservation temperature for a shorter duration will not affect the potency of the serum used in precipitation tests.

The results of the study established that differentiation of chevon from beef could be reasonably done by the precipitation technique using hyperimmune sera raised in rabbits, and by its absorption using freeze-dried antigen to remove the cross-reacting antibodies. Adulteration of chevon with beef and buffalo meat could also be detected by the same method upto 20 per cent level.

SUMMARY

Adulteration and misrepresentation are common fraudulant practices encountered in meat industry. It is difficult to distinguish the meat of different species by their physical character alone. Immunological methods are considered as one of the reliable methods for this purpose. Hence in the present study, differentiation of beef from chevon by serological methods was undertaken.

Hyperimmune sera was raised in rabbits maintained in the laboratory against beef and chevon. Saline extract of beef and chevon were used as antigen for intraperitoneal injection. Five injections, at an interval of four days, were given to the rabbits. All the rabbits developed detectable level of antibodies after third injection, when test bleeding was done. Tube precipitation and gel-diffusion techniques were adopted for the tests. Saline extract of muscle was found to be a competent antigen to elicit sufficient antibody response in rabbits. Saline extracts of the meat samples to be tested were prepared in 1 in 10, 1 in 50 and 1 in 100 dilutions and were used as antigens against antisera. One in fifty dilution was found to be most satisfactory.

The unabsorbed antisera on testing proved to contain

cross-reacting antibodies. This could be removed by absorption with freeze-dried antigen against which the cross-reacting antibodies were present. The antiserum raised against chevon was found more potent than that against beef. Absorption technique slightly reduced the potency of both types of antisera.

Eighty samples of meat from known sources were collected at different periods and false numbered to mask the identity of meat sample. These samples were subjected to tube precipitation and gel-diffusion tests. Forty two samples were identified as beef and thirty eight samples as chevon, which were in agreement with the records. Thus gel-diffusion and tube precipitation test have proved its worthiness as a reliable and dependable method for differentiating between chevon and beef.

Same tests were conducted using buffalo meat and mutton as antigens, in place of beef and chevon, which elicited reactions quite similar to that of beef and chevon respectively.

In mixtures of chevon and beef at different proportions, the adulterants could be identified at levels as low as 20 per cent by both tube precipitation

and gel-diffusion tests. When buffalo meat was substituted in place of beef, it could also be detected upto 20 per cent level as in the case of beef.

The sera preserved at 5°C and -20°C for six months did not apparently affect its potency in spite of occasional electricity failure. It is concluded that the precipitation test by both tube and gel-diffusion methods using absorbed sera are quite reliable to differentiate between chevon and beef.

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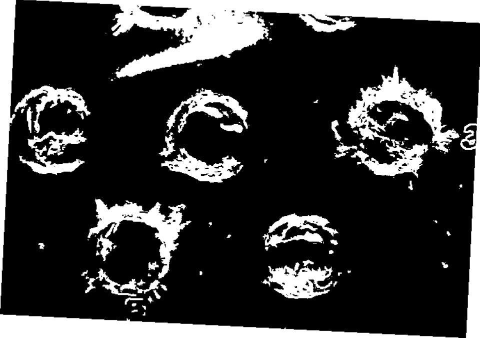

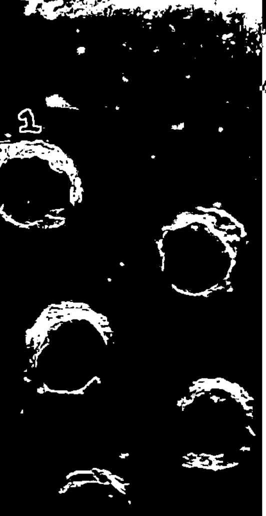
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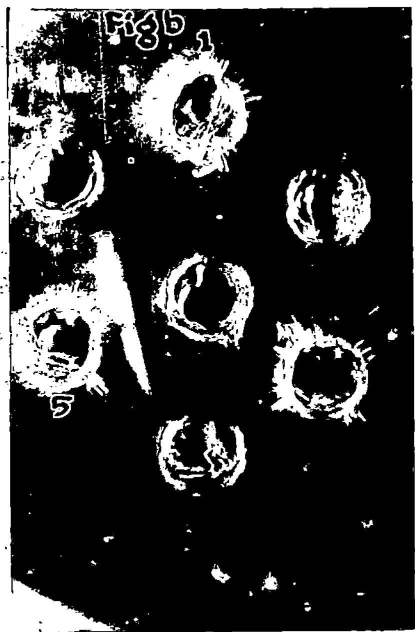
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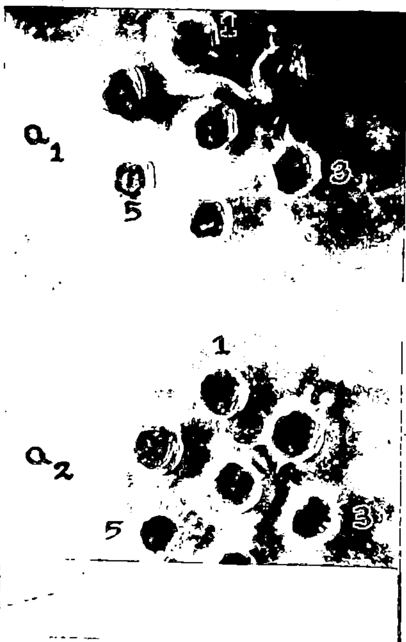
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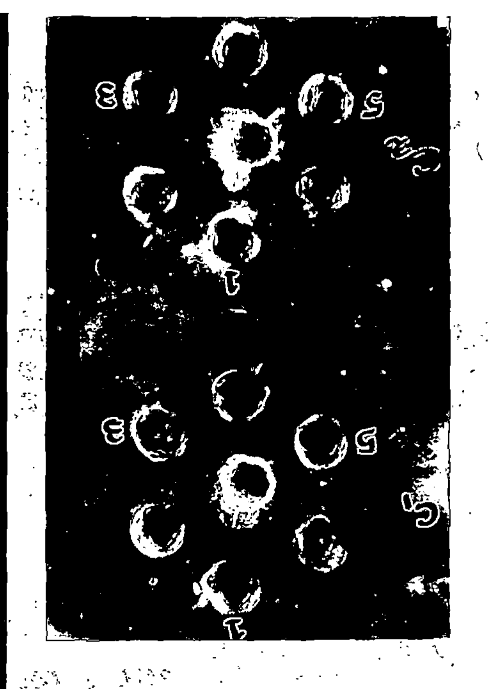

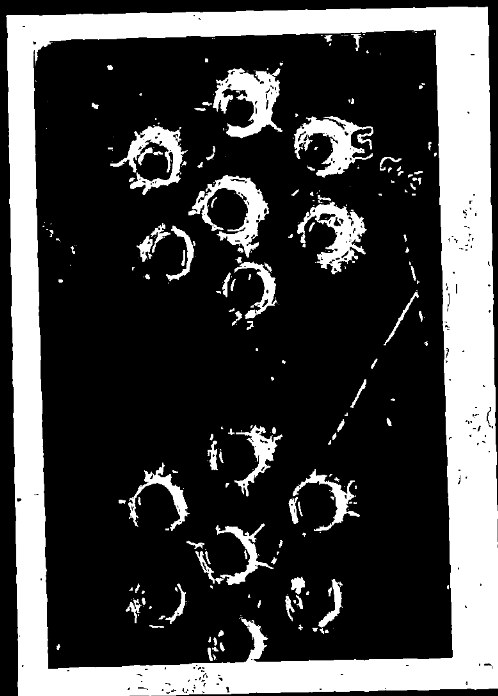

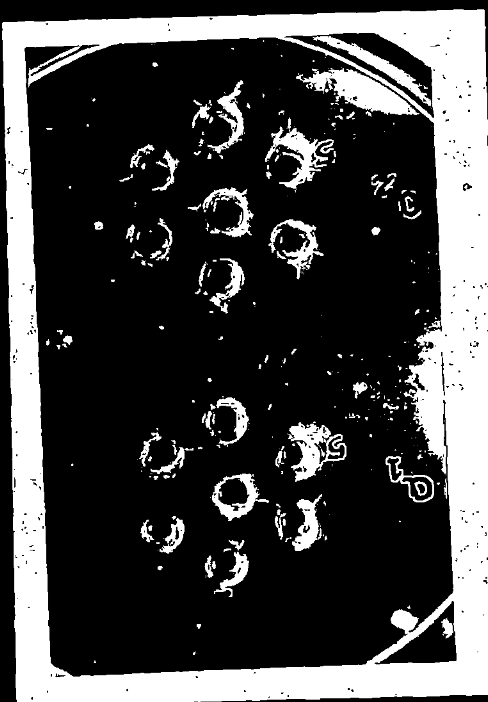

DIFFERENTIATION OF BEEF FROM CHEVON BY SEROLOGICAL METHODS

BY

N. MOHAN DAS

ABSTRACT OF A THESIS

Submitted in partial fulfilment of the requirement for the degree

MASTER OF VETERINARY SCIENCE

Faculty of Veterinary and Animal Sciences
Kerala Agricultural University

Department of Veterinary Public Health
COLLEGE OF VETERINARY AND ANIMAL SCIENCES
MANNUTHY - TRICHUR

1980

ABSTRACT

Identification of meat of different species by physical examination alone is difficult. Serological tests are advocated as one of the reliable methods. In order to differentiate beef from chevon, tube precipitation and gel-diffusion tests were concurrently done. Antisera raised in rabbits against beef and chevon, and saline extracts of meat samples collected from known sources as antigen, were used for the tests. In order to remove cross-reacting antibodies, the sera were absorbed with freeze-dried antigen against which cross-reacting antibodies were present.

Tube precipitation and gel-diffusion tests were adopted for identifying the meat samples. The tests conducted on eighty samples of meat gave cent per cent accuracy in identifying the meat.

Buffalo meat and mutton used in the test as antigen reacted in the same manner as that by beef and chevon respectively. It was also possible to detect the presence of adulterant in a mixed sample of beef and chevon when the proportion or level of adulterant was upto 20 per cent.

The antisera could be preserved for more than six months at 5°C and -20°C without loss of efficacy inspite of occasional electricity failure. It is concluded that tube precipitation and gel-diffusion tests are reliable methods for differentiation of beef and chevon.