

**ASSESSMENT OF CALF HEALTH AND STRESS
ASSOCIATED WITH DISBUDDING IN DAIRY ANIMALS
USING INFRARED THERMOGRAPHY**



**THESIS SUBMITTED TO THE
ICAR-NATIONAL DAIRY RESEARCH INSTITUTE, KARNAL
(DEEMED UNIVERSITY)**

**IN PARTIAL FULFILMENT OF THE REQUIREMENTS
FOR AWARD OF THE DEGREE OF**

MASTER OF VETERINARY SCIENCE

IN

LIVESTOCK PRODUCTION MANAGEMENT

BY

**ASHEQ AHMED MIR
(B.V.Sc. & A.H.)**

**DIVISION OF LIVESTOCK PRODUCTION MANAGEMENT
ICAR- NATIONAL DAIRY RESEARCH INSTITUTE
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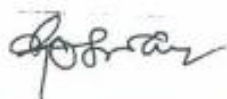
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Approved by:




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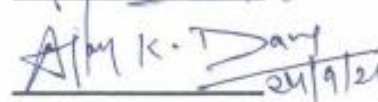
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Date: 18/09/2021

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(Major Advisor and Chairman)

DEDICATED
TO MY
BELOVED PARENTS,
SISTERS
AND GUIDE

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ABBREVIATIONS

%	Percentage
p<0.01	1 % level of significance
p<0.05	5% level of significance
<	Less than
>	Greater than
⁰ C	Degree Celsius
⁰ F	Degree Fahrenheit
ANOVA	Analysis of variance
BRD	Bovine Respiratory Disease
BVD	Bovine Viral Diarrhoea
Cm	Centimetre
Fig	Figure
GM	Gram
Hb	Hemoglobin
ICAR	Indian Council of Agriculture Research
IR	Infra-Red
IRT	Infrared thermography
KG	Kilo gram
LRC	Livestock Research Centre
Mm	Millimetre
MI	Millilitre
mmHg	Millimeter mercury
NCD	Neonatal calf diarrhoea
μ M	Micrometer
NDRI	National Dairy Research Institute
Ng	Nano gram
Nm	Nano metre
OD	Optical density
PCV	Packed cell volume
RBC	Red blood corpuscles
RPM	Revolution per minute
SPSS	Statistical package software system

TDN	Total digestible nutrient
USA	United States of America
S.E	Standard error
AUC	Area under curve
HPA	Hypothalamic pituitary adrenal axis
SAM	Sympathetic adrenal medullary activity
NSAIDs	Non steriodal antiinflammatory drugs
PGE ₂	Prostaglandin E2
PGI ₂	Prostaglandin I2
HR	Heart rate
LA	Local anesthetic
Nmol/l	Nanomole per litre
LRC	Livestock research centre
MG	Miligram
PM	Post meridiem
AM	Ante meridiem
RR	Respiration rate
EDTA	Ethylene diamine tetraactate
NS	Normal saline
HM	Himedia
EMB	Eosin methylene blue
HCL	Hydrochloride
DSLRL	Digital SLR
ELISA	Emzyme linked immunosorbant assay
DMRT	Duncan's Multiple Range test
IBM	International Business Machines
STD	Standard deviation
SEM	Standard error of mean
DAHDF	Department of Animal husbandary Dairying and fisheries
BRSV	Bovine respiratory syncitial virus
PI	Parainfluenza
BHV	Bovine herpes virus
Hb	Heamoglobin

g/dl	Grams per decilitre
TLC	Total leucocyte count
μL	Microlitre
WBC	White blood cells
TEC	Total erythrocyte count
GI	Gastrointestinal
MCV	Mean Corpuscular volume
GRAN	Granulocyte
LYM	Lymphocyte
m/mm ³	Cubic millimeter
CVMA	Canadian Veterinary Medical Association
AVMA	American Veterinary Medical Association
USDA	U.S. Department of Agriculture
Hr	Hour
MNT	Mechanical nociceptive threshold
ADG	Average daily gain
Neu	Neutrophil
EMB	Eosin methylene blue
BGA	Brilliant Green agar
Temp	Temperature
HR	Heart rate
RR	Respiration Rate
BPM	Breaths per minute

ABSTRACT

The study was planned to assess the calf health and stress associated with different methods of disbudding in dairy calves using Infrared Thermography (IRT) with the two objectives i.e. evaluation of calf health in dairy animals using infrared Thermography, under which two experiments were conducted and Evaluation of stress associated with different methods of disbudding using infrared thermography in dairy calves, under which two experiments were conducted. Under first objective in the first experiment data was generated for physiological, haematological, IRT based ocular and body surface temperature of calf diarrhoea (n=19), calf pneumonia (n=6), calf fever (n=4) and healthy dairy calves (n=29). Respiration rate, heart rate, rectal temperature ($^{\circ}\text{C}$) was significantly ($p<0.05$) higher in calves affected with diarrhoea, pneumonia and fever as compared to healthy calves. PCV (%) showed a significant ($p<0.05$) increase in diarrhoea and a significant ($p<0.05$) decrease in pneumonia affected calves as compared to healthy calves. TLC ($\times 10^3/\mu\text{l}$) showed a significant ($p<0.05$) increase in diarrhoea and pneumonia as compared to healthy calves. Neutrophil (%) showed a significant ($p<0.05$) increase in diarrhoea, pneumonia and fever affected calves as compared to healthy calves. Lymphocyte (%) showed a significant increase in diarrhoea and decrease in pneumonia. In diarrhoea, pneumonia and fever significant ($p<0.05$) increase in IR temperature of eyeball (1.07°C , 0.66°C , 0.76°C), peri-anal (1.43°C , 1.16°C , 0.92°C), para-lumbar (0.91°C , 0.99°C) temperature was observed as compared to healthy calves. In experiment 2, for Early detection of diseases in calves, healthy newborn Sahiwal calves (n=46) were taken. Maximum increase of eyeball, para-lumbar, peri-anal and rectal temperature was observed in the case of calves affected with diarrhoea, pneumonia and fever. Significant ($p<0.05$) increase in eyeball, peri-anal and rectal temperature was recorded one day before, on the day and one day after diarrhoea and pneumonia as compared to healthy calves. Significant ($p<0.05$) increase in eyeball, para-lumbar, peri-anal and rectal temperature was recorded only on the day of fever and increase in para-lumbar temperature was recorded only on the day of diarrhoea and pneumonia as compared to healthy calves. Significant ($p<0.05$) increase in the eyeball (0.6°C and 0.66°C), peri-anal (0.85°C and 1.14°C), para-lumbar temperature (0.69°C and 0.73°C), rectal temperature (0.83°C and 0.94°C), was recorded on the day of diarrhoea as compared to two days before diarrhoea as well as previous seven days average temperature before diarrhoea in affected calves. Significant ($p<0.05$) increase in the eyeball (0.56°C and 0.8°C), peri-anal (0.62°C and 0.9°C), para-lumbar temperature (0.9°C and 1.35°C), rectal temperature (0.76°C and 1.16°C), was recorded on the day of pneumonia as compared to two days before pneumonia as well as previous seven days average temperature before pneumonia in affected calves. Significant ($p<0.05$) increase in the eyeball (0.63°C and 1.13°C), peri-anal (0.57°C and 0.95°C), rectal temperature (0.56°C and 0.84°C) was recorded on the day of fever as compared to two days before fever as well as previous seven days average temperature before fever in affected calves. Significant ($p<0.05$) increase in para-lumbar temperature (0.88°C) was recorded on the day of fever as compared to two days before fever. PCV %, TLC (10^3), neutrophil %, lymphocyte % of calves affected with diarrhoea and pneumonia calves and neutrophil % of calves affected with fever showed a significant change as compared to healthy calves. Incidence rate of diarrhoea, pneumonia and fever was 17.39%, 4.34% and 6.52%, respectively and bacteria identified from diarrhoea faecal samples were *E. coli* (75%) and *Salmonella spp.* (25%). In the first experiment of objective 2, 33 Sahiwal calves of different age groups were taken. Significant ($p<0.05$) increase in horn bud diameter for the 11 to 20 days and 21 to 30 days age group as compared to the Birth to 10 days age group. In the second experiment, 30 Sahiwal calves were taken and disbudding

was carried out with different methods i.e. without local anaesthetic (control), with local anaesthetic (Treatment 1) and with local anaesthetic and NSAIDs group (Treatment 2). Significant ($p < 0.05$) drop in eyeball temperature at disbudding time was recorded in the Control as compared before disbudding and a non-significant decrease in Treatment 1 and Treatment 2. Significant ($p < 0.05$) increase in horn bud temperature after disbudding was recorded in Control, Treatment 1 and Treatment 2 as compared to before disbudding. Significant ($p < 0.05$) increase in peri-anal temperature in Control, 30 minutes after disbudding, Treatment 1, 120 minutes after disbudding and in Treatment 2, 150 minutes after disbudding was observed as compared to before disbudding. Heart rate showed a significant ($p < 0.05$) increase at 2 minutes and 3 hrs after disbudding in Control, Treatment 1 and Treatment 2 as compared to baseline. The change in heart rate (bpm) was 20 and 16 in Control, 11 and 10 in Treatment 1 and 8 and 7 in Treatment 2 as compared to baseline. Respiration rate showed a significant ($p < 0.05$) increase in the Control, Treatment 1 and Treatment 2 at 2 minutes and 3 hours after disbudding as compared to before disbudding. A significant increase ($p < 0.05$) in rectal temperature was observed in Control, Treatment 1 and Treatment 2 at 3 hours after disbudding as compared to before disbudding and change of rectal temperature in Control was 0.53°C , Treatment 1, 0.50°C and Treatment 2, 0.31°C . A significant ($p < 0.05$) increase in cortisol concentration (ng/ml) was recorded in the Control and Treatment 1 after 40 minutes of disbudding and 4 hours of disbudding as compared to before 40 minutes of disbudding and a non-significant increase in Treatment 2 after 40 minutes and 4 hrs of disbudding as compared to before 40 minutes of disbudding was recorded. Significant ($p < 0.05$) increase of head shaking and tail shaking was observed in Control as compared to Treatment 1 and Treatment 2. During the healing process significant ($p < 0.05$) decrease in horn bud temperature was observed with time 7th day of 4th week after disbudding in Control, in Treatment 1 and Treatment 2 at time 3rd day of 3rd week as compared to during disbudding. Significant ($p < 0.05$) increase of lesion score in Control was observed in 2nd week and 3rd week as compared to Treatment 1 and Treatment 2. Complete epithelisation in Control and Treatment 1 took place when the lesion score reached 1, 5 weeks after disbudding and in Treatment 2 lesion score reached 1, 4 weeks after disbudding. Significant ($p < 0.05$) increase in weekly average daily gain (gms) was recorded in Treatment 2 and Treatment 1 during 1st week and 2nd week as compared to Control. It can be concluded that IRT can be used as a supportive tool to assess the increase in temperature of eyeball, peri-anal and para-lumbar region of Sahiwal calves affected with diarrhoea, pneumonia and fever on the day of clinical onset and to understand the early change in temperature in case of calves affected with diarrhoea and pneumonia even before appearance of the actual clinical signs. Use of local anesthesia, NSAIDs and Nupatch during disbudding in Sahiwal calves was very effective method to reduce the stress, which can be assessed by non-invasive IRT tool.

Abstract

इन्फ्रारेड थर्मोग्राफी (आईआरटी) द्वारा डेयरी बछड़ों में डिसबडिंग के विभिन्न तरीकों से जुड़े बछड़े के स्वास्थ्य और तनाव का आकलन करने के लिए अध्ययन की योजना बनाई गई थी, जिसमें दो उद्देश्यों यानी इन्फ्रारेड थर्मोग्राफी का उपयोग करके डेयरी जानवरों में बछड़े के स्वास्थ्य का मूल्यांकन किया गया था, जिसके तहत दो प्रयोग किए गए थे और मूल्यांकन किया गया था। डेयरी बछड़ों में इन्फ्रारेड थर्मोग्राफी का उपयोग करके डिसबडिंग के विभिन्न तरीकों से जुड़े तनाव, जिसके तहत दो प्रयोग किए गए थे। पहले प्रयोग में पहले उद्देश्य के तहत बछड़ा दस्त (एन = 19), बछड़ा निमोनिया (एन = 6), बछड़ा बुखार (एन=4) और स्वस्थ डेयरी बछड़ों के शारीरिक, हेमाटोलोजिकल, आईआरटी आधारित ओकुलर और शरीर की सतह के तापमान के लिए डेटा तैयार किया गया था। (एन = 29)। स्वस्थ बछड़ों की तुलना में दस्त, निमोनिया और बुखार से प्रभावित बछड़ों में श्वसन दर, हृदय गति, मलाशय तापमान में ($^{\circ}\text{C}$) काफी ($p<0.05$) अधिक था। पीसीवी (%) ने दस्त में एक महत्वपूर्ण ($p<0.05$) वृद्धि और निमोनिया बछड़ों में एक महत्वपूर्ण ($p<0.05$) कमी, स्वस्थ बछड़ों की तुलना में दिखाई। टीएलसी ($\times 10^3 / \mu\text{l}$) ने दस्त में और निमोनिया में स्वस्थ बछड़ों की तुलना में एक महत्वपूर्ण ($p<0.05$) वृद्धि दिखाई। न्यूट्रोफिल (%) ने स्वस्थ की तुलना में दस्त, निमोनिया और बुखार के बछड़ों में उल्क्ष्मीय ($p<0.05$) वृद्धि दिखाई। लिम्फोसाइट (%) ने दस्त में उल्क्ष्मीय वृद्धि, निमोनिया में कमी देखी। दस्त, निमोनिया और बुखार में नेत्रगोलक तापमान में (1.07°C , 0.66°C , 0.76°C), पेरी-एनल में (1.43°C , 1.16°C , 0.92°C) और पैरा-लम्बर में (0.91°C , 0.99°C) तापमान में स्वस्थ बछड़ों की तुलना में महत्वपूर्ण ($p<0.05$) वृद्धि देखा गया। प्रयोग 2 में, बछड़ों में रोगों का शीघ्र पता लगाने के लिए, स्वस्थ नवजात साहीवाल बछड़ों ($n=46$) को लिया गया। डायरिया, निमोनिया और बुखार से प्रभावित बछड़ों के मामले में नेत्रगोलक, पैरालम्बर, पेरी-एनल और मलाशय तापमान में अधिकतम वृद्धि देखी गई। स्वस्थ बछड़ों की तुलना में डायरिया और निमोनिया के बछड़ों में एक दिन पहले, एक दिन पहले और एक दिन बाद नेत्रगोलक, पेरी-एनल और रेक्टल तापमान में उल्क्ष्मीय ($p<0.05$) वृद्धि दर्ज की गई थी। नेत्रगोलक, पैरालम्बर, पेरी-एनल और मलाशय तापमान में उल्क्ष्मीय ($p<0.05$) वृद्धि केवल बुखार के दिन दर्ज की गई थी और स्वस्थ बछड़ों की तुलना में केवल दस्त और निमोनिया के दिन ही पेरी-एनल के तापमान में वृद्धि दर्ज की गई थी। नेत्रगोलक (0.6 डिग्री सेल्सियस और 0.66 डिग्री सेल्सियस), पेरी-एनल (0.85 डिग्री सेल्सियस और 1.14 डिग्री सेल्सियस), पैरा-लम्बर तापमान (0.69 डिग्री सेल्सियस और 0.73 डिग्री सेल्सियस), मलाशय तापमान में महत्वपूर्ण ($p<0.05$) वृद्धि 0.73 डिग्री सेल्सियस और 0.94 डिग्री सेल्सियस), दस्त के दिन दर्ज किया गया था, जबकि दस्त से पहले दो दिन और साथ ही प्रभावित बछड़ों में दस्त से पहले पिछले सात दिनों का औसत तापमान दर्ज किया गया था। नेत्रगोलक (0.56 डिग्री सेल्सियस और 0.8 डिग्री सेल्सियस), पेरी- एनल (0.62 डिग्री सेल्सियस और 0.9 डिग्री सेल्सियस), पैरा-लम्बर तापमान (0.9 डिग्री सेल्सियस और 1.35 डिग्री सेल्सियस), मलाशय तापमान में महत्वपूर्ण ($p<0.05$) वृद्धि 0.76 डिग्री सेल्सियस और 1.16 डिग्री सेल्सियस) निमोनिया के दिन दर्ज किया गया था, जबकि निमोनिया से पहले दो दिन और प्रभावित बछड़ों में निमोनिया से पहले पिछले सात दिनों का औसत तापमान दर्ज किया गया था। नेत्रगोलक (0.63 डिग्री सेल्सियस और 1.13 डिग्री सेल्सियस), पेरी-एनल (0.57 डिग्री सेल्सियस और 0.95 डिग्री सेल्सियस), मलाशय तापमान (0.56 डिग्री सेल्सियस और 0.84 डिग्री सेल्सियस) में महत्वपूर्ण वृद्धि दर्ज की गई थी। बुखार के दो दिन पहले की तुलना में बुखार के दिन पारालम्बर तापमान (0.88 डिग्री सेल्सियस) में महत्वपूर्ण ($p<0.05$) वृद्धि दर्ज की गई थी। पीसीवी%, टीएलसी (10^3), न्यूट्रोफिल, लिम्फोसाइट, डायरिया और निमोनिया से प्रभावित बछड़ों और बुखार से प्रभावित बछड़ों के न्यूट्रोफिल ने स्वस्थ बछड़ों की तुलना में महत्वपूर्ण परिवर्तन दिखाया। डायरिया, निमोनिया और बुखार की घटना दर 17.39%, 4.34% और 6.52% थी और डायरिया के मल के नमूनों से पहचाने गए बैक्टीरिया ई.

कोलाई (75%), सोबेला एस.पी.पी. (25%) थे। उद्देश्य 2 के पहले प्रयोग में विभिन्न आयु वर्ग के 33 साहीवाल बछड़ों को लिया गया। जन्म से 10 दिन के आयु वर्ग की तुलना में 11 से 20 दिन और 21 से 30 दिन के आयु वर्ग के लिए सींग की कली के व्यास में उल्लेखनीय ($p < 0.05$) वृद्धि हुई। दूसरे प्रयोग में, 30 साहीवाल बछड़ों को लिया गया और उनका डिसबडिंग; स्थानीय संवेदनाहारी (नियंत्रण) के बिना, स्थानीय संवेदनाहारी (उपचार 1) के साथ और स्थानीय संवेदनाहारी और एनएसएआईडी समूह (उपचार 2) के साथ विभिन्न तरीकों के साथ। डिस्बडिंग के समय नेत्रगोलक के तापमान में महत्वपूर्ण ($p < 0.05$) गिरावट को सिंग अवरोधन से पहले की तुलना में कंट्रोल में दर्ज किया गया था और उपचार 1 और उपचार 2 में एक गैर-महत्वपूर्ण कमी दर्ज की गई थी। डिस्बडिंग से पहले की तुलना में नियंत्रण, उपचार 1 और उपचार 2 में हॉर्न बड तापमान में महत्वपूर्ण ($p < 0.05$) वृद्धि दर्ज की गई थी। नियंत्रण में पेरि- एनल तापमान में महत्वपूर्ण ($p < 0.05$) वृद्धि, डिस्बडिंग के 30 मिनट बाद, उपचार 1, डिस्बडिंग के 120 मिनट बाद और उपचार 2 में, डिस्बडिंग के 150 मिनट बाद डिस्बडिंग से पहले की तुलना में देखा गया। शारीरिक मापदण्डों में बेसलाइन की तुलना में नियंत्रण, उपचार 1 और उपचार 2 में डिस्बडिंग के बाद 2 मिनट और 3 घंटे में हृदय गति में उल्लेखनीय ($p < 0.05$) वृद्धि देखी गई। हृदय गति में परिवर्तन (बीपीएम) नियंत्रण में 20 और 16, उपचार 1 में 11 और 10 और उपचार 2 में 8 और 7 था। डिस्बडिंग से पहले की तुलना में श्वसन दर ने नियंत्रण, उपचार 1 और उपचार 2 में 2 मिनट और 3 घंटे बाद महत्वपूर्ण ($p < 0.05$) वृद्धि दिखाई। मलाशय तापमान में उल्लेखनीय वृद्धि ($p < 0.05$) नियंत्रण, उपचार 1 और उपचार 2 में डिस्बडिंग डिस्बडिंग के 3 घंटे बाद देखी गई, जबकि मलाशय तापमान में नियंत्रण में परिवर्तन (0.53°C), उपचार में 1 (0.5°C) और उपचार 2 में (0.31°C) की तुलना में था।। कोर्टिसोल सांद्रता (एनजी/एमएल) में 40 मिनट पहले की तुलना में, नियंत्रण और उपचार 1 में 40 मिनट और 4 घंटे के डिस्बडिंग के विघटन के बाद एक महत्वपूर्ण ($p < 0.05$) वृद्धि दर्ज की गई थी और उपचार में एक गैर-महत्वपूर्ण वृद्धि 40 मिनट बाद और 4 घंटे उपचार 2 और डिस्बडिंग के 4 घंटे पहले की तुलना में डिस्बडिंग के 40 मिनट दर्ज की गई थी। उपचार 1 और उपचार 2 की तुलना में नियंत्रण में महत्वपूर्ण ($p < 0.05$) सिर कांपना और पूंछ का हिलना देखा गया। उपचार प्रक्रिया के दौरान नियंत्रण में डिस्बडिंग के बाद 4 वें सप्ताह के 7 वें दिन समय उपचार 1 और उपचार 2 में तीसरे सप्ताह के तीसरे दिन समय पर वितरण के दौरान की तुलना में के साथ सींग की कली का तापमान महत्वपूर्ण ($p < 0.05$) कम हो गया। उपचार 1 और उपचार 2 की तुलना में नियंत्रण में घाव के स्कोर में महत्वपूर्ण ($p < 0.05$) वृद्धि दूसरे सप्ताह और तीसरे सप्ताह में देखी गई। नियंत्रण और उपचार 1 में उपकलाकरण ने महल ले लिया जब घाव का स्कोर विघटन के 5 सप्ताह बाद 1 तक पहुंच गया और उपचार 2 में घाव का स्कोर डिस्बडिंग के 4 सप्ताह बाद 1, तक पहुंच गया। नियंत्रण की तुलना में पहले सप्ताह और दूसरे सप्ताह के दौरान उपचार 2 और उपचार 1 में साप्ताहिक औसत दैनिक लाभ (ग्राम) में उल्लेखनीय ($p < 0.05$) वृद्धि दर्ज की गई। यह निष्कर्ष निकाला जा सकता है कि आईआरटी को नैदानिक शुरुआत के दिन दस्त, निमोनिया और बुखार से प्रभावित साहीवाल बछड़ों के नेत्रगोलक, पेरि-गुदा और पैरा-लम्बर क्षेत्र के तापमान में वृद्धि का आकलन करने के लिए एक सहायक उपकरण के रूप में इस्तेमाल किया जा सकता है और इसे समझने के लिए इस्तेमाल किया जा सकता है। वास्तविक नैदानिक लक्षणों के प्रकट होने से पहले ही दस्त और निमोनिया से प्रभावित बछड़ों के मामले में तापमान में प्रारंभिक परिवर्तन। साहीवाल बछड़ों में डिस्बडिंग के दौरान स्थानीय एनेस्थीसिया, एनएसएआईडी और न्यूपैच का उपयोग तनाव को कम करने के लिए बहुत प्रभावी तरीका था, जिसका आकलन गैर-आक्रामक आईआरटी उपकरण द्वारा किया जा सकता है।

CHAPTER -1

Introduction

INTRODUCTION

The livestock sector plays a key role in the health and economic growth of rural farmers. It is increasingly organised in long market chains, employing at least 1.3 billion people worldwide and directly supporting the livelihoods of the developing world's 600 million poor smallholder farmers. Dairy animals play an important role in the economic upliftment of small and marginal farmers in the livestock sector (Thornton *et al.*, 2006). The livestock population of India is 535.78 million, according to the 20th livestock census, which shows an increase of 4.6 per cent over the 19th livestock census. The total population of bovine (Cattle, Buffalo, Mithun and Yak) is 302.79 million, indicating a rise of 1.0% over the 19th census of livestock. In 2019, the total number of cattle in the nation is 192.49 million, showing an improvement of 0.8 percent over the 19th census of livestock. The female cattle population is 145.12 million, an increase of 18.0 percent over the 19th livestock census. Total country's buffalo population is 109.85 million, grows at around 1.0 percent than the previous census of livestock. Currently, India is producing 198.4 million metric tonnes of milk, continuing to be the world's largest producing nation (DAHDF, 2019). Most of the milk produced in the country is from buffaloes contributing about 49 percent followed by the cross bred cows 26 percent, indigenous and non-descript cows 21 percent and exotic cows 1 percent. The growth in milk production is technology-driven. The annual growth rate in milk production is 6.47 percent in the year 2019 (DAHDF, 2019). Scientific dairy farming plays an important role to achieve the boom of milk production. It envisages breeding, feeding, weeding and heeding i.e. day to day management of dairy animals playing an important role. Day to day management is the most critical management input and the success of dairy farms depends on efficient farm management. Nowadays, sensors are in use for dairy animal's day to day management, but it has its constraints. The cost of the technology and technological knowledge required for sensor-based technology restricting the adoption of the technology to commercial dairy farms, but in our country maximum milk is contributed by the smallholder dairy farmers. Recently Infrared thermography (IRT) as a supportive cow side diagnostic tool is a point of interest for researchers. Therefore, as an alternative approach, Infrared thermography can be used which can assess the small changes in body surface temperature efficiently and such temperature change is prominently evident under various physio-pathological conditions of dairy animals. The

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dairy sector's success depends on the calves survival rate since it represents a farm's potential breeding stock. Scientific-based breeding performs a vital role in the growth of the dairy industry. Healthy farm animals are not being brought from the market they are reared from birth (Shakya *et al.*, 2017). Calf mortality is one of the main concerns under organised farm and field conditions to achieve the growth of milk production. It is evident in the literature that calf mortality of 20 per cent can decrease the net profit by around 40 percent (Blood and Radostits, 1989; Singh *et al.*, 2009). In dairy farms, calf diarrhoea and pneumonia are the leading causes of calf death. In indigenous cattle and buffalo after the death of the newborn, cow's milk production is decreased and there is also a lack of potential breeding and a waste of investment in feed and care (Wymann *et al.*, 2006). The aim is to provide healthy animals with lower costs so that they can reach their full genetic potential. Wellbeing of the animal is not only influenced due to diseases triggered by a pathogen or metabolic or physical problems but it is also triggered by stress factors like disbudding. Stress results in a change of endocrine responses that directly disturb the metabolism of the body, which in turn affects the immune system, health, growth, reproduction and animal welfare of dairy calves (Hulbert and Moisés, 2016).

Calves are the backbone of the dairy industry, therefore these animals must get the best start in life. Healthy calves are not only important for the survival of dairy herd, but it is also important for the conservation of germplasm (Sreedhar and Sreenivas, 2015). Healthcare and management of calves during initial critical periods of life is important for the prevention of neonatal diseases. Neonatal diseases have a significant effect on the health, growth, longevity and production parameters of dairy animals (Lorenz *et al.*, 2011). Calf diarrhoea is one of the leading causes of calf mortality, it leads to 9.5 percent (Yadav *et al.*, 2019) to 80 percent (Sreedhar and Sreenivas, 2015) of deaths in the dairy farm. 82.2 percent of mortality in the case of calves was also reported due to Calf diarrhoea (Tiwari *et al.*, 2019). The second most common cause of calf mortality in dairy farms is pneumonia, which is diagnosed mostly during post mortem of calves having age 1-5 months. It is a highly contagious disease, affecting more than 50 percent of young calves. The chance of getting calf pneumonia to diarrheic calves is more (Manjari *et al.*, 2015). In commercial dairy farms, death due to pneumonia in calves is 15 percent (Sreedhar and Sreenivas, 2015). While in the case of buffalo calves, 24 percent of death occur due to calf pneumonia (Tiwari *et al.*, 2019). Calf diarrhoea and pneumonia are the

major leading causes of calf death in farms as well as under field conditions. The calf mortality rate is considered one of the most significant indicators of welfare measurement (UETAKE, 2013). The most common symptom of calf diarrhoea is septicemia leading to watery stools, dehydration, sunken eyeballs, increased respiration rate and fever $>39.5^{\circ}\text{C}$. In the case of calf pneumonia initially, there is septicemia after that viremia takes place leads to increased respiration rate, coughing, dullness, increased body temperature over 39.5°C . Change of body surface temperature both in calf diarrhoea and calf pneumonia can be detected by the remote and non-invasive infrared thermography (Schaefer *et al.*, 2007). For the assessment of calf diarrhoea and pneumonia, infrared thermography can be a promising supportive diagnostic method.

Disbudding is a managemental husbandry procedure carried out on young calves commonly between the age of 2-6 weeks, to prevent the increase in horn bud growth (Stafford and Mellor, 2005). Dairy animals that are disbudded or dehorned are easier to manage by the handler, causes less injury to others especially during the transport, management and requires less space for feeding and watering (Stokey and Goonewardene, 1996). Routine managemental practices followed in certain routine farm operations causes small tissue destruction (e.g disbudding, castration) are the potential source to cause pain in the animals. The body of the animal response towards the pain involves a pathway of physiological and behavioural activity. Stress due to the managemental practices is most commonly measured by physiological means. To detect disbudding pain and stress in dairy calves combination of physiological measures and behavioural indicators has been used. The behavioural activity provides a good sign of the duration and unique levels of a painful condition, but might not suggest the maximal depth of an experience in addition to physiological signs (Mellor *et al.*, 2000). Physiological parameters like plasma cortisol concentration, which reflects the hypothalamus-pituitary-adrenal (HPA) activity, has been used to evaluate painful methods (Stafford and Mellor, 2005). An acute response to painful (nociceptive) or stressful conditions is the diversion of blood supply from cutaneous capillary bed via sympathetically mediated vasoconstriction, which subsequently decreases the body surface temperature (Blessing, 2003). So, the effect of vasoconstriction detected by infrared thermography as a temperature change is used for the evaluation of stress associated with disbudding in dairy animals. Infrared thermography and behavioural indicators have been used to assess the disbudding stress in Holstein Friesian dairy

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calves (Stewart *et al.*, 2008). Under Indian conditions use of Infrared thermography to understand the temperature change associated with the calf health during the natural course of infection as well as to assess the stress associated with disbudding in indigenous dairy calves is yet to be explored. Keeping in view the above gaps the following objectives have been devised.

Objectives:

1. Evaluation of calf health in dairy animals using infrared thermography.
2. Evaluation of stress associated with different methods of disbudding using infrared Thermography in dairy calves.

CHAPTER -2

Review of Literature

REVIEW OF LITERATURE

Livestock production management has four fundamental pillars i.e. feeding, breeding, weeding, and heeding. Among them heeding plays a crucial role in the day to day management of dairy animals on the farm. In a dairy farm, technological interventions play a significant role in the health management, reproduction and production of dairy animals. Nowadays, non Invasive IRT Technology has been used to assess the health status of dairy calves and stress associated with disbudding, as these phenomenons are related to the change in the body skin surface temperature (Schaefer *et al.*, 2007, 2012; Adcock and Tucker, 2018). Core body temperature is a precise health indicator of animals. Temperature is a very good indicator of health, as changes of just a few degrees on skin (cutaneous or superficial) temperature can be used as an indicator of possible illness. Changes in body temperature are often the first sign of acute diseases and it occurs earlier than other signs (Sellier *et al.*, 2015). The change in body surface temperature of various body points can be monitored by the non-invasive remote sensing infrared thermography and produces a thermogram. Sound and healthy calfare producedby minimize calf's contact to infection and maximize its defence system against infection. Protecting the health of the calf is important not only for the vitality of the calf, but also for the cow's well-being and future productivity. Assessment of the calf health related to disease conditions and stress associated with the disbudding in dairy calves on farms are important components of farm animal welfare. In the early stages of life, calves are susceptible to diseases like, diarrhoea and pneumonia that affects the health of calves. The survival of calves and their rapid growth is the most important factor responsible for the propagation of dairy animals (Kochewad *et al.*, 2013). In diarrhoea, pneumonia and disbudding there has been a change in the physiological and behavioural responses in the body of dairy calves. Change in the body response of the calves can be used to asses the health status of dairy calves. In calf diarrhoea and pneumonia there is a change in body surface temperature, which can be assessed by the use of infrared thermography (Lowe *et al.*, 2019). In the disbudding of calves, there has been the activation of the autonomic nervous system and the hypothalamic-pituitary-adrenal axis. It coordinates the stress response and is primarily involved in metabolic homeostasis. Stress response results in

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an increase of catecholamines and plasma cortisol concentration, associated with a change in surface blood flow, resulting in a change of body surface temperature of the animal (Cook and Schaefer, 2002). Disbudding of dairy calves is a stressful condition that leads to the change in the surface temperature of the eyes (Stewart *et al.*, 2008). The change in body surface temperature in stress and pain response has been evaluated using infrared thermography as well as change in the behavioural and physiological response of dairy animals. Therefore, understanding the temperature change and monitoring by IRT for early identification of infection and disbudding stress in dairy calves under tropical climate needs scientific validation. Keeping in view the above importance of the study was planned for early identification of infection and disbudding in calves using infrared thermography.

2.1. Infrared thermal camera and principle of working:

A thermographic camera (additionally known as an infrared camera or thermal imaging camera or thermal imager) is a device that generates an image using infrared radiations. The common camera uses a 400-700 nanometre range of visible light, whereas infrared cameras are highly sensitive to wave length from about 1000 nm (1 μm) to about 14000 nm (14 μm). Infrared energy contributes to one part of the electromagnetic spectrum, which comprises radiation from lower wave length to higher wave length i.e gamma rays, x-rays, ultraviolet, a thin region of visible light, infrared, terahertz waves, microwaves and radio waves. The practice of capturing and studying the data by IRT is called thermography. An infrared camera is a non-contact and remote temperature measuring tool. Infrared cameras locate the infrared power emitted, transmitted or contemplated by all substances at a temperature above absolute 0⁰ Kelvin and convert the energy factor into a thermogram. Thermogram is the thermal image displayed by the camera of the substance. The precept of infrared thermography is based on the bodily phenomenon that any frame of a temperature above absolute zero (-273.15 °C) releases electromagnetic radiations. There is a clean correlation between the surface of a frame and the intensity and spectral composition of its emitted radiation. By way of determining its radiation and intensity, the temperature of an object can thereby be decided in a non-contact manner. Measurements of skin temperatures by infrared thermography can reveal various conditions of the body (Speakman and Ward, 1998).

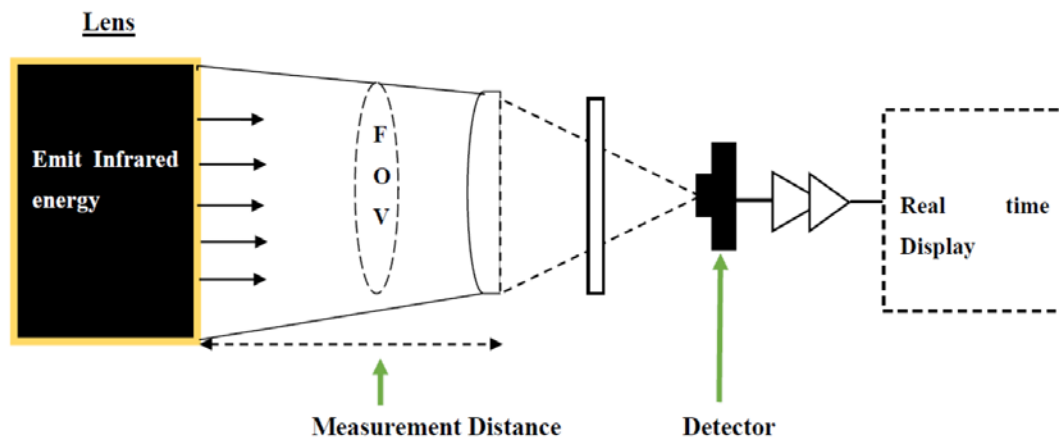


Fig. 2.1: Working Principle of IRT

2.2. History of infrared thermography:

Infrared thermography is equipment, which detects infrared power emitted from an object, converts it to temperature and shows a picture of the temperature distribution. The gadget and the approach must be referred to differently, the device to be known as infrared thermograph and the method to be called infrared thermography. Infrared radiations were discovered by a British astronomer, Herschel in 1800. When he dispersed sunlight using a prism. Herschel accidentally found that there was an invisible light on the outside of the red light, which increases the temperature of an object. It is an electromagnetic wave having a longer wavelength than those of visible light i.e $0.7\mu\text{m}$ or longer wavelength is 1mm or less and frequency is 300GHz or more.

- 1800: William Herschel discovered infrared radiations.
- 1830: Leopoldo Nobili made the first thermopile IR detector.
- 1840: John Herschel produces the first thermal image, called a thermogram.
- 1880: Samuel Pierpont Langley used a bolometer to measure IR or Heat or Radiation.
- 1929: Kalman Tihanyl invented the first infrared-sensitive electronic television camera for anti- aircraft.
- 1956: The first conventional IR camera is the Evaporograph.

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- 1965: Infrared thermography (IRT) was used in veterinary medicine for the first time by Delahanty and Georgi.
- 1981: Palmer, S.E. used a portable infrared thermometer for assessment of the limb surface temperature in the horse.
- 1989: Kajik, C. and Malkova, A. used IRT to monitor the teat surface temperature after machine milking.
- 1990: The use of IRT started in the field of civil engineering.
- 1992: Marr, C.M. assessed the injury of the superficial digital flexor tendon using microwave thermography in the horses.
- 1998: Speakman, J.R. and Ward, S. produced detailed evidence of principle and applications of Infrared thermography.
- 2000: Barth, K. evaluated udder inflammation in cows using a highly sensitive infrared-thermograph technique.
- 2000: Scott, S. L., Schaefer, A.I., Tong, A. K. W. and Lacasse, P. evaluated mastitis in dairy animals using infrared thermography.
- 2004: Schaefer, A. L., Cook, N., Tessaro, S.V., Deregt, D. and Desroches, G. assessed diseases in calves using infrared thermography.
- 2004: Kulezsa, O., Rzczkowski, M. and Kaczorowski, M. established practical use of IRT in equine diagnostics and treatment.
- 2005: Stewart, M., Webster, J., Schaefer, A., Cook, N. and Scott, S. used Infrared thermography to understand animal welfare.
- 2005: Willits, S. used infrared thermography for screening and early detection of mastitis infections in dairy herds.
- 2007: Kunc, P., Knizkova, I., Prikryl, M., Malone, J. used infrared thermography as a tool to study the milking process.
- 2007: Schaefer, A.L., Cook, N.J., Church, J.S., Basarab, J., Perry, B., Miller, C. and Tong, A.K.W. used infrared thermography as an early indicator of bovine respiratory disease complex in calves.

2.3. IRT as a prognostic tool to understand calf health and stress:

Infrared thermography provides dairy farmers, a non-invasive, non-contact, painless management method to distinguish circadian and weekly fluctuations as well as major short and long-term variations in skin surface temperature from various tissues (Nikkhah, 2015). Infrared thermography senses the amount of infrared energy a body radiates; the more the infrared energy, the higher the temperature of the body. Infrared gadgets assign the various colours to various levels of infrared energy to produce a colour picture known as a thermogram (Usamentiaga *et al.*, 2014). Farm animals are very much sensitive to the environmental, physiological and pathological stimuli that lead to the change in the rate of metabolism and blood flow, which is reflected as a change in body surface temperature. The change in body surface temperature can be detected by IRT. Thus, IRT is a useful tool to monitor and quantify the physiological and health status of animals. In the livestock sector IRT is mainly used for disease identification, estrus detection, feed utilization efficiency, birth weight difference in pigs and assessment of welfare. IRT can monitor respiratory disorders (Schaefer *et al.*, 2007), bovine viral diarrhoea in calves (Schaefer *et al.*, 2004), neonatal calf diarrhoea (Lowe *et al.*, 2019), disbudding stress (Stewart *et al.*, 2007), fear-related stress (Stewart *et al.*, 2007) and thermoregulatory capacity of dairy animals (Sevegnani *et al.*, 2016). IRT can also be used for the assessment of various conditions, which has been listed in Table 2.1.

Table 2.1: Uses of IRT in animal production

Use of IRT	References
Early identification of infection in calves	Schaefer <i>et al.</i> , 2007 (Angus Herford cross calves); Lovett <i>et al.</i> , 2009; Schaefer <i>et al.</i> , 2012 (Calves); Lowe <i>et al.</i> , 2019 (Friesian x Hereford's calves).
Disbudding stress	Stewart <i>et al.</i> , 2008 (Holstein Friesian calves); Bates <i>et al.</i> , 2016 (Friesian–Jersey cross calves); Adcock and Tucker, 2018 (Holstein x Jersey calves).

2.4. Calf health:

The future of the dairy industry depends on the efficient raising of dairy calves and replacement heifers (Nor *et al.*, 2015) as calves are the future stock for the dairy farm. In the dairy industry from an economy and management point of view raising healthy calves plays an imperative role. To maintain herd size, calf mortality adversely affects replacement stock and cause financial liabilities to dairy farmers (Yadav *et al.*, 2019). The goal of successful calf rearing is to provide a healthy calf, which is capable of top-rated overall performance throughout its life. To certify a healthy calf, the goal is to minimize the calf's contact with the infection and maximise its body defence system against infection. To minimize the exposure of calf to infection adoption of scientific managerial guidelines in the farm are crucial (Sreedhar and Sreenivas, 2015). A dairy farm, which is managed on the scientific practices has a calf mortality rate not greater than 5% from birth to 1 month age (Radostitis *et al.*, 2007). Healthy calves are not only crucial for the sustenance of dairy farms but also necessary for keeping and retaining the best germplasm for future production. Calfhood disease has a major impact on the average daily growth rate of calves, which in turn affects the weight at puberty, age at sexual maturity (Lorenz *et al.*, 2011) and difficulty at first calving (Rossini *et al.*, 2004; Stanton *et al.*, 2012). Diarrhoea, Bovine Respiratory Disease (BRD), and umbilical infection have all been connected to weight loss (Virtala *et al.*, 1996). Calf diarrhoea and pneumonia are the most common health-related problems of calves causing mortality in a dairy farm (Kharkar *et al.*, 2017). The impact of calf disease can be direct and indirect through increased treatment expenses, decreased lifetime productivity and survivorship (Randhawa *et al.*, 2012). Calf mortality is also associated with welfare and financial loss (Lundborg *et al.*, 2005).

2.5. Calf mortality:

Calf mortality in the dairy farm is considered to be one of the main constraints of herd expansion and genetic improvement (Fentie *et al.*, 2020). 38 to 40 % reduction in the profit of a livestock farm due to 20% calf mortality has been recorded (Radostitis *et al.*, 2007). The farm should maintain a 5% mortality rate as a goal (Amuamutu *et al.*, 2006). Calf mortality rates have significant economic ramifications for the dairy industry around the world. The reports available concerning the rate of calf mortality differ across the countries and production systems, which may be due to different managerial practices followed and herd size. The available literature shows that in India calf mortality under field conditions is 9.8-32.9% (Singh *et al.*, 2009; Mane *et al.*, 2010;

Kalam *et al.*, 2016). In another study higher calf mortality was reported 81.09 and 79.97%, respectively (Tiwari *et al.*, 2007; Sreedhar and Sreenivas 2015). Several authors reported that increased calf mortality with an increase in herd size in commercial dairy farms in India as well as outside (Tiwari *et al.*, 2007; Sreedhar and Sreenivas, 2015; Seppa-Lassila *et al.*, 2016) and also reported that more mortality of calf in larger herds (> 10 to 23) as compared to smaller (1 to 5 animals) or medium (6-10 animals) herds. Gulliksen *et al.* (2009) also reported calf mortality rate tends to increase with increasing herd size and it also increases in winter than summer. The effect of calf mortality rates on the performance of dairy cattle herds should not be ignored (Zhang *et al.*, 2019). Mortality and a decreased growth rate in the calf are associated with a wide range of infectious agents and the lack of scientific management practices. It has been reported that the risk of calf mortality increases drastically with delay in first colostrum feeding (Zucali *et al.*, 2013) due to inadequate passive transfer of readymade antibodies through the gut, which in turn decreases the calf's resistance to fight against the disease (Godden, 2008). The most significant causes of calf mortality under field and farm conditions were neonatal diseases. It has also been reported that deworming of the dam during the last trimester of pregnancy prevents the parasitic infection of calves, which may cause weakness and dehydration as a result increases the chances of calf mortality (Van Der Steen *et al.*, 2014). India is a tropical country, calf mortality in a tropical region is reported as high as 50% (Moran, 2011). Among the diseases, the significant reasons for calf mortality were diarrhoea and respiratory disorders. During the first month of life neonatal calf mortality is reported to be as high as 80-85% of the total mortality and is particularly high in the third week of life (Singh *et al.*, 2009). Calf mortality is one of the most important measures of health status in dairy farms. Combination of scientific practices, adequate care and management of dam during the last stage of pregnancy, intensive care of calf after birth, enhanced management practices such as colostrum feeding, housing and health care can contribute to a large extent to solve the problem of calf mortality (Lorenz *et al.*, 2011; Moran, 2011). The survival of calves and their rapid growth is the most important factor responsible for the propagation of the dairy herd. A higher calf mortality rate is responsible for an increase in the herd replacement cost as well as the time needed to produce replacements (Kochewad *et al.*, 2013). A higher mortality rate in calves is the indicator of poor welfare status associated with disease, lack of care and management (Pelaez *et al.*, 2008). Calf mortality reported by various authors has been documented in Table 2.2.

Table 2.2: Calf Mortality (%) reported by various authors

S. No	Study	Calf mortality (%)	Authors
1	A study on calf mortality in Nili-Ravi buffalo calves	14.59	Kamboj <i>et al.</i> , 2006
2	Pathological basis of a veterinary disease. 4th ed. St. Louise (Missouri): Mosby Elsevier.	75	McGavin <i>et al.</i> , 2007
3	Buffalo calf health care in commercial dairy farms: a field study in UP	81.09	Tiwari <i>et al.</i> , 2007
4	Calf mortality under field condition in India	35.20	Singh <i>et al.</i> , 2009
5	Farmer's attitude towards interventions regarding buffalo calf health care and management practices under field conditions	79.51	Ahmad <i>et al.</i> , 2009
6	Calf mortality under topical climate	50	Moran, 2011
7	The dairy calf mortality: The causes of calf death during ten years at a large dairy farm in Korea	10.7	Hur <i>et al.</i> , 2013
8	Pre-Weaning Morbidity and Mortality of Crossbred Calves in Bahir Dar Zuria and Gozamen Districts of Amhara Region, Northwest Ethiopia	30.7	Ferede <i>et al.</i> , 2014
9	A study on calf mortality and managerial practices in commercial dairy farms- AP	79.97	Sreedhar and Sreenivas, 2015
10	Studies of calf mortality pattern in Gir breed	18.9	Mishra <i>et al.</i> , 2015
11	Factors associated with calf mortality under field condition in Saurashtra region in Gujarat	16.03	Patbhanda <i>et al.</i> , 2017

12	A study of pattern in calf mortality at an organized Murrah buffalo herd in India	9.50	Yadav <i>et al.</i> , 2019
13	Mortality-Culling Rates of Dairy Calves and Replacement Heifers and its Risk Factors in Holstein Cattle	5.5	Zhang <i>et al.</i> , 2019
14	Dairy calves' mortality survey and associated management practices in smallholding, pasture-based herds in southern Brazil	8.5	Fruscalso <i>et al.</i> , 2020
15	Quantitative analysis of calf mortality in Great Britain	6	Hyde <i>et al.</i> , 2020
16	Major causes of calf morbidity and mortality in smallholder dairy farms in Shashemene town, Ethiopia (Local, exotic and crossbred breeds)	6.4	Meribo and Mustefa, 2021

2.6. Calf diarrhoea:

Calf diarrhoea is one of the commonest diseases in young calves and is the greatest single cause of death (Megersa *et al.*, 2009; Romha, 2014). Calf diarrhoea is very common in organized farm and field conditions causing significant production and economic loss in a dairy farm. In a newborn calf, diarrhoea is a complicated, multifactorial and dynamic enteric disease that affects the host's resistance (i.e. active and passive immunity) (Lorenz *et al.*, 2011). It can be infectious and non-infectious in origin (Cho and Yoon, 2014). Newborn calves are susceptible to neonatal calf diarrhoea (calf scours) especially during their first month of life because of poor development of the immune system. Regardless of the cause of calf diarrhoea, absorption of fluids from the intestine is decreased, leading to life-threatening conditions. In case of scouring calf loses fluid rapidly from the body leads to dehydration and suffers from electrolyte imbalance and acidosis. Infectious agents of calf diarrhoea cause initial damage to the intestine causing villous atrophy and inflammation of sub-mucosa (Cho and Yoon, 2014), but death in diarrhoea cases usually occurs due to loss of fluids, acidosis, and loss of electrolytes (Cho and Yoon, 2014; Sevennson *et al.*, 2006). It is the leading cause of calf mortality and the most common health problem affecting young milk-fed calves.

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Calf diarrhoea has long term consequences on the health and productiveness of surviving calves. In the available literature, it is reported that calf diarrhoea accounts for more than 50% of all deaths (Azizzadeh *et al.*, 2012). In a study, it has been found that more than 50% of all neonatal diarrhoeas appear during the first week of life and 15% occur after the second week of life (Bendali *et al.*, 1999). Calves are particularly susceptible during their second week of life. Another study shows that 80% of neonatal calf mortality in a dairy farm is due to calf diarrhoea (Sreedhar and Sreenivas, 2015). Calf mortality due to calf diarrhoea reported by various authors has been presented in Table 2.3.

Table 2.3: Mortality rate due to calf diarrhoea reported by various authors

Sl No.	Mortality %	Authors
1	28	Wymann <i>et al.</i> , 2006
2	83	Tiwari <i>et al.</i> , 2007
3	57	National Animal Health Monitoring System, 2007
4	50	Azizzadeh <i>et al.</i> , 2012
4	74	Shivarudrappa <i>et al.</i> , 2013
5	53.4	Hur <i>et al.</i> , 2013
5	81	Sreedhar and Sreenivas, 2015
6	75	Muktar <i>et al.</i> , 2015
7	79	Asmare and Kiros, 2016
8	82	Shakya <i>et al.</i> , 2017
9	63	Fentie <i>et al.</i> , 2020

2.7. Incidence of calf diarrhoea:

Good health of calves refers to the absence of illness, stress and pain associated with the routine managemental procedures in a dairy farm. The two most common important health problems that affect dairy calves welfare are diarrhoea and respiratory disease. Calf diarrhoea was found to be the predominant calf health problem with an incidence rate of 42.9% as reported by Wudu *et al.* (2008). The different incidence rates of diarrhoea and respiratory disease reported were 23% and 22%, respectively among Canadian dairy farms (Windeyer *et al.*, 2014). In another study incidence risk of

diarrhoea in calves varies from 20.05% to 52.51% (Samad, 2008; Wudu *et al.*, 2008; Megersa *et al.*, 2009; Malik *et al.*, 2012). The most frequent disease syndrome reported was diarrhoea with incidence rates of 10% (Megersa *et al.*, 2009). In another study, Islam *et al.* (2015) reported incidence risk of calf diarrhoea was 34.82% and fever 4.5%. Various reports of the incidence rate of calf diarrhoea have been presented in Table 2.4.

Table 2.4: Incidence rate of calf diarrhoea reported by various authors

Sl.No	Incidence of calf diarrhoea (%)	Researchers
1	Most of the calves	Blood and Radostits, 1989
2	39	Wudu <i>et al.</i> , 2008
3	10	Megers <i>et al.</i> , 2009
3	73.41	Shivarudrappa <i>et al.</i> , 2013
4	21.4	Ferede <i>et al.</i> , 2014
5	34.82	Islam <i>et al.</i> , 2015
6	78.9	Asmare and Kiros, 2016
7	81.25	Shakya <i>et al.</i> , 2017
8	75	Manzoor <i>et al.</i> , 2018

2.8. Etiology of calf diarrhoea:

Calf diarrhoea is a multifactorial syndrome having etiology of infectious and non-infectious, as well as environmental and managemental factors. Infectious factors belong to the different enteric groups of pathogens (Parasite, Virus and Bacteria) (Singh *et al.*, 2009). Signs and symptoms of calf diarrhoea are watery stools, yellow in colour, weak and depressed calves who may lose their desire to nurse, calves develop sunken eye appearance due to dehydration, weight loss, dry muzzle, thick mucus appearing from the nostrils, unable to stand, elevated body temperature (over 39.5°C) (Manzoor *et al.*, 2018). Infectious causes that are linked with the neonatal calf diarrhoea are enterotoxigenic *Escherichia* (*E. coli*), *Cryptosporidium parvum* (*C. parvum*), rotavirus, coronavirus or a combination of these pathogens (Cho and Yoon 2014; Gulliksen *et al.*,

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2009; Svensson *et al.*, 2003; Meganck *et al.*, 2014). Enteric pathogens *Escherichia coli* K99 (*E.coli*), Coronavirus, *Cryptosporidium parvum* (*C. parvum*) and Rotavirus causes 75-95% of intestinal infections in young calves and especially rotavirus accounts for 27-36% (Gumusova *et al.*, 2007; Uhde *et al.*, 2008). It has been reported in the study that organisms isolated from diarrhoeic calves were *E. coli* 22 (72.22%), *proteus* 5 (16.66%), *Klebsiella* 2 (2.833%) and unidentified organisms 1 (2.77%) (Kumar *et al.*, 2010). In another study it has been reported that organisms isolated from calf diarrhoea calves was *E. coli* 44 (38.6%), *Salmonella spp* 25 (21.9%), *Staphylococcus spp* 15 (13.2%), mixed infection 18 (15.8%) and 12 (10.5%) were negative for bacteria (Abdullah *et al.*, 2013). Hemashenpagam *et al.* (2009) reported that micro-organisms isolated from calf diarrhoea was *E. coli* 12 (75%). Bendali *et al.* (1999) also isolated *E. coli* 20.3%, from fecal samples of calf diarrhoea and Khan and Khan (1997) isolated *E. coli* (54-58%), *Staphylococcus* (7-10%) and *Salmonella* (13-14%). Ghanem *et al.* (2012) reported that causative organisms in diarrhoeic buffalo calves was *E. coli* 44 (73.3%) and *Salmonella spp.* 16 (26.7%). Infectious causes of calf diarrhoea are presented in Table 2.5.

Table 2.5: Infectious causes of calf diarrhoea

Sl.No.	Etiology	Causes of calf diarrhoea	Age at which clinical signs most commonly appear
1	Parasite	Cryptosporidia	Ist week of life
		Coccidia	3-6 weeks of age
2	Virus	Rotavirus	1-3 weeks of age
		Coronavirus	1-3 weeks of age
3	Bacteria	<i>Salmonella</i>	2-6 weeks of age
		<i>E.coli</i>	Calves <5 days of age

(Cho *et al.*, 2013)

2.9. Calf pneumonia:

Calf pneumonia is a breathing ailment that occurs due to irritation inside the lungs, normally the alveoli (air sacs). It is a multifactorial disease caused by a wide range of organisms together with viruses, bacteria and mycoplasmas. Environmental factors also are extremely critical in dealing with the disease. Doubtlessly calf pneumonia is an extensive monetary burden to a farm, because of the charges of remedy, mortalities,

additional labour and housing necessities (Andrews, 2000; van der Fels-Klerx *et al.*, 2001). Calf pneumonia is a highly infectious disease, affecting more than 50% of young calves. Calves that had diarrhoea are 3 times more likely to get infected with pneumonia (Manjari *et al.*, 2015). It has been reported that death due to calf pneumonia is about 15% (Azizzadeh *et al.*, 2012). Fever, cough, bilateral mucopurulent nasal discharge, mild depression, inappetence (Mansour 2006; Radostitis *et al.*, 2007) and abnormal sounds (Andrews, 2004) were the symptoms reported by various authors during a clinical examination. The calf mortality due to pneumonia reported by various authors has been presented in Table 2.6.

Table 2.6: Calf mortality due to pneumonia reported by various authors

Sl.No	Mortality %	Author
1	15	Heinrichs and Radostits, 2001
2	40	Sevensson <i>et al.</i> , 2006
3	28	Tiwari <i>et al.</i> , 2007
4	21	Poulsen and McGuirk, 2009
5	15	Azizzadeh <i>et al.</i> , 2012
6	15.32	Shrivastava <i>et al.</i> , 2013
7	15	Sreedhar and Sreenivas, 2015
8	20	Venkestashkumar <i>et al.</i> , 2016
10	17	Fentie <i>et al.</i> , 2020

2.10. Incidence rate of calf pneumonia:

In calves majority of respiratory illness-related problems has been reported at the age of 5-7 weeks (Virtala *et al.*, 1996). Available literature reported that pneumonia is most common in the autumn and winter season (Svensson *et al.*, 2006), and are frequently associated with poorly ventilated housing conditions (Callan and Garry, 2002) and drafts (Lundborg *et al.*, 2005). There are various reports by different authors about the incidence rate of calf pneumonia are mentioned in Table 2.7.

Table 2.7: Incidence rate of calf pneumonia reported by various authors

Sl.No	Incidence rate of pneumonia (%)	Researchers
1	28	Wudu, 2004
2	5.7	Sevenson <i>et al.</i> , 2006
3	9.2	Amuamuta <i>et al.</i> , 2006
4	24	Tiwari <i>et al.</i> , 2007
5	2.5	Gulliksen <i>et al.</i> , 2009
6	18.6	Ferede <i>et al.</i> , 2014
7	15	Sreedhar and Sreenivas, 2015
8	4.9	Assen <i>et al.</i> , 2016
9	11.5	Abdisa and Minda, 2016
10	19.6	Venkateshkumar <i>et al.</i> , 2016

2.11. Etiology of calf pneumonia:

Pneumonia in calves is a multi-factorial disease involving a group of viruses Bovine herpesvirus 1 (BoHV1); Bovine respiratory syncytial virus (BRSV); parainfluenza 3 virus (PI3) and bacteria (*Mycoplasma bovis*, *Pasteurella multocida*, *Mannheimia haemolytica*, *Histophilus somni*), as well as calf-related and environmental risk factors (Ellis, 2009; Brodersen, 2010; Schneider *et al.*, 2010; Autio *et al.*, 2007). It has been reported that viruses are the main causative agent of this disease, these are the first pathogen to intercede, whereas bacteria act as a secondary attacker, which worsens the already-ill animal's condition (Valarcher and Hagglund, 2006; Solis Calderon *et al.*, 2007; Taylor *et al.*, 2010). Bovine mycoplasma has been reported to be of increasing importance as a primary cause of calf pneumonia (Kusiluka *et al.*, 2000), and are often isolated from pneumonic lungs in combination with other pathogens (Booker *et al.*, 2008). Deep nasopharyngeal swabs, positive for *M. haemolytica* and *M. bovis*, in the pneumonic calves, are the representative of isolates present in the lung

(Godinho *et al.*, 2007). The chances of calf pneumonia increase during the combining of different age groups of calves, afterweaning of milk calves and in unfavourable climatic conditions (Lorenz *et al.*, 2011). Signs and symptoms of calf pneumonia are increased respiration rate, dullness and depressed, drooping ears, increased body temperature (over 39.5°C), cough, mucus discharge from the nose and lacrimation (Manjari *et al.*, 2015). Infectious causes of calf pneumonia are given in Table 2.8.

Table 2.8. Infectious causes of calf pneumonia

Sl. No	Etiology	Causes of calf pneumonia
1	Bacterial	<i>Mannheimia haemolytica</i>
		<i>Pasteurella multocida</i>
		<i>Histophilus somni</i>
		<i>Mycoplasma bovis</i>
2	Viral	Bovine respiratory syncytial virus
		Parainfluenza-3 virus(PI-3)
		Bovine herpes virus-1(BHV-1)

(Lorenz *et al.*, 2011)

2.12. Fever:

Fever is a key symptom of a variety of diseases, including abscesses, tuberculosis, urinary tract infections, endocarditis, hepatobiliary infections, osteomyelitis, rickettsia, chlamydia, systemic bacterial infections, parasitic infections, auto-immune diseases (Vanderschueren *et al.*, 2009). it is a high temperature condition that is often accompanied by disease symptoms such as lethargy, depression, anorexia, drowsiness, hyperalgesia, and inability to concentrate (Johnson, 2002). Fever in animals causes anorexia, wasting, sadness, muscle weakness, and a decrease in milk output, causing producers to lose a lot of money. In a study, it was reported that the most common disorders found in water buffaloes were digestive disorders followed by fever and respiratory disorders (Singh *et al.*, 2012). Kumar *et al.* (2015) reported in a study that in fever of unknown origin, mean rectal temperature (104.43±0.16°F), respiration rate (56.57±1.51 bpm), and pulse rate (83.40±1.77 bpm) of animals (n=30) were

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significantly higher as compared to healthy control group's rectal temperature ($101.38 \pm 0.24^\circ\text{F}$), respiration rate (19.10 ± 0.96 bpm) and pulse rate (58.20 ± 2.45 bpm). The haemoglobin and PCV levels of animals suffering from fever of unknown origin were considerably decreased. Lower haemoglobin and haematocrit levels in ill animals were most likely owing to starvation due to prolonged fever, resulting in poor physical condition. The total leukocyte count in diseased animals was shown to be non-significantly greater than in control animals, which could be attributed to some other diseases.

Kumar *et al.* (2015) reported significant ($p < 0.05$) increase in Neutrophils (%) (54.63 ± 5.48 vs. 39.50 ± 1.31) and decrease in Lymphocytes (%) (40.33 ± 5.60 vs. 57.00 ± 0.95), PCV (%) (30.08 ± 1.31 vs. 36.90 ± 1.21), Hb (g/dL) (10.25 ± 0.64 vs. 12.64 ± 0.40), whereas non-significant increase in TLC ($10^3/\mu\text{L}$) (10.57 ± 0.94 vs. 8.33 ± 0.34), Monocytes (%) (3.53 ± 0.93 vs. 2.30 ± 0.45) and Eosinophils (%) (1.5 ± 0.61 vs. 1.20 ± 0.35) was recorded in case of buffaloes affected with fever as compared to control. In another study it was reported that in Babesia infected calves significant decrease ($P < 0.05$) in RBCs, HB, PCV (8.59 ± 0.51 vs. 6.026 ± 0.224 ; 11.05 ± 0.31 vs. 9.56 ± 0.366 ; 32 ± 1.140 vs. 25.892 ± 1.099) and significant increase ($P < 0.05$) in WBC and Monocytes (10.640 ± 0.928 vs. 24.630 ± 0.566 ; 6.00 ± 0.71 vs. 11.10 ± 1.23) was observed. Significant decrease in RBCs, HB, PCV values in diseased calves shows the presence of macrocytic hypochromic anaemia. Leukocytosis in clinically infected calves is due to the ability of protozoan to trigger production of WBC in blood during infection (Salem *et al.*, 2016). Kachhawa *et al.* (2016) reported that significant ($p < 0.01$) decrease in Hb, TEC, PCV and TLC in theileriosis infected calves as compared to healthy calves (5.02 ± 0.27 vs. 11.67 ± 0.3 , 3.32 ± 0.22 vs. 6.57 ± 0.30 , 17.13 ± 0.74 Vs 32.98 ± 0.80 and 4.07 ± 0.12 vs. 6.57 ± 0.24). Decrease in the Hb, TEC and PCV is due to the destruction of RBCs cells by the macrophages and monocyte macrophage system coupled with reduced erythropoietic activity.

2.13. Assessment of calf diseases:

The calf's diseases and mortality were caused by both infectious and non-infectious causes. The intricate combination of management techniques, the environment, infectious organisms, and the animal itself causes calf diseases that cause morbidity and mortality. Diarrhoea (63%) and respiratory illnesses (17%) were the leading causes of calf death among the diseases of calf. Regular on-farm screening of

calves for the disease is rare. Without regular screening of the dairy farms for the calf, it is very difficult to identify diseases, so detection rate is poor (McGuirk and Peek, 2014). For assessing the calf diarrhoea in the dairy farms most promising method is to understand the dehydration status of the animal along with the other physiological parameters like respiration rate, heart rate, rectal temperature and blood parameters as well as the age of the affected animal (Millemann, 2009). Sharma (2013). reported that the mean rectal temperature ($^{\circ}\text{F}$), heart rate (per minute), respiration rate (per minute), fecal consistency score and clinical dehydration score in healthy calves was 101.28 ± 0.93 , 110.20 ± 0.88 , 17.00 ± 0.78 , 0.00 ± 0.00 and 0.00 ± 0.00 , respectively whereas corresponding values in colibacillosis affected diarrhoeic calves were 102.40 ± 0.63 , 117.43 ± 0.82 , 22.10 ± 0.69 , 2.55 ± 0.07 and 1.80 ± 0.08 , respectively. In another study it was reported that significant ($p < 0.05$) increase in respiration rate was observed in diarrhoeic groups as compared to healthy group (38.00 ± 1.20 vs. 24.80 ± 0.64) (Ghanem *et al.*, 2012). It has been reported that significant increase in body temperature ($^{\circ}\text{C}$), heart rate (per minute) and respiration rate (per minute) in pneumonia calves as compared to healthy calves (39.72 ± 0.10 vs. 38.84 ± 0.08 , 101.72 ± 3.16 vs. 85.60 ± 1.75 and 52.40 ± 2.68 vs. 37.60 ± 1.12). In pneumonia inflammation of the lung causes the increase in the core body temperature of the calves and the inflamed lung causes stress which increases heart rate and the symptoms such as cough, fever, chills, and difficulty in breathing (Ozbek and Ozkan, 2020). In another study it was reported that significant increase in Temperature ($^{\circ}\text{C}$), Pulse rate (beats per minute), Respiration rate (breaths per minute) in Bovine Respiratory Disease as compared to control calves (40.615 ± 0.09 vs. 38.94 ± 0.11 , 154.05 ± 2.66 vs. 93.3 ± 1.71 , 54.25 ± 2.75 vs. 29.1 ± 1.15) (Ramadan *et al.*, 2019). In case of fever calves by tropical theileriosis significant increase ($P < 0.01$) in body temperature ($^{\circ}\text{F}$), heart rate and respiration rate was observed as compared to the healthy calves (105.84 ± 0.24 vs. 101.56 ± 0.19 , 146.7 ± 3.08 vs. 107.1 ± 1.58 , 47.4 ± 1.63 vs. 23.6 ± 0.93). Increase in the physiological parameters of the infected calves is because of the infection in the body (Kachhawa *et al.*, 2016).

The results of fecal consistency score (0-3 scale) in diarrhoeic calves for assessing the faecal consistency were reported by McGuirk (2008). Leruste *et al.* (2012) reported that clinical signs of respiratory disease and lung lesion at slaughter in young dairy calves may depend on the severity of disease, timing of screening and duration of disease.

Table 2.9: Faecal consistency score

Animal ID No.	Age (days)	Faecal Score
7200	10	2
7202	9	0
7203	9	2B
7204	9	2
7207	8	1
7209	8	3
7210	7	1

Where faecal score is 0 = normal consistency; 1 = semi-formed or pasty; 2 = loose but enough consistency to remain on bedding; 3= watery feces that sift through bedding material, B = blood is present.

2.14. Temperature change associated with calf health:

In case of fever, calf diarrhoea and pneumonia significant changes in the body surface temperature of calves along with the other clinical signs have been recorded. The change in body surface temperature can be measured by non-invasive technology i.e. infrared thermography. It is reported that infrared temperatures, particularly for a facial region of diseased calves, increased by 1.5°C to 4°C several days to 1 week before the clinical onset of the disease to indicate illness in the infected calves (Schaefer *et al.*, 2004). In another study, it is reported that in mild cases of calf diarrhoea there is an increase in body temperature >39.5°C (Ghanem *et al.*, 2012). Also in bovine viral diarrhoea significant temperature change has been seen in the nose, ear, side and dorsal region of the calf (Schaefer *et al.*, 2004). A decrease in shoulder temperature and a rise in side temperature assessed by IRT was recorded in neonatal calf diarrhoea even before the appearance of clinical symptoms. IRT temperatures of side and shoulder region and behavioural indicators such as lying bouts number and length as well as the duration of time spent on the water trough showed potential as appropriate early indicators of disease (Lowe *et al.*, 2019). In bovine respiratory disease, change has been recorded in the

orbital temperature from 35.1°C on 1st day of disease to 38.4°C on the 8th day of disease (Schafer *et al.*, 2007).

2.15. Temperature change assessed by sensor and clinical thermometer to assess calf health:

Basic physiological parameters like heart rate, respiration rate and rectal temperature are most commonly used to assess the health status of dairy calves. Temperature change takes place during the disease condition in the body and assessment of this change is important for the early detection of the disease (Lowe *et al.*, 2019). The available literature shows that the change in temperature of calves can be sensed by the fever tags for the detection of elevated body temperature as an early predictor of bovine respiratory disease or bovine viral diarrhoea. Mean rectal temperature was recorded 1.5°C higher in the infected pen as compared to the uninfected pen ($39.8 \pm 0.15^\circ\text{C}$ versus $38.2 \pm 0.11^\circ\text{C}$; $P \leq 0.0001$) (McCorkell *et al.*, 2014). Early prediction of disease in animals helps in reducing the cost of treatments in the dairy farm. Infrared thermography has been used in early disease prediction and reported temperature rises from 1.5°C to over 4°C ($P < 0.01$) several days to 1 week before even appearance of clinical scores or serum acute-phase protein concentrations in infected calves (Schafer *et al.*, 2004). Ghanem *et al.* (2012) reported in a study that in neonatal calf diarrhoea significant decrease in temperature was seen in severe diarrhoea group $37.20 \pm 0.50^\circ\text{C}$ and mild diarrhoea group there was an increase in temperature $39.52 \pm 0.22^\circ\text{C}$ when compared with control group $38.46 \pm 0.05^\circ\text{C}$ ($P < 0.05$).

2.16. Use of Infrared thermography to assess calf health:

Infrared thermography has also been used as a non-invasive diagnostic tool in humans and animals to assess physiological conditions and pathological changes in body surface temperature resulting from medication administration, surgical operations, vascularity changes, and both systemic inflammatory responses (fever) and local inflammatory responses associated with disease conditions. Calf diseases have a significant effect on the performance and production level of the animals (Nikkhah, 2015). In calf diarrhoea, disease-producing organisms or their toxin are present in the blood result in septicemia due to inflammation, which in turn leads to the development of fever or change in body temperature. Calves that develop pneumonia before weaning also share the same risk factors as in the case of calf diarrhoea. Deficiency of colostrum

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immunity or incomplete transition, extended exposure to poor ventilation, wide variations in daily temperatures, stress from transport or grouping may lead to the development of pneumonia. In both the events of calf pneumonia and diarrhoea the change in the body surface temperature can be captured by the infrared thermographic cameras. Infrared images taken from various body points helps in detecting the change in surface temperature. It was reported that in bovine viral diarrhoea, IRT can predict the change in body surface temperature before the onset of clinical diseases (Schaefer *et al.*, 2004). In another study, it was reported that IRT can also detect true positive cases of bovine respiratory diseases 4-6 days before the onset of clinical signs (Schaefer *et al.*, 2007). In neonatal calf diarrhoea changes in IRT temperature of $<0.3^{\circ}\text{C}$ before clinical signs appeared were found to be significant ($P < 0.001$). An orbital temperature change of the calf is displayed at the earliest and most consistent sensitive change observed even before the appearance of the clinical signs of disease. A significant decrease in shoulder temperature ($^{\circ}\text{C}$) 28.49 ± 0.18 and an increase in side temperature $25.0.9 \pm 0.09$, but not for the eye, cheek, or back was recorded in neonatal calf diarrhoea (Lowe *et al.*, 2019). So, IRT is a useful tool in the livestock production sector for the early identification of diseases in dairy calves. The temperature change associated with various diseases of the calf has been presented in Table 2.13.

Table 2.10: Temperature change associated with various diseases in calves

Disease in calf	Breed	Temperature increase and decrease	References
Bovine Viral Diarrhoea (BVD)	Angus-Hereford cross	Facial temperature increase by 1.5°C to over 4°C	Schaefer <i>et al.</i> , 2004
Bovine Respiratory Disease (BRD)	Hereford x Angus	Mean peak IRT orbital maximum for True Positive calves 35.7 ± 0.35 and True Negative calves 34.87 ± 0.0025 ($P < 0.01$)	Schaefer <i>et al.</i> , 2012
Neonatal Calf	Friesian males	Change in IRT	Lowe <i>et al.</i> , 2019

Diarrhoea (NCD)	and Hereford females	temperature of $<0.3^{\circ}\text{C}$ for side and shoulder found significant ($P < 0.001$)	
Calf diarrhoea	Murrah calves	Significant ($P < 0.05$) increase in Eyeball (0.47°C) and Rectal temperature (0.92°C) was recorded between the day of diarrhoea and two days before diarrhoea in affected calves	Ragul, 2020

2.17. Calf diarrhoea and blood profile:

Neonatal calf diarrhoea can occur due to various reasons i.e. micro-organisms, like bacteria, protozoa, viruses as well as managemental factors can also play an important role in causing diarrhoea. Diarrhoea causes severe water and electrolyte loss from the gastrointestinal (GI) tract lead to dehydration and death in acute situations (Barua *et al.*, 2018). So, haematological indices are used to evaluate the response of the calves to the fluid therapy, to gage the severity of diarrhoea. Various parameters are known to be useful in assessing the morbidity or mortality predisposition of the calf; some of them are total red blood cell count (tRBC), haemoglobin (Hb) and packed cell volume (PCV) (Brar *et al.*, 2014). Haematological changes showed a significant increase in PCV, white blood cells (WBCs), and a non-significant increase in neutrophil %, lymphocytes %, and monocytes %. On the other hand, a significant decrease in RBCs was found might be associated with long-standing diarrhoea, dehydration and haemoconcentration. Fluid loss from the body of calves results in dehydration. Generally, a significant increase has been reported in the haematological parameters such as haemoglobin (Hb) and packed cell volume (PCV) due to haemoconcentration of blood that is related to dehydration (Singh *et al.*, 2014). The significant increase in WBCs count in diarrhoea calves than control calves might be attributed to the infection by microorganisms that leads to neutrophilia (Ghanem *et al.*, 2012). Various haematological parameters reported by different authors in healthy and diarrhoea calves are mentioned in Table 2.14.

Table 2.11: Changes in haematological parameters in calves affected with calf diarrhoea as compared to healthy calves

Parameter	Calf affected with diarrhoea		Healthy calves	Reference
	Increase	Decrease		
PCV(%)	36.92±4.33*	-	35.52±5.24	Niaz <i>et al.</i> , 2000
	37.88 ±4.30*	-	25.86 ±2.97	Ghanem <i>et al.</i> , 2012
	44.2 ± 0.692	-	37.5 (36.0-49.0)	Malik <i>et al.</i> , 2013
	43.8±0.48**	-	34.08±0.4	Singh <i>et al.</i> , 2014
	44.2 ± 0.692	-	36.0-49.0	Barua <i>et al.</i> , 2018
Hb (g/dl)	-	13.11±1.97*	14.08±2.98	Niaz <i>et al.</i> , 2000
	11.36 ±1.5*	-	8.54 ±0.11	Ghanem <i>et al.</i> , 2012
	12.2 ± 0.221	-	11.3 (9.5-13.5)	Malik <i>et al.</i> , 2013
	-	12.64±0.18	12.76±0.08	Singh <i>et al.</i> , 2014
	12.2 ± 0.221	-	9.5-13.5	Barua <i>et al.</i> , 2018
RBCs (10 ⁶ /µl)	-	8.65±1.84*	8.83±2.60	Niaz <i>et al.</i> , 2000
	-	8.29 ±1.01*	11.16 ±0.11	Ghanem <i>et al.</i> , 2012
WBCs (10 ³ /µl)	15.09 ±1.9 *	-	9.08 ±1.63	Ghanem <i>et al.</i> , 2012
Lymphocytes (%)	-	40.52±3.78*	50.94±6.56	Niaz <i>et al.</i> , 2000
	57.8 ±4.56	-	44.75 ±6.63	Ghanem <i>et al.</i> , 2012
	-	51.6 ± 0.514	68.6 (33-87)	Malik <i>et al.</i> , 2013
	51.6 ± 0.514	-	33-87	Barua <i>et al.</i> , 2018
Monocytes (%)	-	2.75±2.13	3.00±2.24	Niaz <i>et al.</i> , 2000
	1.9 ±0.32	-	1.25 ±0.3	Ghanem <i>et al.</i> , 2012
	-	2.72 ± 0.687	3.4 (1-5)	Malik <i>et al.</i> , 2013
	2.72 ± 0.687	-	1-5	Barua <i>et al.</i> , 2018
Neutrophil (%)	55.35±6.80*	-	44.71±3.04	Niaz <i>et al.</i> , 2000
	52 ±3.8*	-	45.8 ±8.24	Ghanem <i>et al.</i> , 2012
	46.8 ± 0.748*	-	37 (12-38)	Malik <i>et al.</i> , 2013
	46.8 ± 0.748	-	12-38	Barua <i>et al.</i> , 2018

** Significant at P≤0.01, *Significant P ≤0.05

2.17. Calf pneumonia and blood profile:

Pneumonia is an important disease of the respiratory system commonly seen in calves. The disease is caused by various microorganisms like viruses and bacteria, either alone or in combination, as well as by various other factors (Pancieria *et al.*, 2010). The various haematological reports showed a significant decrease in RBCs, Hb, PCV and Mean Corpuscular Volume (Mansour, 2006; Nahed and Allam, 2014) with a significant increase in WBCs, Granulocytes, Lymphocytes (LYM) (Ismael *et al.*, 2017) in case of pneumonia. In pneumonia significant increase in WBC and neutrophil counts is a result of inflammatory processes caused by the disease. In another study statistical comparisons of selected haematological parameters of the healthy group and the pneumonic calves revealed that WBC, Lym, Neutrophils and Hb values in the pneumonic calves were higher than the control group calves ($P < 0.05$) (Akgul *et al.*, 2019). Adaptation and compensatory processes reflecting chronic pulmonary illness, hypoxaemia, and stimulation of erythropoiesis could explain differences in haemoglobin concentration and mean RBC count. Various haematological parameters of healthy and pneumonia calves reported by various authors have been presented in Table 2.15.

Table 2.12: Changes in haematological parameters in calves affected with pneumonia as compared to healthy calves

Parameter	Calf affected with pneumonia		Healthy calves	Reference
	Increase	Decrease		
RBC (m/mm^3)	-	$8.23 \pm 0.22^{**}$	$9.56 \pm 0.52^*$	Almujalli <i>et al.</i> , 2015
RBC ($10^6/\mu l$)	-	$5.61 \pm 0.19^*$	$6.40 \pm 0.16^*$	Ismael <i>et al.</i> , 2017
	$10.80 \pm 2.20^*$	-	$9.69 \pm 2.52^*$	Akgul <i>et al.</i> , 2019
WBC (m/mm^3)	$13.48 \pm 0.08^*$	-	$9.32 \pm 0.17^*$	Ismael <i>et al.</i> , 2017
WBCs ($10^3/\mu l$)	$28.22 \pm 18.10^*$	-	$13.79 \pm 3.41^*$	Akgul <i>et al.</i> , 2019
PCV (%)	-	$22.26 \pm 0.31^*$	$33.73 \pm 0.43^*$	Ismael <i>et al.</i> , 2017
Lym (m/mm^3)	-	$31.25 \pm 2.45^{**}$	$48.25 \pm 1.54^{**}$	Almujalli <i>et al.</i> , 2015

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Lym ($10^3/\mu\text{l}$)	8.83 \pm 0.05*	-	6.11 \pm 0.12*	Ismael <i>et al.</i> , 2017
	-	44.14 \pm 3.68**	75.17 \pm 1.62**	Pradhan and Gupta, 2018
	-	13.18 \pm 8.78*	17.06 \pm 9.73*	Akgul <i>et al.</i> , 2019
Mon (m/mm^3)	1.25 \pm 0.14**	-	1.24 \pm 0.21**	Almujalli <i>et al.</i> , 2015
	-	2.14 \pm 0.25**	2.17 \pm 0.70**	Pradhan and Gupta, 2018
	-	0.70 \pm 0.18*	0.78 \pm 0.2*	Akgul <i>et al.</i> , 2019
Neu (m/mm^3)	68.13 \pm 3.34**	-	44.25 \pm 2.35**	Almujalli <i>et al.</i> , 2015
	31.86 \pm 4.53**	-	16.67 \pm 1.17**	Pradhan and Gupta, 2018
	10.58 \pm 6.54*	-	3.64 \pm 1.14*	Akgul <i>et al.</i> , 2019
Hb (g/dl)	11.0 \pm 1.7	-	9.8 \pm 1.2	Šoltésová <i>et al.</i> , 2015
	-	10.45 \pm 1.32**	12.36 \pm 1.45**	Almujalli <i>et al.</i> , 2015
	14.81 \pm 1.43*	-	11.27 \pm 0.36*	Kabu <i>et al.</i> , 2016
	-	8.34 \pm 0.20*	9.32 \pm 0.22*	Ismael <i>et al.</i> , 2017
	14.40 \pm 3.13*	-	11.96 \pm 1.27*	Akgul <i>et al.</i> , 2019
PLT (m/mm^3)	-	406.32 \pm 272.10*	603.00 \pm 211.90*	Akgul <i>et al.</i> , 2019

* significantly at $p < 0.05$, ** percentage

2.19. Horn bud:

Mostly in dairy animals horns grow naturally that must be removed to protect people and other animals from injury, but if the horn bears the breed characteristics then should not be removed. Horns can be removed from calves when the horn is still in the bud stage (disbudding), or by the method of amputation in older animals (dehorning). The removal of the corium, while it is still in the bud phase, is known as disbudding. When the animals are young and the bud is just emerging, it is the best moment to disbud. This lowers the risk of problems like infection or bleeding. A small patch of hairless, highly pigmented skin that is significantly thicker and has a lustrous sheen appears on newborn

calves. This is where horns begin to form, and it is surrounded by a little rosette of hair. The nascent horn of several weeks old calf can be gripped between two fingers and float freely on the skull bone when it is about one centimetre high. The horn bud attaches to the skull at around 2 months of age and grows as an extension of the skull. Because a portion of the delicate sinus above the eyes fuses with the horn after it is linked to the skull, hot-iron or paste disbudding is no longer an option. Paste disbudding is performed as early as possible, within the first week of life. but when calf having age >1 week, the best method for disbudding is electric hot-iron disbudding. Because the horn is still free-floating at this age, it is the least painful time for this treatment; however, short and long-term pain management is essential (Marquette *et al.*, 2021). Age at disbudding and the procedure followed varies across the nations. Reported literature shows the age at disbudding varies from 3 to 68 days of age (Stanek *et al.*, 2018). Adcock and Tucker (2018) reported that the age of calves at disbudding time was 3 days and 35 days and disbudding at younger age took a long time for the healing process. Despite the age factor visibility of the horn bud being the characteristic, normally adopted by the farmers for the disbudding. Various reports that are available regarding the horn bud diameter, age of disbudding and breed have been documented in Table 2.17.

Table 2.13: Horn bud diameter in the different age groups of calves

Horn bud diameter	The age group of calves	Breed
16.97mm	37days	Holstein Fresian male calves
15.09mm	26 days female and 24 days male	Charolais
13.76mm	28 days female and 26 days male	Limousin
14.41mm	23 days for both male and female	Simmental

(Marquette *et al.*, 2021)

2.20. Disbudding:

Disbudding is the routine managerial procedure followed in the dairy farms, in which removal of horn bud or horn bud cells is carried out at early age of life mostly at the age of 0-2 months before it is attached to the underlying bone (CVMA, 2010; AVMA, 2014; Dairy. USDA, 2018). Disbudding is done by different methods like hot-

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iron disbudding, chemical disbudding and amputation dehorning (Stock *et al.*, 2013). These procedures generate various sorts of tissue injury, which can lead to pain of various types, intensities, and durations (Petrie *et al.*, 1996; Stilwell *et al.*, 2009; Braz *et al.*, 2012). Disbudding is mostly practised without the use of anaesthesia and NSAIDs under Indian condition, that affects the welfare and performance of dairy calves. When performed without enough anaesthetic and analgesia, they are the most painful procedures (Stafford and Mellor, 2011; Stock *et al.*, 2013; Black *et al.*, 2017; Hambleton and Gibsion, 2017). It is a stressful procedure that leads to the activation of stress hormones i.e cortisol in blood and saliva. Most commonly plasma cortisol concentration in the blood and behavioural indicators has been used for assessing the pain, stress and welfare associated with disbudding (Herskin and Nielsen, 2018; Heinrich *et al.*, 2010; Balloua *et al.*, 2013). Disbudding destroys the skin around the horn bud leaving a small bruised area. The available literature shows that most common behavioural responses i.e. head shaking, head rubbing and ear flicking rate vary between disbudded and control calves (Morisse *et al.*, 1995; Graf and Senn, 1999; Grøndahl-Nielsen *et al.*, 1999). Disbudded calves display higher rates of behavioural head shaking, head rubbing and ear flicking post-disbudding as compared with calves treated with NSAIDs before disbudding (Graf and Senn, 1999; Faulkner and Weary, 2000; Stilwell *et al.*, 2012). Behavioural changes are mostly due to the pain and it remains for a period of about 4 hours because the peak of the cortisol is reached within 30 minutes and returns to the baseline levels within 2- 4 hours. Depressed parasympathetic or vagal tone and increased sympathetic activity can be triggered by painful procedures of disbudding. The stressful condition causes the release of catecholamines from sympathetic activity into blood circulation. Catecholamines increase the heart rate and respiration rate of the dairy calves to maintain homeostasis (Stewart *et al.*, 2008). In disbudding, it has been reported that there is a decrease in body weight gain due to the chronic inflammation mediators, which acts against the growth hormone. The available literature shows that the disbudded calves grow at a slower rate of 0.55kg/day than disbudded calves given with NSAIDs 0.65kg/day. In a study, it was reported that NSAIDs helps in reducing the chronic inflammation of disbudding (Bates *et al.*, 2016). Comparison of behavioural and hormonal changes of calves disbudded by hot iron after administration of local anaesthetic vs. local anaesthetic and NSAID has been reported by various authors and the finding are presented in Table 2.16.

Table 2.14. Comparison of behavioural and hormonal changes of calves disbudded by hot iron after administration of local anaesthetic (LA vs. LA+ NSAID) by various authors

Age and no. of calves per treatment	Analgesics used and interval to disbudding	Variables involved	Effects of a combination of NSAID and LA as compared to LA	References
4-8 weeks, n=10	Lidocaine (10min), Ketoprofen (-2, 2, 7hr)	Head shake (3 to 24hr) Ear flick (3 to 24hr) Head rub (3 to 24 hr) ADG (24hr)	↓3-12hr ↓3-24hr ↓4-12hr Tended to↑	Faulkner and Weary, 2000
2-14 days, n=20	Lidocaine (10min), Ketoprofen (10min)	Cortisol (0-6hr) Ear flick (0-8hr) Head shake (0 to 8hr) Head rub (0-8hr) Lying (0-8hr)	↓ change from 0 to 3h No effect No effect No effect No effect	Milligan <i>et al.</i> , 2004
6-12 weeks, n=30	Lidocaine (10min), Meloxicam	Cortisol (0-24hr) Heart rate (0-24hr) Respiration rate (0-24hr)	↓ 0-6hr ↓ overall ↓0-6hr	Heinrich <i>et al.</i> , 2010
4-5.5 weeks, n=8	Lidocaine (10 min), meloxicam (55min)	Heart rate (0-180min) Heart rate variability(0-180min) Ocular temperature (0-180min)	↓increase when LA wanes off Differed from LA No clear effects	Stewart <i>et al.</i> , 2009

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4-8 weeks, n=20	Lidocaine (10min), ketoprofen (10min)	Cortisol (3 to 6hr) Ear flick (0-8hr) Head shake (0-8hr) Head rub (0-8hr) Feed intake (24hr)	No effect ↓ overall No effect No effect Tended to ↑	Duffield <i>et al.</i> , 2010
6-12 weeks, n=30	Lidocaine (10min), meloxicam (10min)	MNT (4hr) Ear flick (-22 to 44hr) Head shake (-22 to 44hr) Head rub (-22 to 44hr) Tail flick (-22 to 44hr) Foot stamp (-22 to 44hr)	↑ threshold ↓ overall ↓ 4-9h No effect No effect No effect	Heinrich <i>et al.</i> , 2010
8-10 weeks, n=6-7	Lidocaine (15min). carprofen (15 min)	Cortisol (0-24hr) Head shaking (0 to 24hr) Ear flicking (0 to 24hr) Head rubbing (0 to 24hr)	Comparable to sham except for 24hr ↓ at 15 min ↓ at 3 and 6 hr ↓ at 3hr	Stilwell <i>et al.</i> , 2012
8-10 weeks, n=10	Lidocaine (10min), meloxicam (0 or 12h)	Cortisol (0-7d) Substance P (0-7d) Haptoglobin (0-7d)	↓ increases when LA wanes off ↓ at 120hr No effect	Allen <i>et al.</i> , 2013

		PGE ₂ (0 TO 72 h) MNT(0-7D) ADG (7d)	↓ up to 48hr ↓ the threshold at 3hr No effect	
5-9 weeks, n=20	Procaine (20min) flunixin (20min, 3t)	Cortisol (-1.25 to 8 hr) Heart rate (-1.25 to 8 hr) Respiration (-1.25 to 8hr) Head shaking (-1.25 to 8 hr) Head rubbing (-1.25 to 8hr) Foot stamping(-1.25 to 8hr) Ear flicking (-1.25 to 8hr)	↓ AUC No effect No effect No effect No effect No effect No effect	Huber <i>et al.</i> , 2013
4-5.5 weeks, n=8	Lidocaine (10min), meloxicam (55min)	Play behaviour at 3 and 27 hr Von frey filaments -1 to 75hr	↑ play at 3hr Thresholds not affected	Mintline <i>et al.</i> , 2013
3-6 weeks, n=51	Lidocaine (0min), meloxicam (0min)	ADG (15d, 30d) Milk consumption (0-11d)	No effect No effect	Bates <i>et al.</i> , 2016

ADG, average daily gain. **MNT**, mechanical nociceptive threshold obtained with a handheld algometer. **PGE₂**, plasma concentration of prostaglandins. **AUC**, the area under the curve.

2.21. Stress and temperature change associated with disbudding:

Disbudding is the stressful and painful procedure that leads to activation of the hypothalamic-pituitary-adrenal (HPA) axis and sympathetic adrenal medullary activity (SAM), which are a primary biological response to stress in animals. The concentration of plasma cortisol in the blood, which reflects the hypothalamus-pituitary-adrenal axis (HPA) activity, has long been used to assess the stress associated with disbudding (Stafford and Mellor, 2005). Salivary cortisol is preferred as it is non-invasive and doesn't create stress in the animals than invasive methods (Stewart *et al.*, 2008). The immediate response of disbudding is the diversion of blood from cutaneous capillary beds via sympathetically mediated vasoconstriction, which subsequently decreases the skin temperature of calves. Infrared thermography can identify the effect of vasoconstriction as a temperature change (Blessing, 2003). Eye temperature responses, assessed by IRT, have been reported to be a helpful non-invasive approach for evaluating stress in animals (Stewart *et al.*, 2005). In response to stress and early disease detection in cattle, eye temperature has been demonstrated to be a more consistent indicator of temperature changes than other anatomical locations such as the nose, ear, body, and feet (Schaefer *et al.*, 2004). It was also reported that eye temperature and plasma cortisol concentration increased in response to velvet antler removal in Elk (Cook *et al.*, 2005). The available literature showed that eye temperature dropped immediately after 2-5 min of disbudding in without local anaesthetic group (-0.27°C , $P < 0.05$), which was lower than all other treatments groups ($P < 0.05$). From 5 min post-disbudding, eye temperature increased and was higher ($P < 0.001$) for both disbudded groups as compared to control groups. It has been observed that as compared to baseline, the eye temperature of a disbudded group of calf without local anaesthetic was 0.60°C and disbudded group of calf with local anaesthetic was 0.66°C higher ($P < 0.001$) during the last 10 min of sampling (Stewart *et al.*, 2008). In another study, it was reported that there was no treatment difference in eye temperature at 2.0 to 2.5 h ($37.9 \pm 0.1^{\circ}\text{C}$, $P = 0.378$) or 2.5 to 3.0 h ($37.8 \pm 0.1^{\circ}\text{C}$, $P = 0.108$). But differences were there between treatments ($P = 0.011$) in the change in eye temperature between 2 and 3 h ($P = 0.011$), the eye temperature decreased by $0.6 \pm 0.1^{\circ}\text{C}$ during this time ($P < 0.001$) following disbudding with a local anaesthetic. No significant differences in eye temperature were recorded between 2 and 3 hr after dehorning for any other treatment ($P \geq 0.129$) (Stewart *et al.*, 2008). In another study, it was reported that the surface temperature of disbudding wounds did not differ

from that of nondisbudded tissue (Disbudded: $37.1 \pm 0.51^\circ\text{C}$; Sham: $37.2 \pm 0.51^\circ\text{C}$; $F_{1,41} = 0.03$; $P = 0.859$). Surface temperature decreased over time, regardless of disbudding (Adcock and Tucker, 2018). Therefore, IRT can be used to measure the change in eye temperature which is a good indicator of stress in dairy calves during disbudding.

2.22. Strategies to reduce disbudding stress:

The goal of disbudding is to facilitate coping with and to increase the protection of animal keepers and other animals. Disbudding is always associated with the stress, pain and welfare of calves. During the disbudding process, change in behaviour and physiological parameters like heart rate, respiratory rate and extended plasma cortisol concentration in blood is well documented (Kupczyński *et al.*, 2014). Horn buds in calves are normally removed using thermal or chemical methods (Faulkner and Weary, 2000). Chemical methods are less painful and are rarely used due to incomplete removal of horn tissue in chemical disbudding that results in deformed horns in older calves (Stewart *et al.*, 2009). In the thermal method, the removal of skin tissues and horn buds is carried out by high temperatures. It simultaneously induces blood vessel cauterization and prevents bleeding of the wound. After disbudding, wound recovery will continue for some time. Various researchers have reported a reduction of production indexes during the wound recovery period after disbudding (Faulkner and Weary, 2000; Knierim *et al.*, 2015; Stafford and Mellor, 2011). Stress in calves after disbudding is primarily because of pain resulting from physical and chemical mediators of inflammation. For effective pain management combination of drugs is always preferred to overcome acute and chronic pain (Herskin and Nielsen, 2018). Disbudded calves that had been exposed to local anaesthesia and given a NAISDS showed more proper patterns of behaviour and lower blood cortisol concentration (Stilwell *et al.*, 2012). Pain-induced distress caused by disbudding has been evaluated by measuring the change in cortisol response before and after disbudding (Sutherland *et al.*, 2002). The peak of the cortisol concentration in blood was reached within 30 min (Allen *et al.*, 2013; Mosher *et al.*, 2013; Balloua *et al.*, 2013) and its response remained for 7-9 hours, for the next 30-60 min the concentration decreases to reach a plateau that persists for 5-6 hours before declining to the baseline level (Sylvester *et al.*, 1998). The available literature showed that various pharmacological agents were used to alleviate pain and stress due to disbudding. It was reported that corneal nerve blockers lidocaine abolished pain induce distress i.e cortisol

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response for 2 hours (Graf and Senn, 1999; Grøndahl-Nielsen *et al.*, 1999; McMeekan *et al.*, 1998), 4 hr (Bupivacaine), 6 hr (lignocaine followed by bupivacaine), and 8 hr (Bupivacaine given twice) (Petrie *et al.*, 1996; Sylvester *et al.*, 1998). Compression band on horns base (Duffield *et al.*, 2010) along with, Non-Steroidal AntiInflammatory Drugs (NSAIDs) stops the formation of the plateau and the plasma cortisol levels returned to pre-treatment values at about 2 hr rather than 8 hr after dehorning (McMeekan *et al.*, 1998). Flunixin meglumine (Stilwell *et al.*, 2009; Duffield *et al.*, 2010; Huber *et al.*, 2013), Ketoprofen (McMeekan *et al.*, 1998; Stilwell *et al.*, 2012), Meloxicam (McMeekan *et al.*, 1998; Duffield *et al.*, 2010; Heinrich *et al.*, 2010; Stilwell *et al.*, 2012), Carprofen (Stilwell *et al.*, 2012), when compared to the other NSAID, carprofen did not reduce cortisol levels to the same extent in the first 8 hours after disbudding. It was reported that when sedatives xylazine was given to calves it did not abolish the first 3 hr of cortisol response after dehorning (Stafford and Mellor, 2011; Mintline *et al.*, 2013), but when xylazine and lignocaine were given together before the dehorning procedure the response of the cortisol was nearly abolished during the first 3 hr (Stafford *et al.*, 2003). Available literature also showed that Prostaglandins E2 (PGE2) and presumably PGI2 exhibit the highest influence on pain signals transformation. PGE2 production is decreased by Non-steroidal anti-inflammatory drugs, which help in reducing the pain induced stress by disbudding (Kupczyński *et al.*, 2014).

2.23. Behaviour monitoring to assess disbudding stress:

Both during and after the hot-iron disbudding leads to pain-related behavioural alterations. These changes in behaviour last for 12 to 44 h (Faulkner and Weary, 2000; Heinrich *et al.*, 2010). The skin around the horn bud is damaged by hot-iron disbudding, creating a third-degree wound. Hot iron disbudding causes a pronounced behavioural response such that physical restraint is necessary to carry out the disbudding procedure. Cortisol is a reliable indicator for acute stress, as its concentration changes when an animal experiencing stress; stress indicates alterations in the hypothalamus-pituitary-adrenal axis (Terlouw *et al.*, 1997) results in an immediate response of glucocorticoids and an increase of cortisol concentrations within minutes. Concentrations reach levels that are several-fold greater than the baseline levels. The response of the glucocorticoids is proportional to the severity of the stress that is experienced by the animal (Greco and Stabenfeldt, 2013). Human presence or restraint can alter cortisol concentrations, even if this includes acutely painful occurrences (Karlen *et al.*, 2021). The stressor determines

the increase in cortisol concentrations in calves (Stilwell *et al.*, 2012) as well as on the individual and anxiety-related behaviour of the animal (Bristow and Holmes, 2007). Management techniques and external environmental factors can influence the rhythm cycle of cortisol. After disbudding, increased levels of circulating corticosteroids are typically seen, indicating alterations in the hypothalamus-pituitary-adrenal axis (Terlouw *et al.*, 1997). The use of a local anaesthetic reduces the early rise in plasma cortisol. Tail wagging, head movements, tripping, head rubbing, head shaking and ear flicking and rearing are all reduced by local analgesics (Graf and Senn, 1999). Stress indispensably accompanies animals on a dairy farm. The methodologies used to quantify stress include direct behavioural observations and evaluation of physiological parameters, such as fluctuations in heart rate, blood pressure or changes in the concentration of stress hormones in the blood (Ayala *et al.*, 2012). The behaviour of calves is most commonly used to assess the disbudding stress, which is considered as a key indicator of calf welfare (Dawkins, 2003; Stafford and Mellor, 2005; Stull and Reynolds, 2008). Behavioural changes are a more sensitive marker for the assessment of pain in comparison with other physiological markers such as cortisol (Anil *et al.*, 2002). Behaviour indices in farm animals have been recorded using videography (Duffield *et al.*, 2010), accelerometers and remote triangulation devices (Theurer *et al.*, 2012). After disbudding, calves jiggle their heads, attempt to suspend the head around pen instruments, sometimes behave apathetically, lying side by side with their heads and do not respond to other people in the group as well as muscle trembling and fear are also detected in the calves (Stilwell *et al.*, 2012). Increased physical activity after disbudding increases the body surface temperature, which influences the eye temperature so that the overall degree of activity is calculated by recording actions before and after disbudding (Yamazaki *et al.*, 2006). Display of behavioural patterns after disbudding may be used to generate ethograms, which involves the study of the rate or duration of predefined behaviours (Graf and Senn, 1999). Calves often display behavioural signs of pain and discomfort after disbudding, which includes increased head shaking, lying bouts, hind leg kicks and decreased grooming, rumination, rubbing, and head jerks. These behavioural measures are most commonly observed during 4 hr after disbudding (Grøndahl-Nielsen *et al.*, 1999). Calves display the majority of behavioural modification during the 2-4 hour period after disbudding (Morisse *et al.*, 1995; Petrie *et al.*, 1996; Graf and Senn, 1999). In hot iron, disbudding causes thermal destruction to the tissue

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surrounding the horn bud area, as a result, there is an increase in head-shaking activity of the calf to eliminate the source of discomfort or reduce pain. Frequent head shaking following disbudding can indicate pain. Administration of non-steroidal anti-inflammatory drugs (NSAID) to disbudded calves can reduce head shaking frequencies similar to that of placebo kids (Ingvast-Larsson *et al.*, 2011). A significant increase in head shaking, ear flicking, vocalizing, grooming and head rubbing behaviour was recorded in disbudded calves (Faulkner and Weary, 2000).

Behavioural responses of the calves are considered a vital measure of their well-being (Dawkins, 2003; Stull and Reynolds, 2008). Calf behaviour has been studied by various authors about routine management practices associated with the tissue-damaging effect (Stafford and Mellor, 2011). Behavioural responses like head shake, head rubs, ear flicks, tail flicks, foot stamps, play behaviour, lying, standing, grooming, kicking, and falling are considered as a marker of pain (Doherty *et al.*, 2007). Several types of research also measured the frequency of non-evoked behavioural states such as movement, self-grooming, or drinking as an indicator of stress (Heinrich *et al.*, 2010; Faulkner and Weary, 2000; Milligan *et al.*, 2004; Duffield *et al.*, 2010). In a study comparing the post-disbudding behaviour of calves administered with NSAID and the non-treated controls groups, it was reported that increased lying period for 1-4 days after disbudding (Theurer *et al.*, 2012). In another study behaviour of NSAID treated calves after disbudding showed an increased occurrence of play behaviour in NSAID-treated calves when the animals were tested 3hr post disbudding (Mintline *et al.*, 2013). In another study, it was reported that compared to stimulation, control calves induce increased frequencies of tail wagging (3.5 ± 0.5 vs. 1.5 ± 0.5 ; $P < 0.05$), head moving (5.3 ± 1.5 vs. 2.9 ± 0.6 ; n.s), tripping (3.1 ± 0.5 vs. 1 ± 0.5 ; $P < 0.05$), and rearing (1.9 ± 0.5 vs. 0.4 ± 0.2 ; $P < 0.05$) as well as more frequent abnormal backwards-locomotion for 1 hr (2.6 ± 1.0 vs. 0 ; $P < 0.05$) and higher frequencies of head shaking (27.4 ± 5.9 vs 1.3 ± 0.6 ; $P < 0.05$). Saline and control calves showed significantly ($P < 0.05$) more frequent backward locomotion as compared to an anaesthetic group of calves within 1 hr after dehorning. Dehorning produces an increase in head-shaking was significantly ($P < 0.05$) higher within 1 hr after dehorning in the control group as compared to the anaesthetic and saline group (Graf and Senn, 1999). Caray *et al.* (2015) reported vigil calves vocalized and struggled more frequently than control calves and xylazine calves during disbudding and following 15 min where the sedative was used (Vigil calves 1.95; Control calves 0.2; and

Xylazine calves, 1.30) but the result was non-significant. Faulkner and Weary (2000) reported less head shaking, ear flicking and head rubbing behaviour in calves after hot-iron disbudding in the ketoprofen treated group as compared to the control group. Both responses peaked 6hr after disbudding, the difference between the treatment groups remained statistically significant for 12 h (head shaking) and 24h (ear flicking) after disbudding ($P < 0.01$). Sylvester *et al.* (2004) reported that disbudded calves without lidocaine were highly restless for the first 6 hr after disbudding and exhibited significantly more tail flicking, head flicking, ear flicking than the control ($P < 0.05$). The behaviour of the lidocaine treated group was similar to that of the control for the first 2 hr. In another study, it was reported that after the first 2 min of disbudding with or without local anaesthetic, both the groups have a higher level of physical activity than the control group ($P < 0.01$). Physical activity was elevated 12.2 ± 4.3 ($P < 0.001$) times from baseline levels during disbudding without local anaesthetic, 9.5 ± 2.9 ($P < 0.001$) times during disbudding with local anaesthetic and 2.0 ± 0.6 ($P < 0.05$) times for controls. During the remaining post-disbudding period control group had a higher level of physical activity than all other treatments ($P < 0.01$), which was 4.4 ± 1.8 ($P < 0.01$) times from baseline levels (Stewart *et al.*, 2008). Duffield *et al.* (2010) reported that Ketoprofen-treated calves displayed significantly fewer total head flicking ($P = 0.046$) and ear-flicking ($P = 0.01$) behaviours than control calves. Heinrich *et al.* (2010) reported that ear flicking behaviour was higher in control calves as compared to meloxicam treated calves on day 0, (1.31 ± 0.66 vs 4.29 ± 1.10 ear flicks/h) and day 1 (0.55 ± 0.50 vs. 3.27 ± 0.89 ear flicks /h), ($F = 10.65$, $df=16$, $P = 0.005$). Vickers *et al.* (2005) reported head shaking was 9 times higher in calves dehorned with a hot iron as compared to the calves dehorned with the caustic paste ($U = 7.5$, $N_1 = 7$, $N_2 = 8$, $P < 0.05$) during the first 4 hr. It is evident in the above literature that behavioural responses in dairy calves get influenced by both methods of disbudding and the use of analgesic, sedative and NSAID during disbudding.

2.24. Physiological indicators and healing process associated with disbudding:

The changes in physiological parameters during disbudding is well documented. The normal range of heart rate (HR) of calves is between 100 and 120bpm (Jacobson and cook, 1998; Despres *et al.*, 2002; Veissier *et al.*, 2002). In a study, it was reported that after the first 5min of disbudding with or without local anaesthetic (LA), HR increased

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but remain elevated above baseline following disbudding without local anaesthetic ($P < 0.001$). Heart rate is showing significant difference in without local anaesthetic group, when compared to local anaesthetic group ($P < 0.001$) (Stewart *et al.*, 2008). In another study heart rate changed significantly with time ($F^{18,954}$ (time) = 8.70, $P < 0.0001$) and the changes varied between treatments ($F^{36,954}$ (time x treatments) = 11.1, $P < 0.0001$). In dairy cattle, heart rate variability is frequently used as a proxy measure of the autonomic stress response. In response to a stressor, the sympathetic and parasympathetic branches of the autonomic nervous system innervate the heart and act antagonistically. The sympathetic nervous system raises the heart rate, while the parasympathetic nervous system lowers it (Rea, 2016). Change in heart rate over time has been used to elucidate the information regarding the autonomic nervous system in response to different on-farm stressors like disbudding (Stewart *et al.*, 2008, 2009). Following disbudding, there was an increase in respiration rate, which is usually associated with an increase in heart rate (Stewart *et al.*, 2013). Heinrich *et al.* (2010) also reported that increase in respiration rate following the disbudding of calves with a local anaesthetic. There is no available literature regarding the rectal temperature change before or after disbudding. The available literature regarding wound healing showed that disbudded wounds take 62 ± 10 d (Mean \pm SD; range: 42-91 d) for complete re-epithelialization. But it was reported that necrotic tissue took a longer time to heal when disbudding was carried out at younger age, in 3 days calves (26 ± 1 d) as compared to 35 days older calves (23 ± 1 d; $F_{1,22} = 7.36$, $P = 0.013$) (Adcock and Tucker, 2018). Tucker *et al.* (2014) reported that hot-iron branding, which takes at least 10 weeks to re-epithelialize in beef calves aged 4 to 7 months, showed a similar healing pattern as that of disbudding. The type of iron utilized did not affect re-epithelialization latency, which took on an average (mean \pm standard deviation) 53 ± 3 days for Portasol wounds and 55 ± 3 days for Rhinehart wounds, respectively (range: 40–70 days) (Adcock *et al.*, 2019). A recent study looked at wound healing in feedlot cattle using various dehorning methods and found that different dehorning methods resulted in different levels of wound infection and healing time, with recovery taking up to four weeks (Neely *et al.*, 2014). Alvarez *et al.* (2019) also reported a 5-9 wk wound healing period for hot iron disbudding. In a recent study, Huebner *et al.* (2017) reported no difference in lesion score between the control group as compared with the aluminium-based aerosol bandage group during the first 2 weeks post-disbudding but the proportion of lesion score on 3rd-week post disbudding was greater (17 vs. 8%, respectively). Control disbudding sites were exhibited 1.42 times delayed

healing at week 3 post-disbudding as compared to aluminum-based aerosol bandage disbudding sites. In 3rd week, the treatment group's wound diameter was 1 mm smaller than the control group, and the treatment group wound diameter declined better throughout the healing process as compared to the control group. The medicated patch acts as a drying or astringent agent to accelerate wound contracture or reduce inflammation associated with coagulative necrosis (Huebner *et al.*, 2017).

2.25. Growth parameters affected by disbudding:

Changes in body weight (or average daily gain) are the key production indicators reported from horn removal in calves (Allen *et al.*, 2013; Black *et al.*, 2017; Stock *et al.*, 2015; Bates *et al.*, 2016). As disbudding is a stressful procedure that leads to increased plasma cortisol concentrations and a decrease in dry matter intake which primarily affect body weight. Weight gain has been quantified as the difference between body weight before disbudding and the bodyweight after few days and the period between weighings varied greatly between experiments (from 24h to 30days). Faulkner and Weary (2000) reported that calves treated with ketoprofen tended to gain more weight (1.2 ± 0.4 kg) during the 24h after dehorning as compared to control calves (0.2 ± 0.4 kg) ($P = 0.07$) and during the next 24h period, weight gains were similar for treated (1.0 ± 0.2 kg) and control calves (1.1 ± 0.4 kg) ($P > 0.1$). Bates *et al.* (2016) reported that from -3 day to the 15 day growth rate of calves disbudded without pain relief 0.53 kg/day (95% CI=0.47-0.60) was less than that of calves disbudded with some form of pain relief 0.65kg/d (95% CI=0.62-0.68, $P = 0.004$) and NSAID treatment increased the growth rate of calves disbudded as compared to calves disbudded without pain relief ($P < 0.05$). Adcock and Tucker (2018) reported no effect of disbudding age at the time of disbudding on average daily gain (Disbudded: 0.59 ± 0.06 kg/d; Sham/control: 0.52 ± 0.06 kg/d; $F_{1, 42} < 0.60$, $P > 0.444$). Calves disbudded or sham-disbudded at 35d of age gained over twice as much weight during the week following the procedure than calves disbudded or sham-disbudded at 3d of age (3d: 0.35 ± 0.06 kg/d; 35d: 0.77 ± 0.06 kg/d; $F_{1, 42} = 22.21$, $P < 0.001$). Duffield *et al.* (2010) reported that ketoprofen-treated calves consumed more calf starter in the 24 hours following dehorning ($P = 0.09$), equating to approximately 0.25 kg more grain and there was also a significant effect of calf weight on starter consumption, with larger calves consuming more feed ($P < 0.001$).

2.26. Cortisol level to assess disbudding stress:

Disbudding procedure in calves causes distress of varying intensities which is reflected through changes in behavioural and plasma cortisol levels (Caray *et al.*, 2015). The most often investigated indication of wellbeing features of horn removal in calves is plasma cortisol levels (Stock *et al.*, 2013). When the body perceives stress, adrenal glands make and release the stress hormone cortisol into the bloodstream causes an increase in heart rate. Various reports are available that have shown removal of horns in calves by any method of disbudding leads to a marked, early increase in the plasma cortisol concentration (Coetzee *et al.*, 2012; Allen *et al.*, 2013; Stewart *et al.*, 2013). After 7 hours, of disbudding the concentration of cortisol in the blood falls (Petrie *et al.*, 1996; McMeekan *et al.*, 1998; Sylvester *et al.*, 1998). Plasma cortisol levels reach a peak in the first 30 minutes in disbudded calves without analgesia and then returning to baseline at approximately 7-8 hr post-disbudding as reported by Stock *et al.* (2013). In chemical disbudding rise in plasma cortisol concentration occur within 1 hour and returns to pre-treatment level 4-24 hours later. Mainau *et al.* (2012) reported that after dehorning plasma cortisol concentration increased immediately within the 30 minutes and return to the pre-treatment levels after 5-9 hours. The available literature regarding the administration of local anaesthetic before disbudding showed a lower cortisol peak as compared to calves dehorned without any pain relief (Morisse *et al.*, 1995; Aubry, 2005; Allen *et al.*, 2013). Various researchers recorded a decrease in cortisol plasma levels in NSAID-administered calves after disbudding (Milligan *et al.*, 2004; Heinrich *et al.*, 2010; Stilwell, 2012; Allen *et al.*, 2013; Huber *et al.*, 2013; Stock *et al.*, 2015). Carry *et al.* (2015) reported that cortisol levels varied markedly between t_{+0} and t_{+90} ($F^{7,304}(\text{time})=13.5$, $P < 0.0001$). The peak in salivary cortisol concentrations was observed between t_{+15} and t_{+45} in vigil calves, whereas a small variation in xylazine or control calves ($F^{14,304}(\text{time} \times \text{treatment})=6.64$, $P < 0.0001$) was observed. Graf and Senn (1999) reported that plasma cortisol levels in the saline and control group were significantly higher than anaesthetic group ($P < 0.05$). Black (2016) reported that cortisol concentration was lowest 4hr after disbudding (1.93 ± 0.49 ng/ml) as compared to -24, 0, and 1hr (6.00 ± 0.48 , 5.23 ± 0.49 , and 6.82 ± 0.49 ng/ml; $P < 0.0001$) and tended to be higher 1 hr after disbudding as compared to 0 hr ($P = 0.09$). In another study at 4 hours following dehorning, meloxicam-treated calves exhibited considerably lower serum cortisol concentrations than placebo-treated controls ($P = 0.0004$). When the meloxicam-pre

disbudding ($P = 0.015$) and meloxicam-post disbudding ($P = 0.002$) calves were compared to the control calves at this point of time, the mean cortisol concentration was considerably lower in both the meloxicam -pre disbudding ($P = 0.015$) and meloxicam post disbudding ($P = 0.002$) (Allen *et al.*, 2013). Stilwell *et al.* (2012) reported that after 1 hr of disbudding only disbudded group of calves showed higher cortisol levels than the other two anaesthesia groups and anaesthesia with carprofen groups ($P < 0.01$). Duffield *et al.* (2010) reported no significant differences in the serum cortisol concentrations (nmol/L) of calves 10 min before dehorning (time 0) and at 3 and 6 h after dehorning in control and ketoprofen-treated calves. Therefore, cortisol level in the blood can act as a good indicator to assess the stress level associated with the disbudding in the dairy calves.

CHAPTER –3

Materials & Methods

MATERIALS AND METHODS

In the new era of life, smart technologies are being incorporated in the production improvement and management of livestock. Thermal images of different body surface points of livestock are being used for measuring problems related to the health condition in dairy animals (Stewart *et al.*, 2007; Colak *et al.*, 2008; Talukder *et al.*, 2014). Therefore, temperature measurement at various body points using Infrared Thermography (IRT) can act as a putative marker of physiological condition and health of the animal. Infrared Thermography (IRT) is a simple, non-invasive, non-contact, very precise instrument to measure the minute changes in surface temperature. It absorbs infrared radiation and based on the amount of heat generated by the object, produce a thermogram. In diseased calves, the infection affects the eyeball temperature, and this is essential to develop a model for the early detection of diseases in calves. During disbudding, different stress levels affect the eyeball temperature of calves, and the change in temperature of the eyeball is essential to evaluate the levels of stress by different methods of disbudding. Therefore, understanding of temperature change and monitoring by IRT for early detection of diseases in Sahiwal calves and evaluation of stress and healing associated with different methods of disbudding in dairy animals under tropical climate needs scientific validation. Keeping in view the above importance, the study was planned for, early detection of diseases and evaluation of stress and healing associated with different methods of disbudding using Infrared Thermography in dairy animals. So, to test this hypothesis experiments were performed in dairy calves. All the animals used in the study were maintained under uniform management conditions at Livestock Research Centre (LRC), ICAR-NDRI, Karnal, Haryana.

3.1. Location of study:

Livestock Research Centre (LRC) is located at ICAR- National Dairy Research Institute (ICAR-NDRI), Karnal, Haryana in a geographic orientation with an elevation (altitude) of 250 meters above the mean sea level, latitude 29°43" North and longitude 77°20" East. This is a tropical climate region, and the annual minimum maximum temperature ranges from 4 to 45°C. Most of the rainfall occurs in July and August with

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an annual average of 70 cm. However, during the hot-humid season (July to September) the relative humidity varies from 41 to 85 percent and vapour pressure from 7.0 to 25 mmHg. Thus, due to a wide range of meteorological factors, the experimental farm receives the extreme hot and cold temperatures.

3.2. Experimental details and selection of experimental animals:

The study was conducted with the following two objectives and a total of four experiments were conducted under two objectives. Under Objective 1: Evaluation of calf health in dairy animals using infrared thermography, two experiments were conducted. In Experiment 1, data was generated for IRT based ocular and body surface temperature of 29 diseased and 29 healthy dairy calves. In Experiment 2, for early detection of diseases in calves using Infrared Thermography, 46 newborn healthy Sahiwal calves were monitored using IRT for 30 days from birth. Under Objective 2: Evaluation of stress associated with different methods of disbudding using infrared thermography in dairy calves, two experiments were conducted. In Experiment 1, data generation of horn bud size in the different age groups of calves was carried out and 33 healthy dairy calves of three different age groups were selected 0-10days, 11-20days, 21-30days. In Experiment 2, evaluation of stress associated with different methods of disbudding using IRT, in this experiment 30 healthy Sahiwal calves of 10-30days age was used.

3.3. Background information about the experimental animals:

3.3.1. Sahiwal:

The Sahiwal originated in the dry Punjab region, which lies along the India-Pakistan border. They were kept in large herds by professional herdsmen called "Junglies". The Sahiwal is one of the best indigenous dairy breeds in India. It has been exported to Australia, where Sahiwal was initially selected as a dual-purpose breed. It played a valuable role in the development of the two Australian tropical dairy breeds, the Australian Milking Zebu and the Australian Friesian Sahiwal. Sahiwal colour can range from reddish-brown to the more predominant red, with varying amounts of white on the neck, and the underline. In males, the colour darkens towards the extremities, such as the head, legs and tail. The breed is tick-resistant, heat-tolerant and noted for its high resistance to parasites, both internal and external. Cows average, milk production 2270kg

during a lactation while suckling a calf and much higher milk yields have been recorded. The Sahiwal is the heaviest milker of all Zebu breeds and displays a well-developed udder. Sahiwals demonstrate the ability to sire small, fast-growing calves and are noted for their hardiness under unfavourable climatic conditions.

3.4. Management practices:

3.4.1. Housing:

Calves were placed in a single room for the period of night hours. The rooms were well ventilated with windows to provide a comfortable environment for the calves. Rooms were maintained under the utmost hygienic condition and cleaning of the room were done early in the morning. The flooring of the room was concrete and covered with a rubber mat. During the day hours, calves were placed in the open paddock region shaded with trees. The feeding of milk was done twice daily individually in the calf pens inside the shed. All the pens and rooms were furnished well with requisite facilities for room heater in winter and fan with cooler in summer.

3.4.2. Feeding:

Calves received the colostrum from birth to 5th day, morning at 7:00 AM and evening at 3:00 PM twice daily. Newborn calves were separated from their dam immediately after birth. The first colostrum was offered within half an hour. Feeding of colostrum was done @ 1/10th of body weight by pail method or bottle method. Normally bottle method was followed when the calf refused to take milk from the pail. After the 5th day calves were offered the whole milk @ 1/10th of body weight two times at 8:00 AM and evening 3:00 PM by pail method. After the 15th-21th day, a little calf starter and good quality green fodder was offered to calves. After 22-35th days whole milk feeding was carried out @ 1/15th of body weight, along with 100 gm of calf starter and ad-lib green fodder. Upto 2 months, whole milk @ 1/20th of body weight, along with 250gm of calf starter and ad-lib green fodder was offered. From 2-3 months milk was gradually reduced and calf starter was offered @ 500gm and ad-lib green fodder.

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Table 3.1: Feeding schedule of calf upto 3 months of age:

Age of calf	Whole milk	Calf starter	Good quality green fodder
1-5 day	Colostrum @ 1/10 th of body weight in 2 feeds	-	-
5-7 day	Whole milk @ 1/10 th of body weight in 2 feeds	-	-
8-14 day	Whole milk @ 1/10 th of body weight	-	-
15-21 day	Whole milk @ 1/10 th of body weight	A little	A little
22-35 day	Whole milk @ 1/15 th of body weight	100gm	Ad lib
Upto 2 months	Whole milk @ 1/20 th of body weight	250gm	Ad lib
2-3 months	Milk is gradually reduced	500gm	Ad lib

Table 3.2: Composition of Calf Starter for Calves:

Feed ingredients	Composition
Maize	42
Groundnut cake	35
Wheat bran or rice bran	10
Skim milk powder	10
Mineral mixture	2
Salt	1

3.4.3. Health management:

To ensure the good health of the calves' routine deworming protocol and the daily treatment of sick calves were done in the calf section. Deworming started from the 5th day with a drenching of liquid Albomar oral Suspension @ 5mg/kg body weight. After this regularly every month deworming was done by liquid Albomar for the first 6 months, thereafter once in three months. Routine vaccination protocol was carried out as per the standard protocol of the farm to maintain herd health.

3.5. Infrared thermal image recordings and analysis:

Infrared thermal images were collected using an infrared thermal camera within the pen, at a distance of 1 m in front of the calf while the calf is standing. In the present investigation, thermographic images of all the animals of various experiments were captured using a hand-held high image resolution (384 X 288 pixels) digital infrared thermal camera (Darvi DTL007 camera, TAK Technologies Pvt. Ltd). The thermal camera can capture a temperature range of -20°C to +650°C. The thermal sensitivity of the IRT camera was < 20°C with an accuracy of ±2%. Before capturing of IRT image, the camera was adjusted to the ambient conditions. The camera was calibrated to ambient temperature and humidity. The reference calibration was exercised using the cap of the camera, which was sorted at ambient temperature as a reference. The value of emissivity and reflected apparent temperature was kept constant for all the images as 0.96 and 20°C, respectively, which is recommended for biological tissue analysis based on the manufacturer's recommendation. The surface temperature of each photographic episode was analysed by using thermal image analysis software (Darvi TI analysis software). Before this, unclear thermal images were excluded from the analysis and the maximum temperature of the particular region of the images were recorded and used in the statistical analysis. An orbital image was obtained by tracing an oval image over the orbital area, which included the eyeball itself plus approximately 1 cm surrounding this area. A square image of the nose is obtained by tracing an area approximately 1 cm square on the hairless frontal surface of the nose. A square image of the cheek traced an area approximately 1 cm square on the lateral surface. A rectangular image of the paralumbar region of the calf traced an area approximately 20 cm by 40 cm on the mid-back surface. Both mean and maximum temperatures of these areas were recorded. IRT images were also taken for the disbudded wound till the healing process is completed.

3.6. Objective 1: Evaluation of calf health in dairy animals using infrared thermography

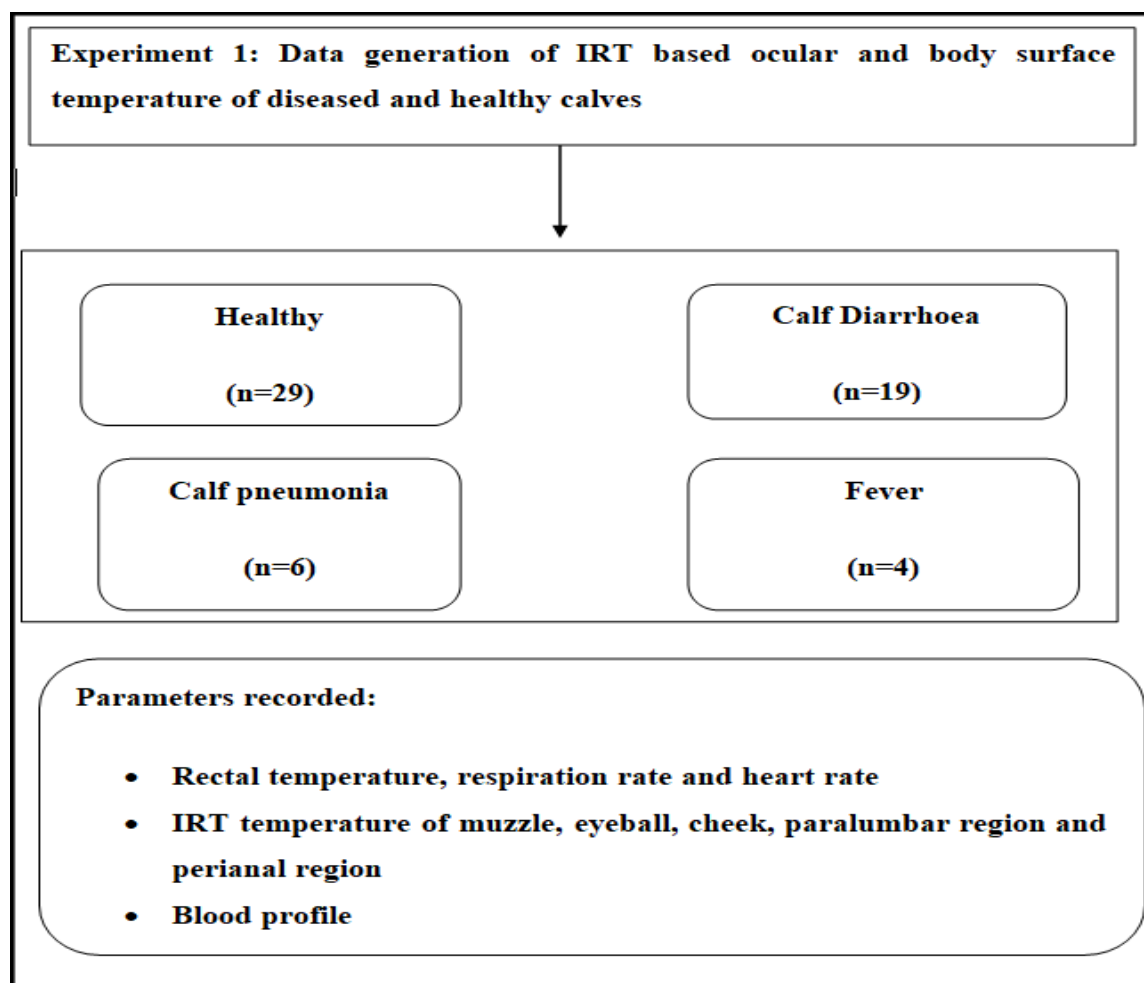
Two experiments were conducted under this objective.

3.6.1. Experiment 1: Data generation of IRT based ocular and body surface temperature of diseased and healthy calves

To generate the data of IRT based ocular and body surface temperature, 29 diseased and 29 healthy dairy calves were used in the present experiment. In routine, the

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calves reported for calf diarrhoea, calf pneumonia, and fever in the Animal Health Complex, Livestock Research Centre, ICAR-NDRI were selected for infrared thermography and in a same-day corresponding number of healthy calves were also selected for infrared thermography as control. IRT images of diseased and healthy calves were taken around the eyeball, muzzle, cheek, para-lumbar region and peri-anal region in the morning from 7:00 to 9:00 AM under the calf shed. The calves having diarrhoea, pneumonia, and fever were confirmed by an experienced veterinarian working in the calf health section and using physiological parameters like heart rate, respiration rate and rectal temperature. Blood samples were taken for the estimation of haematological parameters from the diseased calves that showed symptoms of diarrhoea, pneumonia, fever and similar representative physiological parameters (heart rate, respiration rate and rectal temperature) and blood samples were taken from healthy calves. The diseased calves were considered as experimental group and healthy calves were considered as a control group for the further analysis.



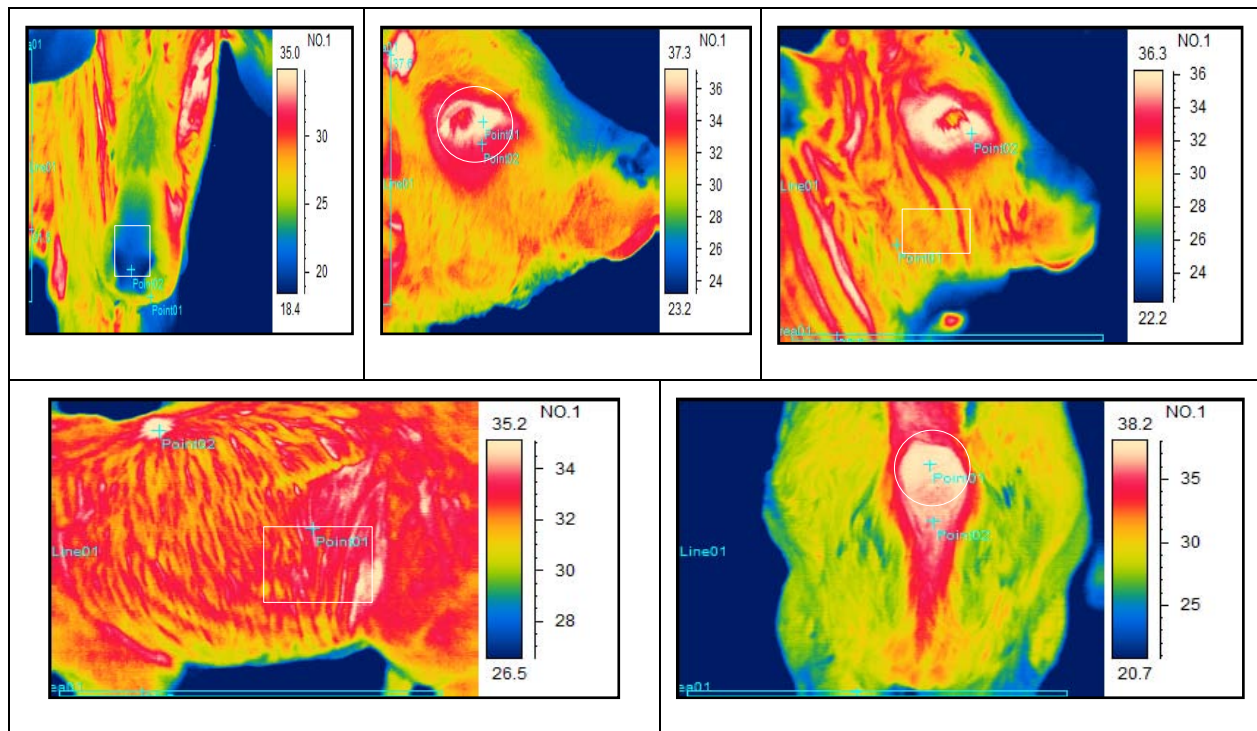


Plate3.1: Infrared thermal image of ocular, muzzle, cheek, para-lumber region and peri-anal region

3.6.2. Physiological parameters:

Health checkups were carried out each morning to assess the calves general well-being and to identify which calf began to display clinical signs of illness. Health check ups are carried out based on their general appearance, coat condition, gut fill and faecal consistency. Dehydration levels were assessed by monitoring calves for sunken eyes and by performing a tent test to measure skin elasticity in which the skin of the neck was pinched and the time for the skin to return to its normal position was recorded. Health checkups were also included monitoring signs of nasal and ocular discharge and navel infection (a disease resulting from bacterial infection via umbilical cord soon after birth). Rectal temperatures were measured once during health check up of each animal using a clinical digital thermometer (MC-343; Omron, Kyoto, Japan). The clinical digital thermometer was inserted into the rectum, in contact with the mucosa until stabilization of the temperature, which was recorded in degree centigrades ($^{\circ}\text{C}$). As part of the health checkup, Respiration Rate was measured by counting the flank movements to record the time taken for each calf to complete 10 breaths; this was then used to calculate RR (breaths/min). The Heart rate of calves was evaluated by the semiotic technique of

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listening to the heart-beat using a stethoscope. The animals were auscultated preferably in a standing position and at rest.

3.6.3. Clinical score of calf diarrhoea and pneumonia:

Calves were defined as being clinically ill when they were observed as being diarrhoeic. To be considered diarrhoeic, a calf had to be witnessed passing malodorous faeces with a loose to a watery consistency, with the possibility of blood present in severe cases (a score of 2 or 3 for faecal consistency). For calves that had not been observed passing faeces but were suspected of being diarrhoea due to loose faecal matter present on the top of the tail or hind legs, a faecal sample was taken to confirm whether the calf was diarrhoeic or not. From all diarrhoeic calves, a faecal sample was collected for analysis to confirm the cause of illness and to identify the specific pathogen responsible for diarrhoea. Once confirmed clinically ill, calves were treated accordingly with electrolytes (NaCl -0.9%; B BRAUN) and antibiotics (Metrogyl; Virbac) as needed to help them to recover from the disease. The calves affected with diarrhoea were monitored daily for clinical signs of illness in the calf pens using the method described by Renaud *et al.* (2020).

Table 3.3: Clinical scores for diarrhoeic calves

Score	Faecal Consistency Score	Clinical Dehydration Score
0	Normal, well-formed faeces	Normal, bright eyes, pliable skin
1	Pasty faeces	Mild dehydration, eyes not recessed into orbits, slight loss of skin elasticity, skin tents <3 seconds
2	Semi liquid faeces still with a solid component	Moderate dehydration, eyes slightly recess into orbit, skin tent > 3 seconds but < 10 seconds
3	Watery faeces	Severe dehydration, eyes markedly recess into orbits, skin tents > 10 seconds

For the respiratory diseases and fever, calves in the pens were monitored daily for clinical signs of illness using methods described below and the protocol was adopted as per the method described in Table by McGuirk and Peek (2014).

Table 3.4: Clinical parameters and point scale used for respiratory clinical scoring

Clinical parameter	Points and description			
	0	1	2	3
Rectal temperature (°F)	100-100.9	101-101.9	102-102.9	≥103
Nasal discharge score	Normal, serous discharge	A small amount of unilateral, cloudy discharge	Bilateral, cloudy, or excessive mucus	Copious, bilateral mucopurulent nasal discharge

3.6.4. Blood parameters:

Calves affected with diarrhoea (n=19), pneumonia (n=6), fever (n=4) and 29 healthy calves were individually restrained for blood collection on specific days when they got affected with the disease and corresponding healthy animals on the same day. 2-3 ml blood was collected using EDTA vacutainer from each calf and immediately subjected to blood profile by using an automatic blood analyser (Nihon kodhencelltaca, Tokoyo, Japan).

3.6.5. Microbiological evaluation to identify the organism responsible for calf diarrhoea:

3.6.5.1. Faecal sample:

The faecal sample used in the study were collected from clinically diarrhoea calves. Representative faecal samples (n=19) were taken in the sterile petri dish plate by the rectal stimulation method before the treatment of the sick calves. Samples were put in the box containing ice packs and taken to the laboratory without delay, for further processing.

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3.6.5.2. Normal saline preparation:

8.5gm of Sodium Chloride (NaCl) was weighed with the help of a weighing scale. Then 500ml of distilled water was taken in the volumetric flask and added 8.5gm of NaCl. The flask swirled gently to mix the contents. When NaCl dissolved completely then distilled water was added and made the final volume of 1 litre. An air-tight stopper was inserted into the mouth of the volumetric flask and shook gently to make the solution homogeneous. Cool the prepared solution to room temperature after autoclaving for 15 minutes at 120°C.

3.6.5.3. Serial dilution of the faecal sample with normal saline:

1. 1ml of faecal sample solution was taken and mixed it well with the 9ml of 0.85ns of test tube 1st.
2. 1 ml from the test tube 1st was taken and mixed well with the 9ml of 0.85ns of test tube 2nd.
3. 1ml from the test tube 2nd was taken and mixed well with the 9ml of 0.85ns of test tube 3rd.
4. 1ml from the test tube 3rd was taken and mixed well with the 9ml of 0.85ns of test tube 4th.
5. 1 ml from the test tube 4th was taken and mixed well with the 9ml of 0.85ns of test tube 5th.
6. From the last test tube, 1ml was discarded



Fig 3.1. Serial dilution of normal Saline

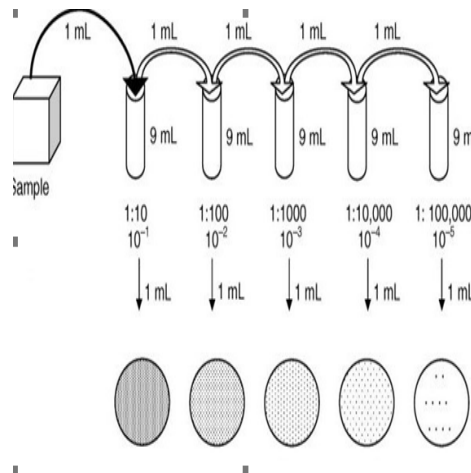


Fig 3.2. Serial dilution of the faecal sample with normal saline

3.6.5.4. Nutrient agar plate:

Nutrient agar are basic culture media used for maintaining microorganisms, cultivating fastidious organisms. Nutrient agar is popular because it can grow a variety of types of bacteria and fungi, and contains many nutrients needed for the bacterial growth.

Table 3.5: Composition of Nutrient agar

Peptones	5.00 gm/l
Sodium chloride	5.00 gm/l
HM peptone B	1.50 gm/l
Yeast extract	1.50 gm/l
Agar	15.00 gm/l
pH after sterilization(at 25°C)	7.40±0.2

3.6.5.4.a. Procedure:

28.0 grams of nutrient agar media was added in 1000 ml distilled water and heated till boiling to dissolve the medium completely. The medium was sterilized by autoclaving at 15 lbs pressure (121°C) for 15 minutes and cooled to 45-50°C. The medium was mixed well and pour into sterile petri plates (Plate 3.2).

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Feecal samples after serial dilution was poured into the nutrient agar plate under laminar air flow kept it in incubator for overnight to check the growth of microorganisms. After that it was subcultured into different differential and selective media.

3.6.5.5. MacConkey agar plate:

MacConkey Agar is recommended for selective isolation of *Escherichia coli*. It is also recommended for selective isolation and differentiation of lactose fermenting and lactose non fermenting enteric bacteria. MacConkey agar used in this study was produced from Himedia.

Table 3.6: Composition of MacConkey agar

Peptones (meat and casein)	3.00 gm/l
Pancreatic digest of gelatin	17.00 gm/l
Lactose monohydrate	10.00 gm/l
Bile salts	1.500 gm/l
Sodium chloride	5.00 gm/l
Crystal violet	0.01 gm/l
Neutral red	0.03 gm/l
Agar	13.50 gm/l
pH after sterilization(at 25°C)	7.10±0.2

3.6.5.5.a. Procedure:

49.53 grams of MacConkey agar dehydrated medium was suspended in 1000 ml distilled water. Then, the suspension was boiled to dissolve the MacConkey agar dehydrated medium completely. It was sterilized by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Then, it was cooled to 45-50°C. It should be mixed well before pouring into sterile petri plates. The surface of the medium should be dry when inoculated (Plate 3.3).

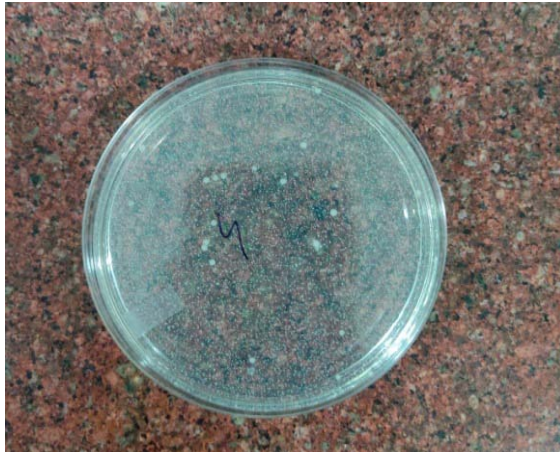


Plate 3.2: Nutrient agar plate with bacteria Plate 3.3. Mac Conkey Agar Plate

3.6.5.6. EMB agar plate:

It is recommended for differential isolation of Gram-negative enteric bacilli from clinical samples. This media was used to differentiate the *E. coli* and *Klebsiella* species. EMB agar used in this study was produced from Himedia.

Table 3.7: Composition of EMB agar

Peptones (meat and casein)	10.00 gm/l
Dipotassium hydrogen phosphate	2.00 gm/l
Lactose	5.00 gm/l
Saccharose (Sucrose)	5.00 gm/l
Eosin - Y	0.40 gm/l
Methylene blue	0.06 gm/l
Agar	13.50 gm/l
pH after sterilization(at 25°C)	7.20±0.2

3.6.5.6.a. Procedure:

35.96 grams EMB agar was dissolved in 1000 ml distilled water and mixed well, until suspension was uniform. The suspension was boiled to dissolve the medium completely. It was sterilized by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Then, it was cooled to 45-50°C. It should be shake well in order to oxidize the methylene blue (i.e. to restore its blue colour) and to suspend the flocculent precipitate.

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3.6.5.7. Blood Agar Plate:

It is recommended as a base to which blood may be added for use in the isolation and culture of fastidious pathogenic microorganisms.

Table 3.8: Composition of Blood agar

HM peptone B	10.00 gm/l
Tryptose	10.00 gm/l
Sodium chloride	5.00 gm/l
Agar	15.00 gm/l
pH after sterilization(at 25°C)	7.2±0.2

3.6.5.7.a. Procedure:

40.0 grams medium was dissolved in 1000 ml distilled water and mixed well until suspension was uniform. The suspension was boiled to dissolve the medium completely. It was sterilized by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cooled to 45-50°C and aseptically added 5% v/v sterile defibrinated blood. It should be mixed well before pouring into sterile petri plates.

3.6.5.8. Baird-Parker Agar Plate:

Baird-Parker Agar Medium is recommended for the selective isolation and enumeration of coagulase positive Staphylococci.

Table 3.9: Composition of Baird- Parker agar

Pancreatic digest of casein	10.00 gm/l
Beef extract	5.00 gm/l
Yeast extract	1.00 gm/l
Glycine	12.00 gm/l
Sodium pyruvate	10.00 gm/l

Lithium chloride	5.00 gm/l
Agar	20.00 gm/l
pH after sterilization(at 25°C)	6.80±0.2

3.6.5.8.a. Procedure:

63 grams medium was dissolved in 950 ml distilled water and mixed well until suspension was uniform. The suspension was boiled to dissolve the medium completely. It was sterilized by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cooled to 45-50°C and aseptically added aseptically 50 ml concentrated Egg Yolk Emulsion (FD045) and 10 ml sterile 1% Potassium Tellurite solution (FD052). It should be mixed well before pouring into sterile petri plates.

3.6.5.9. Brilliant Green Agar Plate:

Brilliant Green Agar Medium was used for selective isolation of Salmonellae other than *Salmonella typhi* from faeces.

Table 3.10: Composition of BGA agar

Peptone	5.00 gm/l
Tryptone	5.00 gm/l
Yeast extract	3.00 gm/l
Lactose	10.00 gm/l
Sucrose	10.00 gm/l
Sodium chloride	5.00 gm/l
Phenol red	0.08 gm/l
Brilliant green	0.01 gm/l
Agar	20.00 gm/l
pH after sterilization(at 25°C)	6.90±0.2

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3.6.5.9.a. Procedure:

58.09 grams of Brilliant Green Agar media was added in 1000 ml distilled water and heated till boiling to dissolve the medium completely. The medium was sterilized by autoclaving at 15 lbs pressure (121°C) for 15 minutes and cooled to 45-50°C. The medium was mixed well and pour into sterile Petri plates.

3.6.5.10. Streaking method:

Bacterial colonies from the nutrient agar were picked up by inoculation loop and subcultured by streaking on different agar plate. After incubation, all the plates were examined for the growth of different bacteria on different agar plates

3.6.5.11. Purification of culture:

The lactose fermenting, pink coloured colonies from the MacConkey agar plate were fished out and subcultured in fresh nutrient agar plate. Further purification of *E. Coli* isolates was carried out in nutrient agar plates. A purified isolated colony was streaked on Eosin Methylene Blue (EMB) agar plates. The inoculated EMB agar media were incubated at 37⁰C for 24 hours. The EMB agar plates were examined for the colonies showed green metallic sheen which indicated the *Escherichia coli* strains.

Subcultures were also performed for Staphylococcus and Salmonella on blood agar, Baird Parker agar and Brilliant green agar to obtain pure culture and study the cultural characteristics of these organisms.

Table 3.11: Normal Characteristics of bacterial pathogens isolated from diarrhoeic calves by Gram's staining technique

Gram Staining			Identification
Shape	Arrangement	Gram's staining reaction (+/-)	
Short plump rods	Single, paired or in short chain	Gram negative	<i>E. coli</i>
Very short plump rods	Single	Gram negative	<i>Salmonella</i> spp.

Cocci arranged	Grape- like clusters	Gram-positive	<i>Staphylococcus</i> spp.
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3.6.6. Thermal image capture:

A similar procedure has been adopted as described in the materials and methods section 3.6.1

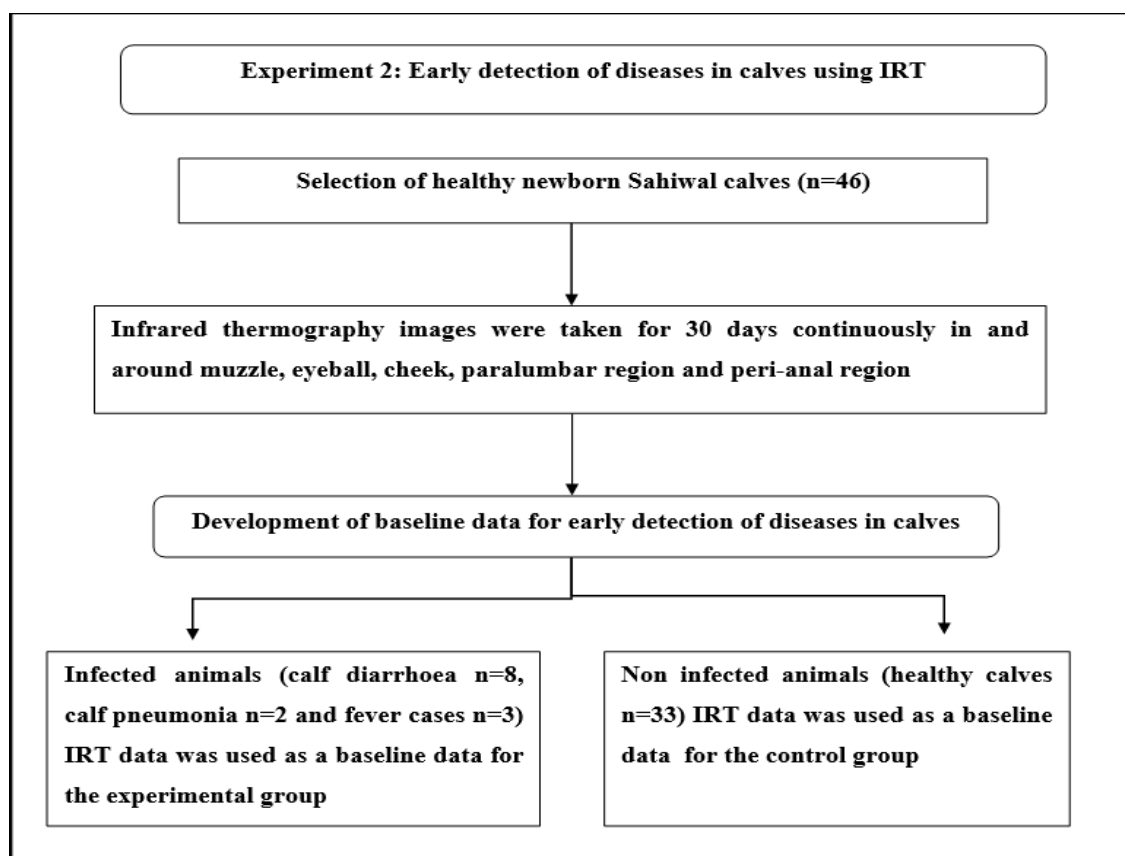
3.6.7. Thermal image analysis:

The eye ball area, muzzle, cheek, para-lumber region and perianal region temperature of each photographic episode was analysed by using thermal image analysis software (Dravi TI analysis software). Before this, unclear thermal images were excluded from the analysis and the maximum temperature of a particular region of images were recorded and used in statistical analysis.

3.6.8. Experiment 2: Early detection of diseases in calves using IRT

In the present experiment, 46 newborn healthy Sahiwal calves were taken from the day of birth. The calves were monitored for 30 days continuously. Infrared thermal images were taken for 30 days continuously around the eye ball area, muzzle, cheek, perianal region and para-lumber region in the morning from 7:00 to 9:00 AM under calf shed. Daily rectal temperatures were also monitored using a clinical digital thermometer. Blood samples were taken for the estimation of haematological parameters from the calves that showed symptoms of calf diarrhoea, calf pneumonia and fever and similar representative blood samples were taken from healthy animals. The infected calves were considered as the experimental group and healthy calves were considered as a control group for further analysis.

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3.6.9. Blood parameters:

A similar procedure has been adopted as described in the materials and methods section 3.6.3

3.6.10. Microbiological evaluation to identify the organism responsible for calf diarrhoea:

Representative faecal samples (n=8) were used for microbiological evaluation to identify the organism responsible for calf diarrhoea. A similar procedure has been adopted as described in the materials and methods section 3.6.5 to 3.6.5.11

3.6.11. Thermal image analysis:

A similar procedure has been adopted as described in the materials and methods section 3.5.

3.7. Objective 2: Evaluation of stress associated with different methods of disbudding using infrared thermography in dairy calves

Two experiments were conducted under this objective.

3.7.1 Experiment 1: Data generation of horn bud size in the different age groups of calves

To generate the data of horn bud size 33 Sahiwal calves of different age groups (0-10 days, 11-20 days, and 21-30 days) were selected for this experiment. Calves were moved to the paddock region after feeding of milk in the morning and then hair around the horn bud was trimmed by scissor and trimmer. Horn bud diameter was measured by vernier calliper daily for 10 days. The tip of the vernier calliper should be touched with the periphery of the horn bud. Jaws of the vernier calliper were parallel to each other. Three groups based on age were considered for further analysis.

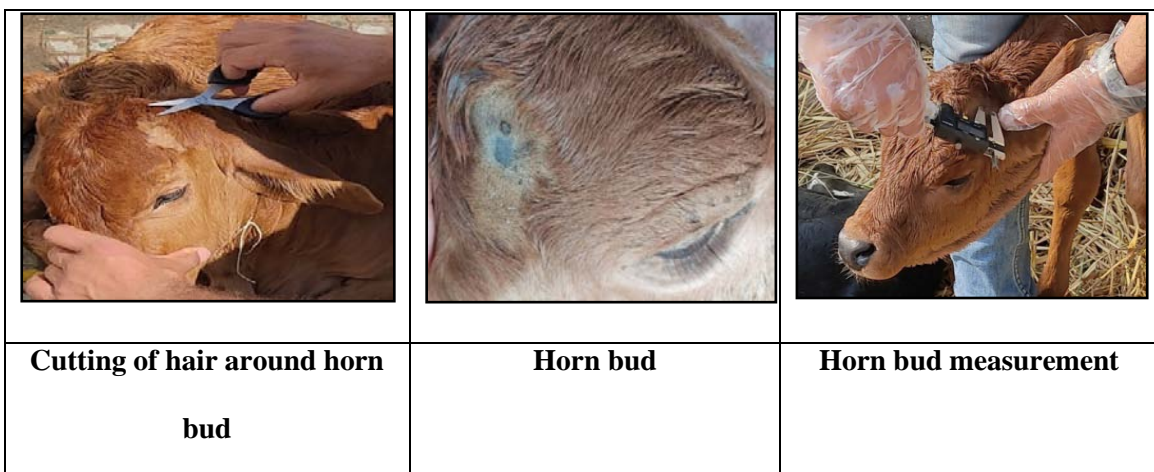
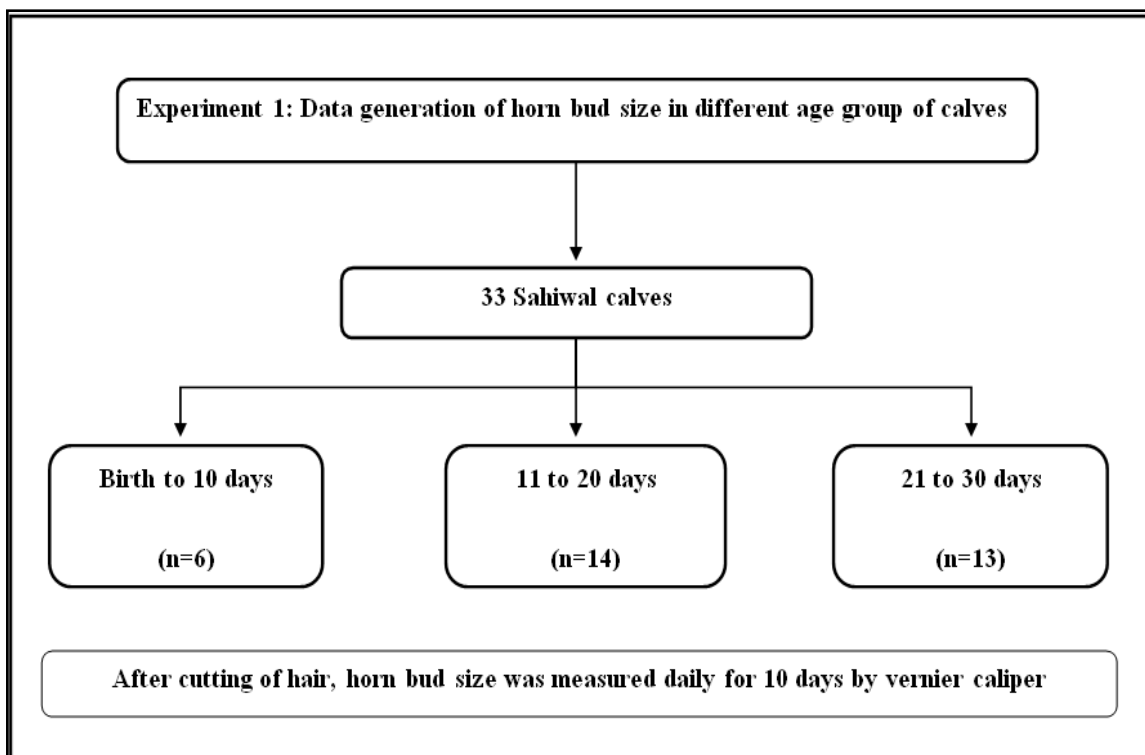


Plate 3.4: Steps of horn bud measurement

3.7.2. Experiment 2: Evaluation of stress associated with different methods of disbudding using IRT

In this experiment, 30 Sahiwal healthy calves, age group of 10-30days were selected randomly assigned into 3 groups each having 10 calves i.e. control (without local anesthesia), T1 (with a local anesthesia) and T2 (with local anesthesia and NSAIDs). On the day of disbudding before the feeding of milk in the morning, calves were moved to the paddock region where they were weighed on the digital electronic balance. After that calves were moved to the individual calf pens before disbudding. Physiological parameters were taken in the calf pens where calves were properly restrained by the attendant. During disbudding hair around the horn bud was trimmed by scissors and trimmer and then an electrically heated hot-iron disbudder at approximately 1000°F (Rhinehart X30, Rhinehart Development Corp., Spencerville, IN) were applied on each horn bud approximately for 10-20 seconds. In the control group, disbudding was done without giving any local anesthesia. In treatment group 1, 10 minutes before disbudding local anesthesia (Lignocaine-HCL 2%; Neon, Tarapur Boisar Dist. Thane), 2 ml of lignocaine was injected subcutaneously at the mid-point between horn bud and external canthus of the eye (to block the corneal nerve) and 3 ml was injected subcutaneously as a ring block around each horn bud along with that medicated Nupatch were applied to the disbudded tissue after disbudding. In treatment group 2, along with local anesthesia lignocaine-HCL, NSAID was given sub-cutaneously @ 1ml/33kg bodyweight for 3 days, along with that medicated nupatch was applied to the disbudded tissue after disbudding. Infrared thermography images were captured for eyeball, horn, muzzle, para-lumber region and peri-anal region 30 minutes before disbudding, immediately after disbudding and after every 30 minutes of disbudding till 180 minutes. The IRT images were taken twice weekly till the healing of horn buds. 2-3 ml blood samples were taken before 40 minutes of disbudding, 40 minutes after disbudding and after 4hr of disbudding for the estimation of plasma cortisol concentration. Disbudded calves were monitored for behaviour through videography by Nikon D3400 DSLR camera for 4 hrs after disbudding. The three groups were considered for further statistical analysis.

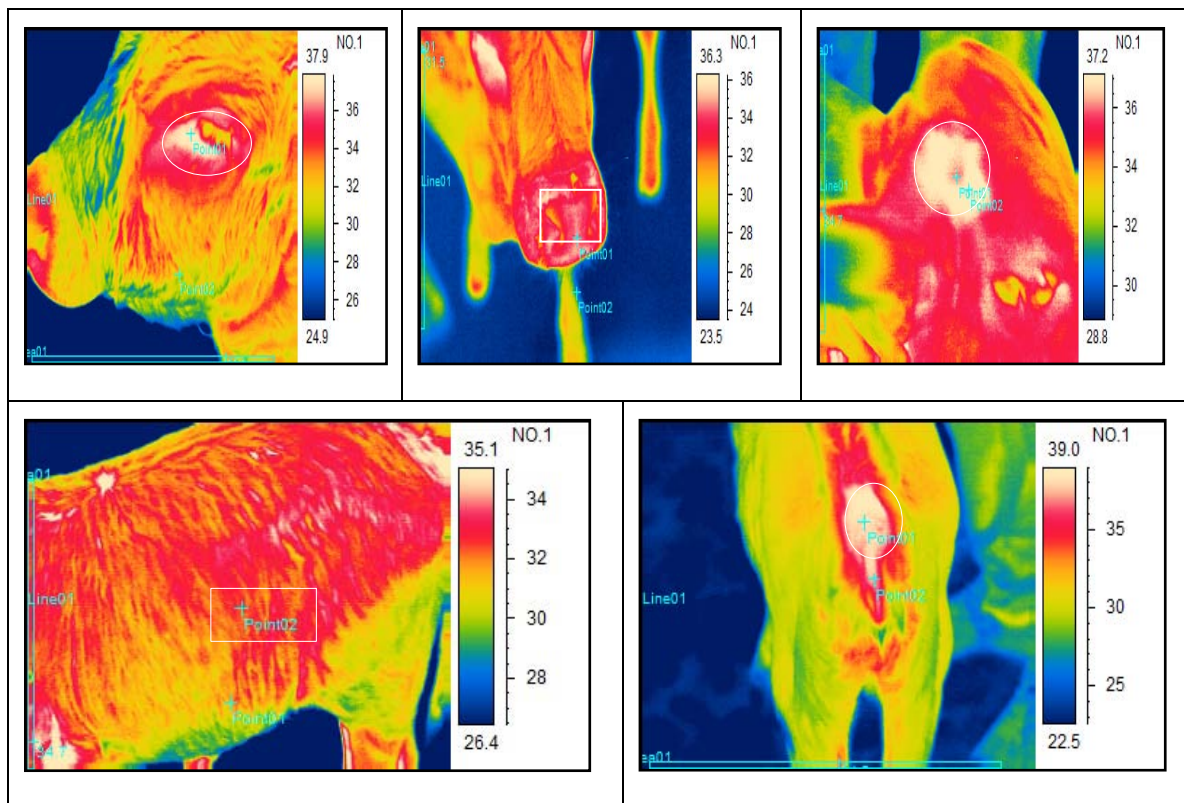
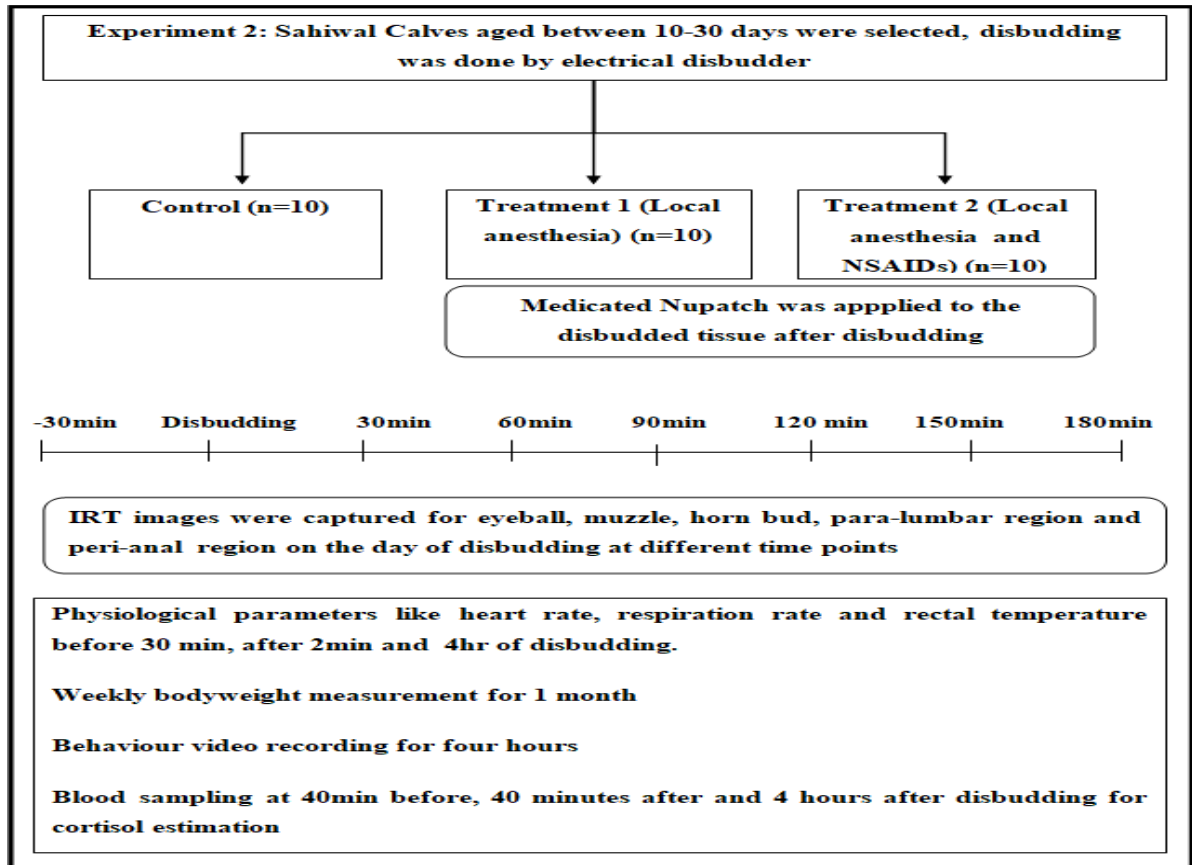


Plate 3.5: Infrared thermal image of ocular, muzzle, horn bud, para-lumbar region and peri-anal region

Materials and Methods

3.7.3. Physiological parameters:

Calves were moved to the individual pens 30 minutes before disbudding to measure the physiological parameters in a stress-free environment. Respiration rate (RR) was measured by observing flank movements to record the time taken for each calf to complete 10 breaths; this was then used to calculate RR (breaths/min). The Heart rate of calves was evaluated by the semiotic technique of listening to heartbeat using a stethoscope at 3th-5th intercostal space for 30 seconds and then multiplying by 2. The animals were auscultated preferably in a standing position and at rest. Rectal temperatures were measured for each animal using a clinical digital thermometer (MC-343; Omron, Kyoto, Japan). The clinical digital thermometer was inserted into the rectum, in contact with the mucosa until stabilization of the temperature, which was recorded in degrees centigrade (°C). Physiological parameters like heart rate were taken before disbudding and twice after the disbudding procedure at 2 minutes and 4 hours of disbudding in the same environment. Respiration rate and rectal temperature were also taken before disbudding and twice after disbudding at 2 minutes and 4 hr of disbudding.

3.7.4. Behaviour Recording:

Behaviour was continuously recorded using a video camera recorder D3400 DSLR camera. The camera was positioned at a height of 1.85 m above the pens so that one camera could monitor three adjacent pens; the cameras were set at an angle of 30 degrees to the vertical attachment pole. Behavioural parameters were selected for inclusion in the ethogram if they were easily identifiable, commonly performed across all calves and were similar at each time of performance. Behaviours that (i) varied widely in intensity and performance characteristics, (ii) were difficult to differentiate from other actions, or (iii) rarely displayed, were omitted. The ethogram was constructed. Descriptions were created based on accurately reflected behaviours exhibited in video recordings, including the duration of the activity and separation times that suggested separate events. The frequency of each behavior was continuously recorded during the 4 hr post-treatment phases. The calves were visible throughout the entire observation period. Approximately 4 hr of video recordings were analyzed using Adobe Premier Pro software (CS6, Version 6.0.0).

Table 3.12: Description of behaviours recorded after disbudding process

Behaviour	Description
Tail wagging frequency	Rapid tail movements from side to side, may include 2–3 movements or last for several seconds, recorded as new event after tail moved slowly or was in resting position
Head moving frequency	Distinct movement or twitch of head despite manual restraint,
Ear flicking frequency	Calf rapidly moves 1 or both ears to the front and back, independent of a head shake, flicking ear with apparent no reason e.g flies
Leg lifting frequency	Lifting of leg, or shifting of weight on leg
Leg to head scratching frequency	Scratching of disbudded wound by the leg

(Stewart *et al.*, 2008)

3.7.5. Body weight:

On the day of disbudding calves were moved to the paddock region where their body weight was taken before the feeding of the morning milk. Bodyweight was taken by the digital electronic weighing balance (Fig 3.3). After disbudding, every week body weight was taken before the feeding of morning milk for one month.

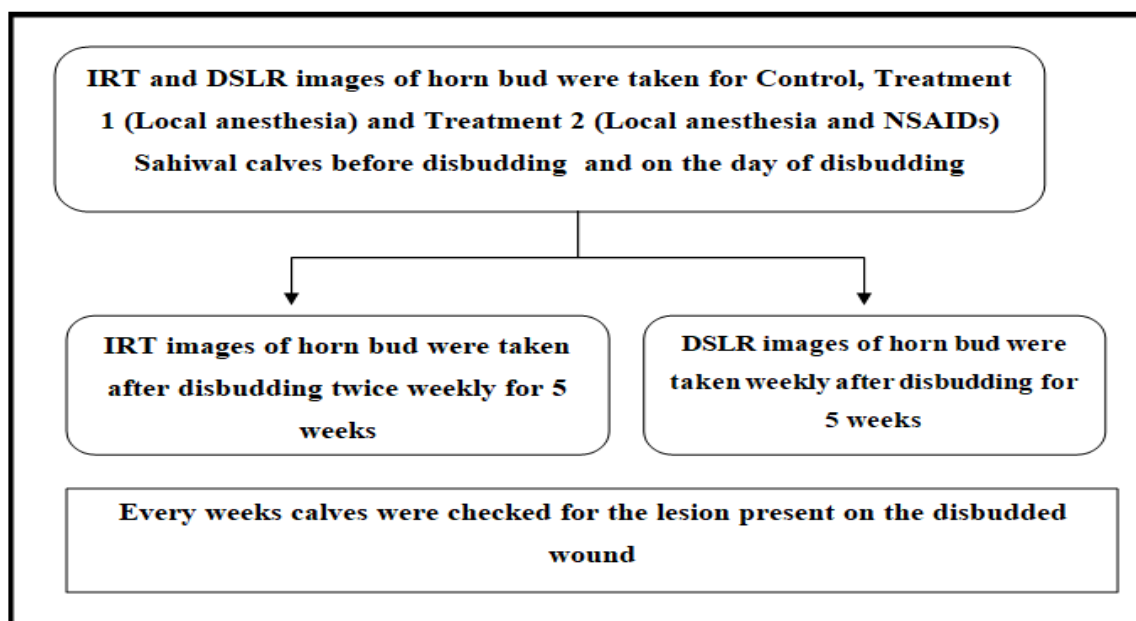


Figure 3.3. Measurement of body weight of calves on an electronic digital balance

Materials and Methods

3.7.6. Healing process:

Calves were moved to the calf shed early in the morning from 7:00 to 9:00 AM to take IR photographs of the wound twice-weekly until the wound was completely re-epithelialized. IRT images of horn bud were also taken before disbudding. Infrared images of horn bud were taken within 1m of distance from the disbudded wound of the calf in a shaded area.



Every week calves were checked for the lesion score (Table 3.13) for the assessment of healing process of horn bud.

Table 3.13: Lesion score used for the healing process of horn buds

Lesion score 1	Normal healing without a scab or discharge present
Lesion score 2	Crusted and scab filled wound or raised scab presents
Lesion score 3	Dried or moist purulent discharge

(Huebner *et al.*, 2017)

3.7.7. Cortisol estimation:

3.7.7.1. Principal:

The kit was based on competitive Enzyme- ELISA detection method [(Bovine COR (Cortisol) ELISA Kit (Wuhan fine biotech Co., Ltd.)]. The micro titer plate provided in with the kit was pre-coated with target. During the reaction, target in the sample or standard competes with a fixed amount of target on the solid phase supporter

for sites on the Biotinylated Detection Antibody. Excess conjugate and unbound sample or standard was washed from the plate, and HRP-Streptavidin (SABC) was added to each micro plate well and incubated. Then TMB substrate solution was added to each well. The enzyme- substrate reaction was terminated by the addition of a sulphuric acid solution and the color change was measured using spectrophotometer at a wavelength of 450nm. The concentration of target in the samples was then determined by comparing the OD of the samples to the standard curve.

3.7.7.2. Sample Collection:

Blood samples were collected thrice in a vacutainer containing anticoagulant (EDTA) before 40 minutes of disbudding, after 40 minutes of disbudding and after 4 hours of disbudding. The samples was centrifuged for 15 minutes at 1000 X g at 2-8 °C within 30 minutes of collection. The supernatant was collected and carried out the assay immediately.

3.7.7.3. Standard preparation:

- ✓ 1 ml sample was added dilution buffer into one standard tube (labeled as zero tube), the tube kept at room temperature for 10 minutes and mixed them thoroughly.
- ✓ Labeled 7 EP tubes with 1/2, 1/4, 1/8, 1/16, 1/32, 1/64 and blank, respectively. Added 0.3ml of the sample with dilution buffer into each tube. Added 0.3ml of the above standard solution (from zero tube) into 1st tube and mixed them thoroughly. Transferred 0.3ml from 1st tube to 2nd tube and mixed them thoroughly. Transferred 0.3ml from 2nd tube to 3rd tube and mixed them thoroughly, and so on. Sample and dilution buffer was used for the blank control.

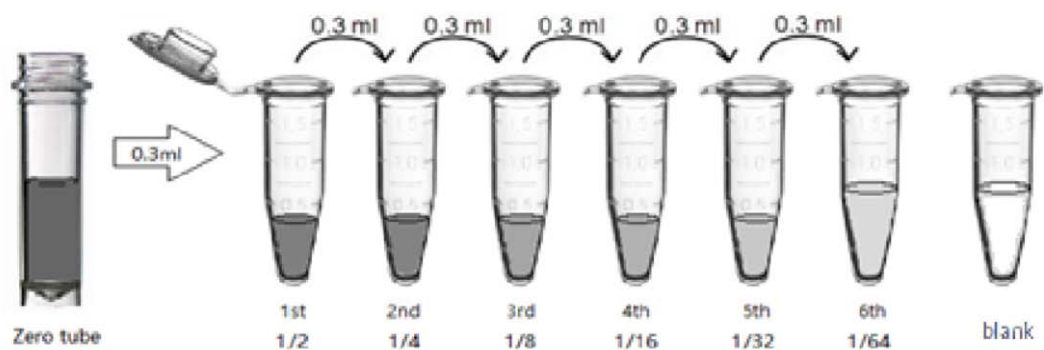


Plate 3.6. Standard Preparation

Materials and Methods

3.7.7.4. Procedure:

During diluting samples and reagents, they must be mixed completely and evenly. Before adding TMB into wells, TMB substrate was equilibrated for 30 minutes at 37°C. A plot as standard curve for each test was devised.

- Standard, test samples, control (blank) wells on the pre-coated plate was loaded, and then recorded their positions. Each standard and sample was measured in duplicate. Plate was washed 2 times before adding standard, sample and control (blank) wells.
- Added 50µl of standard, blank, or sample per well. The blank well added with sample/ standard dilution buffer. Immediately, 50µl Biotin-labeled antibody working solution was added into each well. Covered with the plate sealer of kit. Gently tapped the plate to ensure thorough mixing. Incubated for 45 minutes at 37°C.
- The cover was removed and washed the plate 3 times with wash buffer, and left the wash buffer in the wells for 1 minute each time. After the last wash any remaining wash buffer was removed by aspiration.
- 100 µl HRP-Streptavidin conjugate (SABC) working solution was added into each well. Covered it with a new plate sealer. Incubated for 30 minutes at 37°C.
- Removed the cover and washed the plate 5 times with wash buffer, and left the wash buffer in the wells for 1-2 minutes each time.
- 90µl TMB substrate was added into each well, covered the plate and incubated at 37°C in dark for 10-20 minutes.
- 50µl stop solution was added into each well. The color turned into yellow. Added the stop solution in similar order of adding TMB substrate solution.
- The O.D. was added at 450 nm in micro plate reader immediately after adding the stop solution.

3.7.7.5. Calculation of Result:

A standard curve was constructed by plotting the O.D. at 450 nm of each standard solution (Y) against the respective concentration of the standard solution (X). The target concentration of the samples was interpolated from the standard curve and using the software Curve Expert 1.3.

3.7.8. Thermal image analysis:

The eye, horn, muzzle, para-lumberregion and peri-anal temperature of each photographic episode were analysed by using thermal image analysis software (Dravi TI analysis software). Before this unclear thermal images were excluded from the analysis and the maximum temperature of a particular region of images were recorded and used in the statistical analysis.

3.8. Statistical analysis:

In Experiment 1 of the first objective, the data of eyeball, muzzle, cheek, para-lumbar, peri-anal region temperature and blood profile of healthy calves and calves affected with diseases were analysed by one-way analysis of variance (ANOVA) with post hoc test i.e. Duncan multiple range test (DMRT) to compare between the groups using SPSS 20.0 software (IBM Corporation, Armonk, New York, USA). Distribution of microorganisms in the faecal samples of calf diarrhoea was calculated by percentage and for the clinical signs of the disease score was given.

In Experiment 2, the first objective data of eyeball, muzzle, cheek, paralumbar and peri-anal region temperature from -5 day to +5 day (0 day: affected with disease and clinical sign observed) of healthy calves and calves affected with diseases was analysed by paired sample T-test to compare the difference within the groups using SPSS 20.0 Software (IBM Corporation, Armonk, NewYork, USA). Data of eyeball, muzzle, cheek, para-lumbar and peri-anal region temperature of calves affected with diseases was analysed by paired sample T-test to compare the difference between 0 day (affected with disease and clinical sign observed) and 2 days before as well as seven days average before occurrence of disease. Blood profile of healthy calves and calves affected with diseases were analysed by one-way analysis of variance (ANOVA) with post hoc test i.e. Duncan multiple range test (DMRT) to compare between the groups. Distribution of

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microorganisms in the fecal samples of calf diarrhoea and incidence rate of disease was calculated by percentage and for the clinical signs of the disease score was given.

In Experiment 1, of the second objective, data of horn bud diameter of different age groups were analysed by using one-way analysis of variance (ANOVA) with post hoc Test between the groups using SPSS 20.0 Software (IBM Corporation, Armonk, NewYork, USA). In Experiment 2, of this objective, the data of eyeball, muzzle, horn, para-lumbar, peri-anal region temperature, blood parameters and lesion score during healing process of these groups were analysed by using the two-way analysis of variance (ANOVA) with post hoc test between the groups using SPSS 20.0 Software (IBM Corporation, Armonk, New-York, USA).

CHAPTER -4

Results and Discussion

RESULTS AND DISCUSSION

The results of the present investigation have been presented under the following headings as per the objectives.

4.1. Experimental details and selection of experimental animals:

The study was conducted with the following two objectives and a total of four experiments were conducted under two objectives. Under Objective 1: Evaluation of calf health in dairy animals using infrared thermography, two experiments were conducted. In Experiment 1, data was generated for IRT based ocular and body surface temperature of 29 diseased and 29 healthy dairy calves. In Experiment 2, for early detection of diseases in calves using Infrared thermography, 46 newborn healthy Sahiwal calves were monitored using IRT for 30 days from birth. Under Objective 2: Evaluation of stress associated with different methods of disbudding using infrared thermography in dairy calves, two experiments were conducted. In Experiment 1, data generation of horn bud size in the different age groups of calves was carried out and 33 healthy dairy calves of three different age groups (0-10 days, 11-20 days, 21-30 days) were selected. In Experiment 2, evaluation of stress associated with different methods of disbudding using IRT, 30 healthy Sahiwal calves of 10-30 days age was used.

4.2. Objective 1: Evaluation of calf health in dairy animals using infrared thermography

Two experiments were conducted under this objective 1. In experiment 1, to generate data of IRT based ocular and body surface temperature of 29 diseased (diarrhoea, n=19, pneumonia, n=6 and fever, n=4) and 29 healthy calves were used. In experiment 2, for early detection of diseases in calves using IRT, 46 healthy Sahiwal calves was monitored for 30 days and 13 calves encountered with diarrhoea (n=8), pneumonia (n=2), and fever (n=3) and 33 Sahiwal calves were healthy.

4.2.1. Experiment 1: Data generation of IRT based ocular and body surface temperature of diseased and healthy calves

The experiment was conducted on 29 diseased (diarrhoea, n=19; pneumonia, n=6 and fever, n=4) and 29 healthy calves at Livestock Research Centre, ICAR, NDRI-Karnal, Haryana, India. All Sahiwal calves were maintained under a loose housing system and standard farm management practices.

Results and Discussion

4.2.2. Vital physiological parameters of healthy and calves affected with diarrhoea, pneumonia and fever:

The results of the physiological parameters of the healthy (n=29) and calves affected with diarrhoea (n=19), pneumonia (6) and fever (n=4) have been presented in Table 4.2.1 and Fig 4.1- 4.3.

Table 4.2.1: Mean±S.E. of vital physiological parameters of healthy and calves affected with diarrhoea, pneumonia and fever

Physiological Parameters	Healthy calves (n=29)	Diarrhoea calves (n=19)	Pneumonia calves (6)	Fever calves (n=4)
Respiration rate (Breaths per minute)	26.03 ^a ±0.78	36.42 ^b ±0.69	52.17 ^c ±1.49	52.25 ^c ±1.65
Heart rate (Beats per minute)	104.10 ^a ±1.26	126.74 ^b ±1.85	150.00 ^c ±1.88	116.50 ^d ±2.10
Rectal Temperature (°C)	38.61 ^a ±0.58	39.22 ^b ±0.74	39.89 ^c ±0.03	39.73 ^c ±0.07

Means bearing different superscripts in a row differ significantly ^{abcd}(p<0.05)

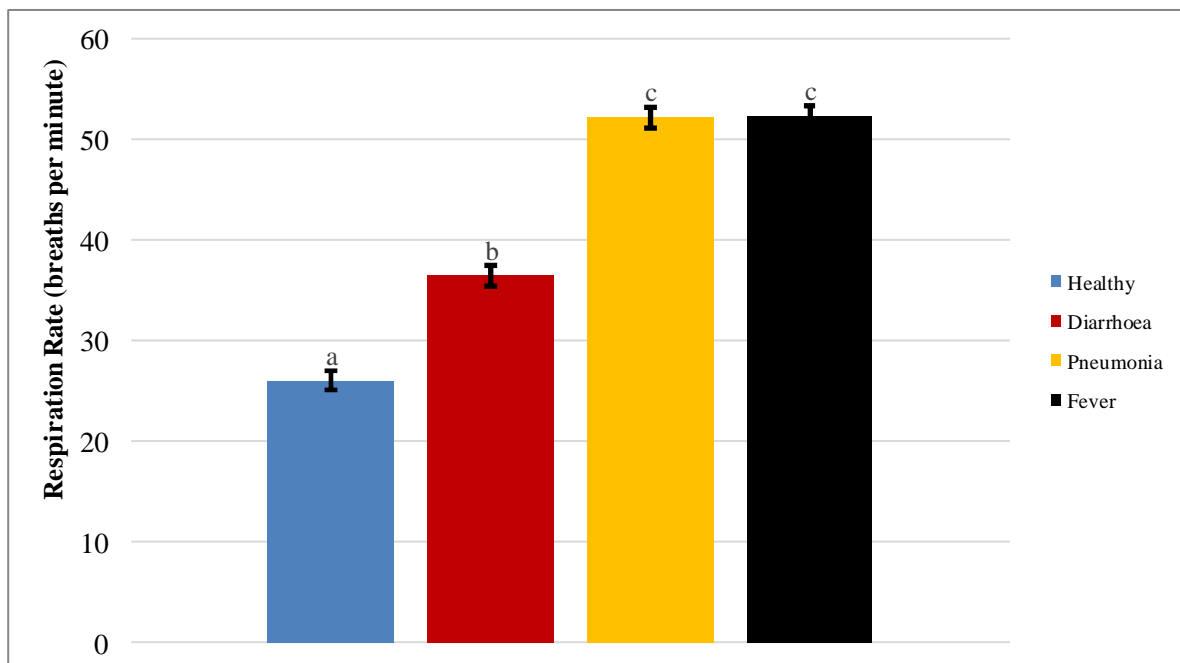


Fig.4.1: Respiration rate (breaths per minute) of healthy calves and calves affected with diarrhoea, pneumonia and fever, Bars bearing different superscripts differ significantly ^{abc}(p<0.05)

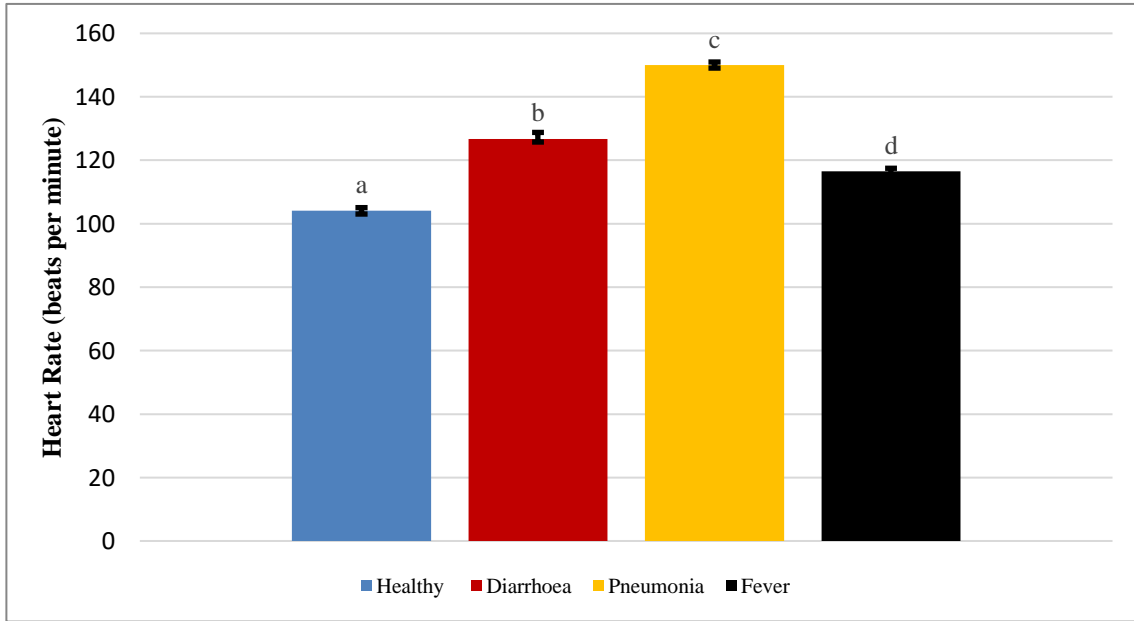


Fig.4.2: Heart rate (beats per minute) of healthy calves and calves affected with diarrhoea, pneumonia and fever, Bars bearing different superscripts differ significantly ^{abcd}($p < 0.05$)

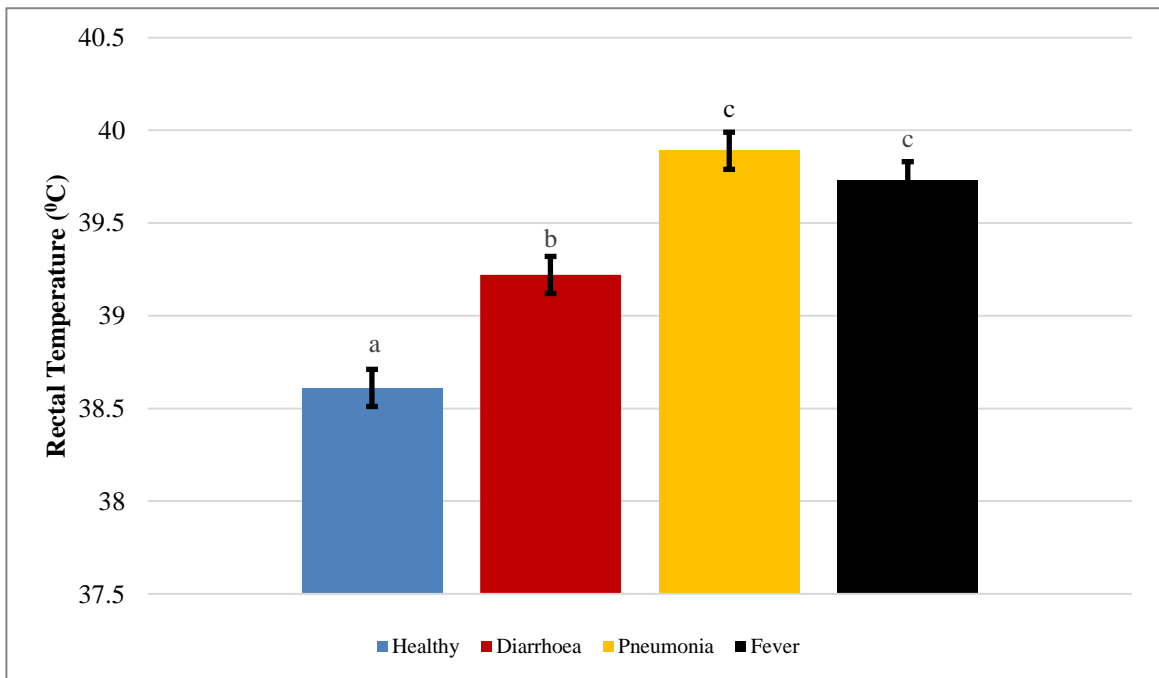


Fig.4.3: Rectal temperature (°C) of healthy calves and calves affected with diarrhoea, pneumonia and fever, Bars bearing different superscripts differ significantly ^{abc}($p < 0.05$)




Results and Discussion

The present study showed a significant change in the physiological parameters of the diseased calves as compared to the healthy calves. Respiration rate (breaths per minute), heart rate (beats per minute) and rectal temperature ($^{\circ}\text{C}$) was significantly ($p<0.05$) higher in calves affected with diarrhoea (36.42 ± 0.69 , 126.74 ± 1.85 , 39.22 ± 0.74), pneumonia (52.17 ± 1.49 , 150.00 ± 1.88 , 39.89 ± 0.03) and fever (52.25 ± 1.65 , 116.50 ± 2.10 , 39.73 ± 0.07) as compared to healthy calves (26.03 ± 0.78 , 104.10 ± 1.26 and 38.61 ± 0.58), respectively. In similar line higher respiration rate, heart rate, and rectal temperature were reported by various researchers in case of calves affected with diarrhoea as compared to healthy calves (Ghanem *et al.*, 2012; Sharma, 2013). Ghanem *et al.* (2012) reported significant increase in the respiration rate (breaths per minute) in diarrhoea calves (38.00 ± 1.20) as compared to healthy (24.80 ± 0.64). Sharma (2013). also reported a significant increase in respiration rate (breaths per minute), heart rate (beats per minute) and rectal temperature ($^{\circ}\text{C}$) in diarrhoea calves (22.10 ± 0.69 , 117.43 ± 0.82 , 102.40 ± 0.63 vs. 17.00 ± 0.78 , 110.20 ± 0.88 , 101.28 ± 0.93) as compared to healthy calves. In pneumonia significantly ($p<0.05$) higher respiration rate, heart rate, and rectal temperature was reported by various researchers (Ramadan *et al.*, 2019; Ozbek and Ozkan, 2020), which is similar to the results of the present findings. Ramadan *et al.* (2019) reported increase in respiration rate (breaths per minute), heart rate (beats per minute) and rectal temperature ($^{\circ}\text{C}$) in pneumonia affected calves (54.25 ± 2.75 , 154.05 ± 2.66 and 40.61 ± 0.09) as compared to healthy calves (29.1 ± 1.15 , 93.3 ± 1.71 and 38.94 ± 0.11). Ozbek and Ozkan (2020) reported a significant increase in respiration rate (breaths per minute), heart rate (beats per minute) and rectal temperature ($^{\circ}\text{C}$) in bovine respiratory diseases (52.40 ± 2.68 , 101.72 ± 3.16 and 39.72 ± 0.10) as compared to healthy calves (37.60 ± 1.12 , 85.60 ± 1.75 and 38.84 ± 0.08). Kachhawa *et al.* (2016) reported significantly ($p<0.05$) higher respiration rate (breaths per minute) (47.4 ± 1.63) and rectal temperature ($^{\circ}\text{F}$) (105.84 ± 0.24) in case of fever, which is similar to the results of the present findings.

The increase in respiration rate of the calves affected with diarrhoea may be due to metabolic acidosis from dehydration or sepsis (Groove-White and White, 2004). Dehydration leads to reduction in blood volume and to maintain the normal pulse rate, heart rate increases as a result of tachycardia. Increase in rectal temperature may be associated with infection agent (Radiostits *et al.*, 2007; Bhalerao *et al.*, 2000).

In pneumonia inflammation of the lung caused by the different organisms increase the core body temperature of the calves and the inflamed lung causes oxidative stress, which has a role in aetiology and pathogenesis along with increase in the heart rate (Ozbek and Ozkan, 2020). The air sacs (alveoli) in the lungs become irritated and fill with fluid or pus as a result of the infection. The oxygen breathing may have a difficult time getting into the bloodstream as a result of infection, which might increase respiration rate in pneumonia (Zambare and Thalkari, 2019).

In fever increased core body temperature leads to increase in respiration rate as body try to reduce the heat caused by fever. Increased respiration is an attempt to increase heat loss by evaporative cooling (Williams and Bellamy, 2008).

<p>Pasty diarrhoea</p>	<p>Sunken eyeball</p>
	
<p>Skin tent test</p>	<p>Active and alert</p>
	





<p>Unilateral nasal discharge</p>	<p>Elevated body temperature</p>
	
<p>Unable to stand</p>	<p>Lacrimation</p>
	

Plate 4.1: Images of different clinical signs in different diseased conditions of Sahiwal calves

4.2.3. Blood parameters of healthy and calves affected with diarrhoea, pneumonia and fever:

Blood parameters of healthy calves (n=29) and calves affected with diarrhoea (n=19), pneumonia (n=6), and fever (n=4) have been presented in Table 4.2.2 and Fig 4.4. Generally, haematological parameters are used to determine general health status; to diagnose, or monitor any diseases and conditions that affect blood cells, such as anaemia, infection and inflammation.

Table 4.2.2: Mean±S.E. of blood parameters of healthy and calves affected with diarrhoea, pneumonia and fever

Blood parameter	Healthy calves (n=29)	Diarrhoea calves (n=19)	Pneumonia calves (n=6)	Fever calves (n=4)
HB (g%)	10.26±0.18	10.39±0.23	10.83±0.16	10.40±0.18
PCV (%)	31.66 ^a ±0.61	38.53 ^b ±0.87	26.67 ^c ±0.67	32.25 ^a ±1.38
TEC (x10 ⁶)	8.70±0.13	8.15±0.17	8.07±0.23	8.41±0.49
TLC (x10 ³)	8.37 ^a ±0.17	17.04 ^b ±0.30	23.22 ^c ±0.94	8.15 ^a ±0.34
Neutrophil (%)	34.69 ^a ±0.49	51.79 ^b ±3.89	64.33 ^{bc} ±0.99	67.50 ^c ±2.63
Lymphocyte (%)	47.55 ^a ±1.15	55.16 ^b ±1.01	32.83 ^c ±0.87	46.75 ^a ±2.02

Means bearing different superscripts in a row differ significantly ^{abc}(p<0.05)

In the present study PCV (%) showed a significant (p<0.05) increase in diarrhoea affected calves (38.53±0.87 vs. 31.66±0.61) as compared to healthy calves. Similar to the present findings significant (p<0.05) increase in PCV (%) was reported by Niaz *et al.* (2000) (36.92±4.33) and Ghanem *et al.* (2012) (37.88 ±4.30) in diarrhoea affected calves. Singh *et al.* (2014) also reported similar findings of significant (p<0.01) increase in PCV (%) (43.8±0.48) might be due to fluid loss from the body of calves in case of dehydration resulted in haemo-concentration of blood. In pneumonia affected calves, a significant (p<0.05) decrease in PCV % as compared to healthy calves (26.67±0.67 vs. 31.66±0.61) was recorded. Ismael *et al.* (2017) had reported similar results of significant decrease in PCV % in case of pneumonia affected calves as compared to the healthy calves (22.26±0.31 vs. 33.73±0.43). The decrease in PCV % in cases of pneumonia might be due to anaemia.

In the present study TLC (x10³/μl) showed a significant (p<0.05) increase in diarrhoea (17.04±0.30 vs. 8.37±0.17) and pneumonia (23.22±0.94 vs. 8.37±0.17) affected calves as compared to healthy calves. In similar line various authors reported significant increase of WBCs in diarrhoea affected calves as compared to healthy calves (15.09 ±1.9 vs. 9.08 ±1.63 10³/μl, Ismael *et al.*, 2017; 28.22±18.10 vs. 13.79±3.41

Results and Discussion

$10^3/\mu\text{l}$, Akgul *et al.*, 2019; 11.36 ± 2.65 vs. 8.91 ± 1.20 $10^3/\mu\text{l}$, Schaefer *et al.*, 2007). Similarly, various authors reported significant increase in WBC in case of pneumonia affected calves as compared to healthy calves (28.22 ± 18.10 vs. 13.79 ± 3.41 $10^3/\mu\text{l}$, Akgul *et al.*, 2019). In case of fever no significant changes was observed for TLC ($\times 10^3$) as compared to healthy calves. In the present study neutrophil (%) showed a significant ($p < 0.05$) increase in diarrhoea (51.79 ± 3.89 vs. 34.69 ± 0.49), pneumonia (64.33 ± 0.99 vs. 34.69 ± 0.49) and fever (67.50 ± 2.63 vs. 34.69 ± 0.49) affected calves as compared to healthy calves. Similar higher neutrophil count was reported by various researchers (Malik *et al.*, 2013; Niaz *et al.*, 2000; Ghanem *et al.*, 2012, Barua *et al.*, 2018). Malik *et al.* (2013) reported that neutrophils (%) count in calves affected with diarrhoea and healthy calves were 46.8 ± 0.75 , and 37 (12-38); Niaz *et al.* (2000) reported 55.35 ± 6.80 and 44.71 ± 3.04 ; Ghanem *et al.* (2012) reported 52 ± 3.8 and 45.8 ± 8.24 ; Barua *et al.* (2018) reported 46.8 ± 0.748 and 12-38, respectively. In pneumonia higher neutrophil count was also reported by various researchers (Almujalli *et al.*, 2015; Pradhan and Gupta, 2018; Akgul *et al.*, 2019). Almujalli *et al.* (2015) reported that neutrophil (%) in calves affected with pneumonia as compared to healthy calves was 68.13 ± 3.34 vs. 44.25 ± 2.35 , Pradhan and Gupta (2018) reported 31.86 ± 4.53 vs. 16.67 ± 1.17 , Akgul *et al.* (2019) reported 10.58 ± 6.54 vs. 3.64 ± 1.14 , respectively. In similar line, Kumar *et al.* (2015) reported a significant ($p < 0.05$) increase in neutrophil (%) in case of fever affected calves as compared to healthy calves (54.63 ± 5.48 vs. 39.50 ± 1.31). In diarrhoea and pneumonia increase in TLC ($\times 10^3$) and neutrophil (%) occurs as a result of infection, inflammatory changes and toxins produced by the microorganism. Increase in TLC count and neutrophil (%) in the blood acts as a defensive mechanism against the pathogen (Mansoor *et al.*, 2018).

Lymphocyte (%) results showed a significant increase in calf diarrhoea (55.16 ± 1.01 vs. 47.55 ± 1.15) and a non-significant decrease in fever affected calves as compared to healthy calves. Various researchers have reported a similar significant increase in lymphocyte (%) in calf diarrhoea (Ghanem *et al.*, 2012; Barua *et al.*, 2018). Ghanem *et al.* (2012) reported significant increase in lymphocyte (%) in calves affected with diarrhoea as compared to healthy calves (57.8 ± 4.56 vs. 44.75 ± 6.63). Barua *et al.* (2018) also reported a significant increase in lymphocyte (%) in calf diarrhoea as compared to healthy calves (51.6 ± 0.514 vs. 33-87). Lymphocyte (%) in calf pneumonia showed a significant decrease as compared to healthy calves (32.83 ± 0.87 vs.

47.55±1.15). In similar line significant decrease in lymphocyte (%) in calves affected with pneumonia as compared to healthy calves was reported by Almujailli *et al.* (2015) (31.25±2.45 vs. 48.25±1.54), Pradhan and Gupta (2018) (44.14±3.68 vs. 75.17±1.62) and Akgul *et al.* (2019) (17.06 ±9.73 vs. 13.18±8.78). In diarrhoea lymphocyte (%) increase might be due to infectious nature of diseases. In case of pneumonia decrease in lymphocyte (%) might be observed due to viral infections and cytotoxic substances released in to the blood stream (Barua *et al.*, 2018).

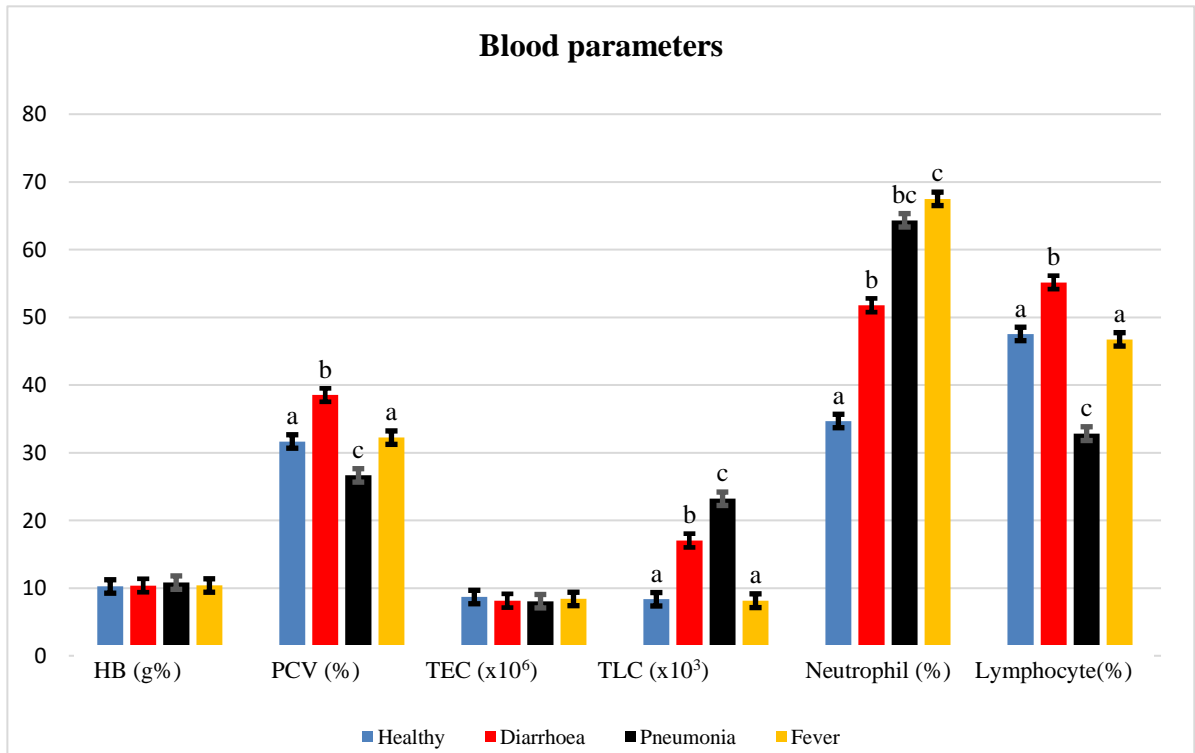


Fig 4.4: Blood parameters of healthy calves and calves affected with diarrhoea, pneumonia and fever, Bars bearing different superscripts differ significantly^{abc}(p<0.05)

4.2.4. IRT muzzle, eyeball, cheek, para-lumbar region, peri-anal region temperatures (°C) of healthy and calves affected with diarrhoea, pneumonia and fever:

IRT temperatures of the eyeball, muzzle, cheek, para-lumbar region and peri-anal region of healthy calves (n=29) and calves affected with diarrhoea (n=19), pneumonia (n=6) and fever (n=4) have been presented in Table 4.2.3 and Fig 4.5. Eyeball temperature showed a significant (p<0.05) increase in calves affected with diarrhoea, pneumonia and fever as compared to healthy calves (37.41±0.18, 37.77±0.09, and 37.25±0.13 vs. 36.34±0.10). Peri-anal region temperature showed a significant (p<0.05)

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increase in calves affected with diarrhoea, pneumonia and fever as compared to healthy calves (38.15 ± 0.08 , 38.65 ± 0.06 , and 38.48 ± 0.26 vs. 37.49 ± 0.08). Para-lumbar region temperature showed a significant ($p < 0.05$) increase in calves affected with diarrhoea and pneumonia as compared to healthy calves (35.22 ± 0.13 and 35.38 ± 0.17 vs. 34.46 ± 0.12). Muzzle and cheek temperature showed non-significant change in case of calves affected with diarrhoea, pneumonia and fever as compared to healthy calves. IR temperature of eyeball showed a significant increase on the day of the clinical onset of diarrhoea (1.07°C), pneumonia (1.43°C) and fever (0.91°C) as compared to healthy calves on the respective day. Significant ($p < 0.05$) increase was observed in case of peri-anal region temperature on the day of the clinical onset of diarrhoea (0.66°C), pneumonia (1.16°C) and fever (0.99°C) as compared to the healthy calves on the respective day. Significant ($p < 0.05$) increase in the para-lumbar region temperature was also recorded on the day of diarrhoea (0.76°C) and pneumonia (0.92°C) as compared to the healthy calves on the respective day. In similar line increase in the eyeball, nose, ear, dorsal and lateral side temperature was reported by Schaefer *et al.* (2004) and Schaefer *et al.* (2007) in case of calves affected with bovine viral diarrhoea and bovine respiratory disease complex. Ragul (2020) also observed significant ($p < 0.01$) increase in eyeball temperature on the day of diarrhoea and a non significant increase of cheek, peri-anal and para-lumbar region temperature as compared to healthy calves. On the contrary significant change for side and shoulder and non-significant change for eye and back in case of induced neonatal calf diarrhoea (NCD) was reported by Lowe *et al.* (2019), but similarity was observed in case of non-significant temperature change for cheek. The differences in the results could be linked to the organisms associated with diseases and their pathogenic pattern in calf diarrhoea, as well as the study's environmental conditions. McCorkell *et al.* (2014) also reported that temperature sensing ear Fever Tag[®] useful for the detection of elevated body temperature as early predictor of BRD.

No published data is available regarding the IR temperature of fever calves to compare findings of the present study. In the present experiment it was observed that IRT was able to detect increase in eyeball, para-lumbar and peri-anal region temperature on the day of clinical onset of diarrhoea, pneumonia and fever. The eyeball temperature is a good indicator of body temperature and it bears lacrimal glands. The eye ball area, had more blood vessels and innervations, were the cause of the rising eye ball

temperature due to pyrexia (Schaefer *et al.*, 2007). The reason for the increase in the para-lumbar region temperature may be due to the inflammation of the intestine or in the surrounding tissue. In similar line, Schaefer *et al.* (2004) reported that the eyeball and para-lumbar region temperature increased before the onset of actual clinical sign in calves affected with bovine viral diarrhoea. In animal's up to 60% of heat loss occurs within the infrared spectrum, the detection of radiated heat loss from the ocular, para-lumbar and peri-anal region served as an early indicator of diseases. They also found that IRT-measured eyeball temperature was more successful in diagnosing bovine viral diarrhoea, with temperature changes occurring as early as one day in the case of the eye, compared to 5-6 days for the nose, neck, body, and hoofs. Before the manifestation of genuine clinical indications, a temperature increases of 1.5 to 4.0°C was noted and it has been well documented that less than 1°C temperature change was clinically significant (P<0.05). Conventional scoring or even other biological tests are often incapable of early detection of infection. But, the minute change in eyeball, peri-anal and para-lumbar region temperature can be observed by using Infrared thermography. Therefore, IRT monitoring of eyeball temperature in case of temperature change related to calf diseases can be used as supportive tool in routine farm management.

Table 4.2.3: Mean±S.E. of IRT muzzle, eyeball, cheek, para-lumbar region, peri-anal region temperature (°C) of healthy and calves affected with diarrhoea, pneumonia and fever

Body point	Healthy calves (n=29)	Diarrhoea calves (n=19)	Pneumonia calves (n=6)	Fever calves (n=4)
Muzzle	34.73±0.11	34.88±0.23	34.45±0.23	34.88±0.20
Eyeball	36.34 ^a ±0.10	37.41 ^b ±0.18	37.77 ^b ±0.09	37.25 ^b ±0.13
Cheek	35.03±0.05	35.17±0.08	35.25±0.15	35.38±0.20
Para-lumbar region	34.46 ^a ±0.12	35.22 ^b ±0.13	35.38 ^b ±0.17	34.53 ^a ±0.21
Peri-anal region	37.49 ^a ±0.08	38.15 ^b ±0.08	38.65 ^b ±0.06	38.48 ^b ±0.26

Means bearing different superscripts in a row differ significantly ^{ab}(p<0.05).

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Infrared thermography has also been used as a non-invasive diagnostic tool in humans and animals to assess physiological conditions and pathological changes in body surface temperature resulting from medication administration, surgical operations, vascularity changes, and both systemic inflammatory responses (fever) and local inflammatory responses associated with disease conditions. Calf diseases have a significant effect on the performance and production level of the animals (Nikkhah, 2015). In calf diarrhoea, disease-producing organisms or their toxin are present in the blood result in septicaemia due to inflammation, which in turn leads to the development of fever or change in body temperature. Calves that develop pneumonia before weaning also share the same risk factors as in the case of calf diarrhoea. Deficiency of colostrum immunity or incomplete transition, extended exposure to poor ventilation, wide variations in daily temperatures, stress from transport or grouping may lead to the development of pneumonia. In the events of calf pneumonia, diarrhoea and fever the change in the body surface temperature can be captured by the infrared thermographic cameras.

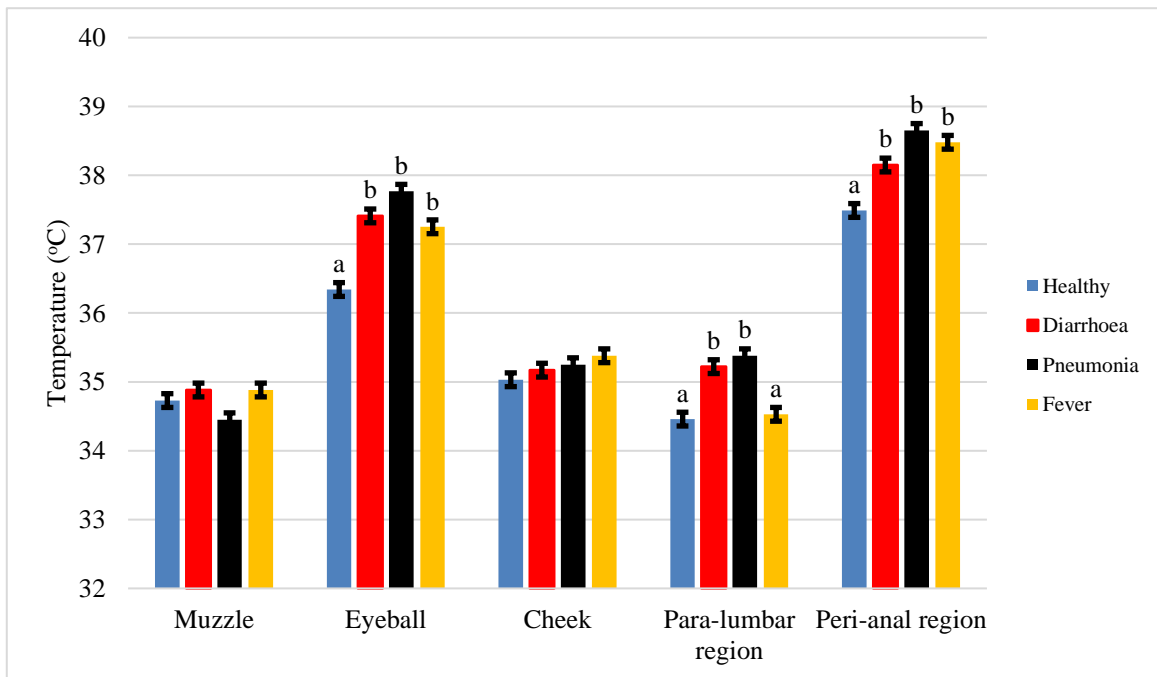


Fig 4.5: IRT muzzle, eyeball, cheek, para-lumbar region and peri-anal region temperatures of healthy and calves affected with diarrhoea, pneumonia and fever, Bars bearing different superscripts differ significantly ^{ab}(p<0.05)

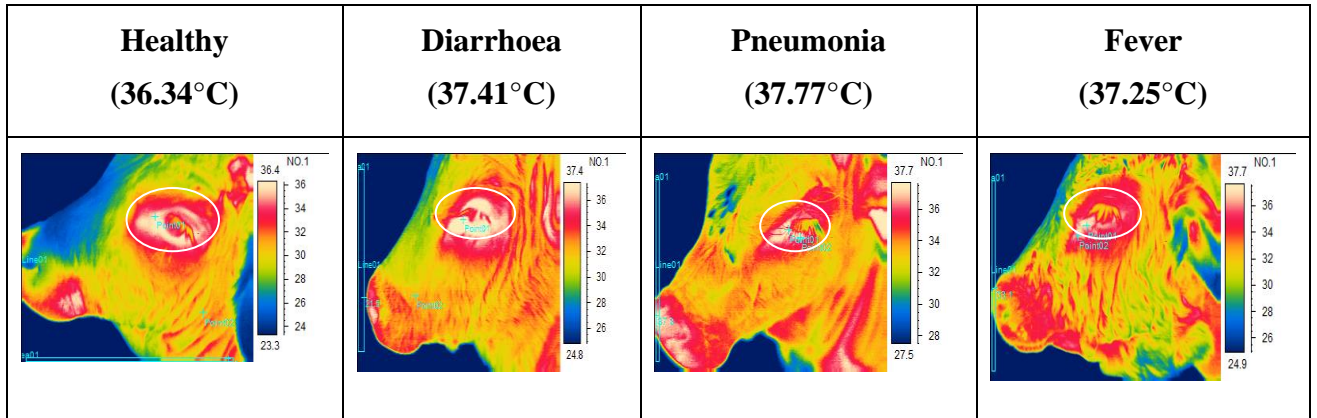


Plate 4.2: IRT eyeball images of healthy calves and calves affected with diarrhoea, pneumonia and fever

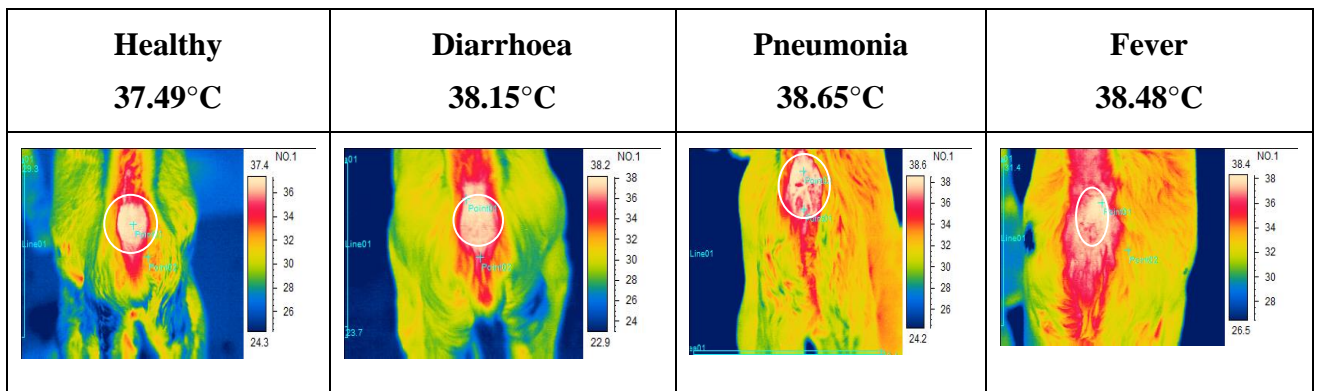


Plate 4.3: IRT peri-anal region images of healthy calves and calves affected with diarrhoea, pneumonia and fever

4.2.5. Clinical signs of diarrhoea and pneumonia in Sahiwal calves:

In the present study, clinical signs of the diarrhoea and pneumonia calves were presented in Table 4.2.4 and Table 4.2.5, respectively. The percentage of these clinical signs was calculated out of the total cases, how many calves showed the specific clinical symptoms of that disease. Clinical findings showed that the most common sign shown by the calves affected with diarrhoea was diarrhoeic faeces (94.73%) ranged from whitish to yellow or greenish colour and tinged with blood or mucus in some cases followed by dehydration (68.42%), anorexia (63.15%), weakness (47.36%) and other signs. Similar clinical signs with varying percentage were recorded by various researchers (Alsaad *et al.*, 2012; Kumar *et al.*, 2010; Devkate *et al.*, 2010). Alsaad *et al.* (2012) reported that calves with BVD showed anorexia (89.39%), profuse watery diarrhoea mixed with mucus/blood (78.78%), dehydration (78.78%), erosive lesions in the oral cavity (65.15%), salivation (60.6%), erosive lesions on the muzzle (48.48%), petechial and

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ecchymotic haemorrhages of the visible mucosa (40.9%), and lacrimation (31.81%). Kumar *et al.* (2010) reported that calves showed dullness, depression with lethargy and anorexia had semisolid to watery faeces with offensive odour, yellowish-white in colour and sometimes blood stained. Manzoor *et al.* (2018) reported signs and symptoms of calf diarrhoea were watery stools, yellow in colour, weak and depressed calves who might lose their desire to nurse, calves developed sunken eye appearance due to dehydration, weight loss, dry muzzle, thick mucus appearing from the nostrils, unable to stand and elevated body temperature (over 39.5°C). Devkate *et al.* (2010) reported anorexia, dullness, sadness, frailty, and halted rumination in case of diarrhoeic animals. The consistency of the faeces ranged from loose to semisolid pasty, with blood tinges suggesting a gastro-intestinal haemorrhage. Drop in tissue fluid levels may create interference or obstruction in tissue metabolism, resulting in a change in appetite in diarrhoea calves (Radostits *et al.*, 2007). The most prevalent cause of dehydration is excessive water and electrolyte loss through the gastrointestinal (GI) tract during diarrhoea and the change in electrolyte balance leads to metabolic acidosis that results in anorexia (Dratwa-Chałupnik *et al.*, 2012). Regardless of the cause of calf diarrhoea, absorption of fluids from the intestine is decreased, leading to life-threatening conditions. Infectious agents of calf diarrhoea cause initial damage to the intestine causing villous atrophy and inflammation of sub-mucosa (Cho and Yoon, 2014), but death in diarrhoea cases usually occurs due to loss of fluids, acidosis, and loss of electrolytes (Cho and Yoon, 2014; Sevennson *et al.*, 2006).

In the present study, the most common signs shown by the calves affected with pneumonia were respiratory distress (83.33%), anorexia (66.66%), nasal discharge (50.00%), congested mucous membrane (50.00%), muzzle dryness (33.33%), and sneezing (16.66%). Various researchers had reported similar clinical findings in pneumonic calves (Ismael *et al.*, 2017; Metwally *et al.*, 2017; Kumar *et al.*, 2018). Major clinical signs of pneumonia affected calves were shallow rapid respiration due to hypoxia and dyspnoea in some cases it might be attributed to severe inflammation in bronchi, bronchioles and alveoli that interfere with gas exchange and respiration. Auscultation of the lung in pneumonia affected calves revealed lung sounds including loud wheezing, crackling sound, moist rales due to the exudates produced by inflammatory cells and goblet cells as a result of pneumonia. In similar line, fever, cough, bilateral muco-purulent nasal discharge, mild depression, in appetence (Mansour

2006; Radostitis *et al.*, 2007) and abnormal sounds (Andrews, 2004) were the symptoms reported by various authors during a clinical examination. Similarly, Manjari *et al.* (2015) reported signs and symptoms of calf pneumonia were increased respiration rate, dullness and depressed, drooping ears, increased body temperature (over 39.5°C), cough, mucus discharge from the nose and lacrimation.

Table 4.2.4: Clinical signs in calves affected with diarrhoea

Clinical signs	No. of cases showing the symptom	No. of diarrhoea cases	Percentage (%)
Diarrhoeic faeces	18	19	94.73
Dehydration	13	19	68.42
Anorexia	12	19	63.15
Weakness	9	19	47.36

Table 4.2.5: Clinical signs in calves affected with pneumonia

Clinical signs	No. of cases showing the symptom	No. of pneumonia cases	Percentage (%)
Respiratory distress	5	6	83.33
Anorexia	4	6	66.66
Nasal discharge	3	6	50.00
Congested mucous membrane	3	6	50.00
Muzzle dryness	2	6	33.33
Sneezing	1	6	16.66

4.2.6. Clinical parameters of healthy and calves affected with diarrhoea and pneumonia

The Mean ± S.E. of clinical parameters of healthy calves and calves with diarrhoea have been presented in Table 4.2.6. Faecal consistency score was significantly ($p < 0.05$) higher in calves with diarrhoea as compared to healthy calves (2.21 ± 0.71 vs.

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0.00±0.00). Clinical examination of calves affected with diarrhoea revealed soft faeces (Score 1) in 1 calf, loose faeces (Score 2) in 13 calves and watery faeces (Score 3) in 5 calves out of 19 calves. Similar changes in fecal consistency of calves affected with diarrhoea have been reported by Bhalerao *et al.* (2000) and Kumar and Mandial (2002). Diarrhoeic calves revealed a wide variety of clinical manifestations. Alteration in the fecal consistency was because of mal-absorption of fluids, electrolytes and other nutrients by the small intestine.

Significant ($p<0.05$) increase in clinical dehydration score in calves affected with diarrhoea as compared to healthy calves (1.16 ± 0.81 vs. 0.00 ± 0.00) was recorded. Clinical examination of dehydration score revealed score 0 in 4 calves, score 1 in 10 calves, score 2 in 4 calves and score 4 in 1 calf. The observation about the dehydration level corroborates with the findings of Kumar and Mandial (2002). Fluid loss from the interstitial and intracellular spaces resulted in loss of normal skin elasticity (Radiostits *et al.*, 2007).

Mean± S.E. of clinical parameters of healthy calves and calves affected with pneumonia has been presented in Table 4.2.7. In the present study significant ($p<0.05$) increase in rectal temperature and nasal discharge score was recorded in calves with pneumonia as compared to healthy calves (2.83 ± 0.40 vs. 0.00 ± 0.00 and 0.67 ± 0.34 vs. 0.00 ± 0.00). Maier *et al.* (2019) reported similar clinical finding of rectal temperature score >2 or ($>39.2^{\circ}\text{C}$) in calves affected with pneumonia. Increase in rectal temperature score was because of the septicaemia caused by the different microorganisms in pneumonia. Nasal discharge might be due to inflammatory changes in the nasal mucus membrane.

Table 4.2.6: Mean ± S.E. of clinical parameters of healthy and calves affected with diarrhoea

Clinical parameters	Healthy (n=19)	Diarrhoea (n=19)
Feecal Consistency score	$0.00^a\pm 0.00$	$2.21^b\pm 0.71$
Clinical Dehydration score	$0.00^a\pm 0.00$	$1.16^b\pm 0.81$

Means bearing different superscripts in a row differ significantly ^{ab}($p<0.05$)

Table 4.2.7: Mean \pm S.E. of clinical parameters of healthy and calves affected with pneumonia

Clinical parameters	Healthy (n=6)	Pneumonia (n=6)
Rectal temperature score	0.00 ^a \pm 0.00	2.83 ^b \pm 0.40
Nasal discharge score	0.00 ^a \pm 0.00	0.67 ^b \pm 0.34

Means bearing different superscripts in a row differ significantly ^{ab}($p < 0.05$)

4.2.7. Micro-organisms associated with calf diarrhoea:

The present research part was conducted to identify and characterize the bacterial pathogens causing calf diarrhoea. The distribution of different micro-organisms in calves affected with diarrhoea has been presented in Table 4.2.8. A total of 19 faecal samples from diarrhoeic calves were collected and then cultured in differential media and examined by using culture properties documented in Table 4.2.9. Result showed that 3 different types of bacteria were isolated from the total of 19 faecal samples collected from diarrhoeic calves. Out of 19 faecal samples, 12 samples were found positive for *E. coli* (63.15%) giving a positive reaction to lactose fermentation on MacConkey agar plate as depicted on Plate 4.5, metallic green sheen colonies on EMB plates as depicted on Plate 4.4. 5 samples were found positive for *Salmonella* (26.31%) producing a negative reaction to lactose fermentation on MacConkey agar plate as depicted in Plate 4.9. The organism produced pale pink colour colonies against a pinkish background on BGA plates as depicted in Plate 4.8. 2 samples were found positive for *Staphylococcus* (10.52%) producing yellowish colonies on Baird Parker agar as depicted on Plate 4.6, haemolysis on Blood agar as depicted on Plate 4.7. Various researchers had reported different frequency distribution of isolates, but the similar bacterial pathogens in case of calves affected with diarrhoea (Ghanem *et al.*, 2012; Kumar *et al.*, 2010; Abdullah *et al.*, 2013; Hemashenpagam *et al.*, 2009; Khan and Khan, 1997) as observed in the present study. Ghanem *et al.* (2012) reported that organisms isolated from diarrhoeic buffalo calves were *E. coli* 44 (73.3%) and *Salmonella spp.* 16 (26.7 %); Kumar *et al.* (2010) reported *E coli* 22 (72.22%), *proteus* 5 (16.66%), *Klebsiella* 2 (2.833%) and unidentified organisms 1 (2.77%). Abdullah *et al.* (2013) reported that organisms isolated from calves affected with diarrhoea was *E. coli* 44 (38.6%), *Salmonella spp* 25 (21.9%), *Staphylococcus spp* 15 (13.2%), mixed infection 18 (15.8%) and 12 (10.5%) were

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negative for bacteria. Hemashenpagam *et al.* (2009) reported that micro-organisms isolated from calves with diarrhoea were *E. coli* 12 (75%), whereas Khan and Khan (1997) isolated *E. coli* (54-58%), *Staphylococcus* (7-10%) and *Salmonella* (13-14%). The variation in the percentage of various organisms causing calf diarrhoea may be due to differences in hygiene management at farm and field level.

Table 4.2.8: Distribution of different micro-organisms in faecal samples of calf diarrhoea

The total no. of samples examined	Name of isolated bacteria	Total no. of positive samples	Frequency of distribution
19	<i>E Coli</i>	12	63.15%
	<i>Salmonella spp.</i>	5	26.31%
	<i>Staphylococcus</i>	2	10.52%

Table 4.2.9: Characterization of isolated bacterial pathogens by culture properties

Name of culture media used	Observation		
	<i>E. coli</i>	<i>Salmonella spp.</i>	<i>Staphylococcus spp.</i>
Nutrient agar	Smooth, circular, white to the greyish colony with peculiar fetid odour	Small, round and smooth colony	Growth of circular, small smooth, convex, and golden yellowish colonies
MacConkey agar	Rose pink lactose fermenter colony	Colourless, pale, translucent colony	No growth (-)
Eosin-Methylene Blue (EMB) agar	Moist circular colonies with dark centres yellow-green metallic sheen	No growth (-)	No growth (-)
Brilliant Green Agar (BGA)	No growth (-)	Pinkish-white or red colonies surrounded by a red halo in the medium	No growth (-)

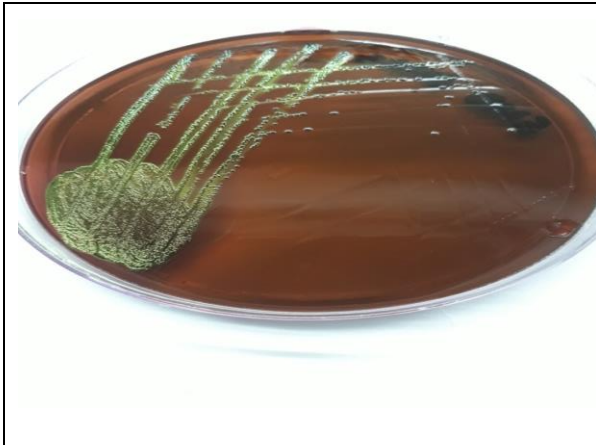


Plate 4.4. Metallic Sheen of *E. coli* on EMB agar



Plate 4.5. Pink colony of *E. coli* on MacConkey agar



Plate 4.6. Staphylococcus on Baird Parker agar



Plate 4.7. Staphylococcus on Blood Agar



Plate 4.8. Salmonella on BGA



Plate 4.9 Colourless colonies of Salmonella on MacConkey agar

4.2.8. Experiment 2: Early detection of diseases in calves using IRT

In Experiment 2, 46 newborn healthy Sahiwal calves were taken from the day of birth. The calves were monitored for 30 days continuously. Infrared thermal images were taken for 30 days continuously around the eyeball area, muzzle, cheek, peri-anal region and para-lumbar region in the morning from 7:00 to 9:00 A.M. under calf shed. Daily rectal temperatures were taken using a clinical digital thermometer. Blood samples were taken for the estimation of haematological parameters from the calves that showed symptoms of calf diarrhoea, pneumonia and fever and similar representative blood samples were taken from the healthy animals. The infected calves were considered as the experimental group and healthy calves were considered as a control group for further analysis.

4.2.9. Day to day variation of the eyeball temperature (°C) between healthy and calves affected with diarrhoea, pneumonia and fever:

The results of eyeball temperature of healthy calves (n=33) and calves affected with diarrhoea (n=8), pneumonia (n=2) and fever (n=3) have been presented in Table 4.2.10. Eyeball temperature of the individual calves varied on day-to-day basis and the variability in a particular day range 36.1 to 37.9°C. No published data is available to reflect the physiologically normal range of the eyeball temperature to compare the result of the present findings. But, the change in eyeball temperature of individual animals was mimicking with the changing pattern of peri-anal, para-lumbar and rectal temperature. The maximum increase of eyeball temperature was observed in case of calves affected with diarrhoea, pneumonia and fever as evident from Fig 4.6 on the day of infection, which was 9th (37.5°C), 12th (37.6°C), 14th(37.5°C), 16th (37.6°C), 20th (37.7°C), 21th (37.6°C), 23th (37.5°C) and 25th (37.6°C) for the calf number 2785, 2882, 2774, 2773, 2771, 2877, 2765 and 2764 in case of diarrhoea, 10th (37.6°C) and 20th (37.8°C) for the calf number 2877 and 2886 in case of pneumonia, 19th (37.9°C), 22th (37.6°C) and 24th (37.7°C) for the calf number 2889, 2783 and 2885 in case of fever respectively. Ragul (2020) reported increased eyeball temperature on the day of clinical onset of diarrhoea in Murrah calves, which is similar to the results of the present finding. Significant increase in the eyeball temperature was also reported by Schafer *et al.*, (2004).The calves affected with diarrhoea, pneumonia and fever showed higher temperature on day to day basis besides the days around diarrhoea, pneumonia and fever.

Table 4.2.10: Mean \pm S.E. of IRT eyeball temperature ($^{\circ}$ C) of healthy and calves affected with diarrhoea, pneumonia and fever

Days	Healthy (n=33)	Calf diarrhoea (n=8)	Calf pneumonia (n=2)	Fever (n=3)
1	35.78 \pm 0.14	36.74 \pm 0.17	37.70 \pm 0.30	37.37 \pm 0.29
2	36.18 \pm 0.11	37.18 \pm 0.14	37.45 \pm 0.55	38.17 \pm 0.13
3	36.37 \pm 0.15	37.11 \pm 0.20	36.80 \pm 0.30	37.50 \pm 0.10
4	36.36 \pm 0.16	37.25 \pm 0.16	37.50 \pm 0.70	37.33 \pm 0.12
5	36.57 \pm 0.11	37.38 \pm 0.32	36.40 \pm 0.30	37.17 \pm 0.85
6	36.60 \pm 0.14	37.09 \pm 0.15	35.95 \pm 0.55	37.5 \pm 0.91
7	36.59 \pm 0.10	37.33 \pm 0.31	36.05 \pm 0.65	37.40 \pm 0.85
8	36.52 \pm 0.15	37.39 \pm 0.21	36.85 \pm 1.25	37.77 \pm 0.55
9	36.45 \pm 0.10	37.09 \pm 0.19	38.30 \pm 0.10	37.90 \pm 0.50
10	36.48 \pm 0.11	37.38 \pm 0.13	36.45 \pm 0.65	37.00 \pm 1.20
11	36.51 \pm 0.17	37.15 \pm 0.38	35.95 \pm 1.25	37.13 \pm 0.95
12	36.58 \pm 0.12	37.50 \pm 0.30	37.65 \pm 0.55	38.27 \pm 0.52
13	36.65 \pm 0.12	37.51 \pm 0.22	37.10 \pm 1.30	38.27 \pm 0.44
14	36.56 \pm 0.12	37.73 \pm 0.28	38.35 \pm 0.55	37.83 \pm 0.54
15	36.75 \pm 0.14	37.25 \pm 0.31	37.10 \pm 0.40	37.63 \pm 0.52
16	36.69 \pm 0.16	37.23 \pm 0.08	38.35 \pm 0.35	38.33 \pm 0.30
17	36.48 \pm 0.13	37.38 \pm 0.30	37.45 \pm 0.25	37.87 \pm 0.43
18	36.68 \pm 0.10	37.38 \pm 0.37	37.40 \pm 0.20	38.67 \pm 0.22
19	36.64 \pm 0.15	37.61 \pm 0.35	37.80 \pm 1.00	39.00 \pm 0.44
20	36.78 \pm 0.13	37.08 \pm 0.37	36.20 \pm 1.80	38.10 \pm 0.25
21	36.73 \pm 0.16	37.41 \pm 0.44	36.65 \pm 1.55	38.20 \pm 0.10
22	36.68 \pm 0.12	37.28 \pm 0.34	37.35 \pm 1.25	39.00 \pm 0.21
23	36.57 \pm 0.13	37.51 \pm 0.28	37.70 \pm 1.10	38.30 \pm 0.44
24	36.53 \pm 0.15	37.23 \pm 0.38	36.90 \pm 1.00	37.93 \pm 0.32
25	36.98 \pm 0.12	37.29 \pm 0.31	36.65 \pm 0.95	38.00 \pm 0.30
26	36.57 \pm 0.17	37.30 \pm 0.46	37.20 \pm 0.70	37.70 \pm 0.15
27	36.92 \pm 0.16	37.64 \pm 0.53	37.45 \pm 1.55	37.83 \pm 0.41
28	36.75 \pm 0.14	37.35 \pm 0.41	37.90 \pm 0.20	37.60 \pm 0.64
29	36.83 \pm 0.12	37.43 \pm 0.22	37.80 \pm 0.7	37.57 \pm 0.27
30	36.60 \pm 0.10	37.12 \pm 0.46	37.65 \pm 0.65	38.03 \pm 0.17

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Rise in eyeball temperature might be due to the septicaemia and viremia, which occurred due to the microorganisms responsible for the disease condition. Lacrimal glands operate as marker for changes in body temperature, according to Schaefer *et al.* (2007). As a result, IRT monitoring of ocular temperature in the event of a temperature change related to calf health can be employed as a supportive tool in routine farm management, albeit more research in a large number of data sets is required. Zanghi (2016) reported that both IR temperature of eye and ear have a strong relationship with rectal temperature, and both were sensitive enough to detect hyperthermia associated with exercise in dogs.

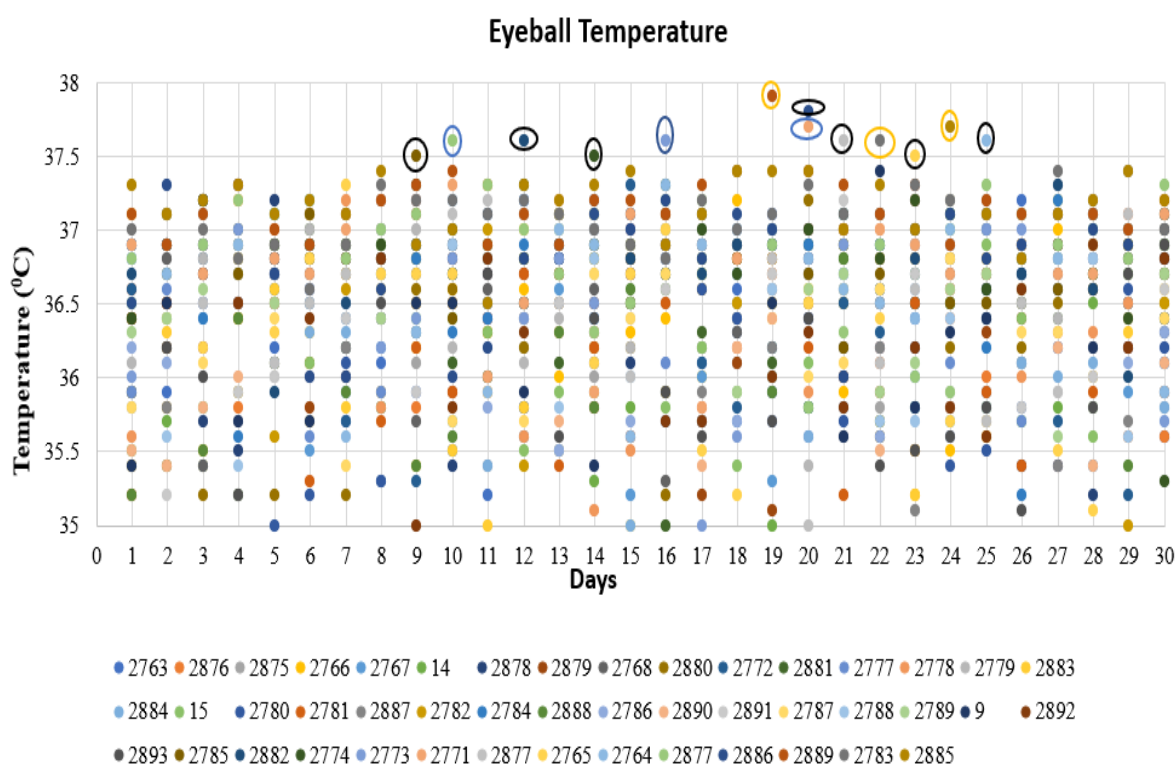


Fig 4.6: IRT eyeball temperature of healthy and calves affected with diarrhoea, pneumonia and fever

4.2.10. Day to day variation of the peri-anal region temperature (°C) between healthy and calves affected with diarrhoea, pneumonia and fever:

The results of peri-anal region temperature of healthy calves (n=33) and calves affected with diarrhoea (n=8), pneumonia (2) and fever (3) have been presented in Table 4.2.11.

Table 4.2.11: Mean \pm S.E. of IRT peri-anal region temperature ($^{\circ}$ C) of healthy and calves affected with diarrhoea, pneumonia and fever

Days	Healthy (n=33)	Calf diarrhoea (n=8)	Calf pneumonia (n=2)	Fever (n=3)
1	37.93 \pm 0.17	38.60 \pm 0.23	38.15 \pm 0.05	38.10 \pm 0.26
2	38.08 \pm 0.18	38.41 \pm 0.33	38.10 \pm 0.80	38.37 \pm 0.12
3	38.23 \pm 0.17	38.46 \pm 0.25	38.55 \pm 0.05	38.43 \pm 0.15
4	38.02 \pm 0.16	38.28 \pm 0.27	38.60 \pm 0.70	38.20 \pm 0.00
5	37.90 \pm 0.15	38.36 \pm 0.25	38.25 \pm 0.35	38.10 \pm 0.53
6	38.03 \pm 0.11	38.20 \pm 0.15	38.35 \pm 0.35	38.33 \pm 0.15
7	38.01 \pm 0.09	38.49 \pm 0.30	38.12 \pm 0.54	38.17 \pm 0.55
8	38.03 \pm 0.15	38.08 \pm 0.26	38.10 \pm 0.40	38.13 \pm 0.22
9	38.20 \pm 0.12	38.21 \pm 0.33	38.70 \pm 0.20	38.53 \pm 0.39
10	38.03 \pm 0.13	38.46 \pm 0.20	38.10 \pm 0.30	38.13 \pm 0.58
11	38.09 \pm 0.14	38.13 \pm 0.37	38.15 \pm 0.05	38.60 \pm 0.06
12	38.14 \pm 0.15	38.23 \pm 0.26	38.60 \pm 0.80	38.30 \pm 0.32
13	38.33 \pm 0.11	38.65 \pm 0.19	38.65 \pm 0.45	38.33 \pm 0.30
14	38.13 \pm 0.13	38.73 \pm 0.28	38.65 \pm 1.05	38.50 \pm 0.45
15	38.06 \pm 0.08	38.19 \pm 0.28	38.10 \pm 0.50	38.40 \pm 0.29
16	37.98 \pm 0.09	38.13 \pm 0.07	38.45 \pm 0.05	38.47 \pm 0.13
17	38.25 \pm 0.14	38.59 \pm 0.20	38.30 \pm 0.10	38.37 \pm 0.44
18	38.27 \pm 0.13	38.56 \pm 0.35	38.30 \pm 0.10	38.87 \pm 0.63
19	38.30 \pm 0.13	38.46 \pm 0.30	38.50 \pm 0.70	38.87 \pm 0.27
20	38.47 \pm 0.09	38.60 \pm 0.15	38.50 \pm 0.30	38.80 \pm 0.50
21	38.15 \pm 0.11	38.40 \pm 0.16	38.40 \pm 0.40	38.27 \pm 0.23
22	38.07 \pm 0.12	38.21 \pm 0.21	38.70 \pm 0.40	38.93 \pm 0.35
23	38.40 \pm 0.15	38.78 \pm 0.21	38.70 \pm 0.60	38.93 \pm 0.37
24	38.28 \pm 0.14	38.59 \pm 0.20	38.35 \pm 0.05	38.73 \pm 0.20
25	38.32 \pm 0.12	38.36 \pm 0.22	38.60 \pm 0.70	38.80 \pm 0.21
26	38.51 \pm 0.09	38.71 \pm 0.17	38.55 \pm 0.15	38.77 \pm 0.38
27	38.58 \pm 0.13	38.60 \pm 0.35	38.90 \pm 0.40	38.60 \pm 0.15
28	38.18 \pm 0.12	38.38 \pm 0.27	38.25 \pm 0.35	38.47 \pm 0.52
29	38.44 \pm 0.13	38.54 \pm 0.26	38.50 \pm 0.10	38.47 \pm 0.43
30	38.10 \pm 0.09	38.15 \pm 0.46	38.25 \pm 0.95	38.50 \pm 0.49

Results and Discussion

The maximum increase of peri-anal region temperature was observed in case of calves affected with diarrhoea, pneumonia and fever as evident from Fig 4.7 on the day of infection, which was 9th (38.8°C), 12th (38.9°C), 14th (38.7°C), 16th (38.8°C), 20th (38.8°C), 21th (38.8°C), 23th (38.9°C) and 25th (38.9°C) for the calf number 2785, 2882, 2774, 2773, 2771, 2877, 2765 and 2764 in case of diarrhoea, 10th (38.6°C) and 20th (38.6°C) for the calf number 2877 and 2886 in case of pneumonia, 19th (38.7°C), 22th (38.8°C) and 24th (38.7°C) for the calf number 2889, 2783 and 2885 in case of fever, respectively. In similar line, Ragul (2020) reported increase in the peri-anal region temperature on the day of diarrhoea. The increase in IRT peri-anal region temperature might be due to the septicaemia and viremia, which occurred due to the microorganisms responsible for the disease condition. No published data is available to compare the results of the present findings with respect to pneumonia and fever.

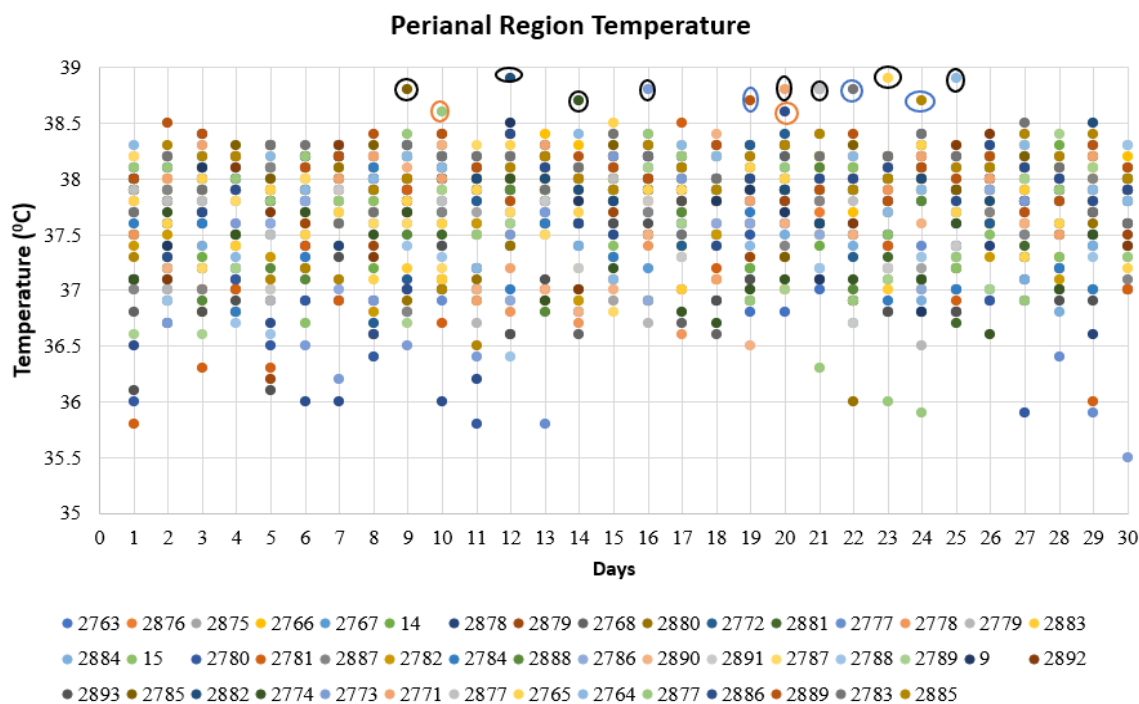


Fig 4.7: IRT peri-anal region temperature of healthy and calves affected with diarrhoea, pneumonia and fever

4.2.11. Day to day variation of the para-lumbar region temperature (°C) between healthy and calves affected with diarrhoea, pneumonia and fever:

The results of para-lumbar region temperature of healthy calves (n=33) and calves affected with diarrhoea (n=8), pneumonia (2) and fever (3) have been presented in Table 4.2.12.

Table 4.2.12: Mean \pm S.E. of IRT para-lumbar region temperature ($^{\circ}$ C) of healthy and calves affected with diarrhoea, pneumonia and fever

Days	Healthy (n=33)	Calf diarrhoea (n=8)	Calf Pneumonia (n=2)	Fever (n=3)
1	34.01 \pm 0.24	34.04 \pm 0.66	34.10 \pm 1.10	34.03 \pm 1.09
2	34.35 \pm 0.21	34.36 \pm 0.42	34.60 \pm 0.10	34.73 \pm 1.11
3	34.14 \pm 0.22	34.58 \pm 0.64	34.50 \pm 0.20	34.50 \pm 0.87
4	34.25 \pm 0.27	34.66 \pm 0.57	34.70 \pm 0.60	34.40 \pm 0.26
5	34.32 \pm 0.23	34.85 \pm 0.50	34.50 \pm 0.70	34.43 \pm 1.21
6	34.61 \pm 0.30	34.69 \pm 0.46	34.70 \pm 1.00	35.53 \pm 0.43
7	34.74 \pm 0.37	34.80 \pm 0.53	35.00 \pm 0.30	34.80 \pm 0.31
8	34.66 \pm 0.23	35.19 \pm 0.75	35.20 \pm 0.20	34.77 \pm 1.10
9	34.96 \pm 0.28	35.09 \pm 0.63	35.30 \pm 0.60	35.17 \pm 0.63
10	34.25 \pm 0.33	34.46 \pm 0.59	34.65 \pm 0.05	35.03 \pm 0.35
11	35.01 \pm 0.27	35.05 \pm 0.38	35.10 \pm 0.10	35.43 \pm 0.18
12	34.90 \pm 0.26	35.01 \pm 0.28	35.40 \pm 1.40	35.57 \pm 0.39
13	34.96 \pm 0.24	35.04 \pm 0.50	35.25 \pm 1.35	35.27 \pm 0.22
14	35.16 \pm 0.15	35.24 \pm 0.34	35.20 \pm 0.80	35.83 \pm 0.37
15	35.00 \pm 0.19	35.06 \pm 0.25	35.40 \pm 0.50	35.60 \pm 0.80
16	34.73 \pm 0.17	34.90 \pm 0.50	35.35 \pm 0.35	35.20 \pm 0.38
17	34.61 \pm 0.25	35.04 \pm 0.52	34.95 \pm 0.05	34.93 \pm 0.74
18	34.76 \pm 0.25	34.89 \pm 0.32	35.00 \pm 0.20	35.00 \pm 0.26
19	34.89 \pm 0.25	35.09 \pm 0.52	34.90 \pm 1.20	34.93 \pm 0.50
20	35.05 \pm 0.25	35.20 \pm 0.32	35.10 \pm 1.40	35.53 \pm 0.38
21	34.94 \pm 0.35	35.10 \pm 0.43	34.95 \pm 0.75	35.57 \pm 0.75
22	34.76 \pm 0.26	35.16 \pm 0.38	34.85 \pm 0.95	35.03 \pm 0.62
23	34.93 \pm 0.20	35.26 \pm 0.50	34.95 \pm 0.05	35.20 \pm 0.47
24	35.02 \pm 0.22	35.14 \pm 0.42	35.10 \pm 1.20	35.53 \pm 0.67
25	34.91 \pm 0.27	35.19 \pm 0.31	35.10 \pm 0.10	35.17 \pm 0.52
26	34.89 \pm 0.27	35.18 \pm 0.67	35.05 \pm 0.15	35.17 \pm 0.61
27	35.05 \pm 0.24	35.10 \pm 0.73	35.35 \pm 0.15	35.83 \pm 1.02
28	35.01 \pm 0.22	35.55 \pm 0.69	35.65 \pm 0.15	35.70 \pm 0.78
29	35.32 \pm 0.24	35.73 \pm 0.54	35.40 \pm 0.00	35.67 \pm 0.86
30	34.91 \pm 0.24	34.94 \pm 0.74	34.95 \pm 1.25	35.00 \pm 1.00

Results and Discussion

The maximum increase in para-lumbar region temperature was observed in case of calves affected with diarrhoea, pneumonia and fever as evident from Fig 4.8 on the day of infection, which was 9th (35.2°C), 12th (35.2°C), 14th (35.3°C), 16th (35.2°C), 20th (35.3°C), 21th (35.5°C), 23th (35.4°C) and 25th (35.3°C) for the calf number 2785, 2882, 2774, 2773, 2771, 2877, 2765 and 2764 in case of diarrhoea, 10th (35.3°C) and 20th (35.2°C) for the calf number 2877 and 2886 in case of pneumonia, 19th (35.3°C), 22th (35.3°C) and 24th (35.3°C) for the calf number 2889, 2783 and 2885 in case of fever, respectively. Ragul (2020) reported increase in para-lumbar region temperature of 0.32°C on the day of diarrhoea as compared to two day before diarrhoea. In similar line increase in side temperature was also reported by Schaefer *et al.* (2004) in case of BVD and Lowe *et al.* (2019) in case of neonatal calf diarrhoea.

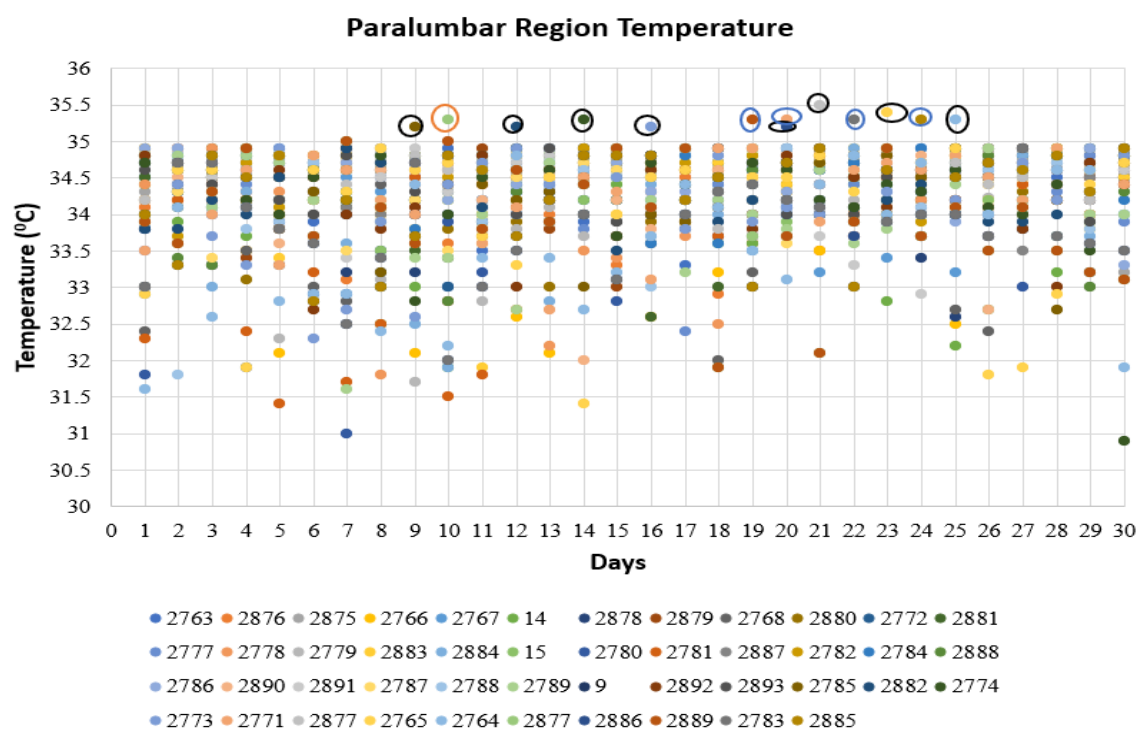


Fig 4.8: IRT para-lumbar region temperature of healthy and calves affected with diarrhoea, pneumonia and fever

4.2.12. Day to day variation of the rectal temperature (°C) by the clinical thermometer between healthy and calves affected with diarrhoea, pneumonia and fever:

The results of the rectal temperature of healthy calves (n=33) and calves affected with diarrhoea (n=8), pneumonia (2) and fever (3) have been presented in Table 4.2.13.

Table 4.2.13: Mean \pm S.E. of rectal temperature ($^{\circ}$ C) by the clinical thermometer of healthy and calves affected with diarrhoea, pneumonia and fever

Days	Healthy (n=33)	Calf Diarrhoea (n=8)	Calf pneumonia (n=2)	Fever (n=3)
1	38.76 \pm 0.06	38.78 \pm 0.11	38.85 \pm 0.05	39.00 \pm 0.06
2	38.94 \pm 0.08	38.96 \pm 0.11	39.06 \pm 0.00	39.04 \pm 0.11
3	39.00 \pm 0.07	39.07 \pm 0.10	39.06 \pm 0.16	39.05 \pm 0.19
4	39.01 \pm 0.06	39.03 \pm 0.12	39.20 \pm 0.13	39.22 \pm 0.14
5	38.89 \pm 0.07	38.99 \pm 0.11	39.04 \pm 0.14	39.02 \pm 0.18
6	39.06 \pm 0.07	39.09 \pm 0.17	39.25 \pm 0.15	39.16 \pm 0.32
7	39.00 \pm 0.05	39.03 \pm 0.12	39.07 \pm 0.23	39.17 \pm 0.17
8	38.93 \pm 0.08	38.97 \pm 0.17	39.05 \pm 0.15	38.98 \pm 0.04
9	38.95 \pm 0.07	39.03 \pm 0.14	39.03 \pm 0.41	39.00 \pm 0.06
10	38.93 \pm 0.06	38.97 \pm 0.13	39.15 \pm 0.25	39.07 \pm 0.17
11	39.03 \pm 0.06	39.24 \pm 0.18	39.44 \pm 0.50	39.10 \pm 0.11
12	38.99 \pm 0.08	39.13 \pm 0.08	39.20 \pm 0.09	39.14 \pm 0.12
13	39.03 \pm 0.08	39.07 \pm 0.16	39.16 \pm 0.05	39.07 \pm 0.20
14	38.95 \pm 0.07	39.10 \pm 0.19	39.31 \pm 0.70	39.19 \pm 0.21
15	38.98 \pm 0.06	39.29 \pm 0.11	39.00 \pm 0.00	39.07 \pm 0.19
16	38.97 \pm 0.07	39.18 \pm 0.16	39.00 \pm 0.28	39.21 \pm 0.26
17	39.00 \pm 0.07	39.11 \pm 0.10	39.06 \pm 0.34	39.91 \pm 0.19
18	38.86 \pm 0.06	39.08 \pm 0.16	39.05 \pm 0.05	39.06 \pm 0.11
19	39.01 \pm 0.06	39.14 \pm 0.09	39.25 \pm 0.25	39.19 \pm 0.15
20	38.97 \pm 0.06	39.31 \pm 0.11	39.09 \pm 0.09	39.13 \pm 0.33
21	38.99 \pm 0.05	39.26 \pm 0.19	39.16 \pm 0.06	39.27 \pm 0.13
22	39.10 \pm 0.04	39.21 \pm 0.13	39.29 \pm 0.01	39.28 \pm 0.09
23	39.00 \pm 0.07	39.19 \pm 0.07	39.20 \pm 0.10	39.15 \pm 0.05
24	38.99 \pm 0.06	39.15 \pm 0.11	39.03 \pm 0.03	39.35 \pm 0.07
25	39.00 \pm 0.07	38.99 \pm 0.10	39.38 \pm 0.39	39.26 \pm 0.13
26	39.00 \pm 0.06	39.12 \pm 0.08	39.05 \pm 0.05	39.38 \pm 0.22
27	39.05 \pm 0.07	39.22 \pm 0.09	39.14 \pm 0.14	39.28 \pm 0.06
28	39.00 \pm 0.06	39.06 \pm 0.13	39.25 \pm 0.15	39.26 \pm 0.17
29	38.90 \pm 0.05	39.08 \pm 0.12	39.20 \pm 0.20	39.28 \pm 0.13
30	38.94 \pm 0.06	38.95 \pm 0.16	39.15 \pm 0.05	39.22 \pm 0.10

Results and Discussion

The maximum increase of rectal temperature was observed in case of calves affected with diarrhoea, pneumonia and fever as evident from Fig 4.9 on the day of infection, which was 9th (38.70°C), 12th (39.80°C), 14th(39.70°C), 16th (39.80°C), 20th (39.70°C), 21th (39.70°C), 23th (39.70°C) and 25th (39.80°C) for the calf number 2785, 2882, 2774, 2773, 2771, 2877, 2765 and 2764 in case of diarrhoea, 10th (39.70°C) and 20th (39.80°C) for the calf number 2877 and 2886 in case of pneumonia, 19th (39.70°C), 22th (39.60°C) and 24th (39.80°C) for the calf number 2889, 2783 and 2885 in case of fever, respectively. Individual calves' rectal temperatures fluctuated from morning to evening within the physiologically normal range of core body temperature. Ragul (2020) reported increase in rectal temperature of 1.12°C on the day of diarrhoea as compared to two days before diarrhoea. In similar line, Sharma (2013) reported significant ($p < 0.05$) increase in rectal temperature (°F) in calves affected with diarrhoea (102.40 ± 0.63). The result of the present findings was in consonance with the observations of various researchers, reported increase in rectal temperature (°C) in calves affected with pneumonia (40.61 ± 0.09 , Ramadan *et al.*, 2019; 39.72 ± 0.10 , Ozbek and Ozkan, 2020). Kachhawa *et al.* (2016) reported significant increase in rectal temperature of calves with fever (105.84 ± 0.24 °F), which is corroborating with the results of the present finding in case of fever. Previous studies (Knauer *et al.*, 2017; Mahendran *et al.*, 2017) used a clinical scoring system for diagnosing ill-health in calves that used a specified rectal temperature threshold (39.5°C) for diagnosis of pyrexia (McGuirk and Peek, 2014). In the present study rectal temperature of calves affected with diarrhoea, pneumonia and fever crossed the threshold (39.5°C).

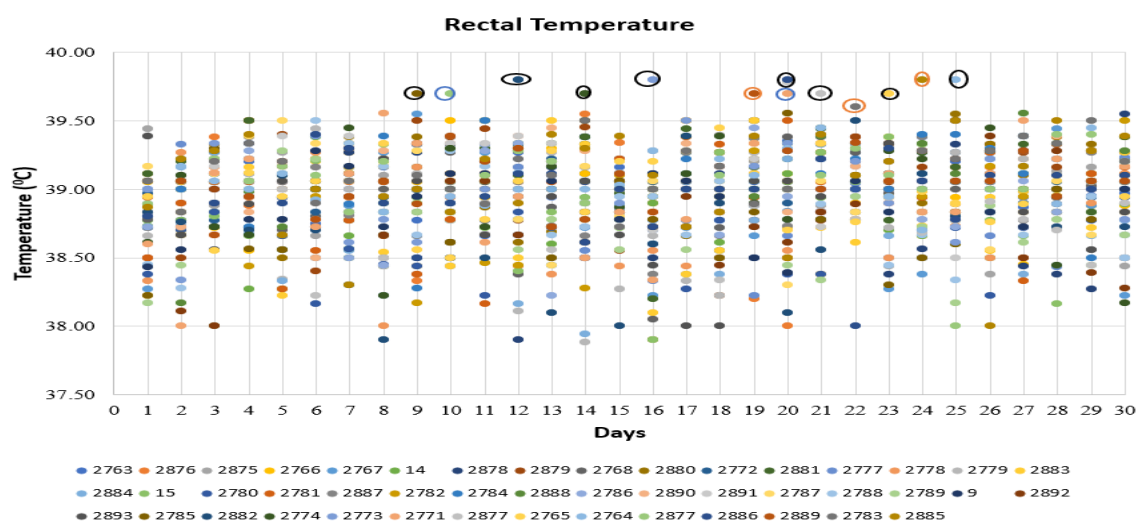


Fig 4.9: Rectal temperature of healthy and calves affected with diarrhoea, pneumonia and fever

4.2.13. Eyeball temperature (°C) of 5 days before and 5 days after diarrhoea between healthy and calves affected with diarrhoea:

Calves affected with diarrhoea on the day were taken as 0 day and the previous five days and after five days data was taken for further analysis. The corresponding day's data of healthy calves were taken as control. The results of eyeball temperature (°C) of 5 days before and 5 days after diarrhoea between healthy and calves affected with diarrhoea have been presented in Table 4.2.14. Clearly depicting from Fig 4.10 and Plate 4.3, eyeball temperature (°C) was significantly ($p < 0.05$) higher, on one day before (37.69 ± 0.08 vs. 36.64 ± 0.21), on the day (37.70 ± 0.06 vs. 36.71 ± 0.18) and one day after diarrhoea (37.48 ± 0.05 vs. 36.79 ± 0.12) in case of calves affected with diarrhoea as compared to healthy calves.

Table 4.2.14: Mean \pm S.E. of IRT eyeball temperature (°C) of 5 days before and 5 days after diarrhoea between healthy (n=33) and calves affected with diarrhoea (n=8)

Days	Healthy	Calf diarrhoea
-5day	36.42 \pm 0.1	36.73 \pm 0.2
-4day	36.73 \pm 0.13	37.04 \pm 0.05
-3day	36.90 \pm 0.07	37.08 \pm 0.06
-2day	36.91 \pm 0.14	37.10 \pm 0.08
-1day	36.64 ^a \pm 0.21	37.69 ^b \pm 0.08
0 day	36.71 ^a \pm 0.18	37.70 ^b \pm 0.06
1day	36.79 ^a \pm 0.12	37.48 ^b \pm 0.05
2day	36.70 \pm 0.11	37.00 \pm 0.11
3day	36.60 \pm 0.15	36.98 \pm 0.08
4day	36.81 \pm 0.14	36.94 \pm 0.08
5day	36.70 \pm 0.18	36.78 \pm 0.05

Means bearing different superscripts in a row differ significantly ^{ab} ($p < 0.05$)

Results and Discussion

Similar to the present findings, Schaefer *et al.* (2004) observed an elevation in ocular temperature in calves affected with bovine viral diarrhoea. Similarly, Ragul (2020) observed that eyeball temperature was significantly ($p < 0.05$) higher, one day before (0.66°C), on the day (0.55°C), and one day after diarrhoea (0.45°C). On the contrary Lowe *et al.* (2019) reported non-significant increase in eyeball temperature in case of induced newborn calf diarrhoea (NCD) prior to actual clinical signs occurs for calves. Differences in the results could be linked to the organisms involved and their pathogenic pattern in calf diarrhoea, severity of diarrhoea as well as the study's environmental conditions. On other days, there was no discernible difference in ocular temperature was observed.

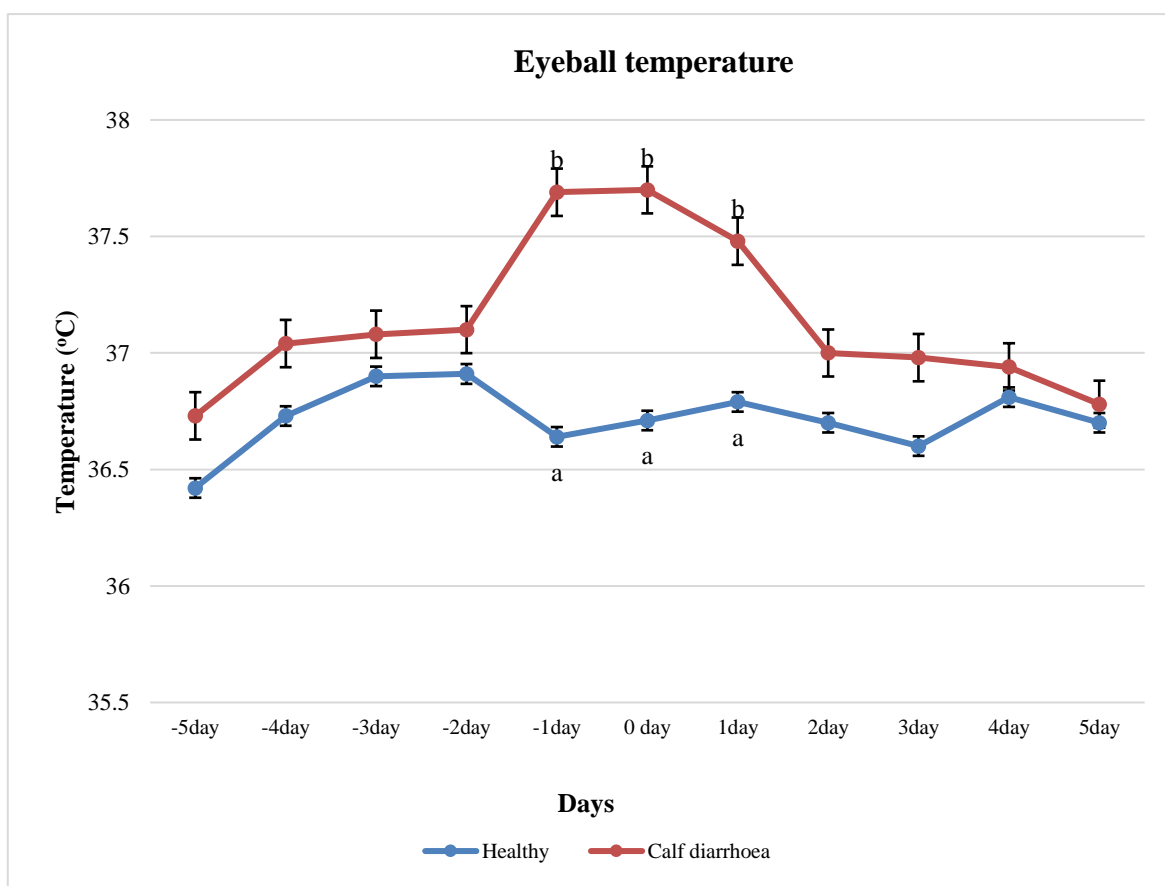


Fig. 4.10: IRT eyeball temperature ($^{\circ}\text{C}$) of 5 days before and 5 days after diarrhoea between healthy and calves affected with diarrhoea, Lines bearing different superscripts differs significantly ^{ab}($p < 0.05$)

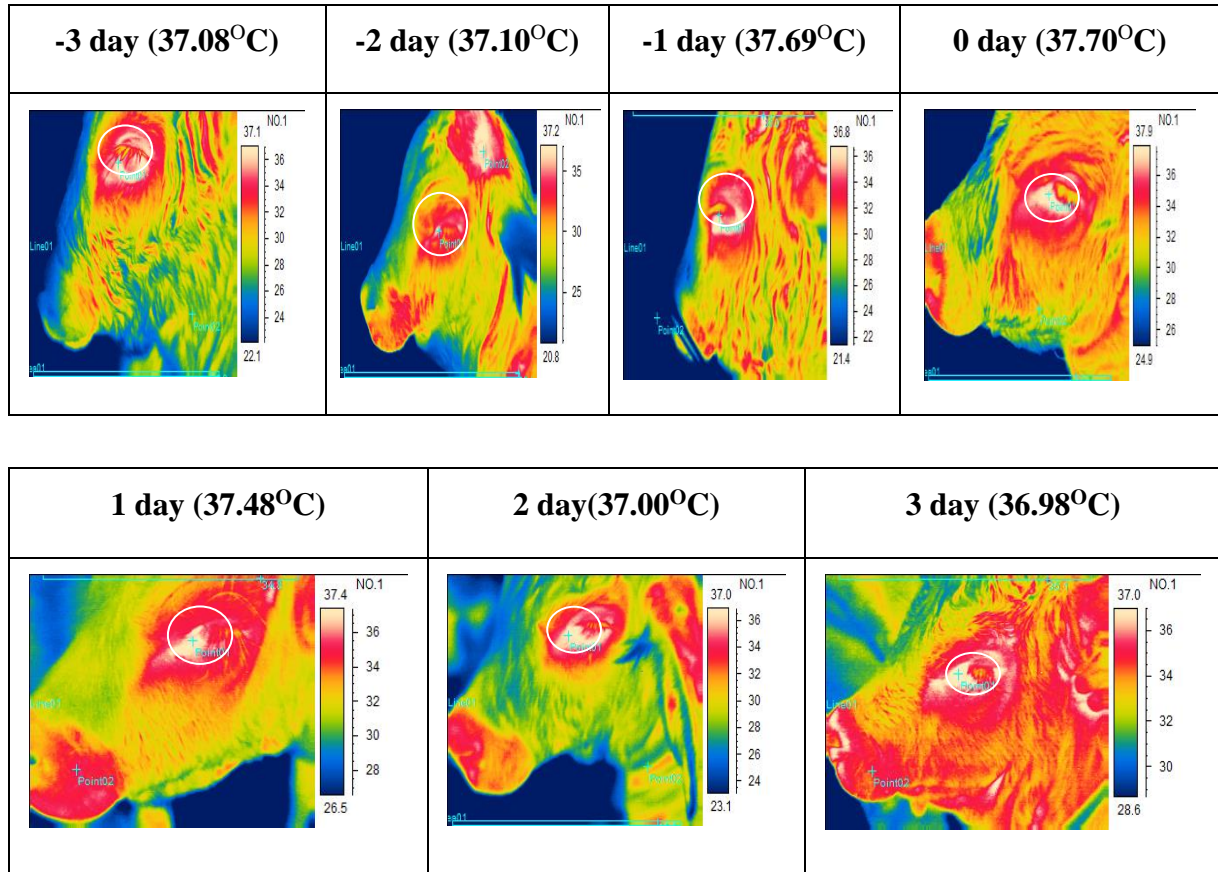


Plate 4.10: IRT eyeball temperature (°C) of calves affected with diarrhoea

4.2.14. Eyeball temperature (°C) of 5 days before and 5 days after pneumonia between healthy and calves affected with pneumonia:

The calves affected with pneumonia on the day were taken as 0 day and the previous five days and after five days data was taken for further analysis. The corresponding day's data of healthy calves were taken as control. The results of eyeball temperature (°C) of 5 days before and 5 days after pneumonia between healthy and calves affected with pneumonia have been presented in Table 4.2.15. Clearly depicting from Fig 4.11, eyeball temperature (°C) was significantly ($p < 0.05$) higher, one day before (37.57 ± 0.05 vs. 36.52 ± 0.13), on the day (37.85 ± 0.35 vs. 36.65 ± 0.25) and one day after pneumonia (37.71 ± 0.06 vs. 36.53 ± 0.00) in case of calves affected with pneumonia as compared to healthy calves. Similar to the findings of the present study, Schaefer *et al.* (2007) observed an elevation in ocular temperature in calves affected with bovine respiratory disease complex. On other days, there was no discernible difference in ocular temperature was observed.

Results and Discussion

Table 4.2.15: Mean \pm S.E. of IRT eyeball temperature ($^{\circ}$ C) of 5 days before and 5 days after pneumonia between healthy (n=33) and calves affected with pneumonia (n=2)

Days	Healthy	Calf pneumonia
-5day	36.76 \pm 0.18	37.05 \pm 0.05
-4day	36.88 \pm 0.22	37.16 \pm 0.04
-3day	36.80 \pm 0.18	37.25 \pm 0.15
-2day	36.60 \pm 0.20	37.29 \pm 0.31
-1day	36.52 ^a \pm 0.13	37.57 ^b \pm 0.05
0 day	36.65 ^a \pm 0.25	37.85 ^b \pm 0.35
1day	36.53 ^a \pm 0.00	37.71 ^b \pm 0.06
2day	36.60 \pm 0.34	37.50 \pm 0.10
3day	36.70 \pm 0.01	37.35 \pm 0.05
4day	36.68 \pm 0.15	37.20 \pm 0.10
5day	36.69 \pm 0.23	37.05 \pm 0.05

Means bearing different superscripts in a row differ significantly ^{ab} (p<0.05)

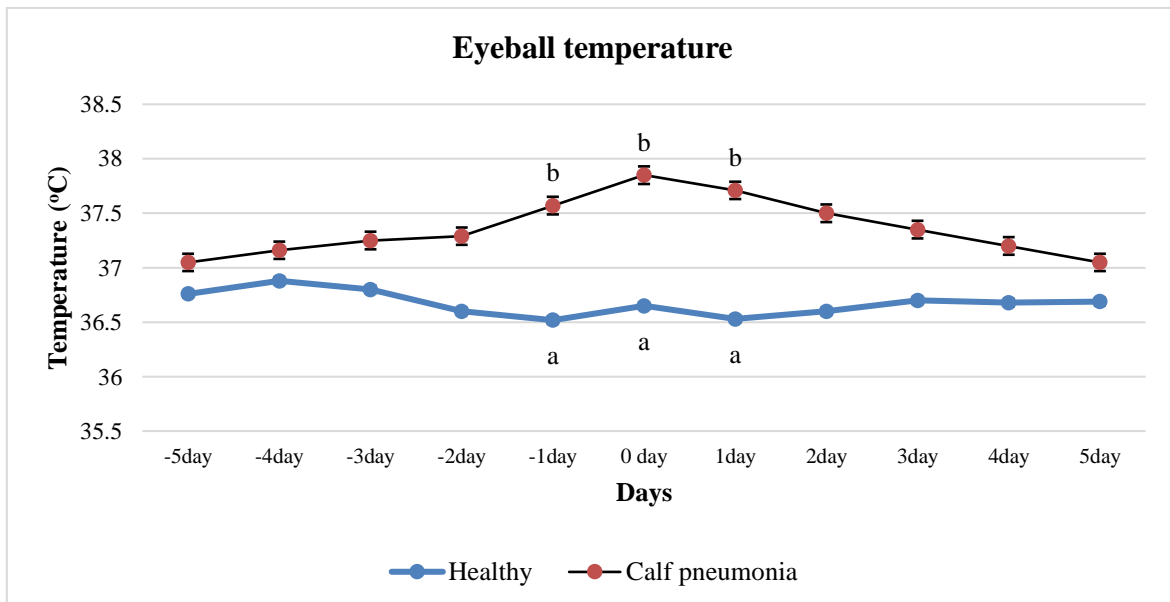


Fig. 4.11: IRT eyeball temperature ($^{\circ}$ C) of 5 days before and 5 days after pneumonia between healthy and calves affected with pneumonia, Lines bearing different superscripts differ significantly ^{ab}(p<0.05)

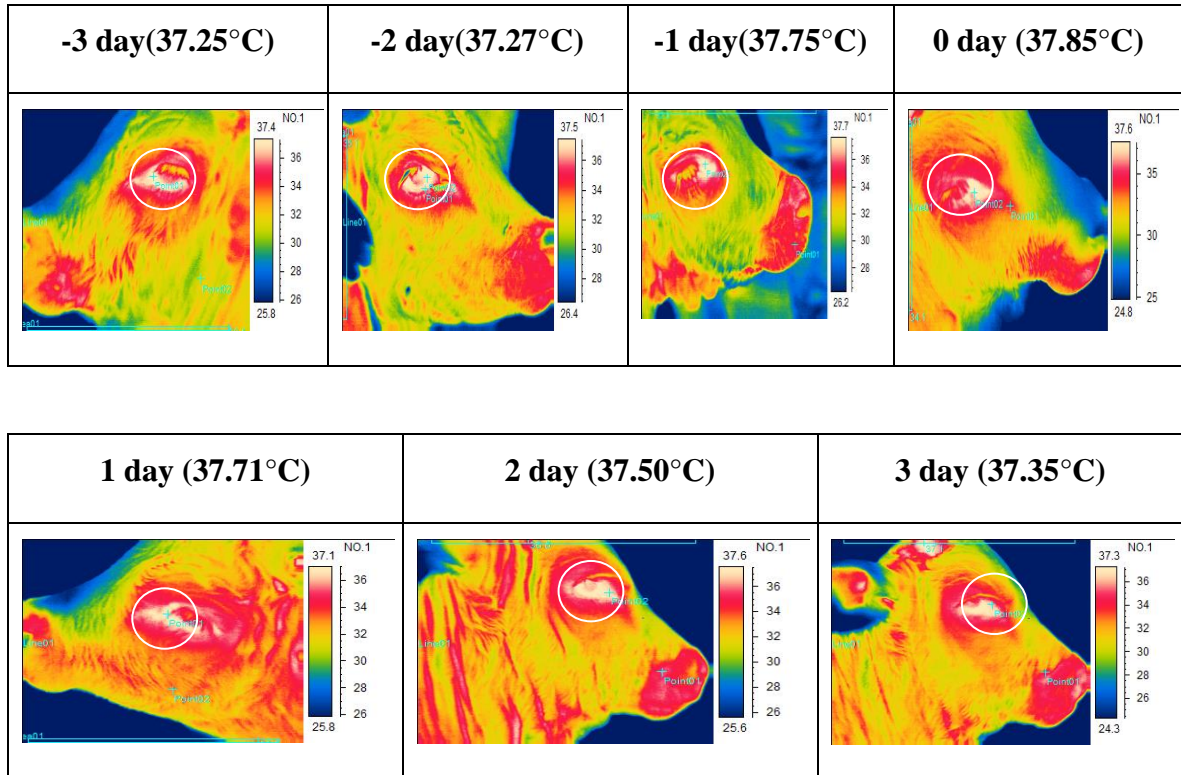


Plate 4.11: IRT eyeball temperature (°C) of calves affected with pneumonia

4.2.15. Eyeball temperature (°C) of 5 days before and 5 days after fever between healthy and calves affected with fever:

Calves affected with fever on the day were taken as 0 day and the previous five days and after five days data was taken for further analysis. The corresponding day’s data of healthy calves were taken as control. The results of eyeball temperature (°C) of 5 days before and 5 days after fever between healthy and calves affected with fever have been presented in Table 4.2.16. Clearly depicting from fig 4.12, Eyeball temperature was significantly ($p<0.05$) higher, on the day (37.73 ± 0.46 vs. 36.60 ± 0.36) of fever as compared to healthy calves. On other days, there was no discernible difference in ocular temperature was observed. In diarrhoea, pneumonia and fever, infection associated with both the bacteria and the virus was linked to septicaemia and viremia, which could explain the rise in orbital temperature of eyeball. No published data is available regarding eye ball temperature of calves with fever to compare the findings of the present study with others.

Results and Discussion

Table 4.2.16: Mean \pm S.E of IRT Eyeball temperature ($^{\circ}$ C) of 5 days before and 5 days after fever between healthy calves (n=33) and calves affected with fever (n=3)

Days	Healthy	Fever
-5day	36.67 \pm 0.19	36.87 \pm 0.28
-4day	36.60 \pm 0.35	37.03 \pm 0.44
-3day	36.80 \pm 0.42	37.09 \pm 0.36
-2day	36.87 \pm 0.20	37.10 \pm 0.06
-1day	36.70 \pm 0.65	37.27 \pm 0.19
0 day	36.60 ^a \pm 0.36	37.73 ^b \pm 0.46
1day	36.70 \pm 0.43	37.22 \pm 0.28
2day	36.71 \pm 0.28	37.10 \pm 0.38
3day	36.56 \pm 0.56	36.80 \pm 0.45
4day	36.39 \pm 0.44	36.81 \pm 0.32
5day	36.40 \pm 0.27	36.88 \pm 0.06

Mean bearing different superscripts in a row differ significantly ^{ab} (p<0.05)

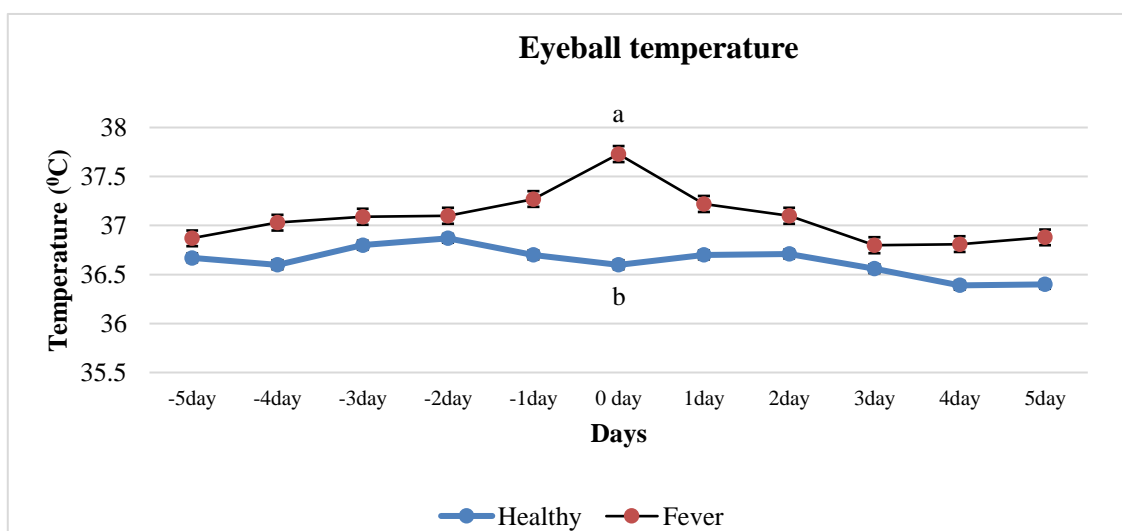


Fig. 4.12: IRT eyeball temperature ($^{\circ}$ C) of 5 days before and 5 days after fever between healthy and calves affected with fever, Lines bearing different superscripts differ significantly ^{ab}(p<0.05)

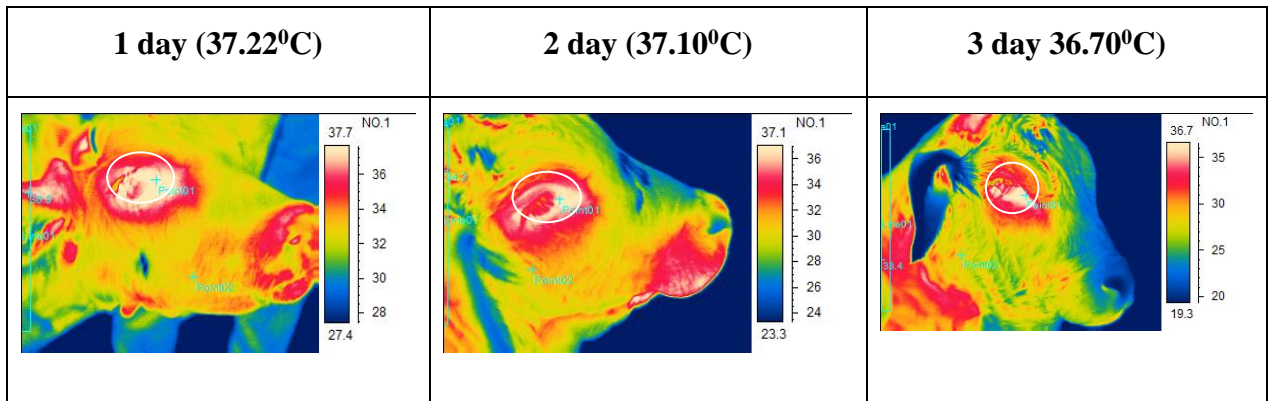
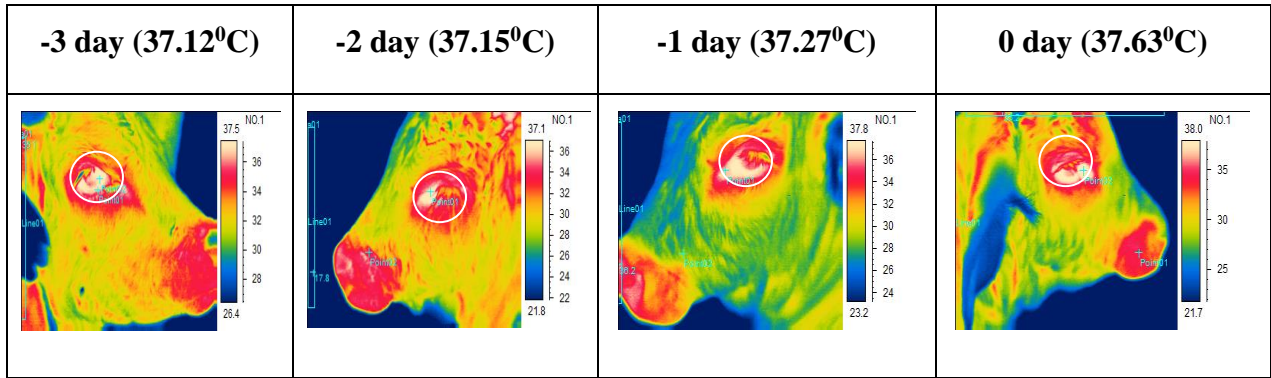


Plate 4.12: IRT eyeball temperature (°C) of calves affected with fever

4.2.16. Peri-anal region temperature (°C) of 5 days before and 5 days after diarrhoea between healthy and calves affected with diarrhoea:

The results of peri-anal region temperature (°C) of 5 days before and 5 days after diarrhoea between healthy calves and calves affected with diarrhoea have been presented in Table 4.2.17 and Fig 4.13. Peri-anal region temperature (Mean±S.E., °C) was significantly ($p<0.05$) higher, one day before (38.98 ± 0.15 vs. 38.10 ± 0.19), on the day (39.25 ± 0.09 vs. 38.13 ± 0.13) and one day after diarrhoea (38.74 ± 0.08 vs. 38.03 ± 0.16) in case of calves affected with diarrhoea as compared to healthy calves. On the contrary, Ragul (2020) observed that non-significant change in peri-anal region temperature, one day before (0.29°C), on the day (0.20°C), and one day after diarrhoea (0.03°C). The variation in the results may be due to species variation, causative agents and pathogenicity of the organisms.

Results and Discussion

Table 4.2.17: Mean ± S.E. of IRT peri-anal region temperature (°C) of 5 days before and 5 days after diarrhoea between healthy (n=33) and calves affected with diarrhoea (n=8)

Days	Healthy	Calf diarrhoea
-5day	38.01±0.10	38.13±0.38
-4day	38.08±0.08	38.26±0.19
-3day	38.29±0.13	38.33±0.21
-2day	38.01±0.23	38.40±0.34
-1day	38.10 ^a ±0.19	38.98 ^b ±0.15
0 day	38.13 ^a ±0.13	39.25 ^b ±0.09
1day	38.03 ^a ±0.16	38.74 ^b ±0.08
2day	38.49±0.10	38.50±0.10
3day	38.30±0.11	38.50±0.19
4day	38.45±0.12	38.46±0.15
5day	38.45±0.14	38.15±0.18

Mean bearing different superscripts in a row differ significantly ^{ab} (p<0.05)

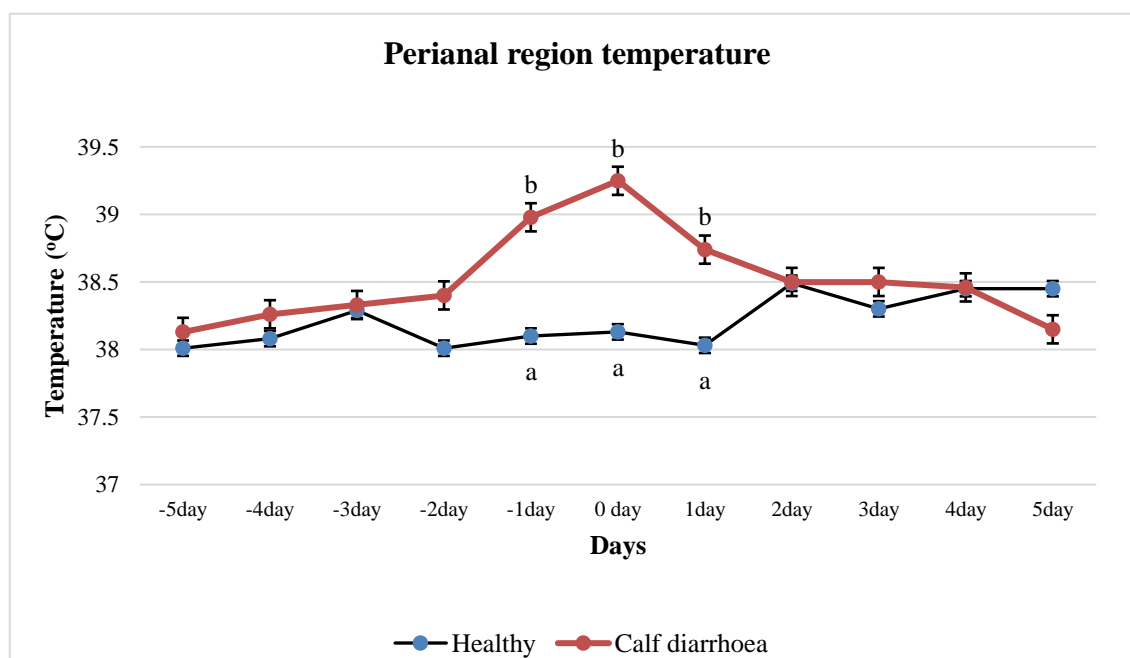


Fig. 4.13: IRT peri-anal region temperature (°C) of 5 days before and 5 days after diarrhoea between healthy and calves affected with diarrhoea, Lines bearing different superscripts differ significantly ^{ab}(p<0.05)

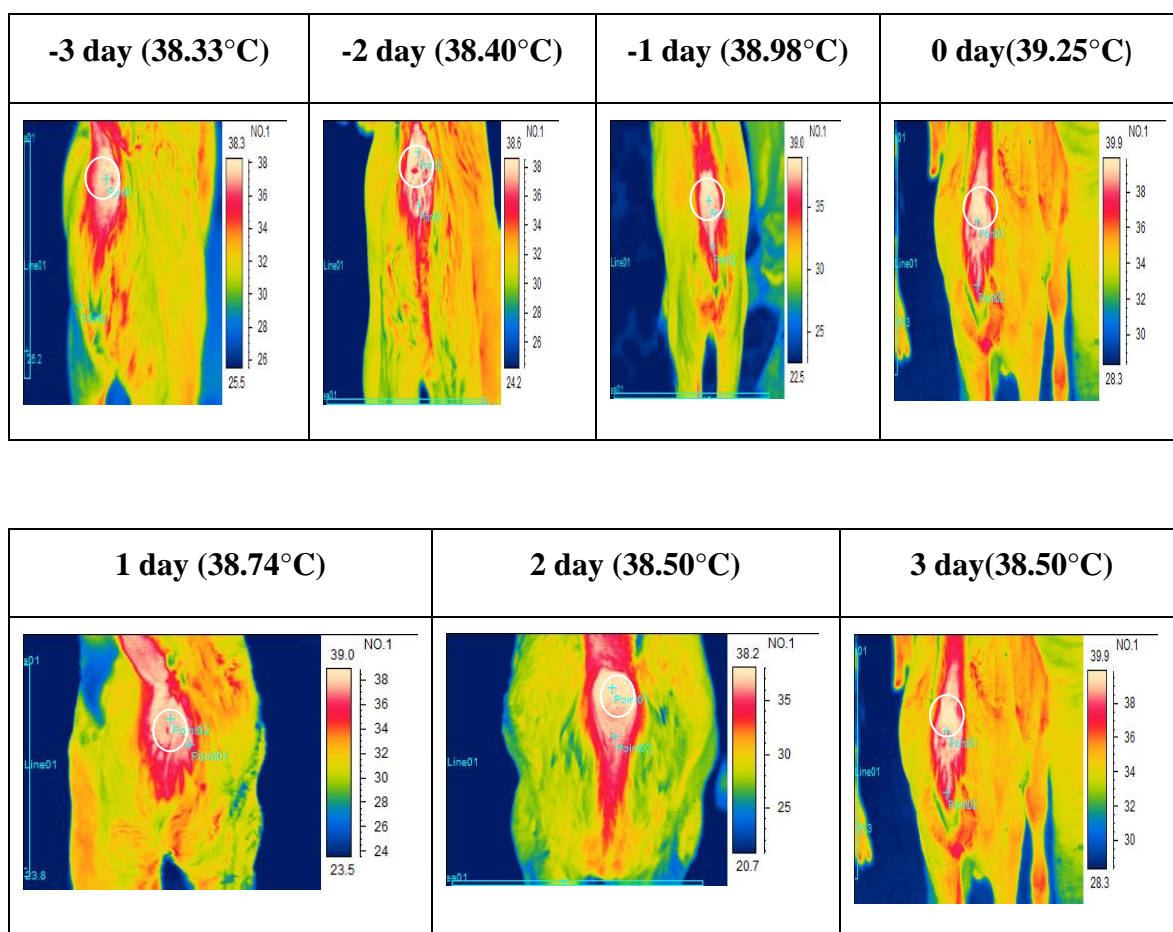


Plate 4.13: IRT peri-anal region temperature (°C) of calves affected with diarrhoea

4.2.17. Peri-anal region temperature (°C) of 5 days before and 5 days after pneumonia between healthy and calves affected with pneumonia:

The results of peri-anal region temperature (°C) of 5 days before and 5 days after pneumonia between healthy and calves affected with pneumonia have been presented in Table 4.2.18 and Fig 4.14. IRT peri-anal region temperature (Mean±S.E., °C) was significantly ($p<0.05$) higher, one day before (39.10 ± 0.10 vs. 38.18 ± 0.03), on the day (39.35 ± 0.05 vs. 38.43 ± 0.03) and one day after pneumonia (39.01 ± 0.01 vs. 38.03 ± 0.02) in case of calves affected with pneumonia as compared to healthy calves. No previously published research data was available regarding IRT peri-anal region temperatures of young calves affected with pneumonia to compare the findings of the present study with others.

Results and Discussion

Table 4.2.18: Mean ± S.E. of IRT peri-anal region temperature (°C) of 5 days before and 5 days after pneumonia between healthy (n=33) and calves affected with pneumonia (n=2)

Days	Healthy	Calf pneumonia
-5day	38.07±0.07	38.25±0.35
-4day	38.43±0.41	38.40±0.10
-3day	38.31±0.06	38.57±0.03
-2day	38.30±0.17	38.73±0.25
-1day	38.18 ^a ±0.03	39.10 ^b ±0.10
0 day	38.43 ^a ±0.03	39.35 ^b ±0.05
1day	38.03 ^a ±0.02	39.01 ^b ±0.01
2day	38.12±0.16	38.45±0.65
3day	38.33±0.07	38.60±0.40
4day	38.20±0.13	38.25±0.15
5day	38.35±0.13	38.45±0.15

Means bearing different superscripts in a row differ significantly ^{ab} (p<0.05)

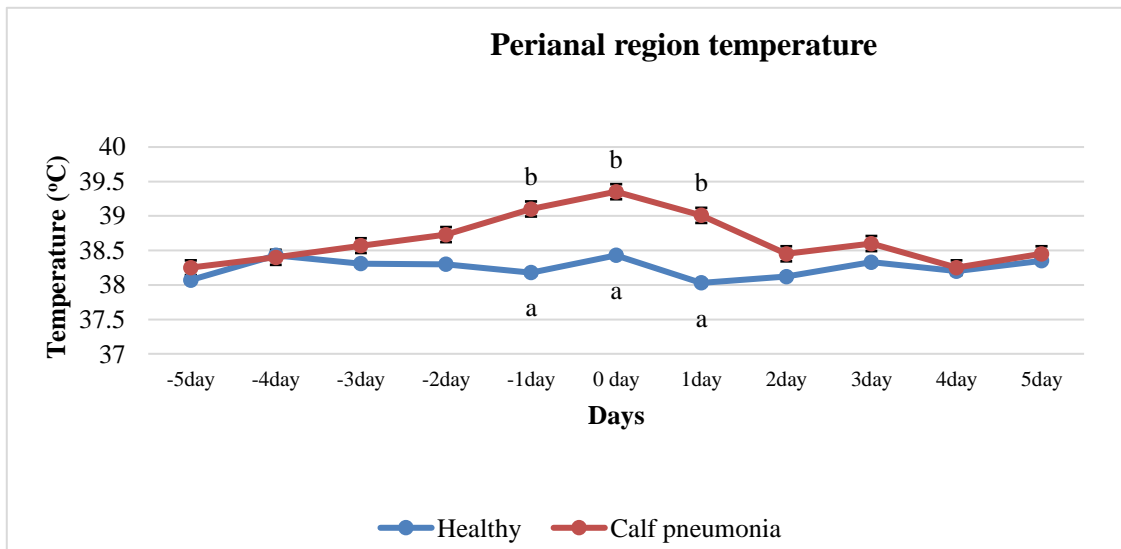


Fig. 4.14: IRT peri-anal region temperature (°C) of 5 days before and 5 days after pneumonia between healthy and calves affected with pneumonia, Lines bearing different superscripts differ significantly ^{ab}(p<0.05)

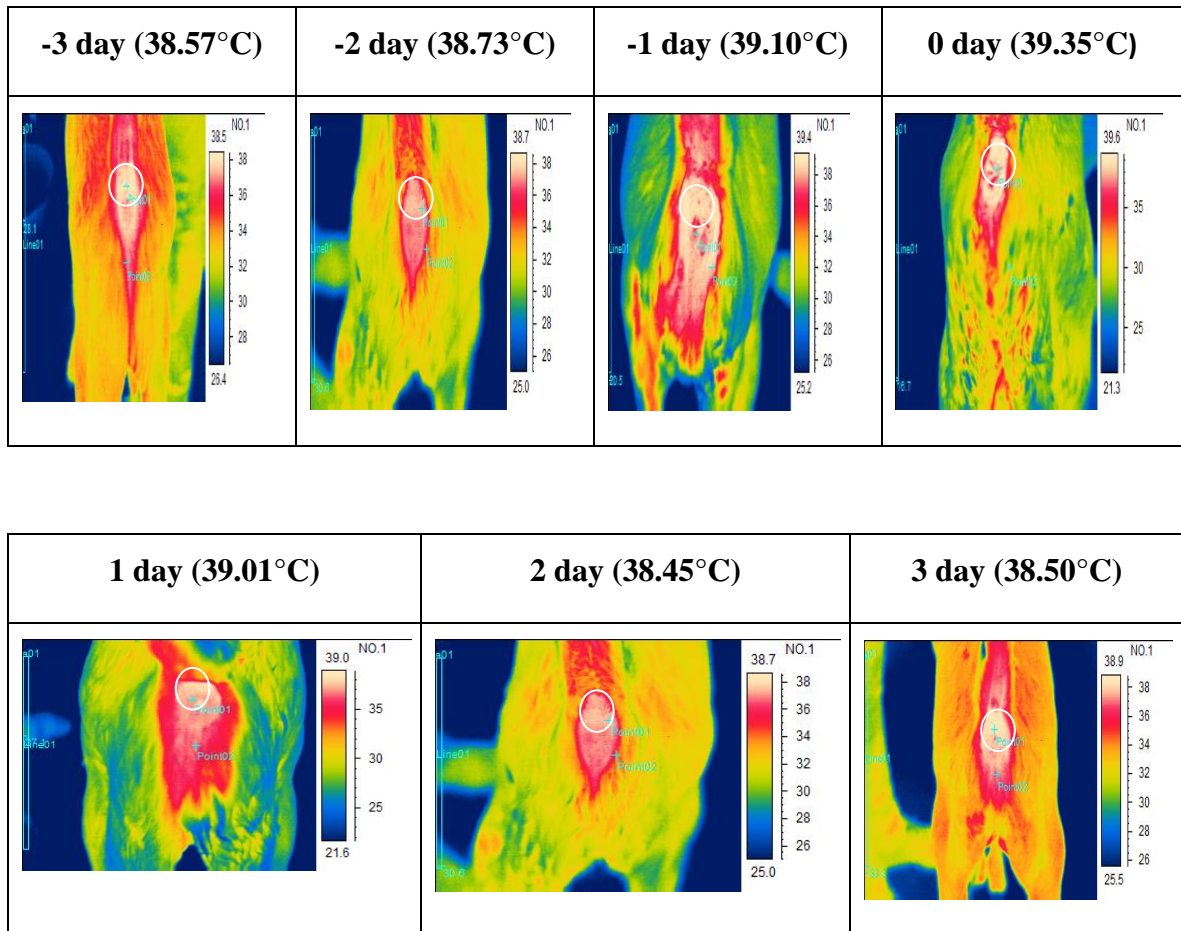


Plate 4.14: IRT peri-anal region temperature (°C) of calves affected with pneumonia

4.2.18. Peri-anal region temperature (°C) of 5 days before and 5 days after fever between healthy and calves affected with fever

The results of peri-anal region temperature (°C) of 5 days before and 5 days after diarrhoea between healthy and calves affected with fever have been presented in Table 4.2.19 and Fig 4.15. Peri-anal region temperature (Mean±S.E, °C) was significantly ($p<0.05$) higher, only on the day (39.25 ± 0.17 vs. 38.19 ± 0.07) in calves affected with fever as compared to healthy calves. Increase in the peri-anal temperature might be due to the increase in the core body temperature in fever. No previously published research data was available regarding IRT peri-anal region temperatures of young calves affected with fever to compare findings of the present study with others.

Results and Discussion

Table 4.2.19: Mean ± S.E. of IRT peri-anal region temperature (°C) of 5 days before and 5 days after fever between healthy (n=33) and calves affected with fever (n=3)

Days	Healthy	Fever
-5day	38.34±0.02	38.40±0.55
-4day	38.55±0.14	38.57±0.52
-3day	38.49±0.11	38.64±0.06
-2day	38.28±0.16	38.68±0.26
-1day	38.30±0.25	38.84±0.20
0 day	38.19 ^a ±0.07	39.25 ^b ±0.17
1day	38.56±0.23	38.89±0.32
2day	38.52±0.10	38.53±0.23
3day	38.26±0.03	38.43±0.26
4day	38.36±0.18	38.57±0.15
5day	38.36±0.28	38.50±0.12

Means bearing different superscripts in a row differ significantly ^{ab} (p<0.05)

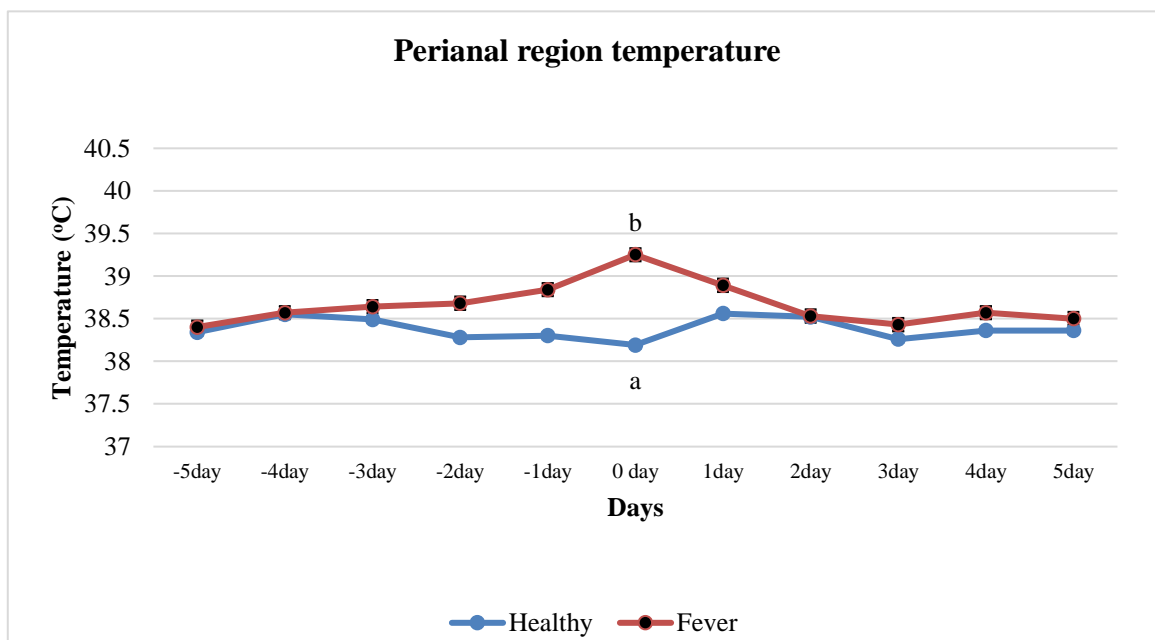


Fig. 4.15: IRT Peri-anal region temperature (°C) of 5 days before and 5 days after fever between healthy and calves affected with fever, Lines bearing different superscripts differ significantly ^{ab}(p<0.05)

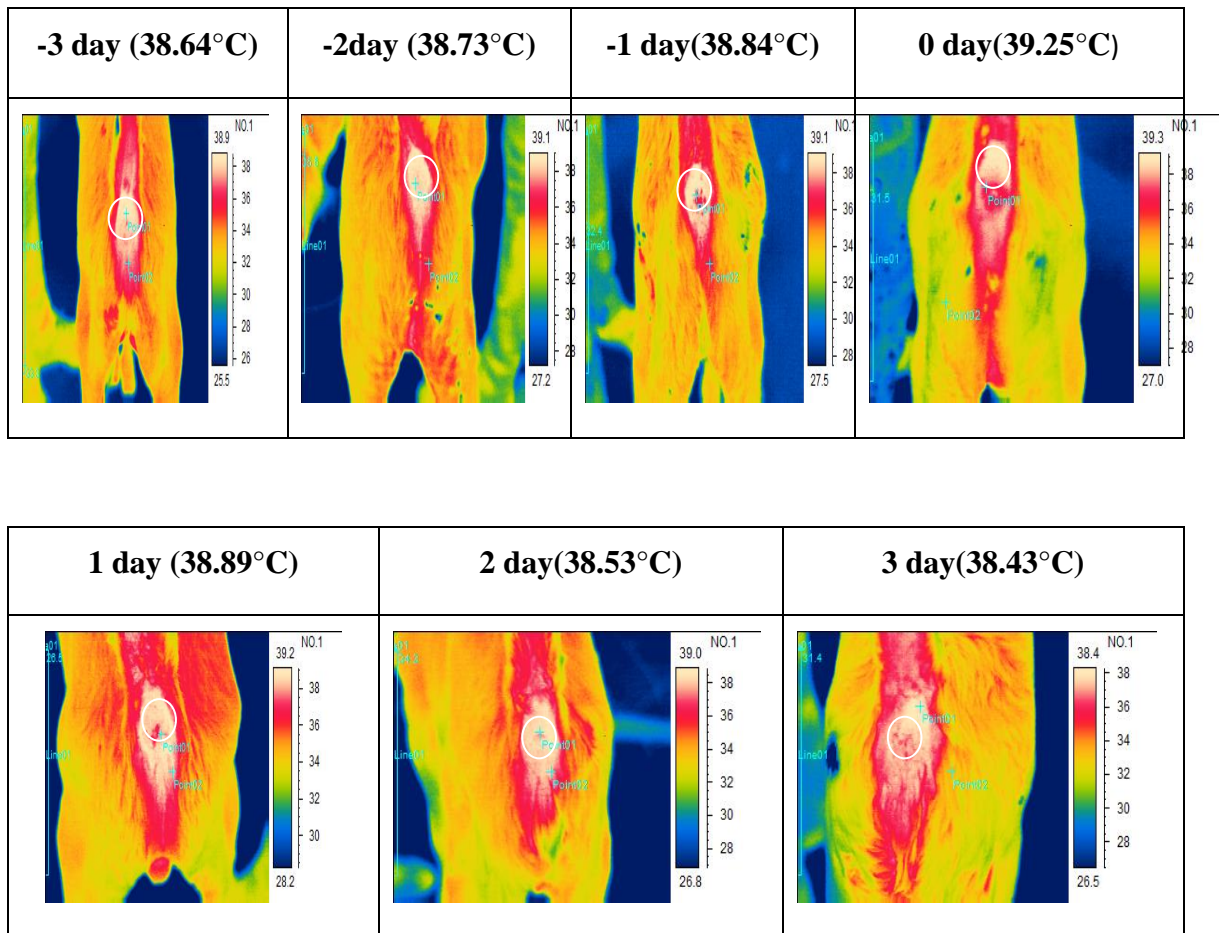


Plate 4.15: IRT Peri-anal region temperature (°C) of calves affected with fever

4.2.19. Para-lumbar region temperature (°C) of 5 days before and 5 days after diarrhoea between healthy and calves affected with diarrhoea:

The results of para-lumbar region temperature (°C) of 5 days before and 5 days after diarrhoea between healthy and calves affected with diarrhoea have been presented in Table 4.2.20 and Fig 4.16. Para-lumbar region temperature (Mean±S.E., °C) was significantly ($p<0.05$) higher only on the day diarrhoea (35.71 ± 0.12 vs. 34.60 ± 0.23) as compared to healthy calves. No significant change in side temperature was observed on other days. Similar to the present finding increase in para-lumbar region temperature was reported in case of calves affected with neonatal calf diarrhoea (NCD) (Lowe *et al.*, 2019). Increase in side temperature may be due to the close proximity of the side area to the site of intestinal inflammation associated with infection (Lowe *et al.*, 2019).

Results and Discussion

Table 4.2.20: Mean \pm S.E of IRT para-lumbar region temperature ($^{\circ}$ C) of 5 days before and 5 days after diarrhoea between healthy (n=33) and calves affected with diarrhoea (n=8)

Days	Healthy	Calf diarrhoea
-5day	34.85 \pm 0.26	34.86 \pm 0.43
-4day	34.67 \pm 0.27	34.94 \pm 0.38
-3day	34.89 \pm 0.19	34.98 \pm 0.49
-2day	34.89 \pm 0.35	35.02 \pm 0.19
-1day	34.61 \pm 0.41	35.46 \pm 0.54
0 day	34.60 ^a \pm 0.23	35.71 ^b \pm 0.12
1day	34.72 \pm 0.18	35.04 \pm 0.42
2day	34.74 \pm 0.18	35.13 \pm 0.29
3day	34.80 \pm 0.27	35.06 \pm 0.60
4day	34.82 \pm 0.25	34.96 \pm 0.50
5day	34.86 \pm 0.38	34.98 \pm 0.30

Means bearing different superscripts in a row differ significantly ^{ab} (p<0.05)

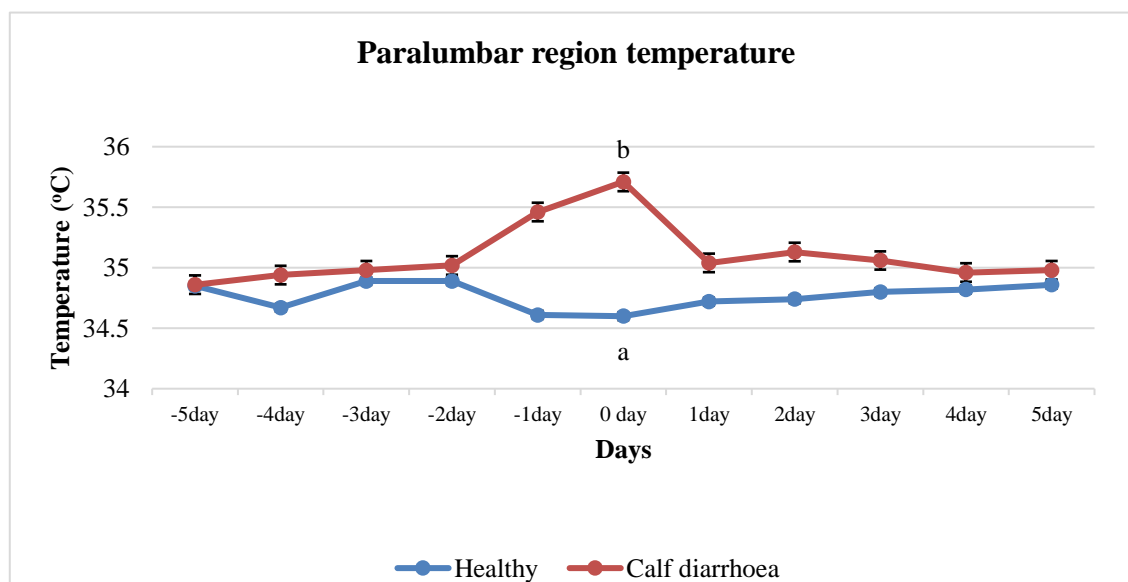


Fig. 4.16: IRT para-lumbar region temperature ($^{\circ}$ C) of 5 days before and 5 days after diarrhoea between healthy and calves affected with diarrhoea, Lines bearing different superscripts differ significantly ^{ab}(p<0.05)

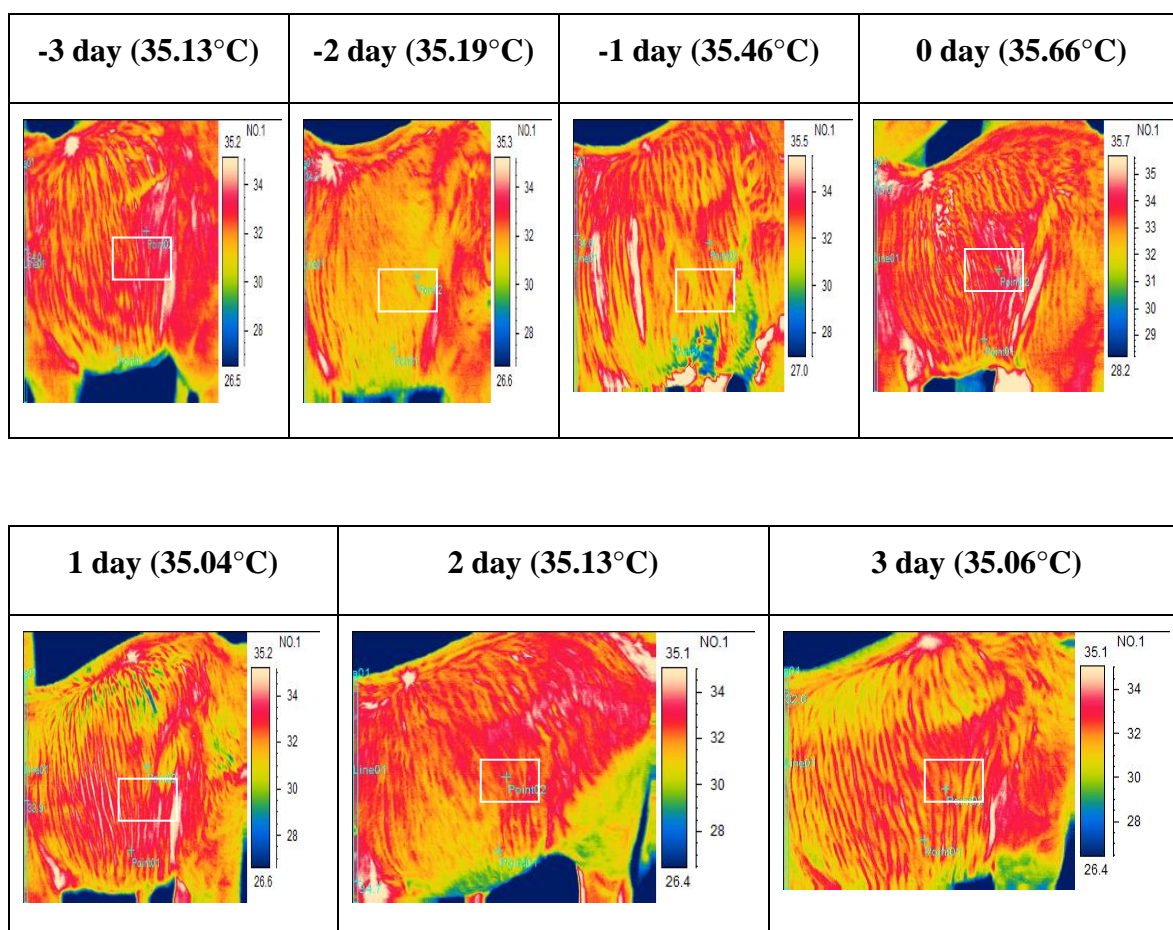


Plate 4.16: IRT para-lumbar region temperature (°C) of calves affected with diarrhoea

4.2.20. Para-lumbar region temperature (°C) of 5 days before and 5 days after pneumonia between healthy and calves affected with pneumonia:

The results of para-lumbar region temperature (°C) of 5 days before and 5 days after pneumonia between healthy calves and calves affected with pneumonia have been presented in Table 4.2.21 and Fig 4.17. Para-lumbar region temperature (Mean±S.E., °C) was significantly ($p<0.05$) higher, only on the day of pneumonia (35.50 ± 0.40 vs. 34.47 ± 0.43) as compared to healthy calves. No significant change in para-lumbar region temperature was observed on other days. Increase in the para-lumbar region temperature might be due to the increase in the core body temperature in case of pneumonia.

Results and Discussion

Table 4.2.21: Mean ± S.E. of IRT para-lumbar region temperature (°C) of 5 days before and 5 days after pneumonia between healthy (n=33) and calves affected with pneumonia (n=2)

Days	Healthy	Calf pneumonia
-5day	34.46±0.09	34.70±0.70
-4day	34.57±0.14	34.85±0.65
-3day	34.50±0.11	35.00±0.00
-2day	34.60±0.45	34.90±0.50
-1day	34.61±0.07	35.00±0.90
0 day	34.47 ^a ±0.43	35.50 ^b ±0.40
1day	34.70±0.48	35.15±0.05
2day	34.53±0.30	34.85±0.25
3day	34.80±0.16	35.10±1.20
4day	34.90±0.20	35.12±0.95
5day	34.90±0.09	35.02±0.05

Means bearing different superscripts in a row differ significantly ^{ab} (p<0.05)

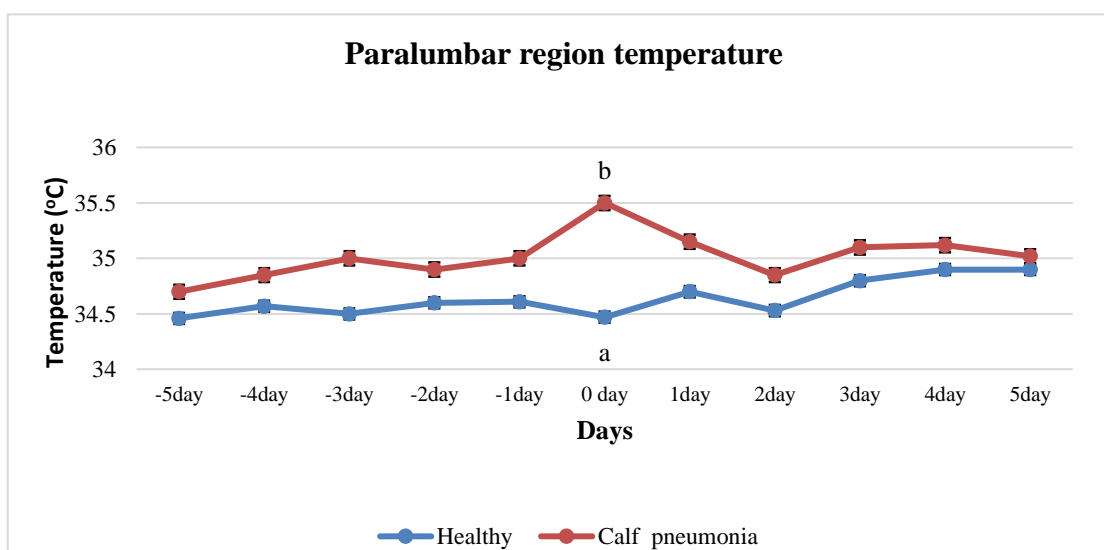


Fig. 4.17: IRT para-lumbar region temperature (°C) of 5 days before and 5 days after pneumonia between healthy and calves affected with pneumonia, Lines bearing different superscripts differ significantly ^{ab}(p<0.05)

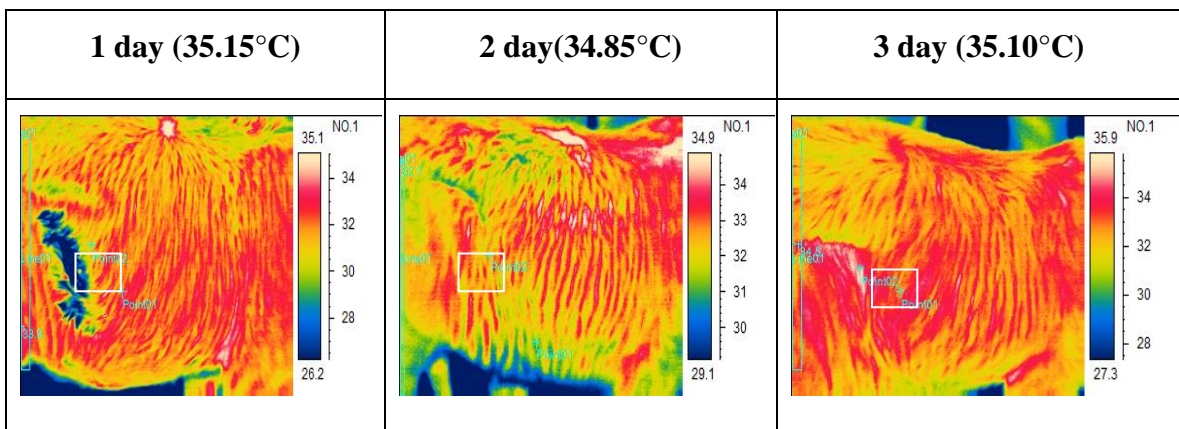
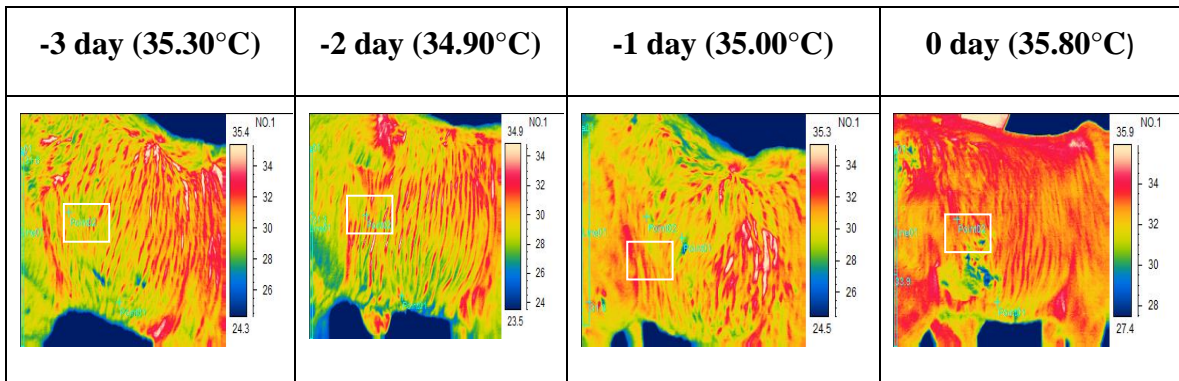


Plate 4.17: IRT para-lumbar region temperature (°C) of calves affected with pneumonia

4.2.21. IRT para-lumbar region temperature (°C) of 5 days before and 5 days after fever between healthy and calves affected with fever:

The results of para-lumbar region temperature (°C) of 5 days before and 5 days after fever between healthy calves and calves affected with fever have been presented in Table 4.2.22 and Fig 4.18. Para-lumbar region temperature (Mean±S.E., °C) was significantly ($p<0.05$) higher, only on the day of fever (35.97 ± 0.18 vs. 34.76 ± 0.08) as compared to healthy calves. No significant change in side temperature was observed on other days. No published data is available regarding para-lumbar region temperature of fever calves to compare the finding of the present study.

Results and Discussion

Table 4.2.22: Mean \pm S.E. of IRT para-lumbar region temperature ($^{\circ}$ C) of 5 days before and 5 days after fever between healthy (n=33) and calves affected with fever (n=3)

Days	Healthy	Fever
-5day	34.99 \pm 0.39	35.27 \pm 0.62
-4day	34.81 \pm 0.58	34.90 \pm 0.96
-3day	34.82 \pm 0.63	34.93 \pm 0.35
-2day	34.66 \pm 0.27	35.09 \pm 0.36
-1day	34.71 \pm 0.30	35.12 \pm 0.28
0day	34.76 ^a \pm 0.08	35.97 ^b \pm 0.18
1day	34.30 \pm 0.58	34.89 \pm 0.67
2day	34.32 \pm 0.88	34.82 \pm 1.08
3day	34.89 \pm 0.45	34.92 \pm 0.60
4day	34.55 \pm 0.43	34.74 \pm 0.58
5day	34.63 \pm 0.36	34.86 \pm 0.99

Means bearing different superscripts in a row differ significantly ^{ab} (p<0.05)

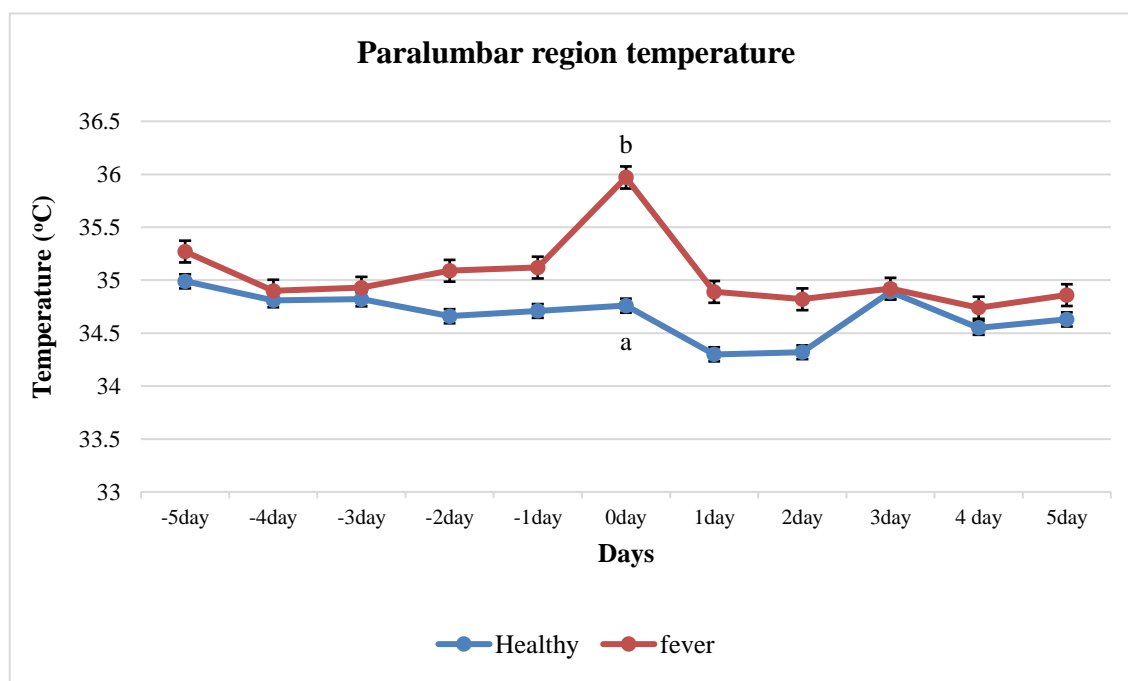


Fig. 4.18: IRT para-lumbar region temperature ($^{\circ}$ C) of 5 days before and 5 days after fever between healthy and calves affected with fever, Lines bearing different superscripts differ significantly ^{ab}(p<0.05)

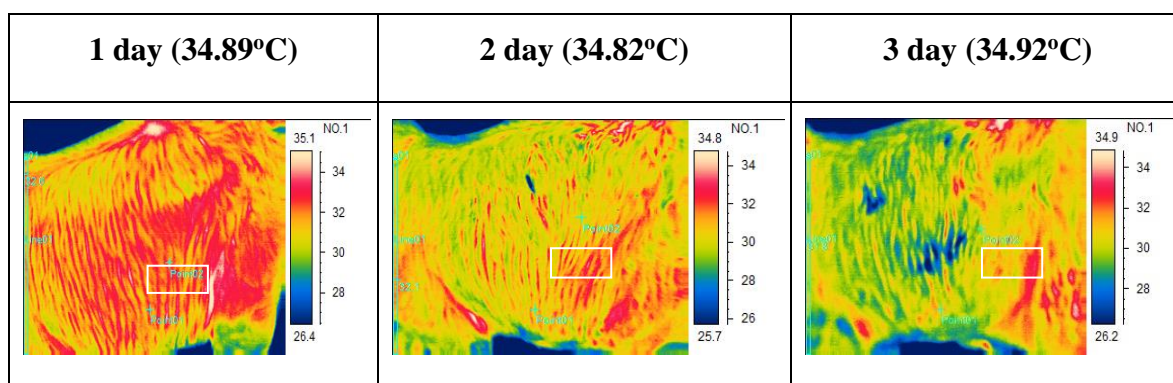
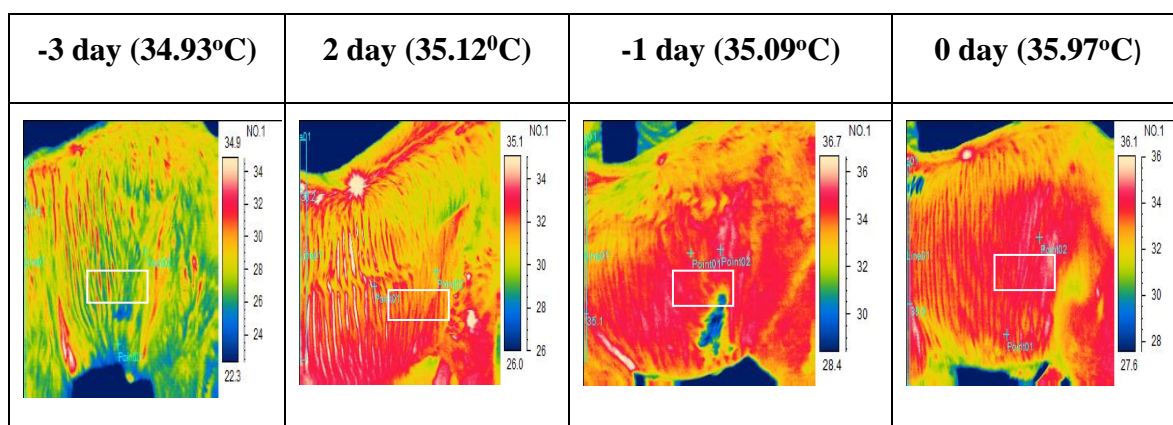


Plate 4.18: IRT Para-lumbar region temperature (°C) of calves affected with fever

4.2.22. Rectal temperature (°C) of 5 days before and 5 days after diarrhoea between healthy and calves affected with diarrhoea:

The results of rectal temperature (°C) of 5 days before and 5 days after diarrhoea between healthy calves and calves affected with diarrhoea have been presented in Table 4.2.23 and Fig 4.19. Rectal temperature (Mean±S.E., °C) was significantly ($p < 0.05$) higher, one day before (39.43 ± 0.10 vs. 38.90 ± 0.12), on the day (39.85 ± 0.03 vs. 38.92 ± 0.07) and one day after diarrhoea (39.35 ± 0.15 vs. 38.62 ± 0.22) in case of calves affected with diarrhoea as compared to healthy calves. Similar to the present observations increase in rectal temperature was reported by Schaefer *et al.* (2004) in case of calves affected with bovine viral diarrhoea. Sharma (2013) also reported a significant increase in rectal temperature of diarrhoea calves ($102.40 \pm 0.63^{\circ}\text{F}$) as compared to healthy calves ($101.28 \pm 0.93^{\circ}\text{F}$). In similar line, Ragul (2020) reported significant ($p < 0.05$) increase in rectal temperature one day before (0.45°C), on the day (0.91°C) and one day after diarrhoea (0.57°C) as compared to healthy calves on that day. No significant change was reported on other days.

Results and Discussion

Table 4.2.23: Mean ± S.E. of rectal temperature (°C) measured by the clinical thermometer of 5 days before and 5 days after diarrhoea between healthy (n=33) and calves affected with diarrhoea (n=8)

Days	Healthy	Calf diarrhoea
-5day	38.80±0.05	38.91±0.11
-4day	38.82±0.05	38.85±0.03
-3day	38.94±0.06	38.93±0.17
-2day	38.87±0.04	39.02±0.07
-1day	38.90 ^a ±0.12	39.43 ^b ±0.10
0 day	38.92 ^a ±0.07	39.85 ^b ±0.03
1day	38.62 ^a ±0.22	39.35 ^b ±0.15
2day	38.95±0.05	38.99±0.13
3day	38.78±0.05	38.82±0.19
4day	38.98±0.07	38.99±0.05
5day	38.90±0.05	39.01±0.21

Means bearing different superscripts in a row differ significantly ^{ab} (p<0.05)

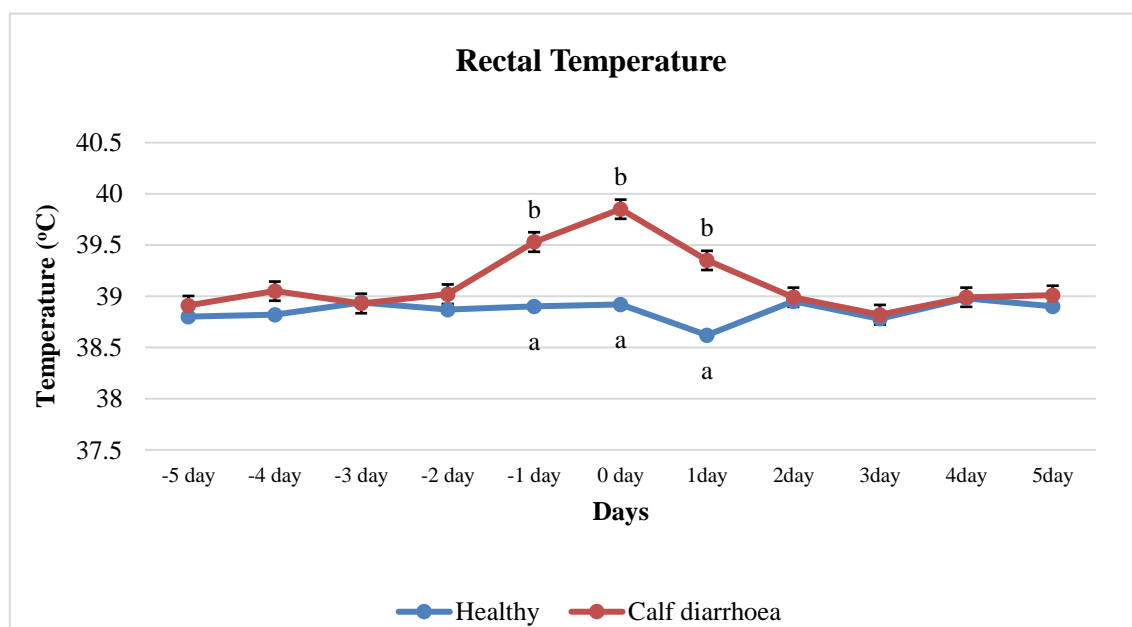


Fig. 4.19: Rectal temperature (°C) of 5 days before and 5 days after diarrhoea between healthy and calves affected with diarrhoea, Lines bearing different superscripts differ significantly ^{ab}(p<0.05).

4.2.23. Rectal temperature (°C) of 5 days before and 5 days after pneumonia between healthy and calves affected with pneumonia

The results of rectal temperature (°C) of 5 days before and 5 days after pneumonia between healthy calves and calves affected with pneumonia have been presented in Table 4.2.24 and Fig 4.20. Rectal temperature (Mean±S.E., °C) was significantly ($p<0.05$) higher, one day before (39.36 ± 0.24 vs. 38.75 ± 0.23), on the day (39.81 ± 0.09 vs. 39.02 ± 0.02) and one day after pneumonia (39.43 ± 0.12 vs. 38.83 ± 0.19) in case of calves affected with pneumonia as compared to healthy calves. Similar to the present findings increase in rectal temperature was reported by Schaefer *et al.* (2007) in case of calves affected with bovine respiratory disease complex. In Similar line, Ramadan *et al.* (2019) reported that in pneumonia increase in rectal temperature (°C) (40.61 ± 0.09) was recorded as compared to healthy calves. Ozbek and Ozkan (2020) reported a significant increase in rectal temperature (°C) in calves affected with bovine respiratory disease (39.72 ± 0.10) as compared to healthy calves.

Table 4.2.24: Mean ± S.E. of rectal temperature (°C) measured by the clinical thermometer of 5 days before and 5 days after pneumonia between healthy (n=33) and calves affected with pneumonia (n=2)

Days	Healthy	Calf pneumonia
-5day	38.81±0.02	38.55±0.15
-4day	38.93±0.15	38.90±0.00
-3day	38.85±0.20	39.00±0.10
-2day	38.87±0.11	39.05±0.20
-1day	38.75 ^a ±0.23	39.36 ^b ±0.24
0 day	39.02 ^a ±0.02	39.81 ^b ±0.09
1day	38.83 ^a ±0.19	39.43 ^b ±0.12
2day	38.96±0.00	39.05±0.35
3day	39.06±0.00	39.25±0.45
4day	38.99±0.13	39.35±0.15
5day	39.00±0.11	39.15±0.05

Means bearing different superscripts in a row differ significantly ^{ab} ($p<0.05$)

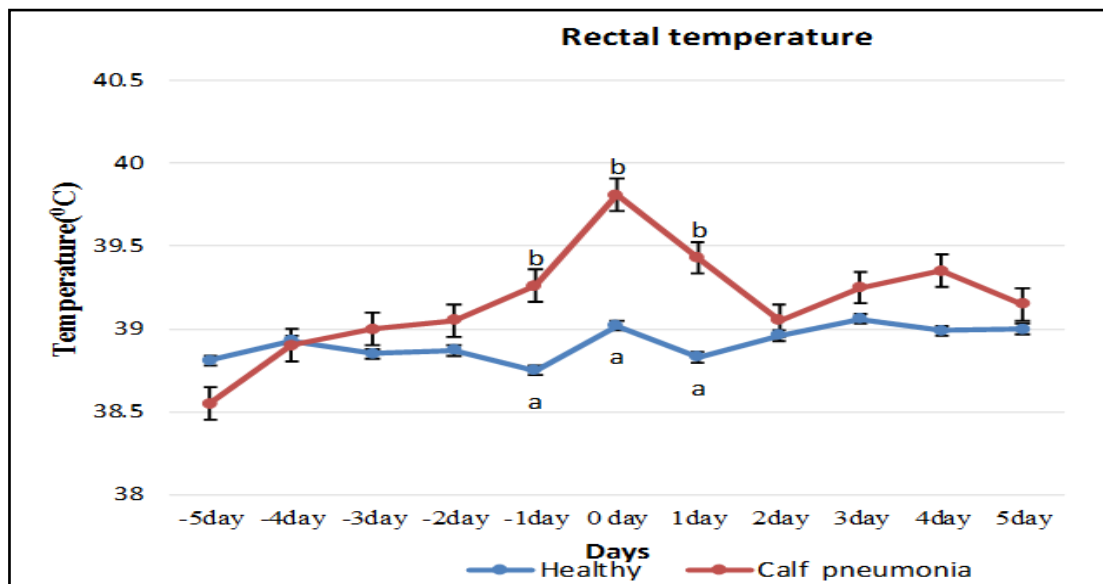


Fig. 4.20: Rectal temperature (°C) of 5 days before and 5 days after pneumonia between healthy and calves affected with pneumonia, Lines bearing different superscripts differs significantly ^{ab}(p<0.05)

4.2.24: Rectal temperature (°C) of 5 days before and 5 days after fever between healthy and calves affected with fever

The results of rectal temperature (°C) of 5 days before and 5 days after fever between healthy calves and calves affected with fever have been presented in Table 4.2.25 and Fig 4.21. Rectal temperature (Mean±S.E., °C) was significantly (p<0.05) higher only on the day (39.76±0.12 vs. 38.87±0.23) in case of calves affected with fever as compared to healthy calves. In similar line Kachhawa *et al.* (2016) reported significant (p<0.05) increase in rectal temperature on the day of fever (105.84±0.24°F), as compared to healthy calves. Fever in calves is one of the most visible physiological responses to infection and inflammation, and changes in the core body temperature are frequently utilised as indicators of ill health (Mahendran *et al.*, 2017).

Table 4.2.25: Mean \pm S.E. of rectal temperature ($^{\circ}$ C) measured by the clinical thermometer of 5 days before and 5 days after fever between healthy (n=33) and calves affected with fever (n=3)

Days	Healthy	fever
-5day	38.80 \pm 0.08	39.13 \pm 0.15
-4day	38.82 \pm 0.13	39.17 \pm 0.15
-3day	38.85 \pm 0.10	39.23 \pm 0.09
-2day	38.92 \pm 0.06	39.20 \pm 0.26
-1day	38.99 \pm 0.13	39.49 \pm 0.07
0 day	38.87 ^a \pm 0.23	39.76 ^b \pm 0.12
1day	38.95 \pm 0.11	39.27 \pm 0.19
2day	38.96 \pm 0.01	39.10 \pm 0.21
3day	39.06 \pm 0.05	39.13 \pm 0.20
4day	38.98 \pm 0.09	39.23 \pm 0.15
5day	38.99 \pm 0.11	39.20 \pm 0.06

Means bearing different superscripts in a row differ significantly ^{ab} (p<0.05)

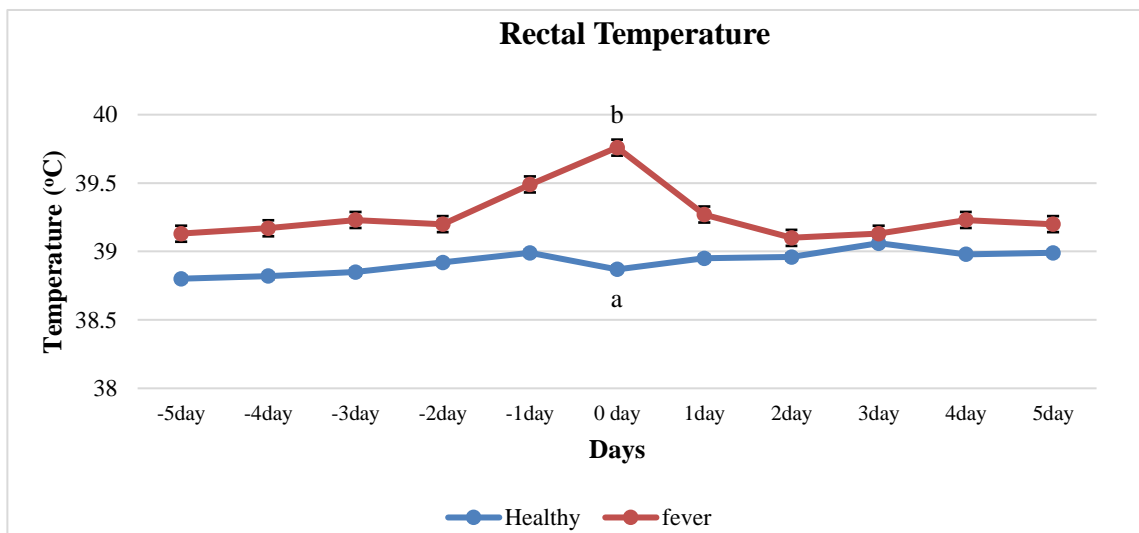


Fig. 4.21: Rectal temperature ($^{\circ}$ C) of 5 days before and 5 days after fever between healthy calves and calves affected with fever, Lines bearing different superscripts differ significantly ^{ab}(p<0.05)

Results and Discussion

4.2.25. IRT muzzle, cheek, para-lumbar region, peri-anal region and rectal temperature (°C) of two day before and day of diarrhoea between healthy and calves affected with diarrhoea

The results of eyeball, muzzle, cheek, para-lumbar region, peri-anal region and rectal temperature (°C) of two days before and on the day of diarrhoea in affected calves have been presented in Table 4.2.26 and Fig 4.22. Eyeball temperature (Mean±S.E., °C) on the day of diarrhoea and two days before diarrhoea was 37.70±0.18 and 37.10±0.21, respectively. Para-lumbar region temperature (Mean±S.E., °C) on the day of diarrhoea and two days before diarrhoea was 35.71±0.12 and 35.02±0.19, respectively. Peri-anal region temperature (Mean±S.E., °C) on the day of diarrhoea and two days before diarrhoea was 39.25±0.09 and 38.40^a±0.34, respectively. Rectal temperature (Mean±S.E., °C) on the day of diarrhoea and two days before diarrhoea was 39.85±0.03 and 39.02±0.07, respectively. Significant (p<0.05) increase in eyeball (0.60°C), para-lumbar (0.69°C), peri-anal (0.85°C), rectal temperature (0.83°C) and non-significant change in muzzle (-0.17°C) and cheek temperature (0.04°C) was recorded between the day of diarrhoea and two days before diarrhoea in affected calves. In similar line Ragul (2020) reported significant (p<0.05) increase in eyeball (0.47 °C) and rectal temperature (0.92°C) between the day of diarrhoea and two days before diarrhoea in affected Murrah buffalo calves.

Table 4.2.26: Mean ± S.E. of IRT muzzle, eyeball, cheek, para-lumbar region, perianal region and rectal temperature by the clinical thermometer of two days before and on the day of diarrhoea in affected calves (n=8)

Body points	Two days before diarrhoea	Day of diarrhoea
IRT muzzle temperature	34.42±0.70	34.25±0.49
IRT eyeball temperature	37.10 ^a ±0.21	37.70 ^b ±0.18
IRT cheek temperature	34.90±0.62	34.94±0.33
IRT para-lumbar region temperature	35.02 ^a ±0.19	35.71 ^b ±0.12
IRT peri-anal temperature	38.40 ^a ±0.34	39.25 ^b ±0.09
Rectal temperature	39.02 ^a ±0.07	39.85 ^b ±0.03

Means bearing different superscripts in a row differ significantly ^{ab} (p<0.05)

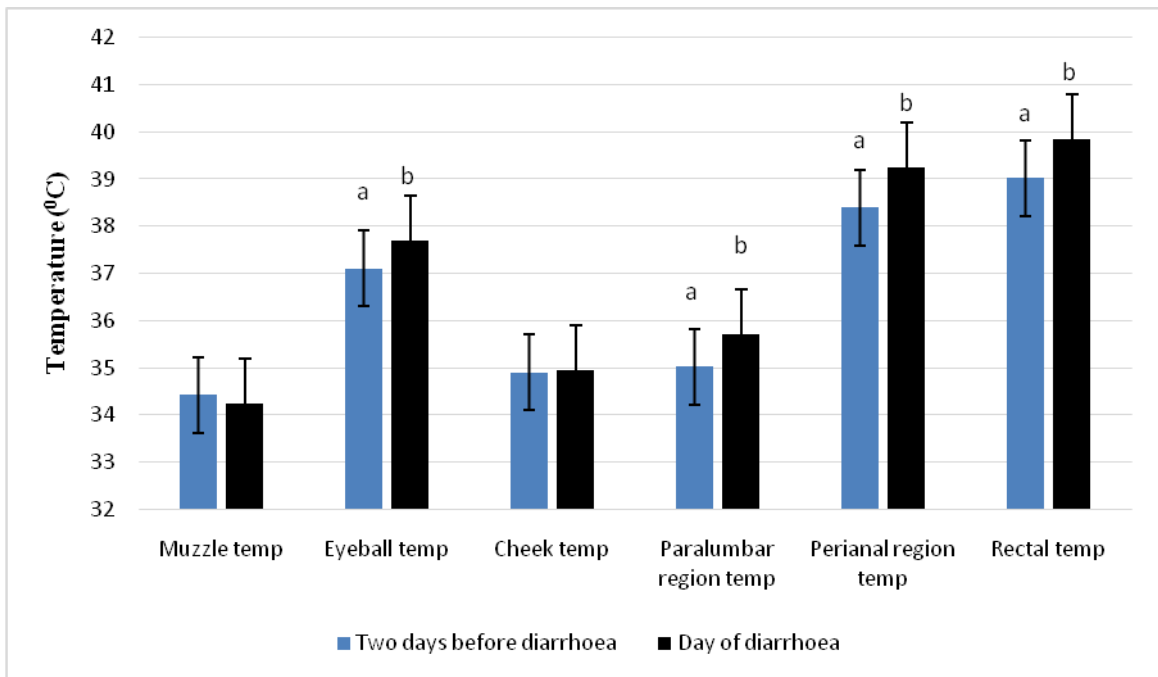


Fig. 4.22: IRT muzzle, eyeball, cheek, para-lumbar region, peri-anal and rectal temperature (°C) by the clinical thermometer of two days before and day of diarrhoea in calves affected with diarrhoea, Bars bearing different superscripts differ significantly ^{ab}(p<0.05)

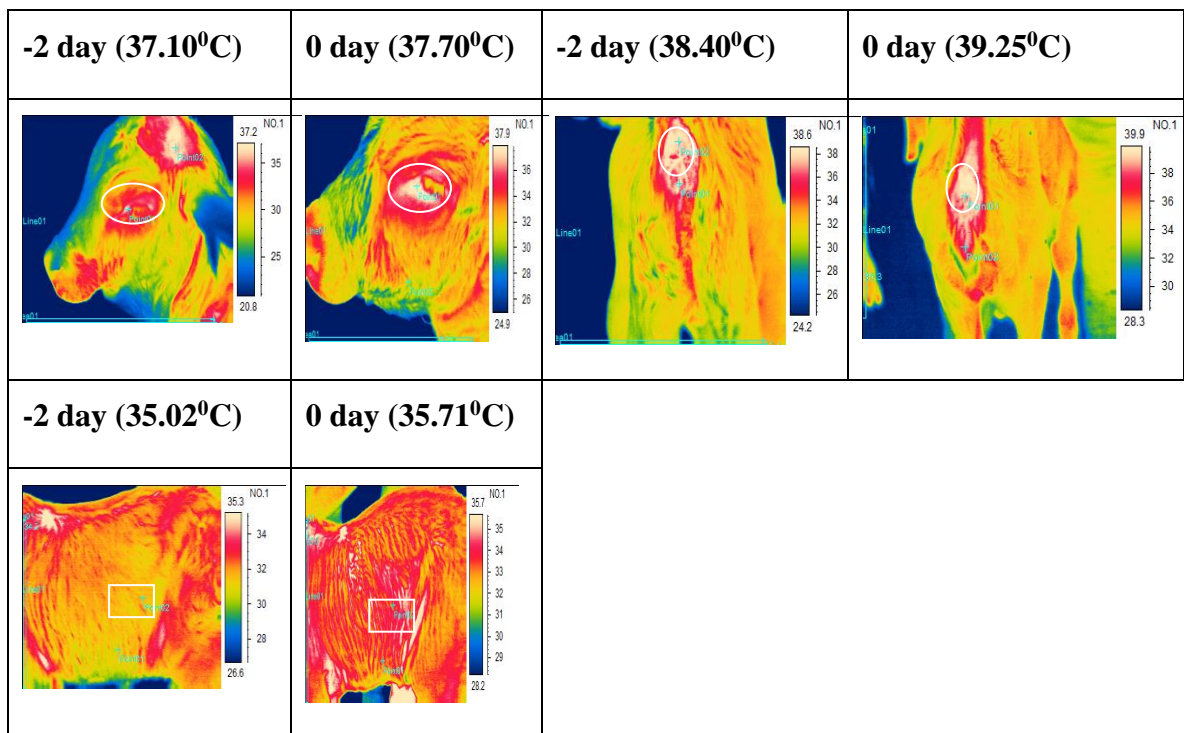


Plate 4.19: IRT images of -2 day and 0 day of diarrhoea affected calves

Results and Discussion

4.2.26. IRT muzzle, cheek, para-lumbar, peri-anal region and rectal temperature (°C) of two day before and day of pneumonia between healthy and calves affected with pneumonia:

The results of eyeball, muzzle, cheek, para-lumbar, peri-anal region and rectal temperature (°C) of two days before and on the day of pneumonia in affected calves have been presented in Table 4.2.27 and Fig 4.23. Eyeball temperature (Mean±S.E., °C) on the day of pneumonia and two days before pneumonia was 37.85±0.35 and 37.29±0.31, respectively. Para-lumbar region temperature (Mean±S.E., °C) on the day of pneumonia and two days before pneumonia was 35.80±0.40 and 34.90±0.50, respectively. Peri-anal region temperature (Mean±S.E., °C) on the day of pneumonia and two days before pneumonia was 39.35±0.05 and 38.73±0.25, respectively. Rectal temperature (Mean±S.E., °C) on the day of pneumonia and two days before pneumonia was 39.81±0.09 and 39.05±0.05, respectively. Significant (p<0.05) increase in Eyeball (0.56°C), para-lumbar (0.90°C), peri-anal (0.62°C), rectal temperature (0.76°C) and non-significant change in muzzle (0.4°C) and cheek temperature (-0.2°C) was recorded on the day of pneumonia and two days before pneumonia in affected calves.

Table 4.2.27: Mean ± S.E. of IRT muzzle, eyeball, cheek, para lumbar region, peri anal region and rectal temperature (°C) by the clinical thermometer of two days before and on the day of pneumonia in affected calves (n=2)

Body points	Two days before pneumonia	Day of pneumonia
IRT muzzle temperature	34.35±0.25	34.75±0.45
IRT eyeball temperature	37.29 ^a ±0.31	37.85 ^b ±0.35
IRT cheek temperature	34.90±0.90	34.70±0.70
IRT para-lumbar region temperature	34.90 ^a ±0.50	35.80 ^b ±0.40
IRT peri-anal temperature	38.73 ^a ±0.25	39.35 ^b ±0.05
Rectal temperature	39.05 ^a ±0.05	39.81 ^b ±0.09

Means bearing different superscripts in a row differ significantly ^{ab} (p<0.05)

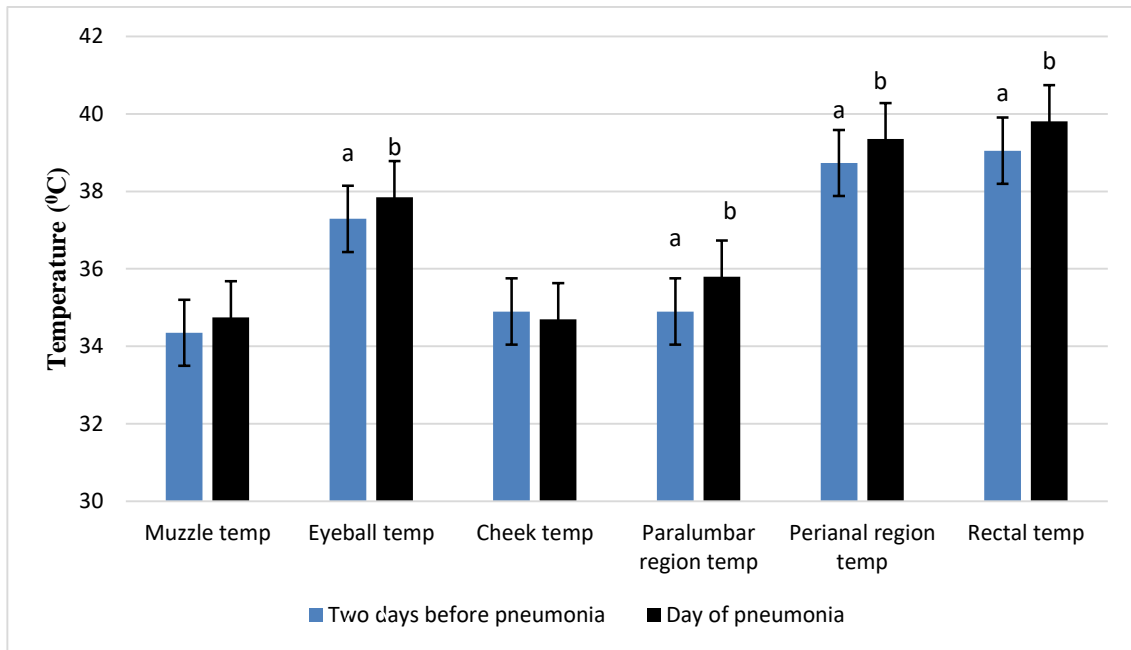


Fig. 4.23: IRT muzzle, eyeball, cheek, para lumbar region, peri-anal region and rectal temperature (°C) by the clinical thermometer of two days before and day of pneumonia in calves affected with pneumonia, Bars bearing different superscripts differ significantly ^{ab}(p<0.05)

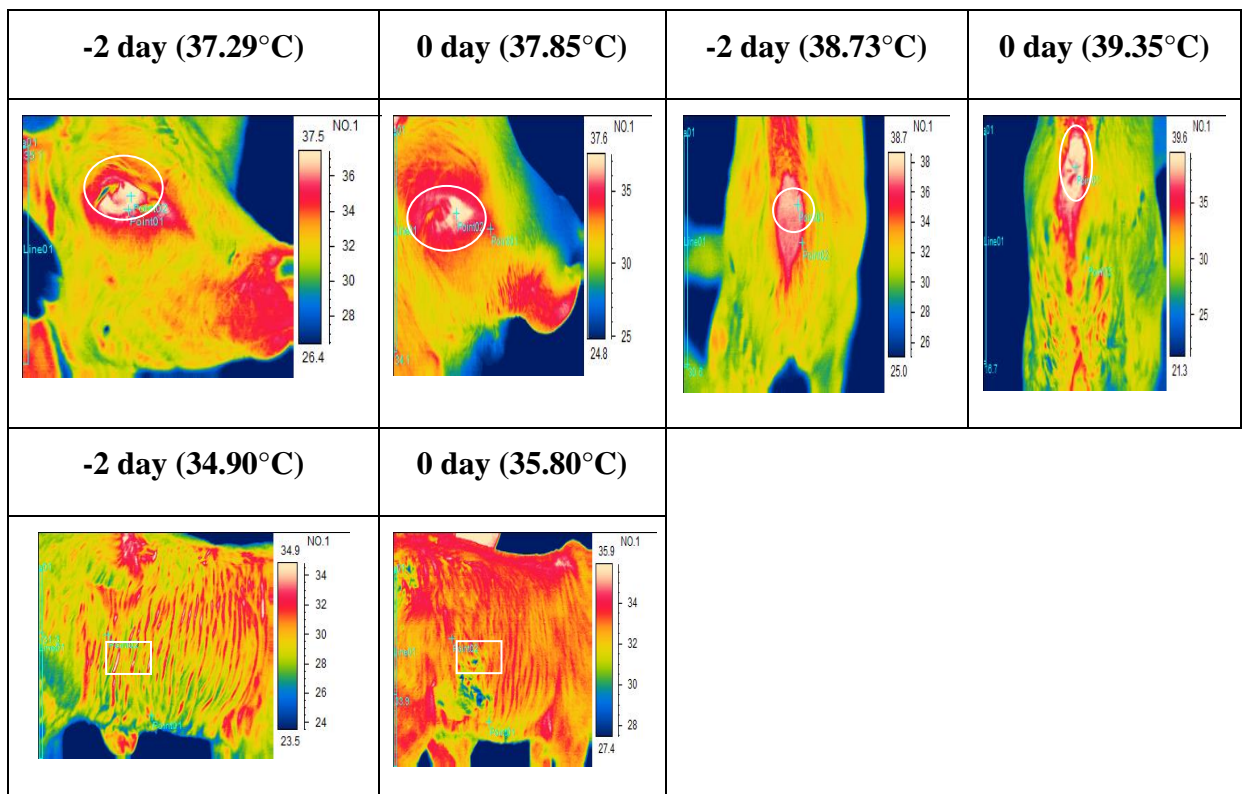


Plate 4.20: IRT images of -2 day and 0 day of pneumonia affected calves

Results and Discussion

4.2.27. IRT muzzle, cheek, para-lumbar, peri-anal region and rectal temperature (°C) of two day before and day of fever between healthy and calves affected with fever:

The results of the eye ball, muzzle, cheek, para-lumbar, peri-anal region and rectal temperature (°C) of two days before and on the day of fever in affected calves have been presented in Table 4.2.28 and Fig 4.24. Eyeball temperature (Mean±S.E., °C) on the day of fever and two days before fever was 37.73±0.46 and 37.10±0.06, respectively. Para-lumbar region temperature (Mean±S.E., °C) on the day of fever and two days before fever was 35.97±0.18 and 35.09±0.36, respectively. Peri-anal region temperature (Mean±S.E., °C) on the day of fever and two days before fever was 39.25±0.17 and 38.68±0.26, respectively. Rectal temperature (Mean±S.E., °C) on the day of fever and two days before fever was 39.76±0.12 and 39.20±0.26, respectively. Significant ($p<0.05$) increase in Eyeball (0.63°C), paralumbar (0.88°C), perianal (0.57°C), rectal temperature (0.56°C) and non-significant change in muzzle (0.09°C) and cheek temperature (0.26°C) was recorded between the day of fever and two days before fever in affected calves.

Table 4.2.28: Mean ± S.E. of IRT muzzle, eyeball, cheek, para lumbar region, peri-anal region and rectal temperature(°C) by the clinical thermometer of two days before and on the day of fever in affected calves (n=3)

Body points	Two days before fever	Day of fever
IRT muzzle temperature	34.59±0.65	34.68±0.46
IRT eyeball temperature	37.10 ^a ±0.06	37.73 ^b ±0.46
IRT cheek temperature	34.70±0.62	34.96±1.03
IRT para-lumbar region temperature	35.09 ^a ±0.36	35.97 ^b ±0.18
IRT peri-anal region temperature	38.68 ^a ±0.26	39.25 ^b ±0.17
Rectal temperature	39.20 ^a ±0.26	39.76 ^b ±0.12

Means bearing different superscripts in a row differ significantly ^{ab} ($p<0.05$)

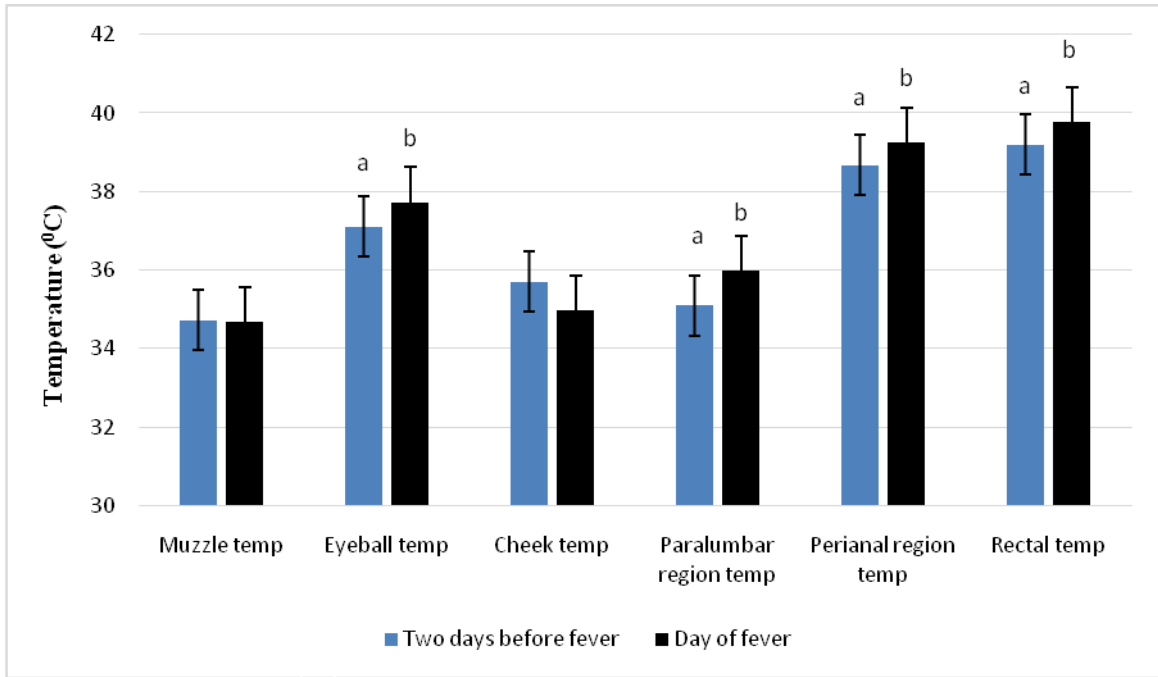


Fig. 4.24: IRT muzzle, eyeball, cheek, para lumbar region, peri-anal region and rectal temperature (°C) by the clinical thermometer of two days before and day of fever in calves affected with fever, Bars bearing different superscripts differ significantly ^{ab}($p < 0.05$)

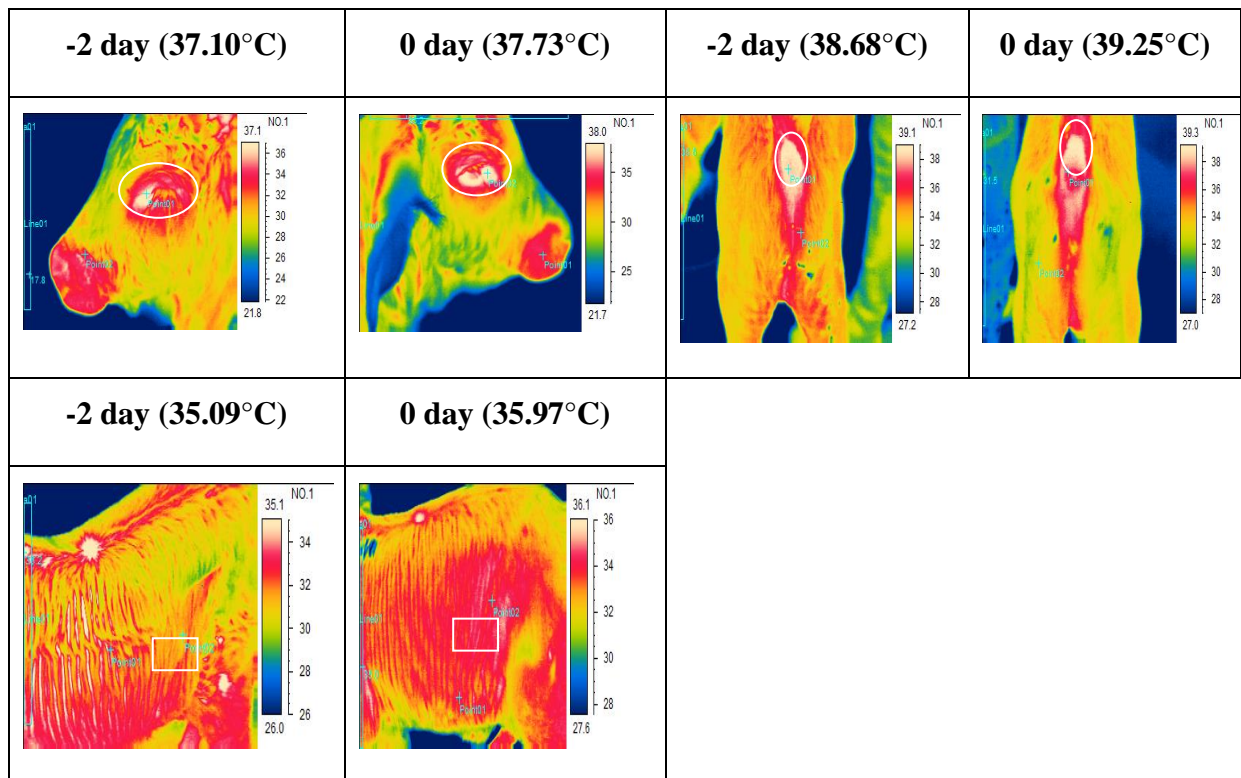


Plate 4.20: IRT images of -2 day and 0 day of fever calves

Results and Discussion

4.2.28. IRT muzzle, eyeball, cheek, para-lumbar region, peri-anal region and rectal temperature (°C) of seven days average before diarrhoea and day of diarrhoea in calves affected with diarrhoea

The results of eyeball, muzzle, cheek, para-lumbar, peri-anal region and rectal temperature (°C) of seven days average before diarrhoea and on the day of diarrhoea in affected calves have been presented in Table 4.2.29 and Fig 4.25. Eyeball temperature (Mean±S.E., °C) on the day of diarrhoea and seven days average before diarrhoea was 37.70±0.18 and 37.04±0.26, respectively. Para-lumbar region temperature (Mean±S.E., °C) on the day of diarrhoea and seven days average before diarrhoea was 35.71±0.18 and 34.98±0.50, respectively. Peri-anal region temperature (Mean±S.E., °C) on the day of diarrhoea and seven days average before diarrhoea was 39.25±0.09 and 38.11±0.30, respectively. Rectal temperature (Mean±S.E., °C) on the day of diarrhoea and seven days average before diarrhoea was 39.85±0.03 and 38.91±0.11, respectively. Significant ($p<0.05$) increase in eyeball (0.66°C), para-lumbar (0.73°C), peri-anal (1.14°C), rectal temperature (0.94°C) and non-significant change in muzzle temperature (0.09°C) and cheek temperature (0.33°C) was recorded between the day of diarrhoea and seven days average before diarrhoea in affected calves. In similar line, Ragul (2020) observed significant ($p<0.05$) increase of 0.70°C in orbital and 1.12°C in rectal temperature on the day of diarrhoea as compared to seven days average before diarrhoea.

Table 4.2.29: Mean ± S.E. of IRT muzzle, eyeball, cheek, para lumbar region, peri-anal region and rectal temperature (°C) by clinical thermometer of seven days average before diarrhoea and on the day of diarrhoea in affected calves (n=8)

Body point	Seven days average before diarrhoea	Day of diarrhoea
IRT muzzle temperature	34.16±0.88	34.25±0.49
IRT eyeball temperature	37.04 ^a ±0.26	37.70 ^b ±0.18
IRT cheek temperature	34.61±0.34	34.94±0.33
IRT para-lumbar region temperature	34.98 ^a ±0.50	35.71 ^b ±0.18
IRT peri-anal region temperature	38.11 ^a ±0.30	39.25 ^b ±0.09
Rectal temperature	38.91 ^a ±0.11	39.85 ^b ±0.03

Means bearing different superscripts in a row differ significantly ^{ab} ($p<0.05$)

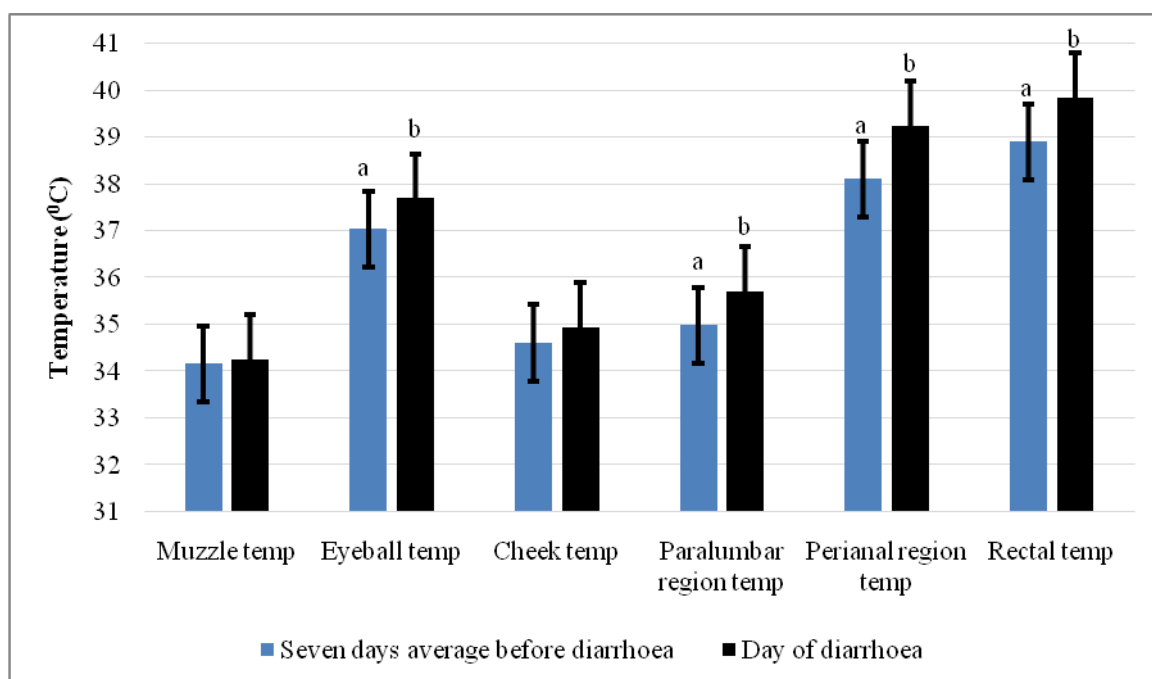


Fig. 4.25: IRT muzzle, eyeball, cheek, para-lumbar region, peri-anal region and rectal temperature (°C) by the clinical thermometer of seven days average before diarrhoea and day of diarrhoea in calves affected with diarrhoea, Bars bearing different superscripts differ significantly ^{ab} (p<0.05)

4.2.29. IRT muzzle, eyeball, cheek, para-lumbar region, peri-anal region and rectal temperature (°C) of seven days average before pneumonia and day of pneumonia between healthy and calves affected with pneumonia

The results of eyeball, muzzle, cheek, para-lumbar, peri-anal region and rectal temperature (°C) of seven days average before pneumonia and on the day of pneumonia in affected calves have been presented in Table 4.2.30 and Fig 4.26. Eyeball temperature (Mean±S.E., °C) on the day of pneumonia and seven days before pneumonia was 37.85±0.35 and 37.05±0.96, respectively. Peri-anal temperature (Mean±S.E., °C) on the day of pneumonia and seven days average before pneumonia was 39.35±0.05 and 38.45±0.15, respectively. Para-lumbar region temperature (Mean±S.E., °C) on the day of pneumonia and seven days average before pneumonia was 35.80±0.40 and 34.45±1.15, respectively. Rectal temperature (Mean±S.E., °C) on the day of pneumonia and seven days average before pneumonia was 39.81±0.09 and 38.65±0.05, respectively. Significant (p<0.05) increase in Eyeball (0.80°C), peri-anal (0.90°C) para-lumbar (1.35°C), rectal temperature (1.16°C) and non-significant change in muzzle temperature (0.18°C) and cheek temperature (0.29°C) was recorded between the day of pneumonia and seven days average before pneumonia in affected calves.

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Table 4.2.30: Mean ± S.E. of IRT muzzle, eye, cheek, para-lumbar region, peri-anal region and rectal temperature (°C) by the clinical thermometer of seven days average before and on the day of pneumonia in affected calves (n=2)

Body points	Seven days average before pneumonia	Day of pneumonia
IRT muzzle temperature	34.57±0.12	34.75±0.45
IRT eyeball temperature	37.05 ^a ±0.96	37.85 ^b ±0.35
IRT cheek temperature	34.41±0.34	34.70±0.70
IRT para-lumbar temperature	34.45 ^a ±1.15	35.80 ^b ±0.40
IRT peri-anal region temperature	38.45 ^a ±0.15	39.35 ^b ±0.05
Rectal Temperature	38.65 ^a ±0.05	39.81 ^b ±0.09

Means bearing different superscripts in a row differ significantly ^{ab} (p<0.05)

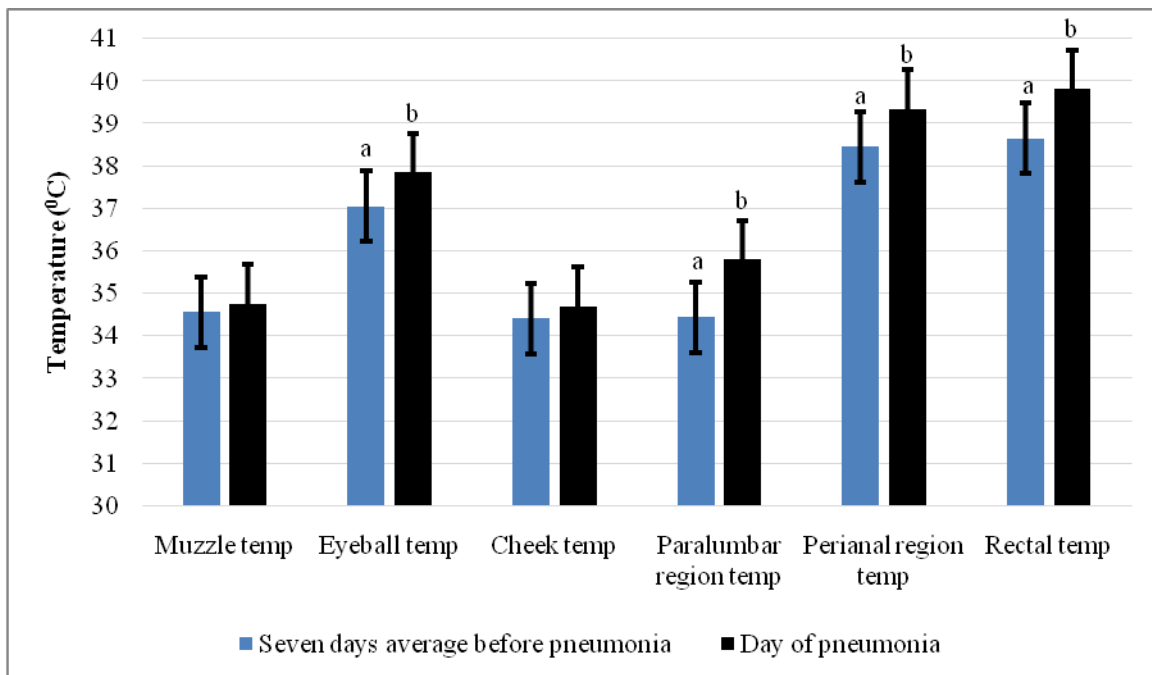


Fig. 4.26: IRT muzzle, eyeball, cheek, para-lumbar region, peri-anal region and rectal temperature (°C) by the clinical thermometer of seven days average before and day of pneumonia in calves affected with pneumonia, Bars bearing different superscripts differ significantly ^{ab}(p<0.05)

4.2.30. IRT muzzle, cheek, para-lumbar, peri-anal region and rectal temperature (°C) of 7 days average before and day of fever between healthy and calves affected with fever:

The results of the eye ball, muzzle, cheek, para-lumbar, perianal region and rectal temperature (°C) of seven days average before and on the day of fever in affected calves have been presented in Table 4.2.31 and Fig 4.27. Eyeball temperature (Mean±S.E., °C) on the day of fever and seven days before fever was 37.73±0.03 and 36.60±0.80, respectively. Peri-anal temperature (Mean±S.E., °C) on the day of fever and seven days average before fever was 39.25±0.17 and 38.30±0.11, respectively. Para-lumbar region temperature (Mean±S.E., °C) on the day of fever and seven days average before fever was 35.97±0.17 and 35.67±0.54, respectively. Rectal temperature (Mean±S.E., °C) on the day of fever and seven days average before fever was 39.67±0.26 and 38.83±0.06, respectively. Significant (p<0.05) increase in eyeball temperature (1.13°C), peri-anal region temperature (0.95°C), rectal temperature (0.84°C) and non-significant change in muzzle temperature (0.35°C), cheek temperature (0.47°C) and para-lumbar region temperature (0.30°C) was recorded between the day of fever and seven days average before fever in affected calves.

Table 4.2.31: Mean ± S.E. of IRT muzzle, eye, cheek, para-lumbar region, peri-anal region and rectal temperature by clinical thermometer (°C) of seven days average before and on the day of fever in affected calves (n=3)

Body point	Seven days average before fever	Day of fever
IRT muzzle temperature	34.33±0.11	34.68±0.46
IRT eyeball temperature	36.60 ^a ±0.80	37.73 ^b ±0.03
IRT cheek temperature	34.50±0.67	34.97±1.04
IRT para-lumbar region temperature	35.67±0.54	35.97±0.17
IRT peri-anal temperature	38.30 ^a ±0.11	39.25 ^b ±0.17
Rectal temperature	38.83 ^a ±0.06	39.67 ^b ±0.26

Means bearing different superscripts in a row differ significantly ^{ab} (p<0.05)

Results and Discussion

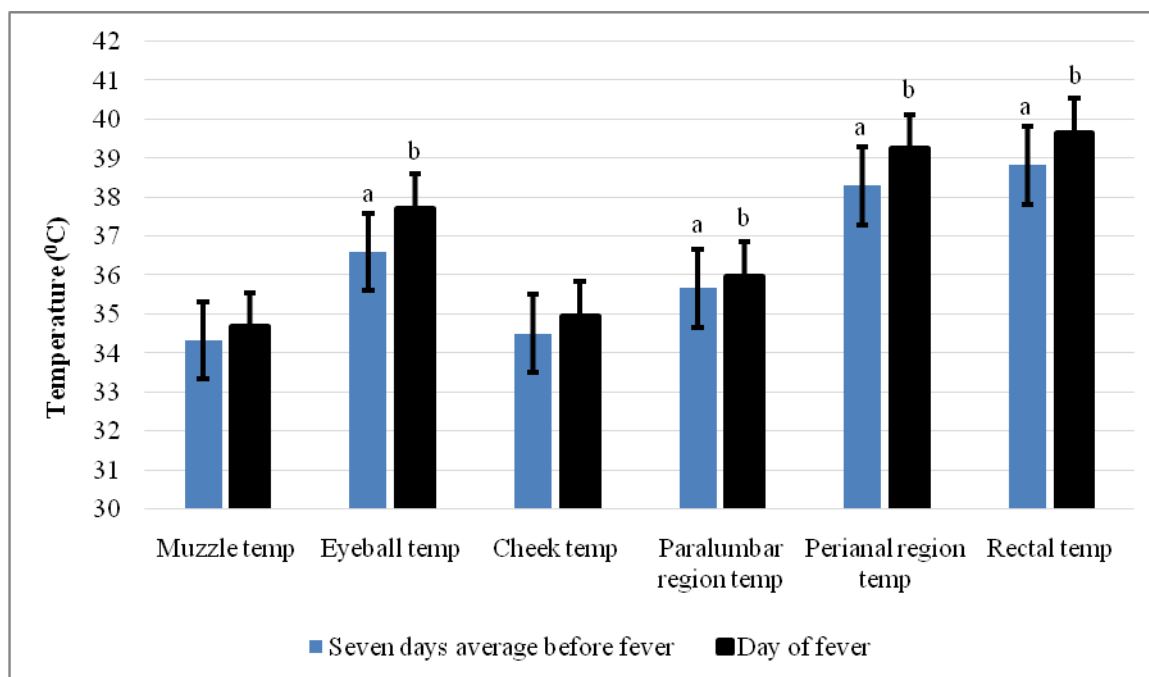


Fig. 4.27: IRT muzzle, eye, cheek, para-lumbar region, peri-anal region and rectal temperature (°C) by the clinical thermometer of seven days average before and day of fever in calves affected with fever, Bars bearing different superscripts differ significantly ^{ab}($p < 0.05$)

Infection with the bacteria and virus together during calf diarrhoea, pneumonia and fever causes septicemia and viremia, which is associated with core body temperature increase and emission of infrared rays, which can be captured by an Infrared camera. Infrared photographs taken over one cm around the eye ball region are useful in identifying temperature variations, since lacrimal glands serve as markers for changes in body temperature (Schaefer *et al.*, 2007). The increase in temperature of orbital region captured by IRT in case of bovine viral diarrhoea (BVD) (Schaefer *et al.*, 2004), bovine respiratory disease complex (BRD) (Schaefer *et al.* 2007, 2012) and side temperature in case of NCD (Lowe *et al.*, 2019) was well documented even before the appearance of actual clinical signs. Conventional scouring or even other biological tests are often incapable of early detection of infection. Some of the pathogens in faeces causing NCD may also found in the healthy calves, further limiting the diagnostic for effective treatment (Abuelo and Alves-Nores, 2016). IRT has been successfully used to monitor temperature change in case of mastitis (Polat *et al.* 2010), subclinical mastitis (Porcionato *et al.*, 2009), lameness (Alsaad *et al.*, 2014), foot and mouth disease (Gloster *et al.*, 2011) and bluetongue disease (Perez de Diego *et al.* 2013). Therefore, IRT may be a promising screening method for early detection of calf diarrhoea,

pneumonia and fever based on assessing the increase in temperature of the orbital region, which is closely associated with rectal temperature (Schaefer *et al.*, 2007).

Early detection of calf diarrhoea, pneumonia and fever or any other disease condition using IRT, where surface and body temperature increase is one of the major phenomena, can help in deciding timely start of the targeted treatment, reduce the use of antibiotics, timely isolation of the animal, prevent spread of infection, decrease animal suffering and improve the welfare of the animal. In the early identification of Bovine respiratory disease (BRD) complex, revaccination can also be a possibility stated by Schaefer *et al.* (2007). Early disease diagnosis helps the farmer in quick decision making and reduces the economic loss associated with veterinary treatment, death and labour. Till now, researchers have used infrared thermography as an early indicator to understand the change in temperature of various anatomical sites in case of bovine viral diarrhoea (Schaefer *et al.*, 2004) and NCD (Lowe *et al.*, 2019) in case of calves using infection induction model under controlled condition. The applicability of such induced models in case of early identification of naturally occurring similar infection under field conditions is still debatable. Therefore, in the present study IRT was used to understand temperature change in various anatomical points as an early indicator to predict calf diarrhoea, pneumonia in naturally occurring infection.

The change in the orbital temperature of an individual animal was mimicking the change in rectal temperature pattern, supported by the findings of Schaefer *et al.* (2004 and 2007) and reported that eye temperature is closely associated with rectal temperature in dairy animals. The lacrimal glands act as markers for change of body temperature as eye ball areas have more blood vessels and innervations (Schaefer *et al.*, 2007). Maximum temperature of orbital area was considered in the present investigation, which is supported by the observation of Lowe *et al.* (2020) who reported that image analyzed manually by software provided by manufacturer and auto analysis based on the algorithm for a maximum temperature of the designated orbital area showed close agreement. The overall higher orbital temperature in calves with diarrhoea, pneumonia and fever compared to healthy calves was supported by the observation of Schaefer *et al.* (2012). They also reported an overall average (3 week period) higher orbital temperature in case of BRD true positive (TP) cases compared to true negative (TN) cases. Similarly, day to day variation of rectal temperature in healthy Sahiwal calves has also been

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reported by various authors (Schaefer *et al.*, 2004; Snodgrass *et al.*, 1986; Mansoor *et al.*, 2018), rise in body temperature over 39.5°C and orbital temperature over 36.5°C was recorded in all the calves affected with diarrhoea, pneumonia and fever in the present study. Similar to the observation of the present study regarding peak orbital temperature of calves with diarrhoea, pneumonia and fever, Schaefer *et al.* (2012) also recorded a significantly higher peak thermal response for the BRD TP cases compared to true TN cases. The increase in temperature on the day of calves with diarrhoea, pneumonia and fever was also reported by various authors (Schaefer *et al.*, 2004; Snodgrass *et al.*, 1986; Mansoor *et al.*, 2018). Calf diarrhoea, pneumonia and fever occur mostly due to infection with bacteria, viruses, and parasites (Mansoor *et al.*, 2018). Infection with the bacteria and virus was associated with septicaemia and viremia, which could be a possible reason for an increase in orbital and rectal temperature as well as in other body points.

An increase in the temperature of orbital, peri-anal and para-lumbar region assessed by IRT and rectal temperature assessed by a clinical thermometer in calves was recorded around days of diarrhoea, pneumonia and fever. In a similar line, Schaefer *et al.* (2004) observed an increase in orbital, ear, nose, dorsal and side temperature in calves affected with bovine viral diarrhoea. Similarly, Schaefer *et al.* (2007) also reported an increase in temperature of various anatomical locations in the case of calves affected with "bovine respiratory disease complex". On the contrary, the non-significant eye temperature change in the case of induced neonatal calf diarrhoea (NCD) was reported by Lowe *et al.* (2019). The significant increase in IRT side temperature and decreased shoulder temperature was recorded even before the disease's clinical identification (Lowe *et al.*, 2019). Schaefer *et al.* (2004) documented that the different anatomical locations of calf regarding temperature change differ with disease conditions. Lowe *et al.* (2019) explained the temperature change of various anatomical location might act as an indicator for specific conditions as the change in temperature of extremities was reported to be a better indicator of feeding efficiency (Montanholi *et al.*, 2010; Martello *et al.*, 2016), whereas Lowe *et al.* (2019) found temperature change of core body area was useful indicators of NCD. The Inconsistencies in results may be associated with organisms involved in calf diarrhoea, pneumonia and fever, their pathogenesis pattern and difference in anatomical location as well as variation in environmental conditions (temperature and humidity) in which the study was conducted.

In the present study it has been observed that IRT was able to detect increase in orbital, perianal and para lumbar region temperature two days before the actual clinical sign appearance of diarrhoea, pneumonia and fever. The results corroborate with findings of Schaefer *et al.* (2004), who documented that less than 1°C temperature change was clinically significant. In a similar line, Schaefer *et al.* (2012) reported more than 1°C increase in orbital temperature in case BRD TP cases compared to TN cases. Lowe *et al.* (2019) reported NCD could be predicted significantly ($P < 0.001$) even before the appearance of clinical signs in case of a change in $<0.3^{\circ}\text{C}$ IRT temperature. Similar to our results, Schaefer *et al.* (2004) reported that the orbital temperature measured by IRT was more effective in detecting bovine viral diarrhoea where temperature change occurred as early as one day in case of eye (2.6°C or 0.26°C per day) compared to 5-6 days for other areas such as the ear (3.9°C or 0.4°C per day), nose (3.5°C or 0.35°C per day), dorsal (1.8°C or 0.18°C per day) and side (1.9°C or 0.19°C per day).

In contrast, clinical signs appeared 8-9 days after BVDV inoculation. The temperature change was more stable in the eye than in other anatomical areas (Schaefer *et al.*, 2004). Similarly, Schaefer *et al.* (2007) detected bovine respiratory disease complex 4 to 6 days earlier than the onset of clinical symptoms using infrared thermography. Similar to the present findings, researchers identified the onset of diseases by monitoring temperature change using infrared thermography (Schaefer *et al.*, 2004; Schaefer *et al.*, 2007; Schaefer *et al.*, 2012; Polat *et al.*, 2010; Hovinen, 2009; Rainwater-Lovett *et al.*, 2009). Schaefer *et al.* (2007) showed that infrared values were as good as or even more efficient than clinical ratings, core temperatures, or hematology in detecting diseased animals before BRD's clinical presentation. HPA axis activity is the first physiological response in case of disease or distress condition, and it is not efficient for energy use, so 40-60% energy is mostly lost as heat within the infrared range (Kleiber, 1975). Considering that up to 60 percent of an animal's heat loss occurred within the infrared spectrum, the finding of radiated heat loss acted as an early predictor of health condition.

Similar to the present study regarding an increase in temperature of the para lumbar fossa, Lowe *et al.* (2019) reported early detection of neonatal calf diarrhoea (NCD) based on a significant ($p < 0.001$) increase in side temperature (0.27°C) using IRT. Similarly, an increase in side temperature was also reported by Schaefer *et al.* (2004) in

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the case of BVD. The temperature change in the para lumbar fossa area may be, the site of infection is closer to the area as it is situated over rumen fossa and the occurrence of inflammation in the intestines. A combination of IRT temperature with feeding and drinking behaviour further can improve the prediction of diseases in calves (Lowe *et al.*, 2020).

The disease prediction using such temperature data generated in the present study can be analysed in several ways, as Galen and Gambino (1975) and Schaefer *et al.* (2004) stated. In the present study, the disease was predicted based on the temperature difference of a particular anatomical site of the affected animal from its own two days before and seven days average before the onset of temperature increase and unaffected control animal. Such models for early prediction of the disease have also been reported in Angus-Hereford cross heifers (Schaefer *et al.*, 2004). The better approach is developing individual animal-specific thresholds to declare the animal healthy so that any deviation from the threshold would be the sign of an early indicator of disease (Lowe *et al.*, 2020). The results depicted a significant increase in eye and rectal temperature in affected animals from its pre-infection temperature and the unaffected control animals of the same breed. Cook *et al.* (2002) reported that even though IRT's sensitivity is higher, the precise use of IRT for early detection of diseases is less. Standard clinical scores as an indicator of infection sometimes showed inconsistency (Wittum *et al.* 1996). The exciting fact besides non-invasiveness that IRT can provide real-time information contrast to confirmatory laboratory-based disease diagnosis (Schaefer *et al.* 2007). The added advantage of non-contact IRT in comparison to invasive monitoring of core body temperature and laboratory diagnostics was demonstrated in the study of Schaefer *et al.* (2007) that individual identification of the calf by RFID and auto capturing of IRT image during water drinking is more effective. Therefore, the IRT monitoring of Orbital temperature in case of temperature change related to calf health can be used as a supportive tool in routine farm management to enhance the clinical score's efficiency. Better eye temperature efficiency may be due to close association with core body temperature as it is closure to blood flow surface (Cook and Schaefer, 2013). To increase the accuracy level, sex, weight, diurnal variation of temperature, season, clinical score and physiological indicators should also be considered (Galen and Gambino 1975; Cook *et al.* 2002). Further, the capturing infrared data without disturbing the animal's natural

behavior, visible under manual handling while capturing IRT image, should be given importance as followed in the study of Schaefer *et al.* (2012), who used RFID enabled IRT scan windows in water trough level. In future, it is a step towards the development of automation to become part of precision farming based on image processing as supported by the recent development of an automated system based monitoring of eye and check the temperature using the algorithm to integrate the IRT image into the system for monitoring health and welfare of the animal (Lowe *et al.*, 2020). Integration of IRT in automatic feed station, water station and machine milking system has enormous opportunity.

4.2.31. Blood parameters of healthy and calves affected with different diseases:

Blood parameters of healthy calves (n=13) and calves affected with diarrhoea (n=8), pneumonia (n=2), and fever (n=3) have been presented in Table 4.2.32 and Fig 4.28. In the present study PCV (%) showed a significant ($p<0.05$) increase in diarrhoea affected calves (42.13 ± 1.46 vs. 32.77 ± 1.02) as compared to healthy calves. Similar to the present findings significant ($p<0.05$) increase in PCV (%) was reported by Niaz *et al.* (2000) (36.92 ± 4.33) and Ghanem *et al.* (2012) (37.88 ± 4.30) in diarrhoea affected calves. Singh *et al.* (2014) also reported similar findings of significant ($p<0.01$) increase in PCV (%) (43.8 ± 0.48) might be due to fluid loss from the body of calves in case of dehydration resulted in haemo-concentration of blood. In pneumonia affected calves, a significant ($p<0.05$) decrease in PCV % as compared to healthy calves (24.50 ± 0.50 vs. 32.77 ± 1.02) was recorded. Ismael *et al.* (2017) had reported similar results of significant decrease in PCV% in case of pneumonia affected calves as compared to the healthy calves (22.26 ± 0.31 vs. 33.73 ± 0.43). The decrease in PCV % in cases of pneumonia might be due to anaemia.

In the present study TLC ($\times 10^3/\mu\text{l}$) showed a significant ($p<0.05$) increase in diarrhoea (13.46 ± 0.39 vs. 8.18 ± 0.11) and pneumonia (24.10 ± 2.40 vs. 8.18 ± 0.11) affected calves as compared to healthy calves. In similar line various authors reported significant increase of WBCs in diarrhoea affected calves as compared to healthy calves (15.09 ± 1.9 vs. $9.08 \pm 1.63 \times 10^3/\mu\text{l}$, Ismael *et al.*, 2017; 28.22 ± 18.10 vs. $13.79\pm 3.41 \times 10^3/\mu\text{l}$; Akgul *et al.*, 2019; 11.36 ± 2.65 vs. $8.91\pm 1.20 \times 10^3/\mu\text{l}$ Schaefer *et al.* 2007). Similarly, various authors reported significant increase in WBC in case of pneumonia affected calves as compared to healthy calves (28.22 ± 18.10 vs. $13.79 \pm 3.41 \times 10^3/\mu\text{l}$,

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Akgul *et al.*, 2019). In case of fever no significant changes was observed for TLC ($\times 10^3$) as compared to healthy calves. In the present study neutrophil (%) showed a significant ($p < 0.05$) increase in diarrhoea (57.25 ± 2.64 vs. 33.00 ± 1.09) pneumonia (62.50 ± 2.50 vs 33.00 ± 1.09) and fever (61.33 ± 2.40 vs. 33.00 ± 1.09) affected calves as compared to healthy calves. Similar higher neutrophil count was reported by various researchers (Malik *et al.*, 2013; Niaz *et al.*, 2000; Ghanem *et al.*, 2012, Barua *et al.*, 2018). Malik *et al.* (2013) reported that neutrophils (%) count in calves affected with diarrhoea and healthy calves were 46.8 ± 0.75 , and 37 (12-38); Niaz *et al.* (2000) reported 55.35 ± 6.80 and 44.71 ± 3.04 ; Ghanem *et al.* (2012) reported 52 ± 3.8 and 45.8 ± 8.24 ; Barua *et al.* (2018) reported 46.8 ± 0.748 and 12-38, respectively. In pneumonia higher neutrophil count was also reported by various researchers (Almujalli *et al.*, 2015; Pradhan and Gupta, 2018; Akgul *et al.*, 2019). Almujalli *et al.* (2015) reported that neutrophil (%) in calves affected with pneumonia as compared to healthy calves was 68.13 ± 3.34 vs. 44.25 ± 2.35 , Pradhan and Gupta (2018) reported 31.86 ± 4.53 vs. 16.67 ± 1.17 , Akgul *et al.* (2019) reported 10.58 ± 6.54 vs. 3.64 ± 1.14 , respectively. In similar line Kumar *et al.* (2015) reported a significant ($p < 0.05$) increase in neutrophil (%) in case of fever affected calves as compared to healthy calves (54.63 ± 5.48 vs. 39.50 ± 1.31). In diarrhoea and pneumonia increase in TLC ($\times 10^3$) and neutrophil (%) occurs as a result of infection, inflammatory changes and toxins produced by the microorganism. Increase in TLC count and neutrophil (%) in the blood acts as a defensive mechanism against the pathogen (Mansoor *et al.*, 2018).

Lymphocyte (%) results showed a non-significant increase in calf diarrhoea and a non-significant decrease in fever affected calves as compared to healthy calves. Lymphocyte (%) in calf pneumonia showed a significant decrease as compared to healthy calves (36.00 ± 3.00 vs. 52.23 ± 1.27). In similar line significant decrease in lymphocyte in calves affected with pneumonia as compared to healthy calves was reported by Almujalli *et al.* (2015) (31.25 ± 2.45 vs. 48.25 ± 1.54), Pradhan and Gupta (2018) (44.14 ± 3.68 and 75.17 ± 1.62) and Akgul *et al.* (2019) (17.06 ± 9.73 vs. 13.18 ± 8.78). In case of pneumonia decrease in lymphocyte (%) might be observed due to viral infections and cytotoxic substances released in to the blood stream (Barua *et al.*, 2018).

Table 4.2.32: Mean \pm S.E. of Blood parameters of healthy and calves affected with diarrhoea, pneumonia and fever

Blood Parameters	Healthy (n=13)	Calf diarrhoea (n=8)	Calf pneumonia (n=2)	Fever (n=3)
Hb (g%)	10.40 \pm 0.26	10.51 \pm 0.24	10.55 \pm 0.05	10.93 \pm 0.20
PCV (%)	32.77 ^a \pm 1.02	42.13 ^b \pm 1.46	24.50 ^c \pm 0.50	33.67 ^a \pm 2.02
TEC (X10 ⁶)	9.10 \pm 0.13	8.75 \pm 0.15	8.83 \pm 0.07	8.86 \pm 0.15
TLC (X10 ³)	8.18 ^a \pm 0.11	13.46 ^b \pm 0.39	24.10 ^c \pm 2.40	8.63 ^a \pm 0.37
Neutrophil (%)	33.00 ^a \pm 1.09	57.25 ^b \pm 2.64	62.50 ^b \pm 2.50	61.33 ^b \pm 2.40
Lymphocyte (%)	52.23 ^a \pm 1.27	55.50 ^a \pm 1.31	36.00 ^b \pm 3.00	51.67 ^a \pm 2.85

Means bearing different superscripts in a row differ significantly ^{abc}(p<0.05)

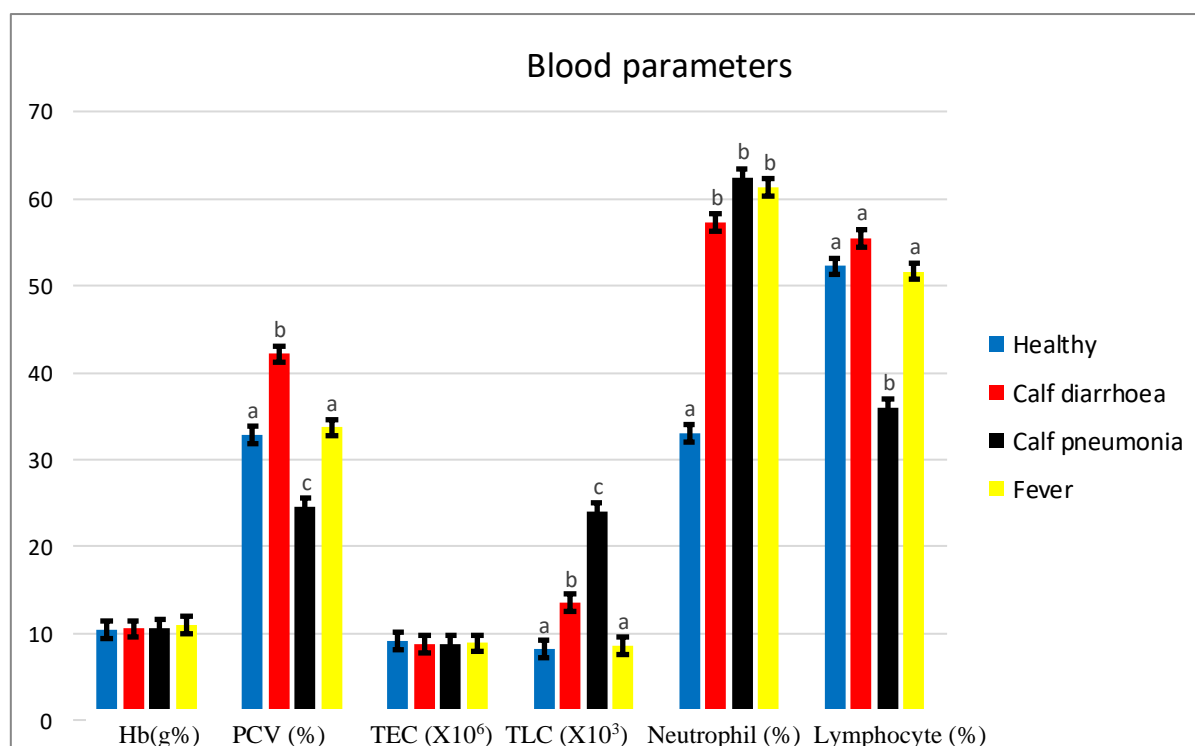


Fig. 4.28: Blood parameters of healthy and calves affected with diarrhoea, pneumonia and fever, Bars bearing different superscripts differ significantly ^{ab}(p<0.05)

4.2.32. Incidence rate of diseases:

In the present study incidence rate of diarrhoea, pneumonia and fever have been presented in Table 4.2.33. Out of 46 calves 8 calves were affected with diarrhoea, 2 calves were affected with pneumonia and 3 calves were affected with fever within one month after birth. The incidence rate of calf diarrhoea in the present study was 17.39%. Ferede *et al.* (2014) had reported nearly similar incidence rate of calf diarrhoea (21.4%). The calf diarrhoea incidence rate reported by other researchers was 79.50% (Ahmad *et al.*, 2009), 73.41% (Shivarudrappa *et al.*, 2013), 78.9% (Asmare and Kiros, 2016), 81.25% (Shakya *et al.*, 2017) and 75% (Manzoor *et al.*, 2018), respectively, which was higher than the present findings. Good health of calves refers to the absence of illness, stress and pain associated with the routine managerial procedures in a dairy farm. The two most common important health problems that affect dairy calf welfare are diarrhoea and respiratory disease. Calf diarrhoea was found to be the predominant calf health problem with an incidence rate of 42.9% as reported by Wudu *et al.* (2008). The different incidence rates of diarrhoea and respiratory disease reported were 23% and 22%, respectively among Canadian dairy farms (Windeyer *et al.*, 2014). In another study incidence risk of diarrhoea in calves varies from 20.05% to 52.51% (Samad, 2008; Wudu *et al.*, 2008; Megersa *et al.*, 2009; Malik *et al.*, 2012). The most frequent disease syndrome reported was diarrhoea with incidence rates of 10% (Megersa *et al.*, 2009). In another study, Islam *et al.* (2015) reported incidence risk of calf diarrhoea was 34.82%. The difference in the results might be due to difference in management involved in the rearing of the calves, causative organism and age groups.

The incidence rate of calf pneumonia in the present study was 4.34%. In similar line Severson *et al.* (2006) reported incidence rate of calf pneumonia 5.7%, Gulliksen *et al.* (2009) reported 2.5% and Assen *et al.* (2016) reported 4.9%. On the contrary various researchers reported a higher incidence rate of calf pneumonia 28% (Wudu, 2004), 18.6% (Ferede *et al.*, 2004), 9.2% (Amuamuta *et al.*, 2006), 15% (Sreedhar and Sreenivas, 2015), 11.5% (Abdisa and Minda, 2016), 19.6% (Venkateshkumar *et al.*, 2016) and 24% (Tiwari *et al.*, 2007), respectively. The increased incidence rate of calf pneumonia might be due to variation in seasons or poorly ventilated housing conditions (Callan and Garry, 2002). The higher incidence rate than the present findings might be due to difference in management involved in the rearing of the calves, herd size, causative organisms, age groups and the environment in which study was conducted.

Pneumonia in calves is a multi-factorial disease involving a group of viruses Bovine herpesvirus 1 (BoHV1); Bovine respiratory syncytial virus (BRSV); parainfluenza 3 virus (PI3) and bacteria (*Mycoplasma bovis*, *Pasteurella multocida*, *Mannheimia haemolytica*, *Histophilus somni*), as well as calf-related and environmental risk factors (Ellis, 2009; Brodersen, 2010; Schneider *et al.*, 2010; Autio *et al.*, 2007). It has been reported that viruses are the main causative agent of this disease, these are the first pathogen to intercede, whereas bacteria act as a secondary attacker, which worsens the already-ill animal's condition (Valarcher and Hagglund, 2006; Solis Calderon *et al.*, 2007; Taylor *et al.*, 2010). Bovine mycoplasma has been reported to be of increasing importance as a primary cause of calf pneumonia (Kusiluka *et al.*, 2000), and are often isolated from pneumonic lungs in combination with other pathogens (Booker *et al.*, 2008). Deep nasopharyngeal swabs, positive for *M. haemolytica* and *M. bovis*, in the pneumonic calves, are the representative of isolates present in the lung (Godinho *et al.*, 2007).

The incidence rate of fever in the present study was 6.52%. Islam *et al.* (2015) reported incidence risk of fever 4.5%, which was nearly similar to the present finding. Fever is a key symptom of a variety of diseases, including abscesses, tuberculosis, urinary tract infections, endocarditis, hepatobiliary infections, osteomyelitis, rickettsia, chlamydia, systemic bacterial infections, parasitic infections, autoimmune diseases (Vanderschueren *et al.*, 2009). It is a high-temperature condition that is often accompanied by disease symptoms such as lethargy, depression, anorexia, drowsiness, hyperalgesia, and inability to concentrate (Johnson, 2002). Fever in animals causes anorexia, wasting, sadness, muscle weakness, and a decrease in milk output, causing producers to lose a lot of money. In a study, it was reported that the most common disorders found in water buffaloes were digestive disorders followed by fever and respiratory disorders (Singh *et al.*, 2012).

Table 4.2.33: Incidence rate of different diseases

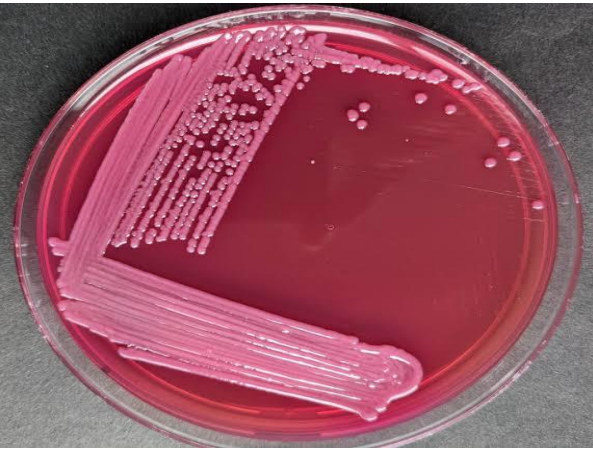

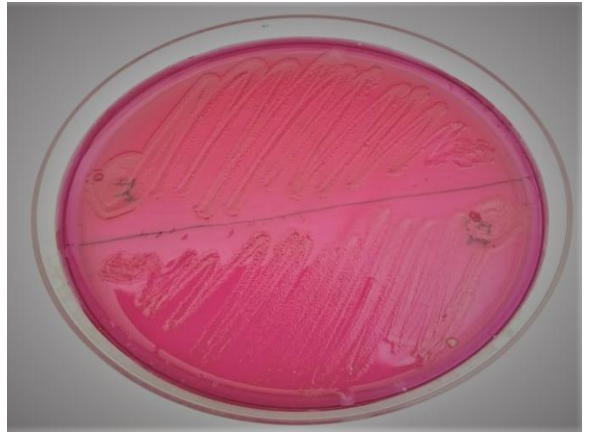
Disease condition	No. of cases	No. of Sahiwal calves	Incidence rate (%)
Diarrhoea	8	46	17.39
Pneumonia	2	46	4.34
Fever	3	46	6.52

4.2.33. Micro-organisms associated with calf diarrhoea:

The present research part was conducted to identify and characterize the bacterial pathogens causing calf diarrhoea. The distribution of different micro-organisms in calves affected with diarrhoea has been presented in Table 4.2.34. A total of 8 faecal samples from diarrhoeic calves were collected then cultured in differential media and examined by using culture properties documented in Table 4.2.9. Result showed that 2 different types of bacteria were isolated from the total of 8 faecal samples collected from diarrhoeic calves. Out of 8 faecal samples, 6 samples were found positive for *E. coli* (75%) giving a positive reaction to lactose fermentation on MacConkey agar plate as depicted on Plate no 4.21, metallic green sheen colonies on EMB plates as depicted on Plate 4.22. 2 samples were found positive for *Salmonella* (25%) producing a negative reaction to lactose fermentation on MacConkey agar plate as depicted in Plate 4.9. The organism produced pale pink colour colonies against a pinkish background on BGA plates as depicted in Plate 4.23. Various researchers had reported different frequency distribution of isolates, but the similar bacterial pathogens in case of calves affected with diarrhoea (Ghanem *et al.*, 2012; Kumar *et al.*, 2010; Abdullah *et al.*, 2013; Hemashenpagam *et al.*, 2009; Khan and Khan, 1997) as observed in the present study. Ghanem *et al.* (2012) reported that organisms isolated from diarrhoeic buffalo calves were *E. coli* 44 (73.3%) and *Salmonella spp.* 16 (26.7 %); Kumar *et al.* (2010) reported *E coli* 22 (72.22%), *proteus* 5 (16.66%), *Klebsiella* 2 (2.833%) and unidentified organisms 1 (2.77%). Abdullah *et al.* (2013) reported that organisms isolated from calves affected with diarrhoea was *E. coli* 44 (38.6%), *Salmonella spp* 25 (21.9%), *Staphylococcus spp* 15 (13.2%), mixed infection 18 (15.8%) and 12(10.5%) were negative for bacteria. Hemashenpagam *et al.* (2009) reported that micro-organisms isolated from calves with diarrhoea were *E. coli* 12 (75%), whereas Khan and Khan (1997) isolated *E. coli* (54-58%), *Staphylococcus* (7-10%) and *Salmonella* (13-14%). The variation in the percentage of various organisms causing calf diarrhoea may be due to differences in hygiene management at farm and field level.

Table 4.2.34: Distribution of different micro-organisms in faecal samples of calf diarrhoea

The total no. of samples examined	Name of isolated bacteria	Total no. of positive samples	Frequency of distribution
8	<i>E Coli</i>	6	75 %
	<i>Salmonella spp.</i>	2	25%

	
<p>Plate 4.22. Pink colony of <i>E.coli</i> on MacConkey agar</p>	<p>Plate 4.23. Metallic Sheen of <i>E. coli</i> on EMB agar</p>
	
<p>Plate 4.24. <i>Salmonella</i> spp on BGA</p>	

4.3: Objective 2: Evaluation of stress associated with different methods of disbudding using infrared thermography in dairy calves

Two experiments were conducted under this objective.

4.3.1. Experiment 1: Data generation of horn bud size in the different age groups of calves

To generate the data of horn bud size 33 Sahiwal calves of different age groups (Birth-10 days, 11-20 days, and 21-30 days) were selected for this experiment. Calves were moved to the paddock region after feeding of milk in the morning and then hair around the horn bud was trimmed by scissor and trimmer. Horn bud diameter was measured by vernier calliper daily for 10 days. The tip of the vernier calliper should be

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touched with the periphery of the horn bud. Jaws of the vernier calliper were parallel to each other. Three groups based on age were considered for further analysis.

4.3.2. Horn bud diameter of different age group of Sahiwal calves:

Mean \pm S.E. of age group wise horn bud diameter (mm) of Sahiwal calves have been presented in Table 4.3.1. In the present experiment significant ($p < 0.05$) increase in horn bud diameter was depicted in Fig 4.29 for the 11 to 20 days and 21 to 30 days age group as compared to the Birth to 10 days age group (10.21 ± 0.25 and 8.59 ± 0.24 vs. 8.16 ± 0.23). The increase in horn bud diameter with increasing age was evident. In similar line, Stafford and Mellor (2005) reported that horn bud size 5-10 mm long at 3-6 weeks of age. Marquette *et al.* (2021) reported that at 37 days of age horn bud size was 16.97 mm (Holestein Fresian male calves), at 24 to 26 days of age horn bud size was 15.09 mm (Charolais), at 26 to 28 days of age horn bud size was 13.76 mm (Limousin) and at 23 days of age horn bud size was 14.41 mm (Simmental). The reported horn bud size was higher than the present finding, might be due to the difference in breed and age group of calves used for the study.

Table 4.3.1: Mean \pm S.E. of age group wise horn bud diameter (mm) of Sahiwal calves

Parameter	Birth to 10 days (n=6)	11 to 20 days (n=14)	21 to 30 days (n=13)
Horn bud diameter	$8.16^a \pm 0.23$	$8.59^b \pm 0.24$	$10.21^b \pm 0.25$

Means bearing different superscripts in a row differ significantly ^{ab} ($p < 0.05$)

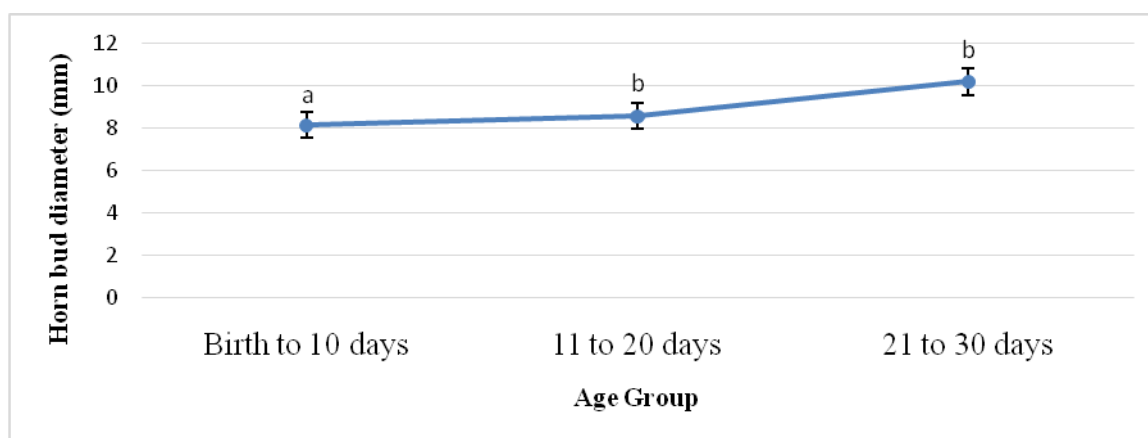


Fig. 4.29: Age group-wise horn bud diameter (mm) of Sahiwal calves. Lines bearing different superscripts differ significantly ^{ab} ($p < 0.05$)

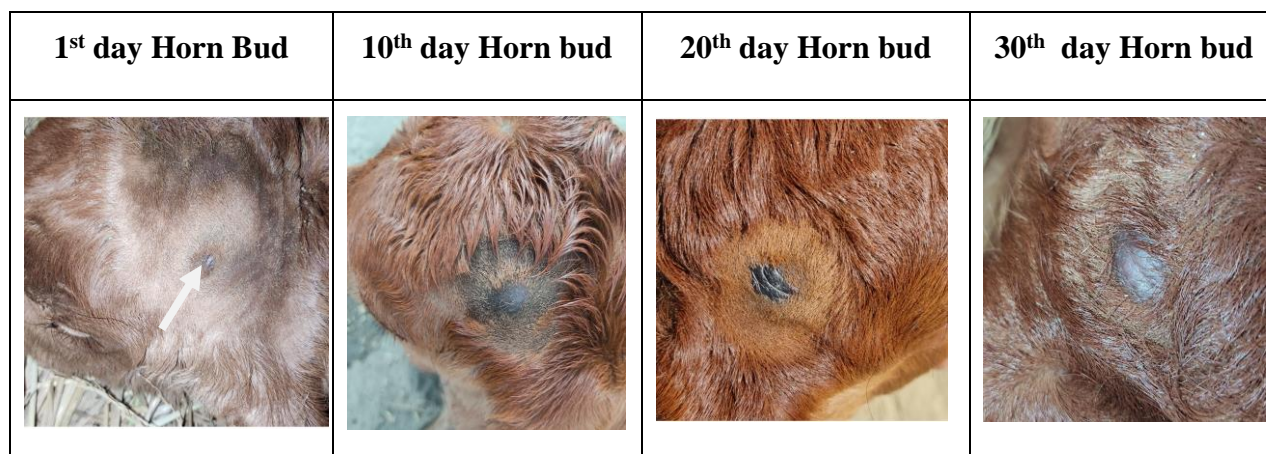





Plate 4.25: Images of horn bud on different days in Sahiwal calves

4.3.3. Experiment 2: Evaluation of stress associated with different methods of disbudding using IRT

In this experiment, 30 Sahiwal healthy calves, age group of 10-30 days were selected randomly assigned into 3 groups each having 10 calves i.e. Control (without local anaesthetic), T1 (with a local anaesthetic) and T2 (with local anaesthetic and NSAID). In T1 and T2 medicated nupatches were applied to the disbudded tissue.

Parameters	Control (n=10)	Treatment 1 (Lignocaine) (n=10)	Treatment 2 (Lignocaine and NSAID) (n=10)
Image of local anaesthetic application	-		
Image of NSAID application	-	-	

<p>Image of medicated nupatches application</p>	<p>-</p>		
<p>Disbudder used</p>			

Plate 4.26. Images of local anesthesia, NSAIDs and Nupatches

4.3.4. Body weight of Sahiwal calves in case of different methods of disbudding:

Mean \pm S.E. of body weight of Sahiwal calves in case of different methods of disbudding has been present in Table 4.3.2. and Fig 4.30. The body weight of the calves in all the groups increased with increasing age. The average of body weight of the calves in Control, Treatment 1 and Treatment 2 was 25.51 ± 1.60 , 25.09 ± 0.27 and 25.35 ± 0.34 , respectively at the time of disbudding and 33.45 ± 1.17 , 34.28 ± 0.37 and 34.80 ± 0.37 , respectively at 4th week after disbudding. In the first week body weight increase of 1.43 kg in Control, 2.11 kg in Treatment 1 and 2.27 kg in Treatment 2 was recorded. Significant increase in weekly average daily gain (ADG) (gms) was recorded in 1st week and 2nd week in Treatment 1 and Treatment 2 (324 ± 0.99 , 301 ± 0.16 vs. 204 ± 0.93 and 341 ± 0.15 , 328 ± 1.49 vs. 264 ± 1.11) as compared to Control. Non-significant increase in average daily gain was observed between the different treatment groups after 2nd week. But, in the first week after disbudding 0.68 kg higher in Treatment 1 and 0.84 kg higher in Treatment 2 as compared to control was recorded. In similar line, Faulkner and Weary (2000) reported that calves treated with ketoprofen tended to gain more weight (1.2 ± 0.4 kg) during the 24h after dehorning as compared to control calves (0.2 ± 0.4 kg) ($P=0.07$) and during the next 24h period, weight gains were similar for treated

(1.0±0.2kg) and control calves (1.1±0.4kg) (P>0.1). Bates *et al.* (2016) also reported that from -3day to the 15day growth rate of calves disbudded without pain relief 0.53 kg/day (95% CI=0.47-0.60) was less than that of calves disbudded with some form of pain relief 0.65kg/d (95% CI=0.62-0.68, P=0.004) and NSAID treatment increased the growth rate of calves disbudded as compared to calves disbudded without pain relief (p<0.05), increase in more weight gain per day than our results may be due to the age differences of calves, breed, sex and management differences of the farm. In similar line, Duffield *et al.* (2010) reported that ketoprofen-treated calves consumed more calf starter in the 24 hours following dehorning (P = 0.09), equating to approximately 0.25 kg more grain and there was also a significant effect of calf weight on starter consumption, with larger calves consuming more feed (P <0.001). Although calves had access to milk through-out the day but there was a daily limit on the volume that could be consumed, under this management these young calves may not able to express their full maximum potential growth rate (Bates *et al.*, 2016).

Changes in body weight (or average daily gain) are the key production indicators reported from horn removal in calves (Allen *et al.*, 2013; Black *et al.*, 2017; Stock *et al.*, 2015; Bates *et al.*, 2016). As disbudding is a stressful procedure that leads to increased plasma cortisol concentrations and a decrease in dry matter intake, which primarily affect body weight. At initial few weeks similar trend was observed as reported by Faulkner and Weary (2000) and Bates *et al.* (2016), but after that weight gain was non-significant might be due to better management of the calves at farm level helped to gain the body weight efficiently in control calves also.

Table 4.3.2: Mean ± S.E. of Bodyweight (Kg) of Sahiwal calves in case of different methods of disbudding

Time point	Control (n=10)	Treatment 1 (Lignocaine) (n=10)	Treatment 2 (Lignocaine and NSAID) (n=10)
Day of disbudding	25.51±1.60	25.09±0.27	25.35±0.34
1st week	26.94±1.43	27.20±0.46	27.62±0.39
2nd week	28.79±1.41	29.50±0.49	30.01±0.45

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3rd week	31.10±1.33	31.86±0.46	32.38±0.39
4th week	33.45±1.17	34.28±0.37	34.80±0.37

Table 4.3.3: Weekly average daily gain (gms) of Sahiwal calves in case of different methods of disbudding

Weekly ADG (gms)	Control (n=10)	Treatment 1 (Lignocaine) (n=10)	Treatment 2 (Lignocaine and NSAID) (n=10)
1st week	204 ^a ±0.93	301 ^b ±0.16	324 ^c ±0.99
2nd week	264 ^a ±1.11	328 ^b ±1.49	341 ^b ±0.15
3rd week	330±0.65	337±0.16	338±0.49
4th week	335±0.78	345±0.57	345±0.37

Means bearing different superscripts as ^{abc}(p<0.05) in a row differ significantly

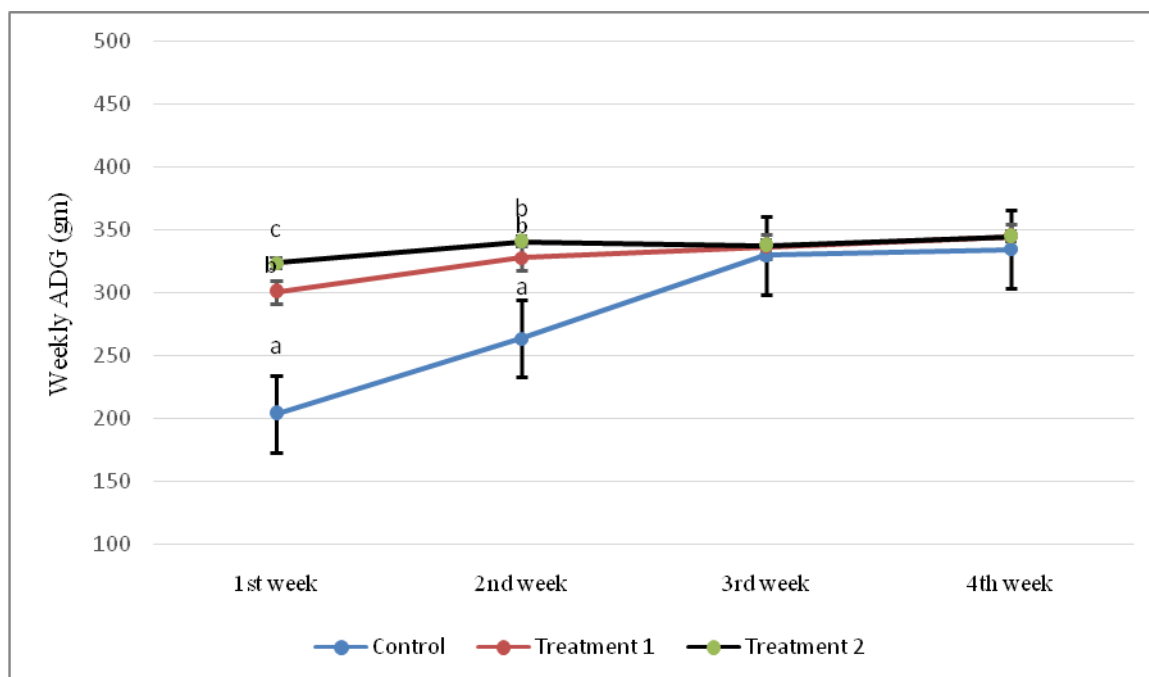


Fig. 4.30: Weekly ADG (gm) of Sahiwal calves in case of different methods of disbudding. Lines bearing different superscripts as ^{abc}(p<0.05) in a row differ significantly

4.3.5. Heart rate of Sahiwal calves in case of different methods of disbudding:

The heart rate (beats per minute) of Sahiwal calves with different methods of disbudding during different time points have been presented in Table 4.3.3. Heart rate (beats per minute) significantly ($p < 0.05$) increased during the 2 minutes following disbudding in Control and Treatment 1 as compared to baseline (136.60 ± 0.92 and 125.60 ± 3.97 vs. 116.1 ± 3.17 and 114.90 ± 3.46) as clearly depicted from Fig 4.31 and it remain elevated above the baseline for the remaining sampling period. In the Treatment 2 group also significant ($p < 0.05$) increase in heart rate (beats per minute) during 2 minutes following disbudding was observed as compared to baseline (123.60 ± 2.93 vs. 115.90 ± 2.89) and remained higher up to 3hrs after disbudding (122.90 ± 1.63). Heart rate increase in control group, 2 minutes after disbudding was 20 beats per minute, in Treatment 1 group was 11 beats per minute and in Treatment 2 group was 8 beats per minute as compared to before disbudding. Across different treatment groups heart rate (beats per minute) significantly ($p < 0.05$) increased during the 2 minutes after disbudding in control (136.60 ± 0.92) as compared to Treatment 1 (125.60 ± 3.97) and Treatment 2 (123.60 ± 2.93) as well as also significant increase at 3 hours after disbudding in control (134.70 ± 1.31) as compared to Treatment 1 (124.50 ± 1.18) and Treatment 2 (122.90 ± 1.63) was observed. Heart rate change in control group at 3 hours after disbudding was 16 beats per minute, in Treatment 1 was 10 beats per minute and in Treatment 2 was 7 beats per minute as compared to before disbudding. Without local anaesthetic, there was a greater change in heart rate, indicating higher levels of autonomic arousal in these calves, possibly symptomatic of acute pain. In similar line, Stewart *et al.* (2008) reported significant ($p < 0.01$) increase in heart rate in disbudded group without local anaesthetic (128.8 ± 8.2) and disbudded with local anaesthetic (110.9 ± 6.5) as compared to before disbudding (94.0 ± 6.8 and 90.1 ± 5.4). In similar line, Carray *et al.* (2015) also reported significant increase of heart rate after disbudding in control (150 ± 2.25) and xylazine (140 ± 2.24) as compared to before disbudding (139 ± 4.69 , 138 ± 4.94). In another study Schwartzkopf-Genswein *et al.* (2005) also reported that significant increase in heart rate in control (120 ± 2.85) and Treatment group (98.3 ± 2.63) as compared to before disbudding (102 ± 2.85 and 94.9 ± 2.83). NSAIDs abolishes the pain induced behaviour and the cortisol concentration which, reduces the stress response and heart rate in Treatment 2. In similar line Grondahl-Nielsen *et al.* (1999) reported that heart rate was raised for 3.5 hours after hot-iron dehorning without

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local anaesthetic. Heinrich *et al.* (2010) reported that meloxicam lowered the stress response following dehorning, as evidenced by changes in heart rate, which was corroborating with the present findings

The changes in physiological parameters during disbudding are well documented. The normal range of heart rate (HR) of calves is between 100 and 120 bpm (Jacobson and cook, 1998; Despres *et al.*, 2002; Veissier *et al.*, 2002), which is similar to the present finding before disbudding in all the three groups. In dairy cattle, heart rate variability is frequently used as a proxy measure of the autonomic stress response. In response to a stressor, the sympathetic and parasympathetic branches of the autonomic nervous system innervate the heart and act antagonistically. The sympathetic nervous system raises the heart rate, while the parasympathetic nervous system lowers it (Rea, 2016). Change in heart rate over time has been used to elucidate the information regarding the autonomic nervous system in response to different on-farm stressors like disbudding (Stewart *et al.*, 2008, 2009).

Table 4.3.4: Mean \pm S.E. of Heart Rate (Beats per minute) of Sahiwal calves in case of different methods of disbudding

Time point	Control (n=10)	Treatment 1 (Lignocaine) (n=10)	Treatment 2 (Lignocaine and NSAID) (n=10)
Before disbudding	116.1 ^X \pm 3.17	114.90 ^X \pm 3.46	115.90 ^X \pm 2.89
2 minutes after disbudding	136.60 ^{aY} \pm 0.92	125.60 ^{bY} \pm 3.97	123.60 ^{bY} \pm 2.93
3 hours after disbudding	134.70 ^{aY} \pm 1.31	124.50 ^{bY} \pm 1.18	122.90 ^{bY} \pm 1.63

Means bearing different superscripts as ^{ab}($p < 0.05$) in a row and as ^{XY}($p < 0.05$) in a column differ significantly

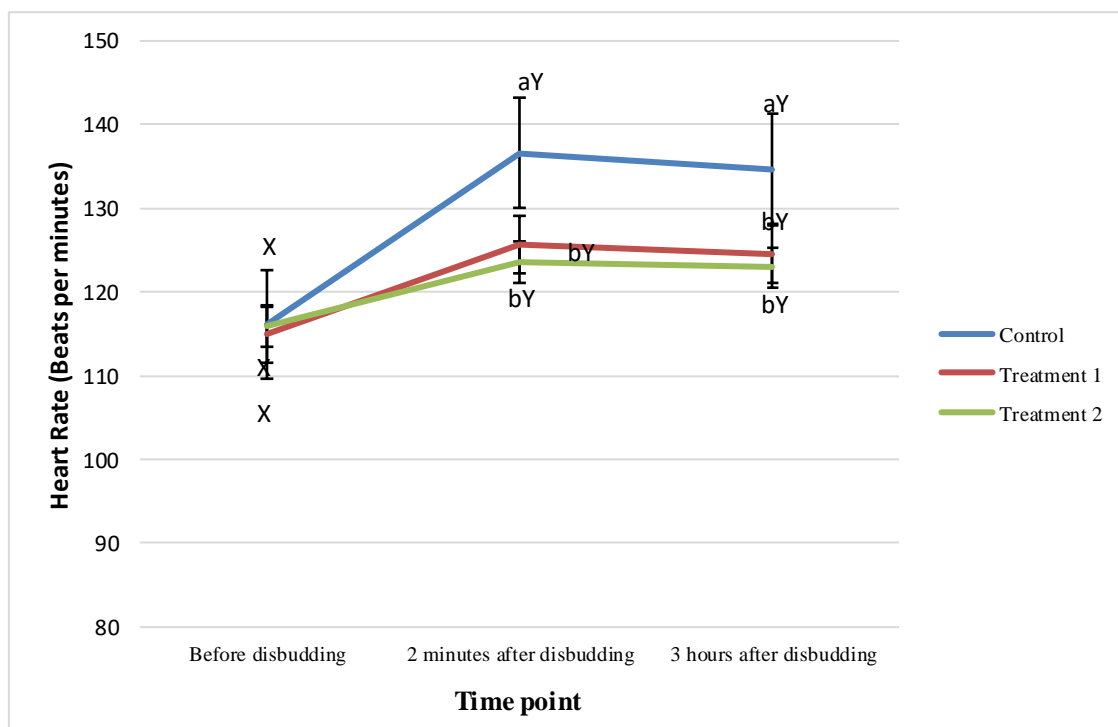


Fig. 4.31: Heart Rate (Beats per minute) of Sahiwal calves in case of different methods of disbudding, Lines bearing different superscripts as ^{ab}($p < 0.05$) between treatment and as ^{XY}($p < 0.05$) between time points differ significantly

4.3.6. Respiration rate of Sahiwal calves in case of different methods of disbudding:

The respiration rate (breaths per minute) of Sahiwal calves with different methods of disbudding during different time points have been presented in Table 4.3.4 and Fig 4.32. Respiration rate showed significant ($p < 0.05$) increase in without local anaesthetic group (33.30 ± 0.68 and 32.40 ± 0.85 vs. 23.50 ± 0.78), with local anaesthetic group (28.90 ± 1.28 and 27.80 ± 0.94 vs. 24.40 ± 0.96) and with local anaesthetic and NSAIDs group (28.10 ± 0.95 and 27.10 ± 0.74 vs. 25.80 ± 0.83) at 2 minutes after and 3 hours after disbudding as compared to before disbudding. Significantly ($p < 0.05$) lower respiration rate (breaths per minute) was observed in case of Treatment 1 and Treatment 2 at 2 minutes after disbudding (28.90 ± 1.28 , 28.10 ± 0.95 vs. 33.30 ± 0.68) and at 3 hours after disbudding (27.80 ± 0.94 , 27.10 ± 0.74 vs. 32.40 ± 0.85) as compared to control. No significant change was observed between Treatment 1 and Treatment 2. Local anaesthetic reduces the pain at the time of disbudding decreases respiration rate of Treatment 1 and NSAIDs decrease the respiration rate of calves during disbudding, so it reduces the respiration rate of Treatment 2. The results of the present study corroborating

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with the findings of Heinrich *et al.* (2010) and Stewart *et al.* (2013). Heinrich *et al.* (2010) reported that increase in respiration rate after calves were disbudded with a local anaesthetic. There was an increase in respiration rate after disbudding, which is normally linked with an increase in heart rate (Stewart *et al.*, 2013).

Table 4.3.5: Mean \pm S.E. of Respiration Rate (Breaths per minute) of Sahiwal calves in case of different methods of disbudding

Time point	Control (n=10)	Treatment 1 (Lignocaine) (n=10)	Treatment 2 (Lignocaine and NSAID) (n=10)
Before disbudding	23.50 ^X \pm 0.78	24.40 ^X \pm 0.96	25.80 ^X \pm 0.83
2 minutes after disbudding	33.30 ^{aY} \pm 0.68	28.90 ^{bY} \pm 1.28	28.10 ^{bY} \pm 0.95
3 hours after disbudding	32.40 ^{aY} \pm 0.85	27.80 ^{bY} \pm 0.94	27.10 ^{bY} \pm 0.74

Means bearing different superscripts as ^{ab}(p<0.05) in a row and as ^{XY}(p<0.05) a column differs significantly

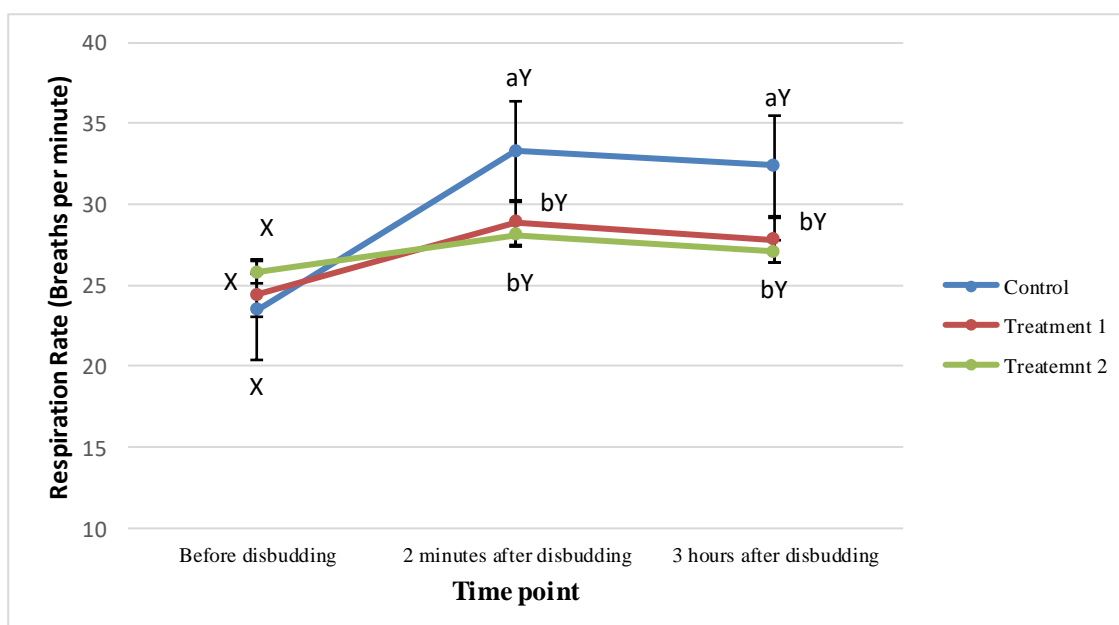


Fig. 4.32: Respiration Rate (Breaths per minute) of Sahiwal calves in case of different methods of disbudding, Lines bearing different superscripts as ^{ab}(p<0.05) between treatment and as ^{XY}(p<0.05) between time points differs significantly

4.3.7. Rectal temperature (°C) of Sahiwal calves in case of different methods of disbudding:

Rectal temperature (°C) of Sahiwal calves with different methods of disbudding during different time points have been presented in Table 4.3.5 and Fig 4.33. A significant increase ($p < 0.05$) in rectal temperature was observed at 3 hours after disbudding when compared with the before disbudding in without local anaesthetic group (39.32 ± 0.11 vs. 38.79 ± 0.12), with local anaesthetic group (39.30 ± 0.18 vs. 38.80 ± 0.19) and in with local anaesthetic and NSAIDs group (39.20 ± 0.12 vs. 38.89 ± 0.09). No significant difference was observed between the different treatment groups at any point of time. Change in rectal temperature in without local anaesthetic group (0.53°C), with local anaesthetic group (0.50°C), with local anaesthetic and NSAIDs group (0.31°C) at 3 hours after disbudding as compared to before disbudding. There is no literature available regarding the rectal temperature change before or after disbudding to compare the findings of the present study. In control group physical activity was more as compared to Treatment 1 and Treatment 2. Change in rectal temperature might be due to the increased physical activity of the calves after disbudding (Yamazaki *et al.*, 2006).

Table 4.3.6: Mean \pm S.E. of Rectal temperature (°C) of Sahiwal calves in case of different methods of disbudding

Time point	Control (n=10)	Treatment 1 (Lignocaine) (n=10)	Treatment 2 (Lignocaine and NSAID) (n=10)
Before disbudding	$38.79^X \pm 0.12$	$38.80^X \pm 0.19$	$38.89^X \pm 0.09$
2 minutes after disbudding	$38.97^X \pm 0.08$	$38.95^X \pm 0.18$	$38.90^X \pm 0.06$
3 hours after disbudding	$39.32^Y \pm 0.11$	$39.30^Y \pm 0.18$	$39.20^Y \pm 0.12$

Means bearing different superscripts in a column differs significantly $^{XY}(p < 0.05)$

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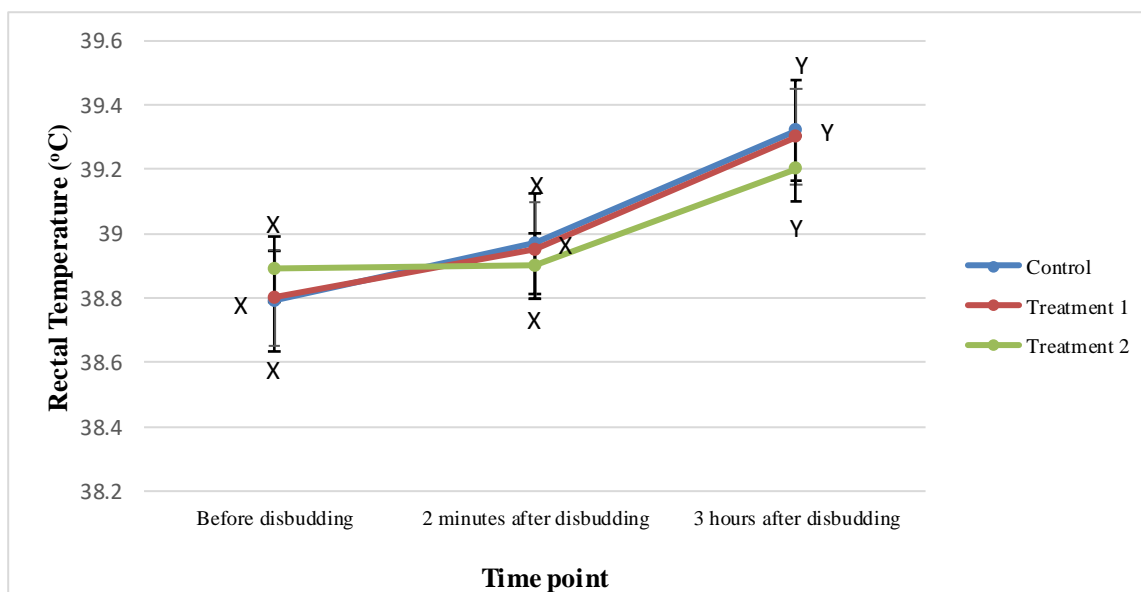


Fig. 4.33: Rectal temperature (°C) of Sahiwal calves in case of different methods of disbudding, Lines bearing different superscripts as ^{XY}($p < 0.05$) between time points differs significantly

4.3.8. Frequency of different behaviours in case of different methods of disbudding:

The behaviour of Sahiwal calves in case of different methods of disbudding during 4 hours time point have been presented in Table 4.3.6 and Fig. 4.34. Significant ($p < 0.05$) change in frequencies of pain-related behaviour after disbudding was observed between the treatment. The frequency of head shaking was significantly ($p < 0.05$) higher in control (33.63 ± 2.27) as compared to Treatment 1 (24.50 ± 1.61) and Treatment 2 (13.50 ± 1.25). The frequency of tail wagging was significantly ($p < 0.05$) higher in the control (13.13 ± 1.06) as compared to Treatment 1 (9.63 ± 0.75) and Treatment 2 (6.00 ± 0.57). Frequency of ear flicking, leg to head scratching, and leg lifting was higher but non-significant in control group (12.88 ± 1.76 , 2.88 ± 0.35 , 5.50 ± 0.73) as compared to Treatment 1 (11.88 ± 1.77 , 2.75 ± 0.95 , 4.63 ± 0.86) and Treatment 2 (10.38 ± 0.89 , 2.63 ± 0.52 , 2.63 ± 0.56). Various researchers reported that calves display the majority of behavioural modification during the 2-4 hours period after disbudding (Morisse *et al.*, 1995; Petrie *et al.*, 1996; Graf and Senn, 1999; Grøndahl-Nielsen *et al.*, 1999) therefore in the present study 4 hrs video recording was carried out for behavioural study. The use of a local anaesthetic and NSAIDs reduces the early rise in plasma cortisol concentration. Tail wagging, head movements, tripping, head rubbing, head shaking, ear flicking and rearing are all reduced by local analgesics (Graf and Senn, 1999). In similar

line, Faulkner and Weary (2000) reported less head shaking, ear flicking and head rubbing behaviour in calves after hot-iron disbudding in the ketoprofen treated group as compared to the control group. In similar line, Duffield *et al.* (2010) reported that Ketoprofen-treated calves displayed significantly fewer total head flicking ($P = 0.046$) and ear-flicking ($P = 0.01$) behaviours than control calves. Sylvester *et al.* (2004) also reported that disbudded calves without lidocaine were highly restless for the first 6 hr after disbudding and exhibited significantly more tail flicking, head flicking, ear flicking than the control ($P < 0.05$). Vickers *et al.* (2005) reported head shaking was 9 times higher in calves dehorned with a hot iron as compared to the calves dehorned with the caustic paste ($U = 7.5$, $N_1 = 7$, $N_2 = 8$, $P < 0.05$) during the first 4 hr. Heinrich *et al.* (2010) reported that ear flicking behaviour was higher in control calves as compared to meloxicam treated calves on day 0, (1.31 ± 0.66 vs 4.29 ± 1.10 ear flicks/h) and day 1 (0.55 ± 0.50 vs 3.27 ± 0.89 ear flicks /h), ($F = 10.65$, $df = 16$, $P = 0.005$), whereas in the present study similar higher ear flicking behaviour observed in control, but it was non-significant. In another study NSAID treated calves after disbudding showed an increased occurrence of play behaviour in NSAID-treated calves when the animals were tested 3 hr post disbudding (Mintline *et al.*, 2013). It is well evident that behavioural responses in dairy calves get influenced by both methods of disbudding and the use of analgesic, sedative and NSAID during disbudding.

Behavioural responses of the calves are considered a vital measure of their well-being (Dawkins, 2003; Stull and Reynolds, 2008). Calf behaviour has been studied by various authors about routine management practices associated with the tissue-damaging effect (Stafford and Mellor, 2011). Behavioural responses like head shake, head rubs, ear flicks, tail flicks, foot stamps, play behaviour, lying, standing, grooming, kicking, and falling are considered as a marker of pain (Doherty *et al.*, 2007). Several types of research also measured the frequency of non-evoked behavioural states such as movement, self-grooming, or drinking as an indicator of stress (Heinrich *et al.*, 2010; Faulkner and Weary, 2000; Milligan *et al.*, 2004; Duffield *et al.*, 2010). During and after the hot-iron disbudding leads to pain-related behavioural alterations. These changes in behaviour last for 12 to 44 hr (Faulkner and Weary, 2000; Heinrich *et al.*, 2010). The skin around the horn bud is damaged by hot-iron disbudding, creating a third-degree wound. Hot iron disbudding causes a pronounced behavioural response such that physical restraint is necessary to carry out the disbudding procedure. Tail wagging, head

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movements, tripping, head rubbing, head shaking and ear flicking and rearing are all reduced by local analgesics (Graf and Senn, 1999). Stress indispensably accompanies animals on a dairy farm. The methodologies used to quantify stress include direct behavioural observations and evaluation of physiological parameters, such as fluctuations in heart rate, blood pressure or changes in the concentration of stress hormones in the blood (Ayala *et al.*, 2012). The behaviour of calves is most commonly used to assess the disbudding stress, which is considered as a key indicator of calf welfare (Stull and Reynolds, 2008; Dawkins, 2003; Stafford and Mellor, 2005). Behavioural changes are a more sensitive marker for the assessment of pain in comparison with other physiological markers such as cortisol (Anil *et al.*, 2002). Behaviour indices in farm animals have been recorded using videography (Duffield *et al.*, 2010), accelerometers and remote triangulation devices (Theurer *et al.*, 2012). In the present study 4 hrs video recording has been used to study the behavioural changes after disbudding. After disbudding, calves jiggle their heads, attempt to suspend the head around pen instruments, sometimes behave apathetically, lying side by side with their heads and do not respond to other people in the group as well as muscle trembling and fear are also detected in the calves (Stilwell *et al.*, 2012). Increased physical activity after disbudding increases the body surface temperature, which influences the eye temperature so that the overall degree of activity is calculated by recording actions before and after disbudding (Yamazaki *et al.*, 2006). Calves often display behavioural signs of pain and discomfort after disbudding, include increased head shaking, lying bouts, hind leg kicks and decreased grooming, rumination, rubbing, and head jerks. These behavioural measures are most commonly observed during 4 hr after disbudding (Grøndahl-Nielsen *et al.*, 1999). Calves display the majority of behavioural modification during the 2-4 hour period after disbudding (Morisse *et al.*, 1995; Petrie *et al.*, 1996; Graf and Senn, 1999). In hot iron, disbudding causes thermal destruction to the tissue surrounding the horn bud area, as a result, there is an increase in head-shaking activity of the calf to eliminate the source of discomfort or reduce pain. Frequent head shaking following disbudding can indicate pain. Administration of non-steroidal anti-inflammatory drugs (NSAID) to disbudded calves can reduce head shaking frequencies similar to that of placebo kids (Ingvast-Larsson *et al.*, 2011). A significant increase in head shaking, ear flicking, vocalizing, grooming and head rubbing behaviour was also recorded in disbudded calves by Faulkner and Weary (2000).

Table 4.3.7: Mean ± S.E. of frequency of behavior of Sahiwal calves in case of different methods of disbudding

Group	Tail wagging	Head shaking	Ear flicking	Leg to head-scratching	Leg lifting
Control (n=10)	13.13 ^a ±1.06	33.63 ^a ±2.27	12.88±1.76	2.88±0.35	5.50±0.73
Treatment 1 (Lignocaine) (n=10)	9.63 ^b ±0.75	24.50 ^{ab} ±1.61	11.88±1.77	2.75±0.95	4.63±0.86
Treatment 2 (Lignocaine and NSAID) (n=10)	6.00 ^c ±0.57	13.50 ^b ±1.25	10.38±0.89	2.63±0.52	2.63±0.56

Means bearing different superscripts in a column differ significantly ^{abc}(p<0.05)

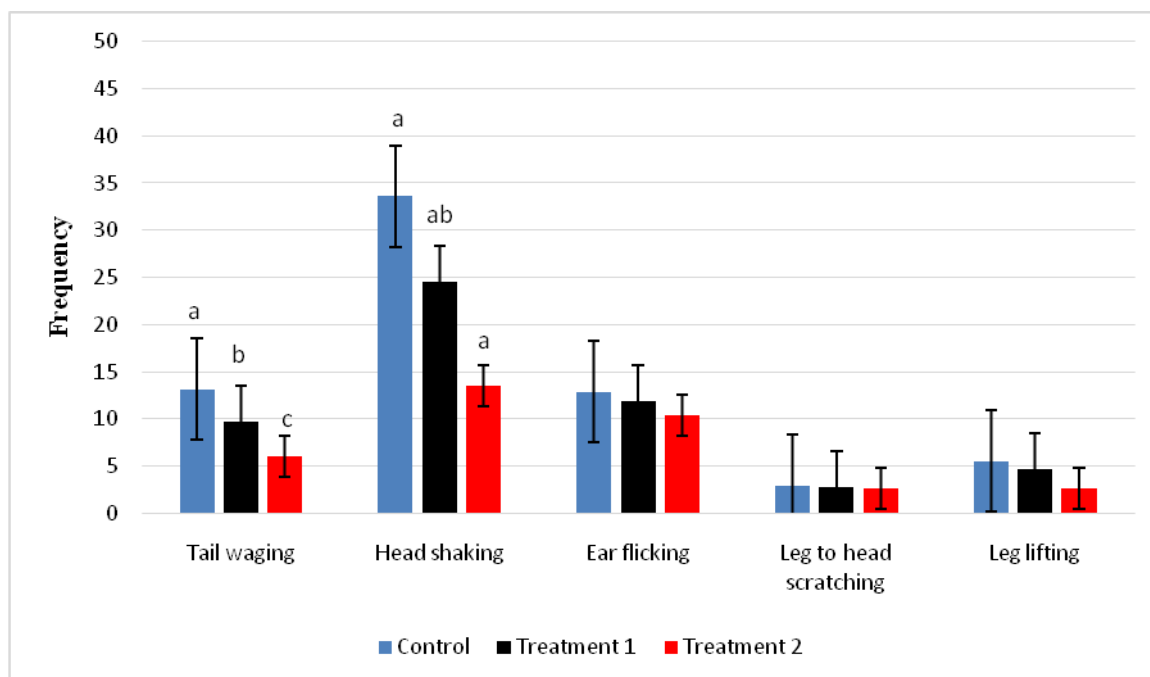


Fig. 4.34: Frequency of behavior of Sahiwal calves in case of different methods of disbudding, Bars bearing different superscripts differ significantly ^{abc}(p<0.05).

4.3.9. IRT eyeball temperature of Sahiwal calves in case of disbudding with different methods:

Eyeball temperature ($^{\circ}\text{C}$) of Sahiwal calves in case of different method of disbudding at different time points has been presented in Table 4.3.7 and Fig 4.35. Significant ($p < 0.05$) drop in eyeball temperature (1.57°C) after disbudding was recorded in without local anaesthetic group as compared to before disbudding (35.45 ± 0.25 vs. 37.02 ± 0.18) after that eyeball temperature returned to the baseline 90 minutes after disbudding (37.22 ± 0.20) and thereafter increase was non-significant for up to 180 minutes. In case of local and local anaesthetic and NSAIDs eyeball temperature had dropped non-significantly from baseline, between 2-5 minutes post disbudding (0.64°C and 0.18°C) as compared to before disbudding (36.50 ± 0.20 vs. 37.14 ± 0.21 and 36.92 ± 0.34 vs. 37.10 ± 0.09) after that eyeball temperature reached to baseline level at 90 minutes (37.18 ± 0.11) in local anaesthetic group and at 30 minutes (37.00 ± 0.17) in local anaesthetic and NSAIDs group after that increase was non-significant for up to 180 minutes. In similar lines, Stewart *et al.* (2008) reported that rapid drop of eyeball temperature (0.27°C) during the 5 minutes following disbudding without local anaesthetic ($p < 0.05$) and only a small non significant decrease of eyeball temperature in local anaesthetic group then there was increase in eyeball temperature which was higher than baseline for the remaining sampling periods in without and with local anaesthetic groups. Similar to the present finding no significant differences in eyeball temperature were recorded between 2 and 3 h after dehorning for any other treatment ($P \geq 0.129$) (Stewart *et al.*, 2008). Significant drop in eyeball temperature in without local anaesthetic may be due to the change in blood flow from the capillary bed via sympathetically- mediated vasoconstriction in the acute pain. Drop in eyeball temperature was consistent with the finding of various authors (Blessing, 2003; Stewart *et al.*, 2008; Vianna and Carrive, 2005; Nakayama *et al.*, 2005). Disbudding is the stressful and painful procedure that leads to activation of the hypothalamic-pituitary-adrenal (HPA) axis and sympathetic adrenal medullary activity (SAM), which are a primary biological response to stress in animals. The concentration of plasma cortisol in the blood, which reflects the hypothalamus-pituitary-adrenal axis (HPA) activity, has long been used to assess the stress associated with disbudding (Stafford and Mellor, 2005). The immediate response of disbudding is the diversion of blood from cutaneous capillary beds via sympathetically mediated vasoconstriction, which subsequently decreases the skin

temperature of calves. Infrared thermography can identify the effect of vasoconstriction as a temperature change (Blessing, 2003). Eye temperature responses, assessed by IRT, have been reported to be a helpful non-invasive approach for evaluating stress in animals (Stewart *et al.*, 2005). In response to stress and early disease detection in cattle, eye temperature has been demonstrated to be a more consistent indicator of temperature changes than other anatomical locations such as the nose, ear, body, and feet (Schaefer *et al.*, 2004). Therefore, IRT can be used to measure the change in eye temperature, which is a good indicator of stress in dairy calves during disbudding.

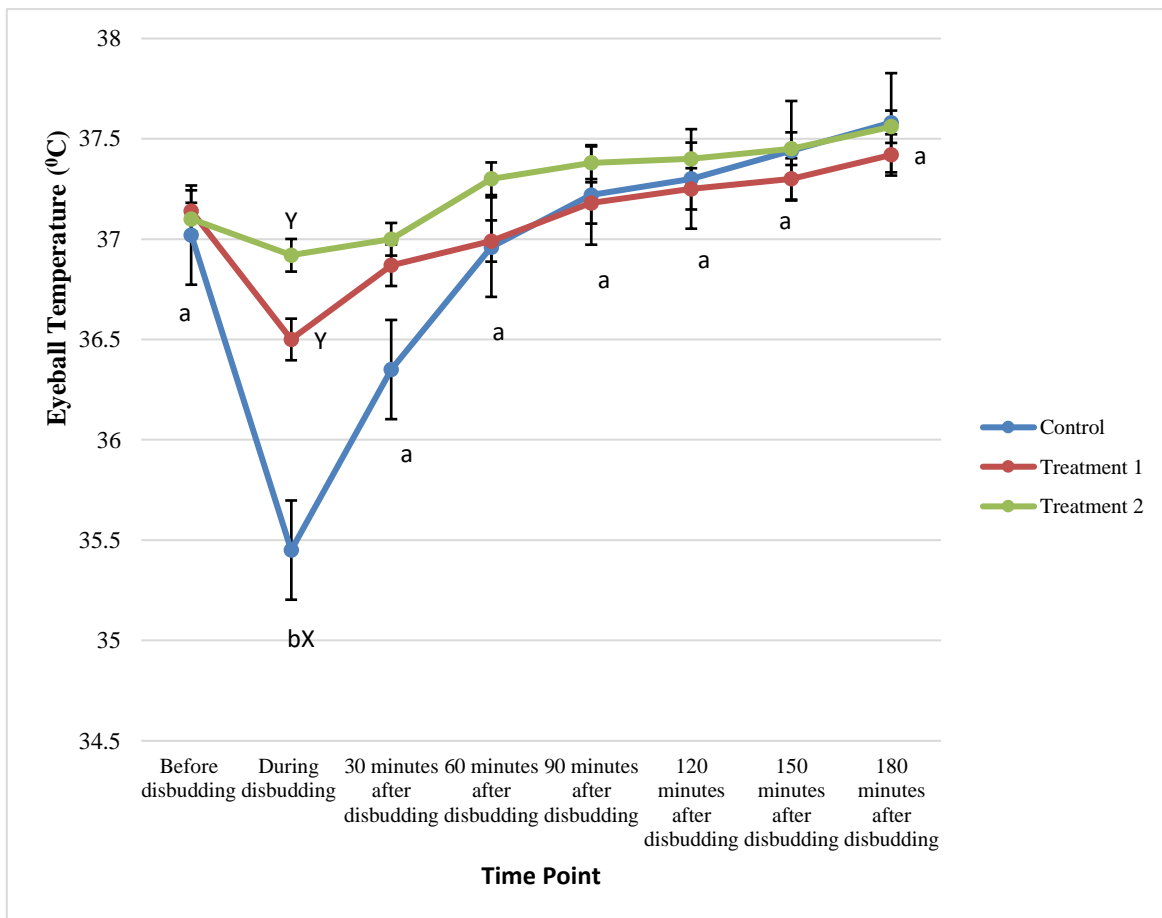


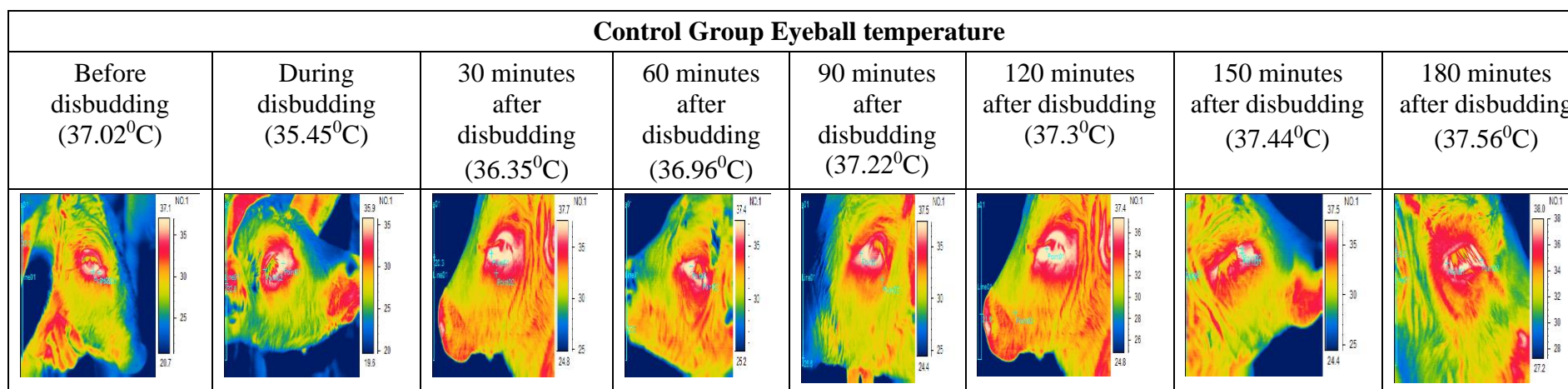
Fig. 4.35: IRT eyeball temperature (°C) of Sahiwal calves in case of different methods of disbudding, Lines bearing different superscripts as ^{XY}($p < 0.05$) between treatment and as ^{ab}($p < 0.05$) between time points differ significantly

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Table 4.3.8: Mean ± S.E. of IRT eyeball temperature (°C) of Sahiwal calves in case of different methods of disbudding

Groups	Time point							
	Before disbudding	At disbudding	30 minutes after disbudding	60 minutes after disbudding	90 minutes after disbudding	120 minutes after disbudding	150 minutes after disbudding	180 minutes after disbudding
Control (n=10)	37.02 ^a ±0.18	35.45 ^{bX} ±0.25	36.35 ^a ±0.22	36.96 ^a ±0.14	37.22 ^a ±0.20	37.3 ^a ±0.18	37.44 ^a ±0.11	37.58 ^a ±0.11
Treatment 1 (Lignocaine) (n=10)	37.14±0.21	36.50 ^Y ±0.20	36.87±0.10	36.99±0.10	37.18±0.11	37.25±0.13	37.3±0.16	37.42±0.11
Treatment 2 (Lignocaine and NSAID) (n=10)	37.10±0.09	36.92 ^Y ±0.34	37.00±0.17	37.30±0.29	37.38±0.20	37.4±0.17	37.45±0.18	37.56±0.14

Means bearing different superscripts as ^{ab}(p<0.05) in a row and as ^{XY}(p<0.05) in a column differ significantly



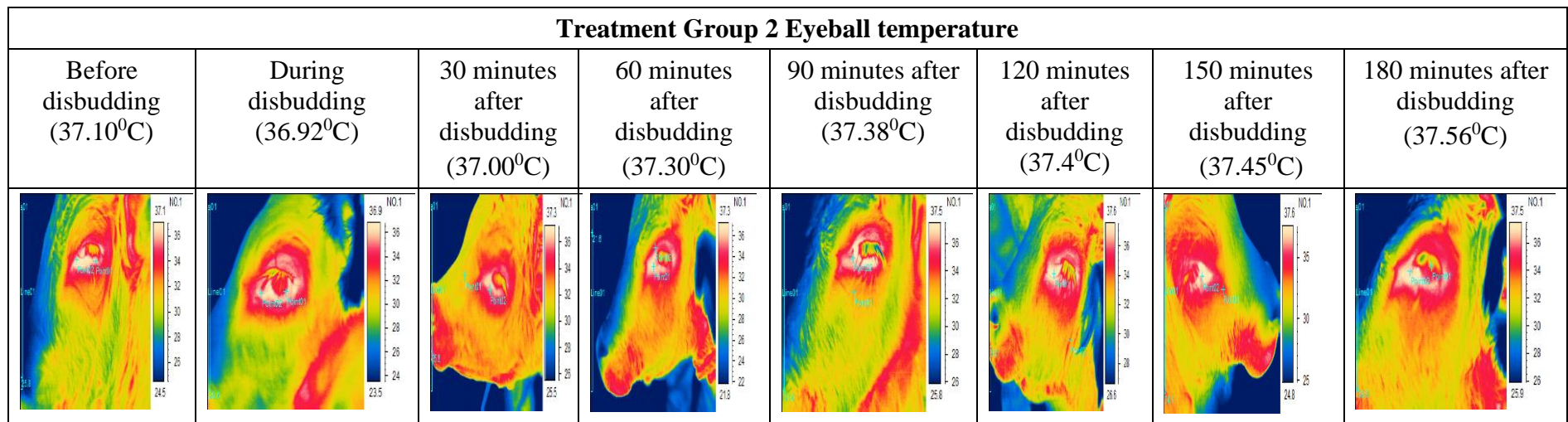
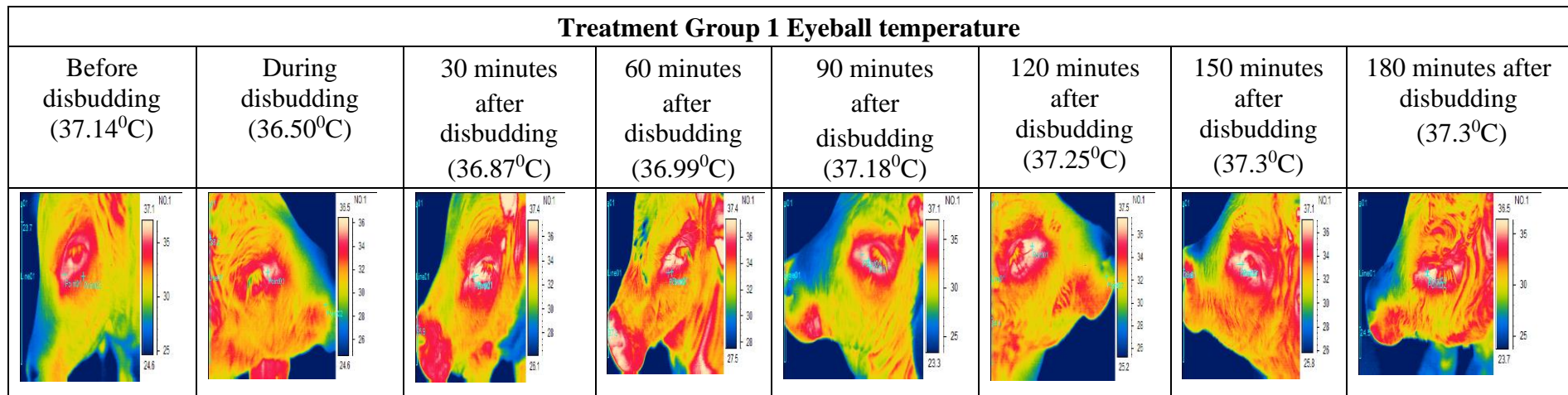


Plate 4.25: IRT images of the eyeball during different methods of disbudding

4.3.10. IRT horn bud temperature of Sahiwal calves in case of disbudding with different methods:

Horn bud temperature (°C) of Sahiwal calves in case of different method of disbudding at different time points has been presented in Table 4.3.8 and Fig 3.36. Significant ($p < 0.05$) increase in horn bud temperature (°C) after disbudding was recorded in without lignocaine (37.40 ± 0.22 vs. 35.26 ± 0.41), with lignocaine (37.35 ± 0.35 vs. 35.31 ± 0.26) and with lignocaine and NSAIDs (37.07 ± 0.46 vs. 35.19 ± 0.13) as compared to before disbudding (30 minutes before). After that horn bud temperature decreased non-significantly in all the groups. No significant difference was observed across the different treatment groups and time points after disbudding. Horn bud temperature returned to the baseline temperature 180 minutes after disbudding in all the groups. In similar lines, Scherf *et al.* (2020) reported a significant ($p < 0.01$) increase in horn bud temperature immediately after disbudding as compared to before disbudding (67.8 ± 1.90 vs 35.6 ± 0.30). Cwynar *et al.* (2017) also reported that significant increase in horn bud temperature after 1 minute of disbudding (38.1°C) as compared to before disbudding (26.9°C). In another study Karlen *et al.* (2021) reported that increase in horn bud temperature at disbudding time was greater in control (36.75°C) and M1 group (36.72°C) than sham disbudded group (34.57°C) and also significant increase in horn bud temperature was observed when compared to 5 minutes before disbudding (25.46°C , 25.19°C and 25.99°C). The surface temperature of the horn bud increased due to the application of hot iron during disbudding. After 30 minutes of disbudding, the temperature of the horn bud had decreased by up to 50 % Scherf *et al.* (2020).

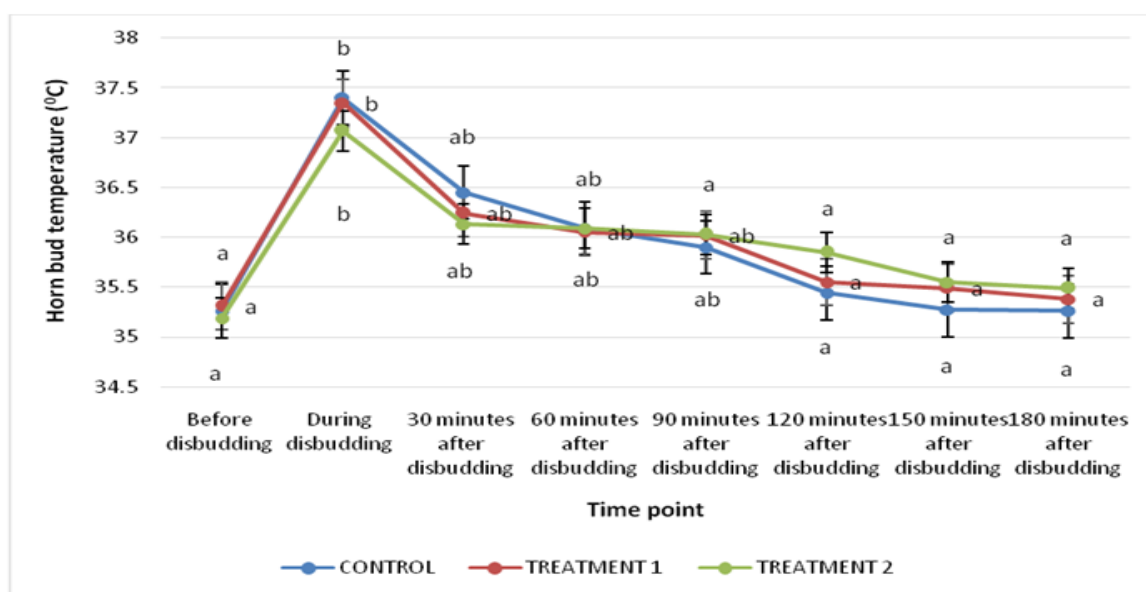
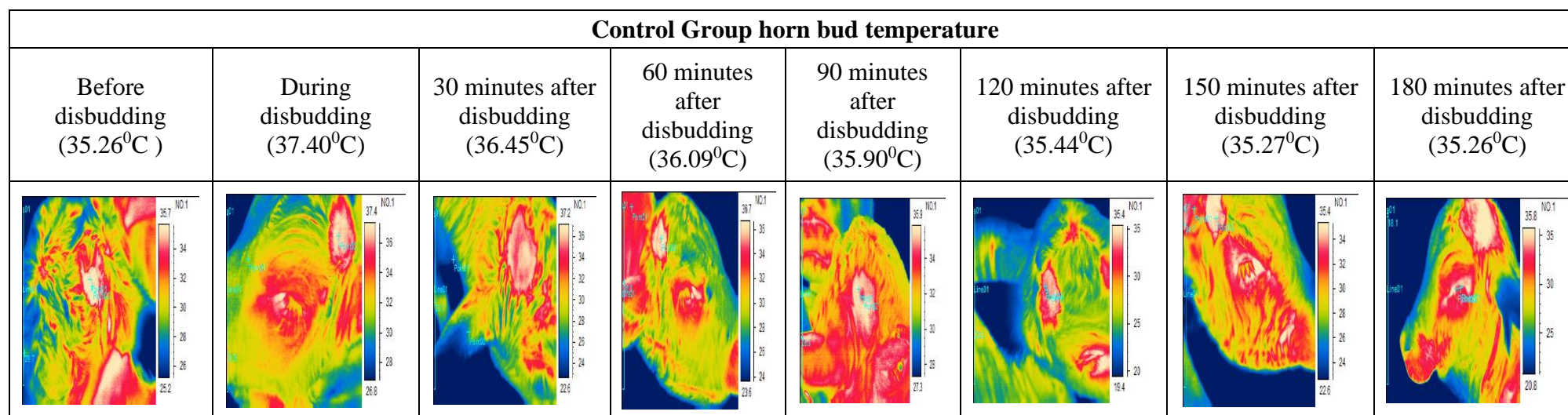


Fig.4.36: IRT horn bud temperature (°C) of Sahiwal calves in case of different methods of disbudding, Lines bearing different superscripts as ^{ab}($p < 0.05$) in different time points differ significantly

Table 4.3.9: Mean ± S.E. of IRT horn bud temperature (°C) of Sahiwal calves in case of different methods of disbudding

Groups	Timepoint							
	Before disbudding	At disbudding	30 minutes after disbudding	60 minutes after disbudding	90 minutes after disbudding	120 minutes after disbudding	150 minutes after disbudding	180 minutes after disbudding
Control (n=10)	35.26 ^a ±0.41	37.40 ^b ±0.22	36.45 ^c ±0.38	36.09 ^c ±0.28	35.90 ^c ±0.27	35.44 ^a ±0.36	35.27 ^a ±0.32	35.26 ^a ±0.24
Treatment 1 (Lignocaine) (n=10)	35.31 ^a ±0.26	37.35 ^b ±0.35	36.24 ^c ±0.42	36.05 ^c ±0.32	36.02 ^c ±0.32	35.55 ^a ±0.22	35.49 ^a ±0.23	35.38 ^a ±0.26
Treatment 2 (Lignocaine and NSAID) (n=10)	35.19 ^a ±0.13	37.07 ^b ±0.46	36.13 ^c ±0.19	36.09±0.23	36.03 ^c ±0.23	35.85 ^a ±0.23	35.55 ^a ±0.33	35.49 ^a ±0.24

Means bearing different superscripts as ^{abc}(p<0.05) in a row differ significantly



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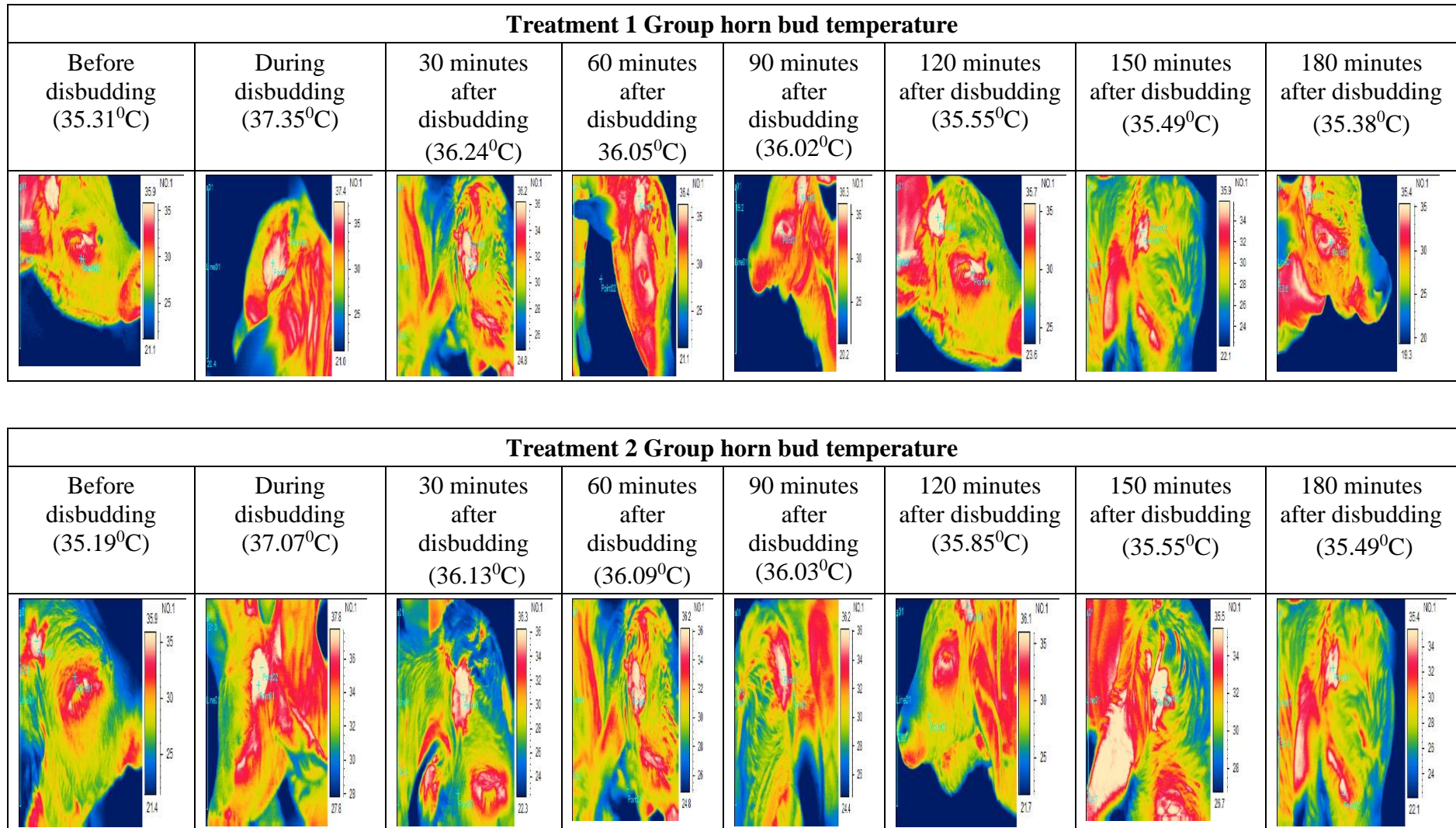


Plate 4.28: IRT images of horn bud with different methods of disbudding

4.3.11. IRT peri-anal region temperature of Sahiwal calves in case of disbudding with different methods:

IRT peri-anal region temperature ($^{\circ}\text{C}$) of Sahiwal calves in case of different methods of disbudding at different time points has been presented in Table 4.3.9 and Fig 4.37. Significant ($p<0.05$) increase in peri-anal region temperature in without local anaesthetic group 30 minutes after disbudding (37.90 ± 0.20 vs. 37.11 ± 0.22), with local anaesthetic 120 minutes after disbudding (37.90 ± 0.21 vs. 37.23 ± 0.31) and with local anaesthetic and NSAIDs 150 minutes after disbudding (37.90 ± 0.19 vs. 37.25 ± 0.24) was observed as compared to before disbudding. Hoffmann *et al.* (2013) reported that tendency for the IR temperatures to increase with increasing rectal temperature was observed in the calves. No published data is available regarding peri-anal region temperature of disbudded calves to compare the findings of the present study. The reason could be a physiological reaction of the body when it is trying to cool down the body temperature over the skin as a type of regulation.

4.3.12. IRT horn bud temperature of Sahiwal calves in case of disbudding with different methods during healing process:

Horn bud temperature ($^{\circ}\text{C}$) of Sahiwal calves in case of different method of disbudding at different time points has been presented in Table 4.3.10 and Fig 4.38. Significant ($p<0.05$) increase in horn bud temperature on the day of disbudding without local anaesthetic (37.40 ± 0.22 vs. 35.26 ± 0.41), with local anaesthetic (37.35 ± 0.35 vs. 35.31 ± 0.26) and with local anaesthetic and NSAIDs (37.07 ± 0.46 vs. 35.19 ± 0.22) group was recorded, as compared with one day before disbudding. Horn bud temperature significant ($p<0.05$) decrease was observed at time 7th day of 4th week after disbudding in control (36.30 ± 0.30), in Treatment 1 and Treatment 2 at time 3rd day of 3rd week (36.34 ± 0.36 and 36.44 ± 0.35) as compared to during disbudding (37.40 ± 0.22 , 37.35 ± 0.35 , 37.07 ± 0.46). No significant difference was observed across the treatment groups. Results of the present study are in consonance with the Tucker *et al.* (2014) and Mirra *et al.* (2018). Adcock and Tucker (2018) reported non-significant difference in temperature between disbudded wound and the non disbudded tissue with respect to time. Alvarez *et al.* (2019) reported a 5-9 wk wound healing period for hot iron disbudding. Adcock and Tucker (2018) reported that wound healing takes 9 weeks (62 ± 10 d). Adcock *et al.* (2019) reported that wound healing takes 40-70 days. The healing duration in the present experiment was less as compared to reports of Alvarez *et al.* (2019); Adcock and Tucker (2018) and Adcock *et al.* (2019). The difference in time for wound healing may be due the difference in the age group of calves, breed and the size of the disbudder and method of disbudding used for conducting the experiment.

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Table 4.3.10: Mean \pm S.E. of IRT peri-anal region temperature ($^{\circ}$ C) of Sahiwal calves in case of different methods of disbudding

Groups	Timepoint							
	Before disbudding	At disbudding	30 minutes after disbudding	60 minutes after disbudding	90 minutes after disbudding	120 minutes after disbudding	150 minutes after disbudding	180 minutes after disbudding
Control (n=10)	37.11 ^a \pm 0.22	37.70 ^{ab} \pm 0.20	37.90 ^b \pm 0.20	38.03 ^b \pm 0.25	38.13 ^b \pm 0.17	38.10 ^b \pm 0.16	38.19 ^b \pm 0.12	38.20 ^b \pm 0.15
Treatment 1 (Lignocaine) (n=10)	37.23 ^a \pm 0.31	37.67 ^{ab} \pm 0.23	37.78 ^{ab} \pm 0.09	37.80 ^{ab} \pm 0.21	37.87 ^{ab} \pm 0.19	37.90 ^b \pm 0.21	37.98 ^b \pm 0.17	38.07 ^b \pm 0.12
Treatment 2 (Lignocaine and NSAID) (n=10)	37.25 ^a \pm 0.24	37.61 ^{ab} \pm 0.17	37.69 ^{ab} \pm 0.14	37.75 ^{ab} \pm 0.26	37.86 ^{ab} \pm 0.25	37.89 ^{ab} \pm 0.25	37.90 ^b \pm 0.19	38.00 ^b \pm 0.16

Means bearing different superscripts as ^{ab}($p < 0.05$) in a row differ significantly

Table 4.3.11: Mean \pm S.E. of IRT horn bud temperature ($^{\circ}$ C) of Sahiwal calves in case of different methods of disbudding during the healing process

Time	Before disbudding	During disbudding	1 st week after disbudding		2 nd week after disbudding		3 rd week after disbudding		4 th week after disbudding		5 th week after disbudding	
			3 rd day	7 th day	3 rd day	7 th day	3 rd day	7 th day	3 rd day	7 th day	3 rd day	7 th day
Control (n=10)	35.26 ^a \pm 0.41	37.40 ^b \pm 0.22	36.92 ^b \pm 0.34	36.81 ^b \pm 0.29	36.69 ^{bc} \pm 0.34	36.50 ^{bc} \pm 0.41	36.49 ^{bc} \pm 0.51	36.47 ^{bc} \pm 0.20	36.40 ^{bc} \pm 0.37	36.30 ^c \pm 0.30	36.20 ^c \pm 0.25	36.07 ^c \pm 0.17
Treatment T1 (Lignocaine) (n=10)	35.31 ^a \pm 0.26	37.35 ^b \pm 0.35	36.80 ^b \pm 0.24	36.71 ^{bc} \pm 0.68	36.60 ^{bc} \pm 0.43	36.43 ^{bc} \pm 0.31	36.34 ^c \pm 0.36	36.2 ^c \pm 0.40	36.19 ^c \pm 0.51	36.11 ^c \pm 0.48	36.07 ^c \pm 0.24	36.00 ^c \pm 0.23
Treatment T 2 (Lignocaine and NSAID) (n=10)	35.19 ^a \pm 0.22	37.07 ^b \pm 0.46	36.70 ^b \pm 0.40	36.64 ^b \pm 0.36	36.54 ^b \pm 0.20	36.44 ^b \pm 0.35	36.30 ^c \pm 0.36	36.23 ^c \pm 0.33	36.16 ^c \pm 0.23	36.09 ^c \pm 0.22	36.02 ^c \pm 0.27	35.98 ^c \pm 0.20

Means bearing different superscripts in a row differ significantly ^{abc}($p < 0.05$)

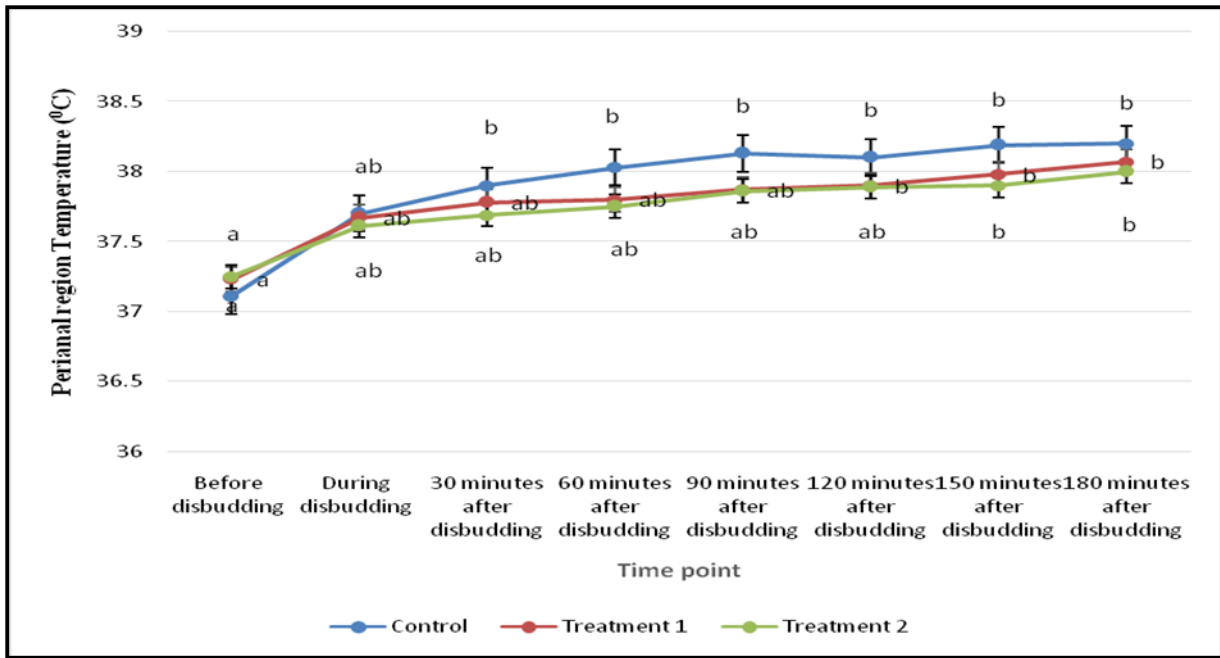


Fig. 4.37: IRT peri-anal region temperature (°C) of Sahiwal calves in case of different methods of disbudding, Lines bearing different superscripts as ^{ab}(p<0.05) in different time points differ significantly

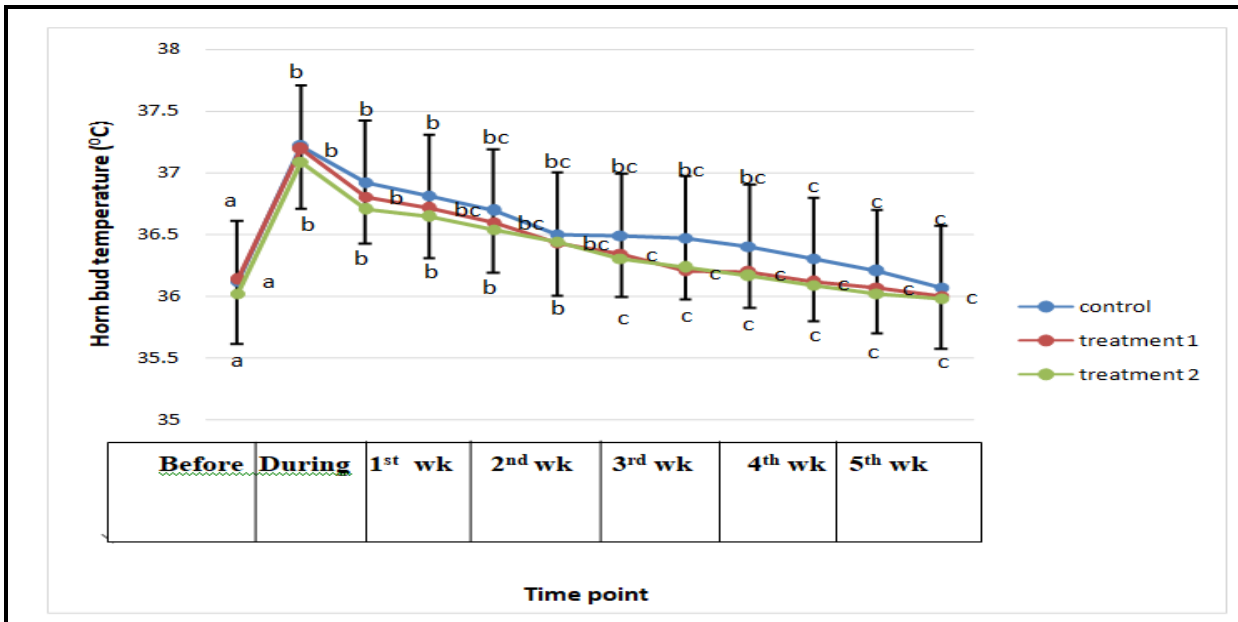


Fig. 4.38: IRT Horn bud temperature (°C) of Sahiwal calves in case of different methods of disbudding during healing process, Lines bearing different superscripts as ^{ab}(p<0.05) between time points differ significantly.

4.3.13. Lesion score of the horn bud of Sahiwal calves in case of disbudding with different methods during healing process:

The lesion score of Sahiwal calves in case of different methods of disbudding have been presented in Table 4.3.11. No significant difference in the lesion score between the different treatments was recorded in 1st week after disbudding as depicted from Plate 4.26. Significant ($p < 0.05$) increase of lesion score in without local anaesthetic group was observed in 2nd week and 3rd week (1.80 ± 0.25 vs. 1.30 ± 0.15 and 1.20 ± 0.13) and (1.80 ± 0.25 vs. 1.30 ± 0.15 and 1.10 ± 0.10) as compared to with local anaesthetic and with local anaesthetic and NSAIDs group, respectively. Complete epithelisation in control and Treatment 1 group took place when lesion score reached to 1, 5 weeks after disbudding and in Treatment 2 lesion score reached 1, 4 weeks after disbudding. In the similar line, Huebner *et al.* (2017) reported that lesion score 3 on 3rd week in control group was greater than aluminum-based aerosol bandage (ALU) group 17% vs. 8%. In a recent study, Huebner *et al.* (2017) reported no difference in lesion score between the control group as compared with the aluminium-based aerosol bandage group during the first 2 weeks post-disbudding but the proportion of lesion score on 3rd-week post disbudding was greater (17 vs. 8%, respectively). In Control group, disbudding sites were exhibited 1.42 times delayed healing at week 3 post-disbudding as compared to aluminium-based aerosol bandage disbudding sites. In 3rd week, the treatment group's wound diameter was 1 mm smaller than the control group, and the treatment group wound diameter declined better throughout the healing process as compared to the control group. The medicated patch acts as a drying or astringent agent to accelerate wound contracture or reduce inflammation associated with coagulative necrosis (Huebner *et al.*, 2017). Stilwell and Laven (2020) reported that presence of granulation tissue and a reduction in crusting of disbudding wounds treated with topical anaesthetic gel compared to those treated with antimicrobial spray implies that topical anaesthetic gel may expedite wound healing. The wound healing after disbudding took 5 week for control, 4 week for Treatment 1 and 4 week for Treatment 2, respectively. The available literature regarding wound healing showed that disbudded wounds take 62 ± 10 d (Mean \pm SD; range: 42-91 d) for complete re-epithelialisation. But it was reported that necrotic tissue took a longer time to heal when disbudding was carried out at younger age, in 3 days calves (26 ± 1 d) as compared to 35 days older calves (23 ± 1 d; $F_{1,22} = 7.36$, $P = 0.013$) (Adcock and Tucker, 2018). Tucker *et al.* (2014) reported that hot-iron branding, which takes at least 10 weeks to reepithelialise in beef calves aged 4 to 7 months, showed a similar healing pattern as that of disbudding. The type of iron








utilized did not affect re-epithelialisation latency, which took on an average (mean \pm standard deviation) 53 ± 3 days for Portasol wounds and 55 ± 3 days for Rhinehart wounds, respectively (range: 40–70 days) (Adcock *et al.*, 2019). A recent study looked at wound healing in feedlot cattle using various dehorning methods and found that different dehorning methods resulted in different levels of wound infection and healing time, with recovery taking up to four weeks (Neely *et al.*, 2014), which was similar to the findings of the present study. Alvarez *et al.* (2019) also reported a 5-9 wk wound healing period for hot iron disbudding. The difference in the wound healing time after disbudding or dehorning may be associated with the difference in age group, breed, methods of disbudding or dehorning and climatic condition at the time of disbudding.








Table 4.3.12: Mean \pm S.E. of lesion Score of horn bud during the healing process

Time	1st week after disbudding	2nd week after disbudding	3rd week after disbudding	4th week after disbudding	5th week after disbudding
Control (n=10)	1.00 \pm 0.00	1.80 ^a \pm 0.25	1.80 ^a \pm 0.25	1.40 \pm 0.16	1.00 \pm 0.00
Treatment 1 (Lignocaine) (n=10)	1.00 \pm 0.00	1.30 ^b \pm 0.15	1.30 ^b \pm 0.15	1.20 \pm 0.13	1.00 \pm 0.00
Treatment 2 (Lignocaine and NSAID) (n=10)	1.00 \pm 0.00	1.20 ^b \pm 0.13	1.10 ^b \pm 0.10	1.00 \pm 0.00	1.00 \pm 0.00

Means bearing different superscripts in column differ significantly ^{ab} (p<0.05)

Results and Discussion

Control						
Before disbudding	After disbudding	1st week	2nd week	3rd week	4th week	5th week
						

Treatment 1						
Before disbudding	After disbudding	1st week	2nd week	3rd week	4th week	5th week
						








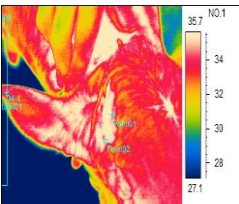
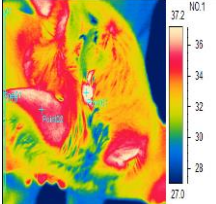
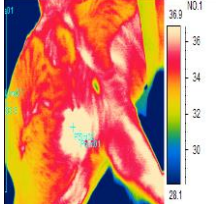
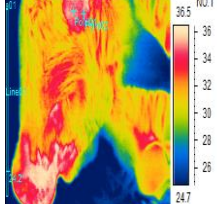
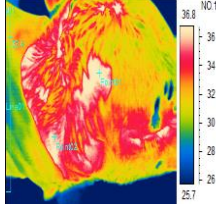
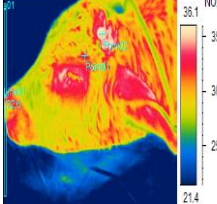
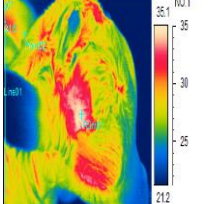
Treatment 2						
Before disbudding	After disbudding	1st week	2nd week	3rd week	4th week	5th week
						

Plate 4.29: Images of horn bud healing process in case of different methods of disbudding

Control						
Before disbudding (35.26°C)	During disbudding (37.40°C)	1st week (36.81°C)	2nd week (36.50°C)	3rd week (36.47°C)	4th week (36.30°C)	5th week (36.07°C)
						

Results and Discussion

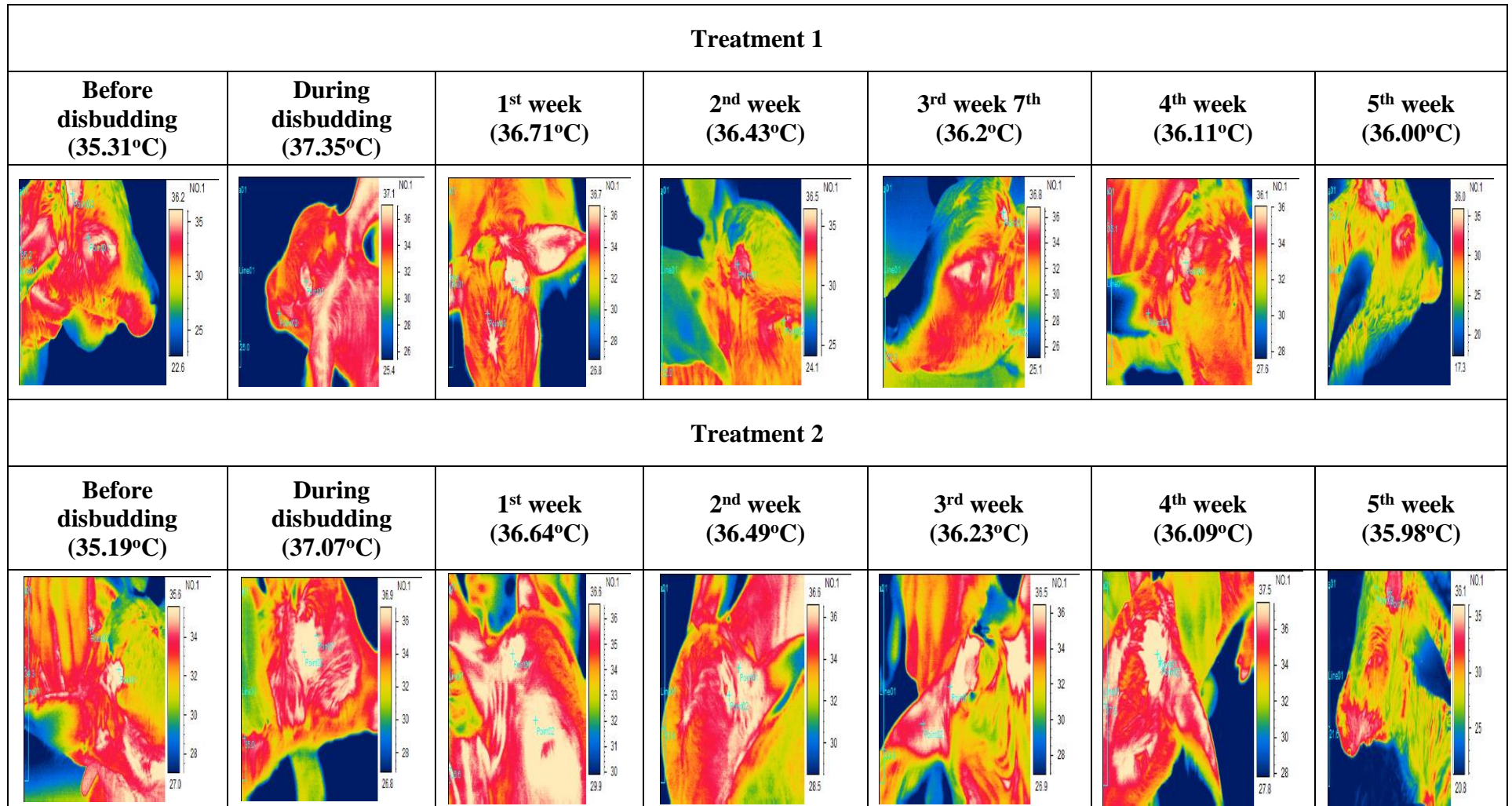


Plate 4.30: IRT images of horn bud during healing process

4.3.14. Cortisol concentration (ng/ml) of Sahiwal calves in case of disbudding with different methods:

Mean \pm S.E. of cortisol concentration (ng/ml) in Sahiwal calves in case of different methods of disbudding have been presented in Table 4.3.12 and Fig 3.39. A significant increase in cortisol concentration (ng/ml) was recorded in the Control and Treatment 1 after 40 minutes of disbudding (7.49 ± 0.56 and 5.43 ± 0.34) and 4 hours of disbudding (5.56 ± 0.12 and 4.11 ± 0.45) as compared to before 40 minutes of disbudding (3.54 ± 0.56 and 3.29 ± 0.12). A non significant increase in Treatment 2 after 40 minutes of disbudding (4.07 ± 0.62) and 4 hours of disbudding (3.92 ± 0.88) as compared to before 40 minutes of disbudding (3.67 ± 0.67) was recorded. Across different groups significant ($p < 0.05$) increase was recorded in control and Treatment 1 after 40 minutes of disbudding (7.49 ± 0.56 and 5.43 ± 0.34) as compared to Treatment 2 (4.07 ± 0.62) and also significant increase at 4 hours of disbudding in control (5.56 ± 0.12) as compared to Treatment 1 and Treatment 2 (4.11 ± 0.45 and 3.92 ± 0.88). In a similar line, Stillwell *et al.*, (2012) reported that significant increase in cortisol concentration in control group at 150 minutes (11.9 ± 16.4) as compared to treatment group with lignocaine (5.8 ± 7.6). Morisse *et al.* (1995) also reported that increase in cortisol in control at 4 hr after disbudding (5.0 ± 5.4) as compared to 0 hours after disbudding (3.2 ± 4.1). In a similar line, Stewart *et al.* (2009) also reported that increase in plasma concentration of cortisol in control calves at 20 minutes and 40 minutes (6.4 ± 1.4 and 3.9 ± 0.8) as compared to 0 minutes after treatment (3.5 ± 1.1).

Pain-induced distress caused by disbudding has been evaluated by measuring the change in cortisol response before and after disbudding (Sutherland *et al.*, 2002; Caray *et al.*, 2015). The most often investigated indication of wellbeing features of horn removal in calves is plasma cortisol levels (Stock *et al.*, 2013). When the body perceives stress, adrenal glands make and release the stress hormone cortisol into the bloodstream causes an increase in heart rate. Various reports are available that have shown removal of horns in calves by any method of disbudding leads to a marked, early increase in the plasma cortisol concentration (Coetzee *et al.*, 2012; Allen *et al.*, 2013; Stewart *et al.*, 2013). After 7 hours of disbudding the concentration of cortisol in the blood falls (Petrie *et al.*, 1996; McMeekan *et al.*, 1998; Sylvester *et al.*, 1998). Plasma cortisol levels reach a peak in the first 30 minutes in disbudded calves without analgesia and then returning to baseline at approximately 7-8 hr post-disbudding as reported by Stock *et al.* (2013). In chemical disbudding rise in plasma cortisol concentration occur within 1 hour and

Results and Discussion

returns to pre-treatment level 4-24 hours later. Mainau *et al.* (2012) reported that after dehorning plasma cortisol concentration increased immediately within the 30 minutes and return to the pre-treatment levels after 5-9 hours. In similar line, the available literature regarding the administration of local anaesthetic before disbudding showed a lower cortisol peak as compared to calves dehorned without any pain relief (Allen *et al.*, 2013; Morisse *et al.*, 1995; Aubry, 2005). In consonance with the present study, various researchers recorded a decrease in cortisol plasma levels peak in NSAID-administered calves after disbudding (Stilwell, 2012; Allen *et al.*, 2013; Huber *et al.*, 2013; Milligan *et al.*, 2004; Heinrich *et al.*, 2010; Stock *et al.*, 2015). In similar line, Carry *et al.* (2015) reported that cortisol levels varied markedly between t_{+0} and t_{+90} ($F^{7,304}$ (time) = 13.5, $P < 0.0001$). The peak in salivary cortisol concentrations was observed between t_{+15} and t_{+45} in vigil calves, whereas a small variation in xylazine or control calves ($F^{14,304}$ (time x treatment) = 6.64, $P < 0.0001$) was observed. Similarly, Graf and Senn (1999) reported that plasma cortisol levels in the saline and control group were significantly higher than anaesthetic group ($P < 0.05$). Black (2016) reported that cortisol concentration was lowest 4 hr after disbudding (1.93 ± 0.49 ng/ml) as compared to -24, 0, and 1hr (6.00 ± 0.48 , 5.23 ± 0.49 , and 6.82 ± 0.49 ng/ml; $P < 0.0001$) and tended to be higher 1 hr after disbudding as compared to 0 hr ($P = 0.09$). In another study at 4 hours following dehorning, meloxicam-treated calves exhibited considerably lower serum cortisol concentrations than placebo-treated controls ($P = 0.0004$). When the meloxicam-pre disbudding ($P = 0.015$) and meloxicam-post disbudding ($P = 0.002$) calves were compared to the control calves at this point of time, the mean cortisol concentration was considerably lower in both the meloxicam -pre disbudding ($P = 0.015$) and meloxicam post disbudding ($P = 0.002$) (Allen *et al.*, 2013). The trend of cortisol increases after disbudding and then decrease was corroborating with the present study. In similar line, Stilwell *et al.* (2012) reported that after 1 hr of disbudding only disbudded group of calves showed higher cortisol levels than the other two anaesthesia groups and anaesthesia with carprofen groups ($p < 0.01$). Duffield *et al.* (2010) reported no significant differences in the serum cortisol concentrations (nmol/L) of calves 10 min before dehorning (time 0) and at 3 and 6 h after dehorning in control and ketoprofen-treated calves. It was reported that when sedatives xylazine was given to calves it did not abolish the first 3 hr of cortisol response after dehorning (Mintline *et al.*, 2013; Stafford and Mellor, 2011), but when xylazine and lignocaine were given together before the dehorning procedure the response of the cortisol was nearly abolished during the first 3

hr (Stafford *et al.*, 2003). The variation in the results may be due to use of various types of anaesthetic and NSAIDs, methods of disbudding, breeds and age group. The cortisol level in the blood can act as a good indicator to assess the stress level associated with the disbudding in the dairy calves.

Table 4.3.13: Mean ± S.E. Cortisol concentration (ng/ml) in Sahiwal calves in case of different methods of disbudding

Group	40 minutes before disbudding	40 minutes after disbudding	4 hours after disbudding
Control (n=10)	3.54 ^a ±0.56	7.49 ^{bX} ±0.56	5.56 ^{cX} ±0.12
Treatment 1 (Lignocaine) (n=10)	3.29 ^a ±0.12	5.43 ^{bY} ±0.34	4.11 ^{cY} ±0.45
Treatment 2 (Lignocaine and NSAID) (n=10)	3.67±0.67	4.07 ^Z ±0.62	3.92 ^Y ±0.88

Means bearing different superscripts as ^{abc}(p<0.05) in a row and as ^{XYZ}(p<0.05) in a column differ significantly

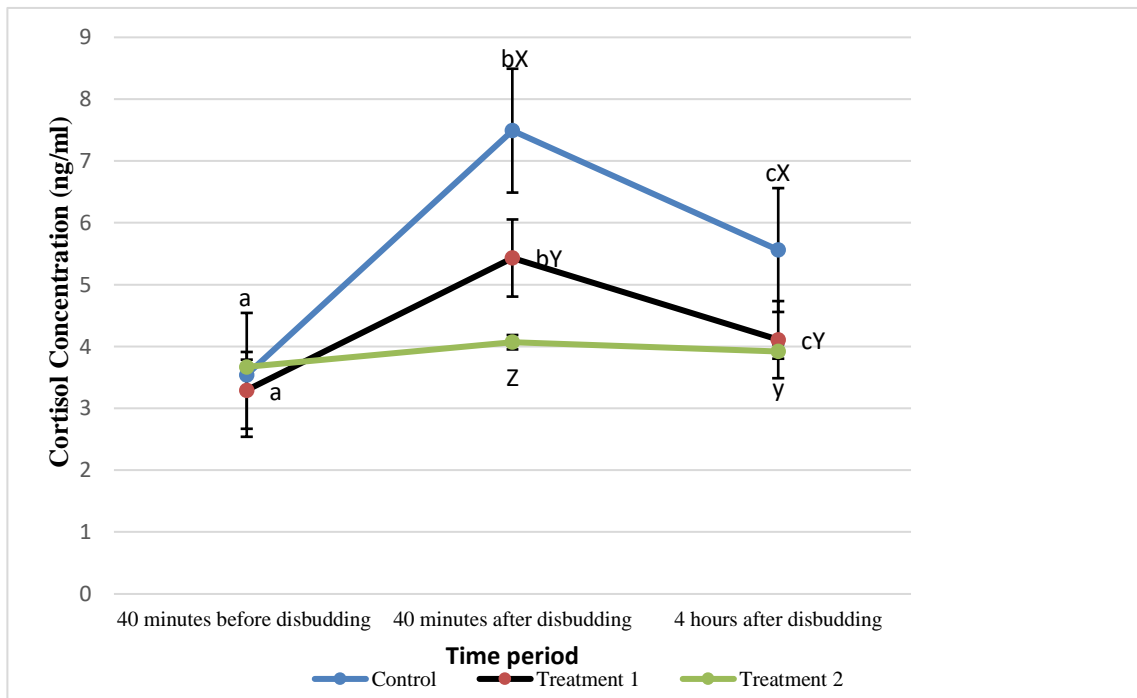


Fig 4.39: Cortisol hormone concentration (ng/ml) in Sahiwal calves in case of different methods of disbudding, Lines bearing different superscripts as ^{abc}(p<0.05) between time points and as ^{XYZ}(p<0.05) between treatments differ significantly

Results and Discussion

The goal of disbudding is to facilitate coping with and to increase the protection of animal keepers and other animals. Disbudding is always associated with the stress, pain and welfare of calves. During the disbudding process, change in behaviour and physiological parameters like heart rate, respiratory rate and extended plasma cortisol concentration in blood is well documented (Kupczyński *et al.*, 2014). Horn buds in calves are normally removed using thermal or chemical methods (Faulkner and Weary, 2000). Chemical methods are less painful and are rarely used due to incomplete removal of horn tissue in chemical disbudding that result in deformed horns in older calves (Stewart *et al.*, 2009). In the thermal method, the removal of skin tissues and horn buds is carried out by high temperatures. It simultaneously induces blood vessel cauterization and prevents bleeding of the wound. After disbudding, wound recovery will continue for some time. Therefore, in the present study electrical disbudding procedure has been adopted. Various researchers have reported a reduction of production indexes during the wound recovery period after disbudding (Faulkner and Weary, 2000; Knierim *et al.*, 2015; Stafford and Mellor, 2011). Stress in calves after disbudding is primarily because of pain resulting from physical mediators of inflammation. For effective pain management combination of drugs is always preferred to overcome acute and chronic pain (Herskin and Nielsen, 2018). Disbudded calves that had been exposed to local anaesthesia and given a NAISDS showed more proper patterns of behaviour and lower blood cortisol concentration (Stilwell *et al.*, 2012), which is also evident in the present study. Available literature also showed that Prostaglandins E2 (PGE2) and presumably PGI2 exhibit the highest influence on pain signals transformation. PGE2 production is decreased by Non-Steroidal Anti-Inflammatory Drugs, which help in reducing the pain induced stress by disbudding (Kupczyński *et al.*, 2014).

Disbudding is the routine managerial procedure followed in the dairy farms, in which removal of horn bud or horn bud cells is carried out at early age of life mostly at the age of 0-2 months before it is attached to the underlying bone (CVMA, 2010; AVMA, 2014; USDA, 2018), therefore in the present study disbudding has been carried out at the age of 10 to 30 days. Disbudding procedures generate various sorts of tissue injury, which can lead to pain of various types, intensities, and durations (Petrie *et al.*, 1996; Stilwell *et al.*, 2009; Braz *et al.*, 2012). Disbudding is mostly practised without the use of anaesthesia and NSAIDs under Indian condition, which affects the welfare and performance of dairy calves. When performed without enough anaesthetic and analgesia,

they are the most painful procedures (Stafford and Mellor, 2011; Stock *et al.*, 2013; Black *et al.*, 2017; Hambleton and Gibsion, 2017). It is a stressful procedure that leads to the activation of stress hormones i.e cortisol in blood and saliva. Most commonly plasma cortisol concentration in the blood and behavioural indicators has been used for assessing the pain, stress and welfare associated with disbudding (Herskin and Nielseen, 2018; Heinrich *et al.*, 2010; Balloua *et al.*, 2013). Therefore, in the present study anaesthetic and NSAIDs has been tried to understand the relief and stress of the disbudded calves based on the behavioural, physiological indicators and cortisol level in the plasma.

Disbudding destroys the skin around the horn bud leaving a small bruised area. The available literature shows that most common behavioural responses i.e. head shaking, head rubbing and ear flicking rate vary between disbudded and control calves (Morisse *et al.*, 1995; Graf and Senn, 1999; Grøndahl-Nielsen *et al.*, 1999). Disbudded calves display higher rates of behavioural head shaking, head rubbing and ear flicking post-disbudding as compared with calves treated with NSAIDs before disbudding (Graf and Senn, 1999; Faulkner and Weary, 2000; Stilwell *et al.*, 2012), which is also observed in the present study. Behavioural changes are mostly due to the pain and it remains for a period of about 4 hours because the peak of the cortisol is reached within 30 minutes and returns to the baseline levels within 2- 4 hours. Depressed parasympathetic or vagal tone and increased sympathetic activity can be triggered by painful procedures of disbudding. The stressful condition causes the release of catecholamines from sympathetic activity into blood circulation. Catecholamines increase the heart rate and respiration rate of the dairy calves to maintain homeostasis (Stewart *et al.*, 2008). In disbudding, it has been reported that there is a decrease in body weight gain due to the chronic inflammation mediators, which acts against the growth hormone. The available literature shows that the disbudded calves grow at a slower rate of 0.55kg/day than disbudded calves given with NSAIDs 0.65kg/day. On the contrary, no significant body weight change was observed in the present study, but lower growth was evident in initial two weeks after disbudding. In a study, it was reported that NSAIDs helps in reducing the chronic inflammation of disbudding (Bates *et al.*, 2016). Use of anaesthetic along with NSAIDs is useful in reducing the stress to the disbudded calves.

CHAPTER -5

Summary and Conclusions

SUMMARY AND CONCLUSION

The present study was carried out on Sahiwal breed of calves maintained at LRC, ICAR-NDRI, Karnal, Haryana, India. The study was conducted with the following two objectives and a total of four experiments were conducted under two objectives. Under Objective 1: Evaluation of calf health in dairy animals using infrared Thermography, two experiments were conducted. In Experiment 1, data was generated for IRT based ocular and body surface temperature of 29 diseased and 29 healthy dairy calves. In Experiment 2, for early detection of diseases in calves using Infrared Thermography, 46 newborn healthy Sahiwal calves were monitored using IRT for 30 days from birth. Under Objective 2: Evaluation of stress associated with different methods of disbudding using infrared thermography in dairy calves, two experiments were conducted. In Experiment 1, data generation of horn bud size in the different age groups of calves was carried out and 33 healthy dairy calves of three different age groups were selected 0-10 days, 11-20 days, 21-30 days. In Experiment 2, evaluation of stress associated with different methods of disbudding using IRT, 30 healthy Sahiwal calves of 10-30 days age were used.

Objective 1: Evaluation of calf health in dairy animals using infrared Thermography

- Vital physiological parameters respiration rate (breaths per minute), heart rate (beats per minute), rectal temperature ($^{\circ}\text{C}$) was significantly ($p < 0.05$) higher in calves affected with diarrhoea (36.42 ± 0.69 , 126.74 ± 1.85 , 39.22 ± 0.74), pneumonia (52.17 ± 1.49 , 150.00 ± 1.88 , 39.89 ± 0.03) and fever (52.25 ± 1.65 , 116.50 ± 2.10 , 39.73 ± 0.07) as compared to healthy calves (26.03 ± 0.78 , 104.10 ± 1.26 , 38.61 ± 0.58) respectively.
- PCV (%) showed a significant ($p < 0.05$) increase in diarrhoea calves (38.53 ± 0.87) and significant ($p < 0.05$) decrease in pneumonia calves (26.67 ± 0.67) as compared to healthy calves (31.66 ± 0.61) was recorded.
- TLC ($\times 10^3/\mu\text{l}$) showed a significant ($p < 0.05$) increase in diarrhoea (17.04 ± 0.30) and pneumonia (23.22 ± 0.94) as compared to healthy calves (8.37 ± 0.17). In the case of fever, no significant changes were observed for TLC ($\times 10^3$) as compared to healthy calves.

Summary and Conclusions

- Neutrophil (%) showed a significant ($p < 0.05$) increase in diarrhoea (51.79 ± 3.89), pneumonia (64.33 ± 0.99) and fever (67.50 ± 2.63) calves as compared to healthy (34.69 ± 0.49) calves.
- Lymphocyte (%) results showed a significant increase in calf diarrhoea (55.16 ± 1.01 vs. 47.55 ± 1.15), a significant decrease in calf pneumonia (32.83 ± 0.87 vs. 47.55 ± 1.15) and a non-significant decrease in fever calves as compared to healthy calves.
- In diarrhoea, pneumonia and fever significant ($p < 0.05$) increase in IR temperature of eyeball (1.07°C , 0.66°C , 0.76°C), peri-anal (1.43°C , 1.16°C , 0.92°C), para-lumbar (0.91°C , 0.99°C) temperature and a non-significant change in para-lumbar region temperature of fever calves as compared to healthy calves.
- Muzzle and cheek temperature showed a non-significant change in case of calves affected with diarrhoea, pneumonia and fever as compared to healthy calves.
- Most common clinical sign recorded in diarrhoea calves was diarrhoeic faeces (94.73%), dehydration (68.42%), anorexia (63.15%) and weakness (47.36%). Clinical faecal consistency score and dehydration score was significantly ($p < 0.05$) higher in diarrhoea (2.21 ± 0.71 vs. 0.00 ± 0.00 , 1.16 ± 0.81 vs. 0.00 ± 0.00) as compared to healthy calves.
- Most common clinical sign recorded in pneumonia calves was respiratory distress (83.33%), anorexia (66.66%), nasal discharge (50.00%), congested mucous membrane (50.00%), muzzle dryness (33.33%) and sneezing (16.66%).
- Significant ($p < 0.05$) increase in clinical rectal temperature and nasal discharge score in pneumonia calves (2.83 ± 0.40 vs. 0.00 ± 0.00 and 0.67 ± 0.34 vs. 0.00 ± 0.00) as compared to healthy calves was observed.
- The result of microbial examination showed that 3 different types of bacteria were isolated from 19 faecal samples of calf diarrhoea was *E. coli* (63.15%), *Salmonella* (26.31%) and *Staphylococcus* (10.52%).
- Calves affected with diseases showed higher temperatures on day to day basis besides the days around the onset of diseases.
- Eyeball temperature ($^\circ\text{C}$) was significantly ($p < 0.05$) higher, on one day before (37.69 ± 0.08 vs. 36.64 ± 0.21), on the day (37.70 ± 0.06 vs. 36.71 ± 0.18) and one day

after diarrhoea (37.48 ± 0.05 vs. 36.79 ± 0.12) in case of calves affected with diarrhoea as compared to healthy calves.

- Eyeball temperature ($^{\circ}\text{C}$) was significantly ($p < 0.05$) higher, one day before (37.57 ± 0.05 vs. 36.52 ± 0.13), on the day (37.85 ± 0.35 vs. 36.65 ± 0.25) and one day after pneumonia (37.71 ± 0.06 vs. 36.53 ± 0.00) in case of calves affected with pneumonia as compared to healthy calves
- Eyeball temperature was significantly ($p < 0.05$) higher, only on the day (37.73 ± 0.46 vs. 36.60 ± 0.36) of fever as compared to healthy calves.
- Peri-anal region temperature ($^{\circ}\text{C}$) was significantly ($p < 0.05$) higher, one day before (38.98 ± 0.15 vs. 38.10 ± 0.19), on the day (39.25 ± 0.09 vs. 38.13 ± 0.13) and one day after diarrhoea (38.74 ± 0.08 vs. 38.03 ± 0.16) in case of calves affected with diarrhoea as compared to healthy calves.
- Peri-anal region temperature ($^{\circ}\text{C}$) was significantly ($p < 0.05$) higher, one day before (39.10 ± 0.10 vs. 38.18 ± 0.03), on the day (39.35 ± 0.05 vs. 38.43 ± 0.03) and one day after pneumonia (39.01 ± 0.01 vs. 38.03 ± 0.02) in case of calves affected with pneumonia as compared to healthy calves.
- Peri-anal region temperature ($^{\circ}\text{C}$) was significantly ($p < 0.05$) higher, only on the day (39.25 ± 0.17 vs. 38.19 ± 0.07) in case of calves affected with fever as compared to healthy calves.
- Para-lumbar region temperature ($^{\circ}\text{C}$) was significantly ($p < 0.05$) higher only on the day diarrhoea (35.71 ± 0.12 vs. 34.60 ± 0.23) as compared to healthy calves.
- Para-lumbar region temperature ($^{\circ}\text{C}$) was significantly ($p < 0.05$) higher, only on the day pneumonia (35.50 ± 0.40 vs. 34.47 ± 0.43) as compared to healthy calves.
- Para-lumbar region temperature ($^{\circ}\text{C}$) was significantly ($p < 0.05$) higher, only on the day fever (35.97 ± 0.18 vs. 34.76 ± 0.08) as compared to healthy calves.
- Rectal temperature ($^{\circ}\text{C}$) was significantly ($p < 0.05$) higher, one day before (39.43 ± 0.10 vs. 38.90 ± 0.12), on the day (39.85 ± 0.03 vs. 38.92 ± 0.07) and one day after diarrhoea (39.35 ± 0.15 vs. 38.62 ± 0.22) in case of calves affected with diarrhoea as compared to healthy calves.

Summary and Conclusions

- Rectal temperature ($^{\circ}\text{C}$) was significantly ($p<0.05$) higher, one day before (39.36 ± 0.24 vs. 38.75 ± 0.23), on the day (39.81 ± 0.09 vs. 39.02 ± 0.02) and one day after pneumonia (39.43 ± 0.12 vs. 38.83 ± 0.19) in case of calves affected with pneumonia as compared to healthy calves.
- Rectal temperature ($^{\circ}\text{C}$) was significantly ($p<0.05$) higher only on the day (39.76 ± 0.12 vs. 38.87 ± 0.23) in case of calves affected with fever as compared to healthy calves.
- Significant ($p<0.05$) increase in the eyeball (0.6°C), para-lumbar (0.69°C), perianal (0.85°C), rectal temperature (0.83°C) and non-significant change in muzzle (-0.17°C) and cheek temperature (0.04°C) was recorded between the day of diarrhoea and two days before diarrhoea in affected calves.
- Significant ($p<0.05$) increase in the eyeball (0.56°C), para-lumbar (0.9°C), perianal (0.62°C), rectal temperature (0.76°C) and non-significant change in muzzle (0.4°C) and cheek temperature (0.2°C) was recorded on the day of pneumonia and two days before pneumonia in affected calves.
- Significant ($p<0.05$) increase in the eyeball (0.63°C), para-lumbar (0.88°C), perianal (0.57°C), rectal temperature (0.56°C) and non-significant change in muzzle (0.09°C) and cheek temperature (0.26°C) was recorded between the day of fever and two days before fever in affected calves.
- Significant ($p<0.05$) increase in the eyeball (0.66°C), para-lumbar (0.73°C), perianal (1.14°C), rectal temperature (0.94°C) and non-significant change in muzzle temperature (0.09°C) and cheek temperature (0.33°C) was recorded between the day of diarrhoea and seven days average before diarrhoea in affected calves.
- Significant ($p<0.05$) increase in the eyeball (0.8°C), perianal (0.9°C) para-lumbar (1.35°C), rectal temperature (1.16°C) and non-significant change in muzzle temperature (0.18°C) and cheek temperature (0.29°C) was recorded between the day of pneumonia and seven days average before pneumonia in affected calves.
- Significant ($p<0.05$) increase in eyeball temperature (1.13°C), perianal region temperature (0.95°C), rectal temperature (0.84°C) and non-significant change in muzzle temperature (0.35°C), cheek temperature (0.47°C) and para-lumbar

region temperature (0.30°C) was recorded between the day of fever and seven days average before fever in affected calves.

- PCV (%) showed a significant ($p < 0.05$) increase in diarrhoea calves as compared to healthy calves (42.13 ± 1.46 vs. 32.77 ± 1.02) and a significant decrease in pneumonia calves (24.50 ± 0.50 vs. 32.77 ± 1.02) as compared to healthy calves.
- TLC ($\times 10^3/\mu\text{l}$) showed a significant ($p < 0.05$) increase in diarrhoea (13.46 ± 0.39), pneumonia (24.10 ± 2.40) and no significant change in fever (8.15 ± 0.34) calves as compared to healthy (8.18 ± 0.11) calves.
- Neutrophil (%) showed a significant ($p < 0.05$) increase in diarrhoea (57.25 ± 2.64), pneumonia (62.50 ± 2.50) and fever (61.33 ± 2.40) calves as compared to healthy (33.00 ± 1.09) calves
- Lymphocytes (%) showed a significant decrease in calf pneumonia as compared to healthy calves (36.00 ± 3.00 vs. 52.23 ± 1.27) and a non-significant change in calf diarrhoea and fever.
- The incidence rate of diarrhoea, pneumonia and fever was 17.39%, 4.34% and 6.52%.
- Bacteria identified from calf diarrhoea in fecal samples were *E. coli* (75%) and *Salmonella spp.* (25%).

Objective 2: Evaluation of stress associated with different methods of disbudding using infrared thermography in dairy calves

- Significant ($p < 0.05$) increase in horn bud diameter for the 11 to 20 days and 21 to 30 days age group as compared to the Birth to 10 days age group (10.21 ± 0.25 and 8.59 ± 0.24 vs. 8.16 ± 0.23) was observed.
- The average body weight of the calves in Control, Treatment 1 and Treatment 2 was 25.51 ± 1.60 , 25.09 ± 0.27 and 25.35 ± 0.34 , respectively at the time of disbudding and 33.45 ± 1.17 , 34.28 ± 0.37 and 34.80 ± 0.37 , respectively at 4th week after disbudding.
- Significant increase in weekly average daily gain (gms) in body weight was recorded in 1st week (324 ± 0.99), 2nd week (341 ± 0.15) in Treatment 2 and 1st week

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(301±0.16), 2nd week (328±1.49) in Treatment 1 as compared to Control (204±0.93 and 264±1.11).

- Heart rate showed a significant ($p<0.05$) increase during the 2 minutes following disbudding in Control and Treatment1 as compared to baseline (136.60±0.92 and 125.60±3.97 vs. 116.1±3.17 and 114.90±3.46) and remain elevated above the baseline for the remaining sampling time. The change in heart rate in the Control group was 20 beats per minute, in the Treatment1 group was 11 beats per minute.
- In the Treatment 2 group significant increase in heart rate during 2 minutes following disbudding as compared to baseline (123.60±2.93 and 115.90±2.89) was observed. Heart rate change in this group was 8 beats per minute.
- Heart rate change in the control group at 3 hours after disbudding was 16 beats per minute, in Treatment 1 was 10 beats per minute and in Treatment 2 was 7 beats per minute as compared to before disbudding (baseline).
- Respiration rate showed significant ($p<0.05$) increase was observed in the Control group at 2 minutes and 3 hours after disbudding as compared to before disbudding (33.30±0.68 and 32.40±0.85 vs. 23.50±0.78) and similar trend was also observed in case of Treatment 1 (28.90±1.28 and 27.80±0.94 vs. 24.40±0.96) and Treatment 2 group (28.10±0.95 and 27.10±0.74 vs.25.80±0.83).
- A significant ($p<0.05$) increase in rectal temperature was observed at 3 hours after disbudding when compared with before disbudding in the Control group (39.32±0.11 vs.38.79±0.12), Treatment 1 group (39.30±0.18 vs. 38.80±0.19) and in Treatment 2 group (39.20±0.12 vs. 38.89±0.09).
- Change of rectal temperature in Control group was 0.53°C, Treatment 1, 0.50°C and Treatment 2, 0.31°C.
- Significant ($p<0.05$) increase in frequencies of pain-related behaviour after disbudding was observed between the treatments. Frequency of head shaking (33.63±2.27 and 24.50±1.61 vs. 13.50±1.25) and tail shaking (13.13±1.06 and 9.63±0.75 vs. 6.00±0.57) was significantly ($p<0.05$) higher in Control as compared to Treatment 1 and Treatment 2.

- Significant ($p<0.05$) drop in eyeball temperature (1.57°C) after disbudding was recorded in the Control group as compared to before disbudding (35.45 ± 0.25 vs. 37.02 ± 0.18) after that eyeball temperature returned to the baseline 90 minutes after disbudding (37.22 ± 0.20) and after that increase was non-significant for up to 180 minutes.
- In Treatment 1 and Treatment 2, eyeball temperature had dropped non-significantly from baseline, between 2-5 minutes post disbudding (0.64°C and 0.18°C) as compared to before disbudding (36.50 ± 0.20 vs. 37.14 ± 0.21 and 36.92 ± 0.34 vs. 37.10 ± 0.09) after that eyeball temperature reached to baseline level at 90 minutes (37.18 ± 0.11) in Treatment 1 and at 30 minutes (37.00 ± 0.17) in Treatment 2 group after that increase was non-significant for up to 180 minutes.
- Significant ($p<0.05$) increase in horn bud temperature after disbudding was recorded in Control (37.40 ± 0.22 vs. 35.26 ± 0.41), Treatment 1 (37.35 ± 0.35 vs. 35.31 ± 0.26) and Treatment 2 (37.07 ± 0.46 vs. 35.19 ± 0.13) as compared to before disbudding.
- Significant ($p<0.05$) increase in peri-anal region temperature in Control group 30 minutes after disbudding (37.90 ± 0.20 vs. 37.11 ± 0.22), Treatment 1 group 120 minutes after disbudding (37.90 ± 0.21 vs. 37.23 ± 0.31) and in Treatment 2 group 150 minutes after disbudding (37.90 ± 0.19 vs. 37.25 ± 0.24) was observed as compared to before disbudding.
- In the healing process significant ($p<0.05$) increase in horn bud temperature on the day of disbudding in Control (37.40 ± 0.22 vs. 35.26 ± 0.41), Treatment 1 (37.35 ± 0.35 vs. 35.31 ± 0.26) and Treatment 2 (37.07 ± 0.46 vs. 35.19 ± 0.22) group was recorded, as compared to before disbudding.
- Significant ($p<0.05$) decrease in horn bud temperature after disbudding in Control (36.30 ± 0.30) at 4th week, in Treatment 1 (36.34 ± 0.36) at 3rd week and in Treatment 2 (36.44 ± 0.35) at 3rd week as compared to during disbudding (37.40 ± 0.22 , 37.35 ± 0.35 and 37.07 ± 0.46).
- Significant ($p<0.05$) increase of lesion score without local anaesthetic was observed in 2nd week (1.80 ± 0.25 vs. 1.30 ± 0.15 and 1.20 ± 0.13) and 3rd week (1.80 ± 0.25 vs. 1.30 ± 0.15 and 1.10 ± 0.10) as compared to with local anaesthetic

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and with local anaesthetic and NSAIDs group, respectively. Complete epithelisation in the control and Treatment 1 group took place when lesion score reached to 1, 5 weeks after disbudding and in Treatment 2 lesion score reached 1, 4 weeks after disbudding.

- A significant ($p < 0.05$) increase in cortisol concentration (ng/ml) was recorded in the Control and Treatment 1 after 40 minutes of disbudding (7.49 ± 0.56 and 5.43 ± 0.34) and 4 hours of disbudding (5.56 ± 0.12 and 4.11 ± 0.45) as compared to before 40 minutes of disbudding (3.54 ± 0.56 and 3.29 ± 0.12).
- A non-significant increase in cortisol concentration (ng/ml) in Treatment 2 after 40 minutes of disbudding (4.07 ± 0.62) and 4 hours of disbudding (3.92 ± 0.88) as compared to before 40 minutes of disbudding (3.67 ± 0.67) was recorded. Across different groups significant ($p < 0.05$) increase was recorded in control and Treatment 1 after 40 minutes of disbudding (7.49 ± 0.56 and 5.43 ± 0.34) as compared to Treatment 2 (4.07 ± 0.62). Cortisol concentration (ng/ml) also increased significantly ($p < 0.05$) at 4 hours of disbudding in control (5.56 ± 0.12) as compared to Treatment 1 and Treatment 2 (4.11 ± 0.45 and 3.92 ± 0.88).

Conclusion:

- IRT can be used as a supportive tool to assess the increase in temperature of eyeball, peri-anal and para-lumbar region of Sahiwal calves affected with diarrhoea, pneumonia and fever on the day of clinical onset.
- IRT can be used as a supportive tool to understand the early change in temperature in case of calves affected with diarrhoea and pneumonia even before appearance of the actual clinical signs, whereas onset of fever can be assessed on the day of clinical onset.
- Use of local anesthesia, NSAIDs and Nupatch during disbudding in Sahiwal calves was very effective method to reduce the stress, which can be assessed by non-invasive IRT tool.

Future research:

- Infrared thermography can be explored to develop baseline database for various diseases condition of dairy calves under control conditions to monitor calf health in dairy animals.

- IRT can be explored to understand different kinds of stress, laminitis, feed efficiency, metabolic disorders, disease diagnosis, healing process of disbudded wound and reproduction etc. in different breeds and seasons for better understanding.
- In future, mobile based application of IRT with precise output can play a crucial role in the livestock management.

Implications:

Infrared thermography is a good supportive tool for assessment of calf health and evaluation of stress associated with different methods of disbudding in dairy animals, but there is need of more scientific validation in large number of dataset and under various environmental conditions.

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