
بسم الله الرحمن الرحيم

Human Population ,Land, Socio- Economics

Human Population	141.3 million
Population in agriculture	71.9 million (50.9%)
Agriculture land	269,600sqkm (35%)
Agriculture GDP	17,979 million (26.3%)
Livestock GDP	9523 million (11.7%) (60.6%)
Human Population Trend growth rate	Rural 2.2% Urban 3.4% of total 2.6%
Livestock Population Growth rate	Cattle & Buffalo 2.5% Sheep and Goat 1.6% Poultry 6.5%
LU per 100 people agriculture population	40-45

Gross value addition of livestock has increased from Rs 1,430 billion (2018-19) to Rs 1,466 billion (2019-20), showing an increase of 2.5 percent over the same period of last year.

TREND -Livestock Population

Species (Million Nos.)	2017-18	2018-19	2019-20
Cattle	46.1	47.8	49.6
Buffalo	38.8	40.0	41.2
Sheep	30.5	30.9	31.2
Goat	74.1	76.1	78.2
camel	1.1	1.1	1.1

Source: Ministry of National Food Security & Research

Constraints in Domestic Livestock Supply and Value Chain

- Meat Production as a Secondary Livestock Activity
- Low Productivity per Animal
- Inappropriate Feed and Nutritional Management
- Issues in Meat Processing
- Insufficient Veterinary Services
- Inefficient Breed Management
- Stakeholders' Awareness and Training
- International Standards, Compliance and Traceability Issues

SERO-MOLECULAR DETECTION OF BORRELIOSIS IN DROMEDARY CAMEL

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Department of Veterinary Medicine

Pak –US S& T Project: Capacity building of vector–borne neglected diseases of livestock

INTRODUCTION

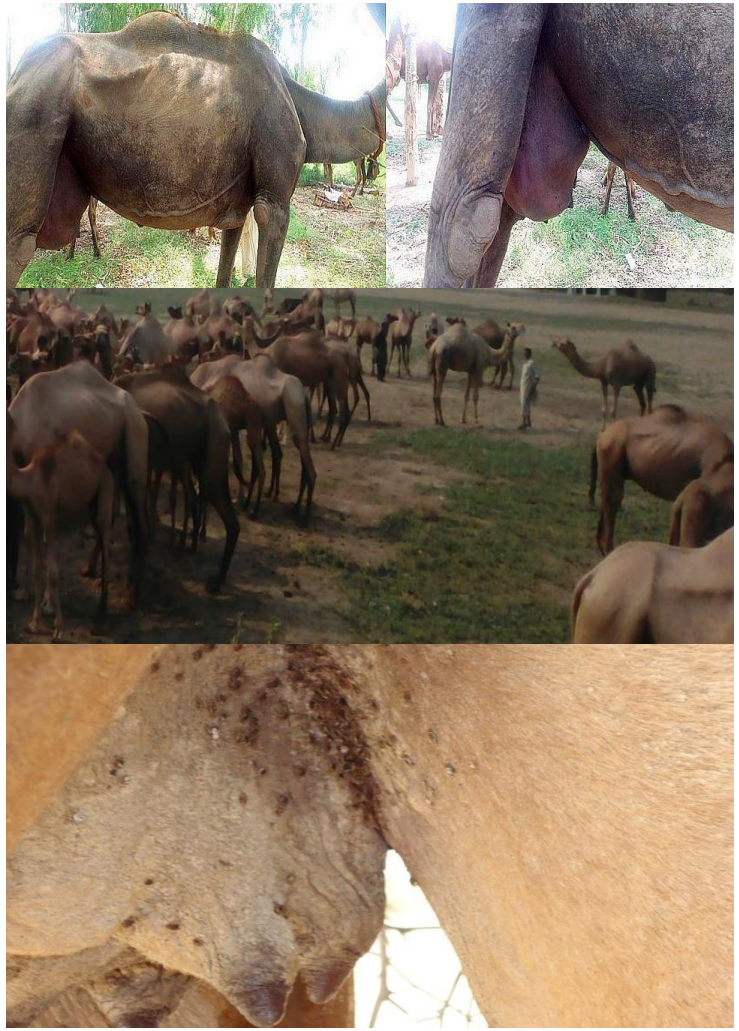
Borreliosis, is an infectious and zoonotic disease caused by Borrelia spp.

It is transmitted by hard ticks

The most common sign of infection: Erythema migrans, fever, anorexia, weight loss, arthritis and nervous signs.

Diagnosis on the basis of clinical signs, ELISA and Polymerase chain reaction (PCR)

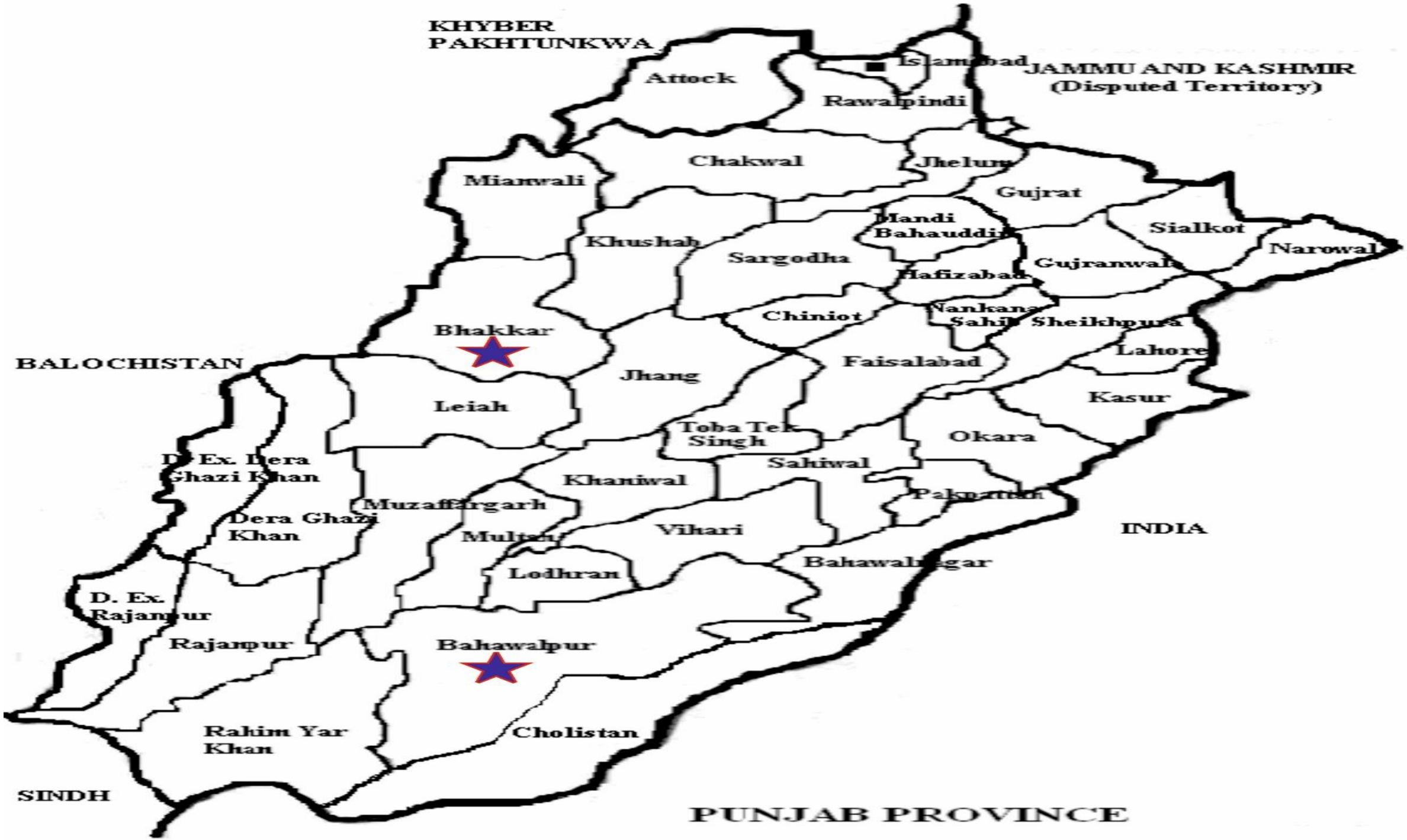
Doxycycline



- (Wasiluk et al.,2011)(Burbelo et al., 2011)

OBJECTIVE

- Detection of borrelia species in dromedary camel along in ticks with sero-molecular techniques and phylogenetic analysis
- Identification of ticks found on dromedary camel in study area
- Comparative efficacy of various therapeutic regimens against Borreliosis in experimental animals


KHYBER
PAKHTUNKWA
Attock
Islamabad
Rawalpindi
JAMMU AND KASHMIR
(Disputed Territory)
Chakwal
Jhelum
Miamwali
Gujrat
Mandi
Bahauddin
Sialkot
Khushab
Sargodha
Narowal
Gujranwala
Hafizabad
Chiniot
Nankana
Sahib
Sheikhpura
Bhakkar
Lahore
BALOCHISTAN
Faisalabad
Jhang
Kasur
Leiah
Okara
Toba Tek
Singh
Sahiwal
Khaniwal
Muzaffargarh
Vihari
INDIA
Dera Ghazi
Khan
Multan
Lodhran
Bahawalnagar
D. Ex.
Rajanpur
Rajanpur
Bahawalpur
Rahim Yar
Khan
Cholistan
SINDH
PUNJAB PROVINCE

Samples collection:

1. Blood

405 samples were collected from camels (Bhakkar n=270 and Bahawalpur n=135).

- 5ml of blood collected in EDTA vacutainer
  - GIEMSA Staining
  - PCR
- 5ml blood collected in none EDTA vacutainer
  - ELISA

2. Ticks

Two hundred ticks were also collected (Bhakkar n=100, Bahawalpur n=100)

- The collected ticks was stored in absolute ethanol in falcon tube
  - Ticks identification
  - PCR

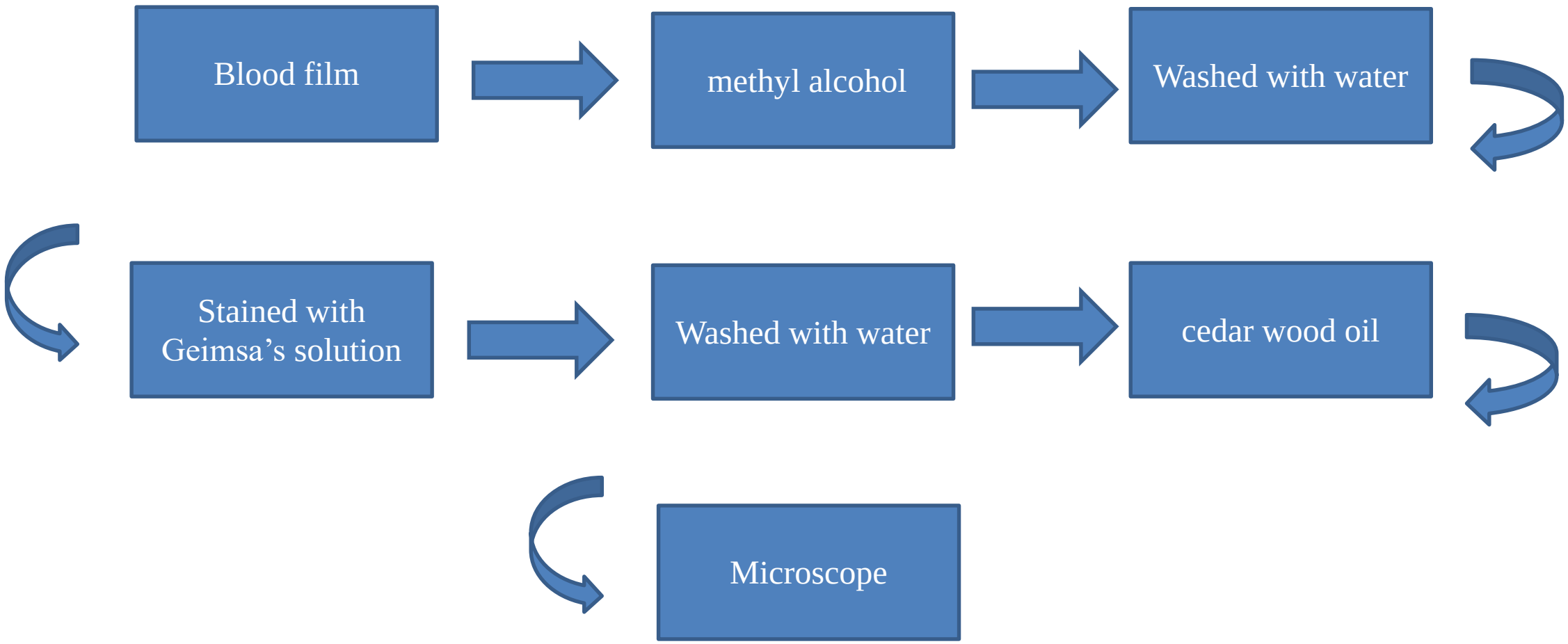
RISK FACTORS ANALYSIS

Questionnaire

Data were collected from sampling sites regarding:

- Age
- Gender
- Species
- Ticks infestation
- Herd size

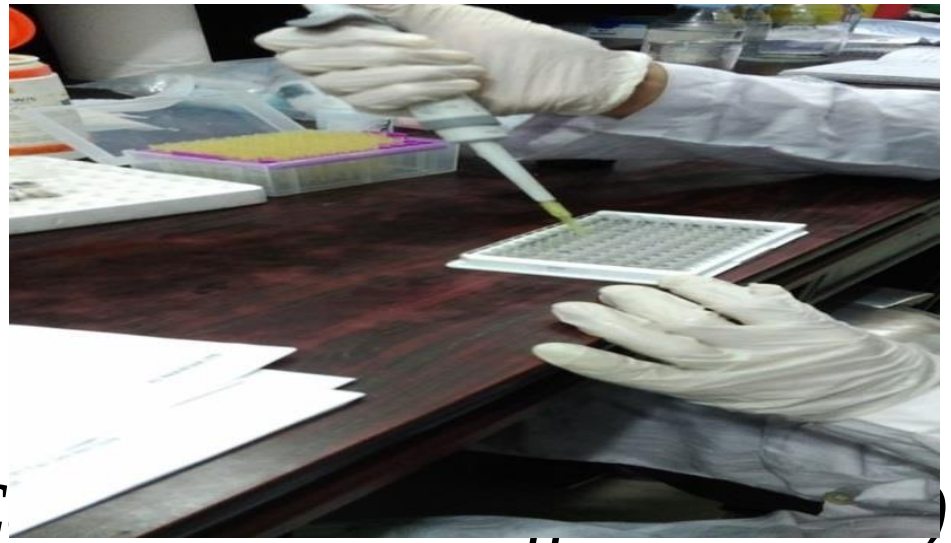
1. MICROSCOPIC EXAMINATION



2. SEROLOGICAL EXAMINATION

- Indirect ELISA
- MAP (1423-2) IDEXX ELISA kit.
- Preparation of reagents temp. and dilutions
- Conjugate concentrate temp & time
- Negative and positive control
- Pouring of serum samples
- Incubation
- Washings
- During incubation

- Pouring of conjugate
- 2nd incubation
- washings.
- Substrate time
- Stop solution.



*Cutoff OD Value (*optical density)

=(CF x mean OD of C

Negative Specimens <0.90, Equivocal Specimens 0.91 to 1.09,
Positive Specimens ≥1.10

3. MOLECULAR EXAMINATION

1. DNA extraction

DNA was extracted using commercial QIAamp DNeasy Blood and tissue kit (QIAGEN, Maryland, and USA), according to the manufacturer's instructions.

2. PCR Protocol

Forward primer BSL 5′ AATAGGTTCTAATAATAGCCTTAATAGC 3′
Reverse primer BSL 5′ CTAGTGTTTTGCCATCTTCTTTGAAA 3′

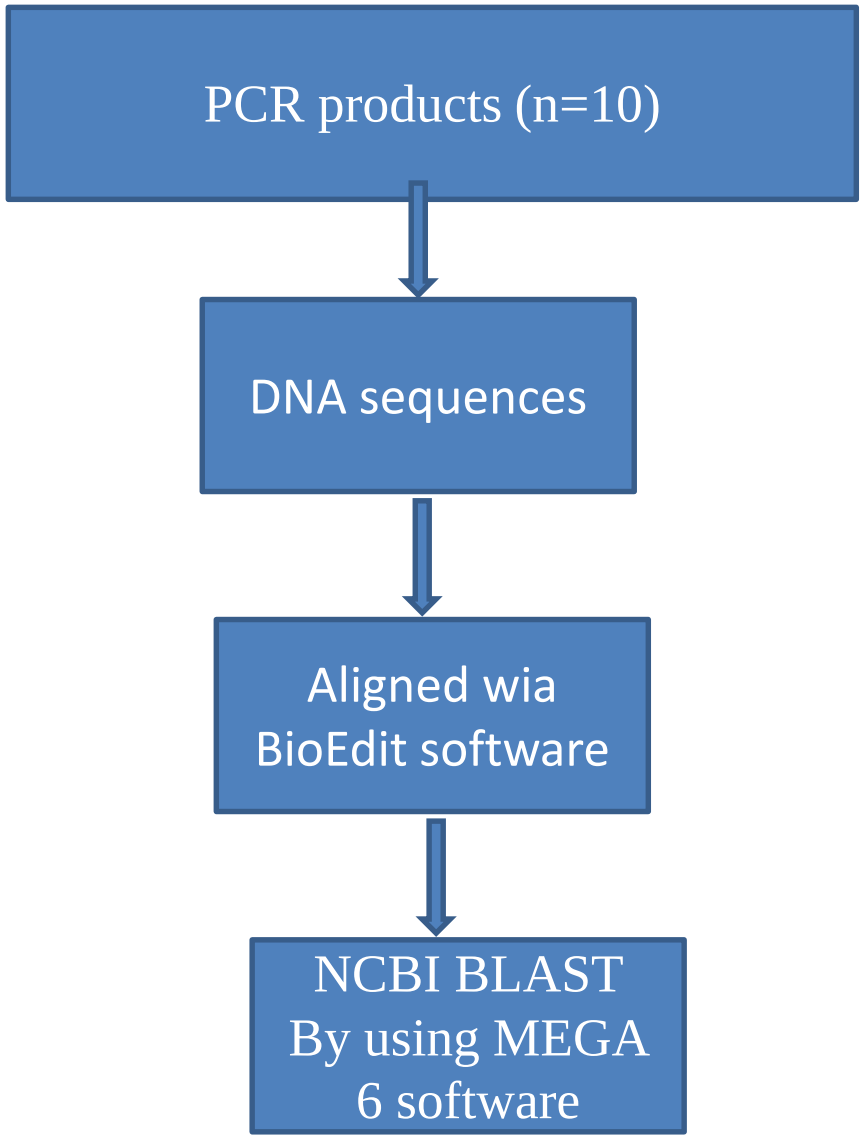
- In PCR nuclease free tube forward Primer 3ul and reverse primer 3ul was added
- Master Mix 15ul was added
- Extracted DNA sample 1.5ul was added
- Then 7.5ul deionized water were added
- It was 30ul total volume became required
- Then it was placed into thermocycler for copying at specific time and specific temperature at following conditions
- AMPLIRUN BORRELIA BURGDORFERI DNA control(catalogues number MB076)

Conditions for PCR in thermocycler for copying of DNA

Initial Denaturation	Denaturation	Annealing	Extension	Cycles	Final extension
95 ^{O}C for 3 minutes	95 ^{O}C for 3 second	56 ^{O}C for 30 minutes	72 ^{O}C for 1 minute	32	72 ^{O}C for 15 minutes

The product size was 300 bp seen on Gel Electrophoresis

PHYLOGENETIC ANALYSIS



Culture and infection protocol

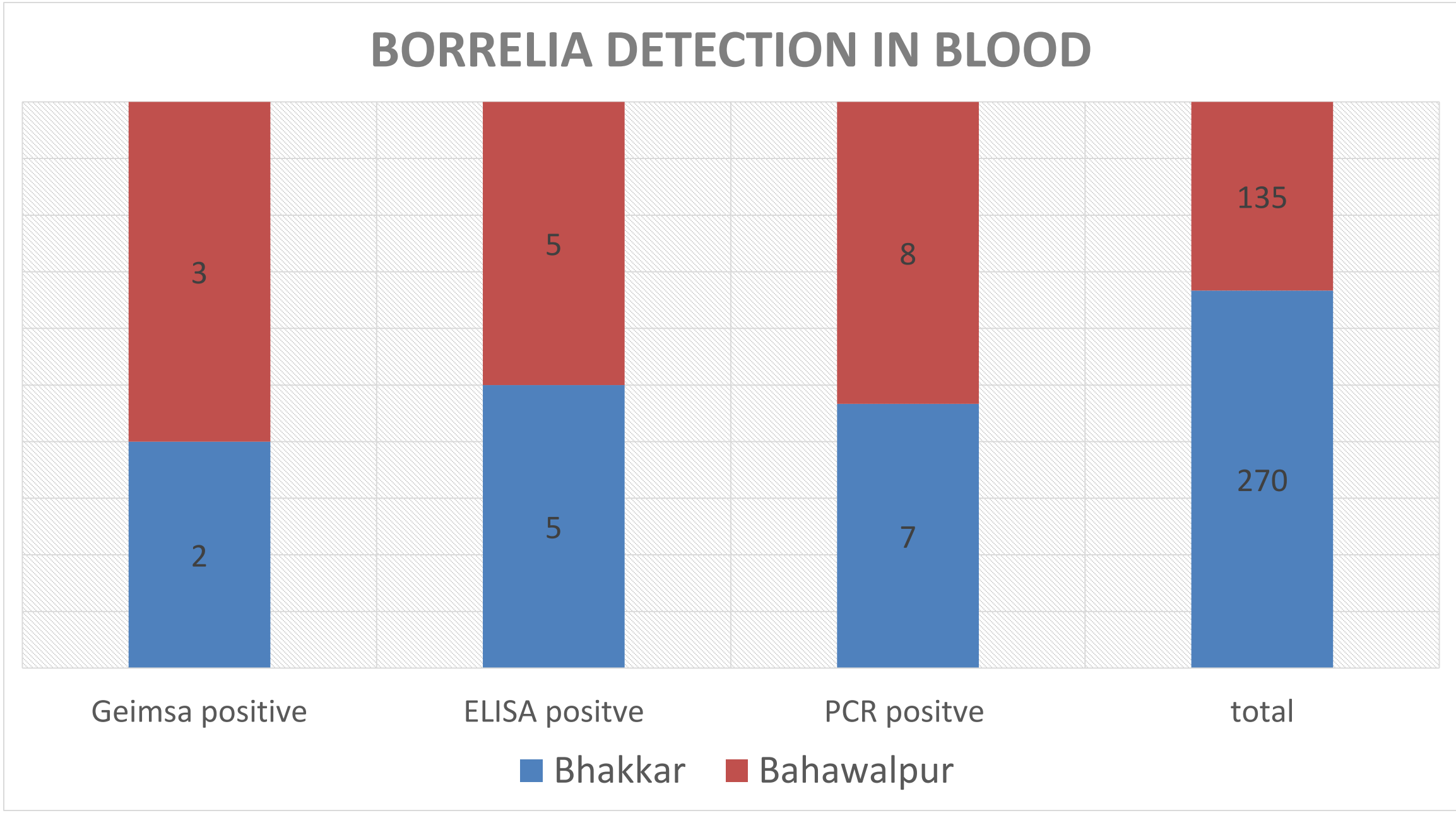
- Blood samples confirmed for Borrelia were used in experimental study
- Samples were kept in BSK-H medium overnight at room temperature
- Borrelia burgdorferi sensu lato were used for all infections
- Hematology and molecular techniques before inoculation were done in rabbits that are guaranteed free from borrelia pathogens.
- Twenty four Rabbits of irrespective sex were inoculated with the dose rate of 0.1 ml (1×10^5 organisms per rabbit) with tuberculin syringe through subcutaneous route.

(Blaho et al. 2011)

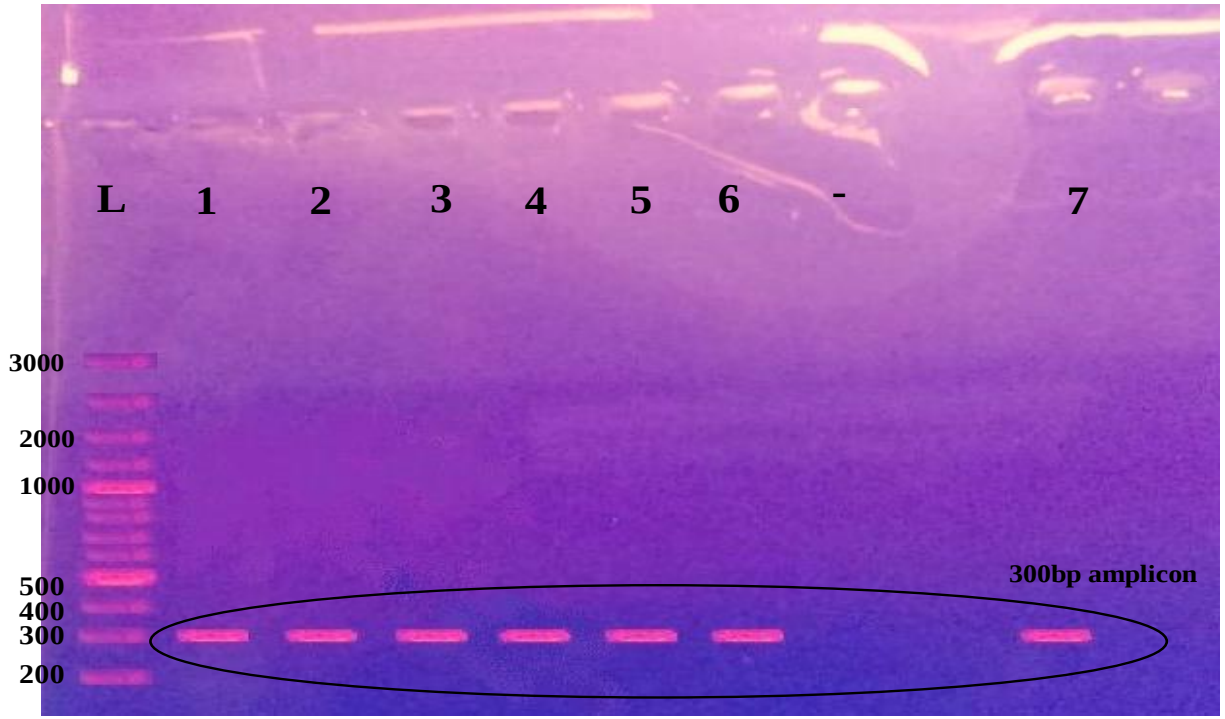
Groups	Medication	Dose / route
Group A (n=6)	Turmeric along with immune booster	Turmeric= 3mg/kg, oral vitamin E=3mg/kg, oral Vitamin C=2mcg/kg, oral selenium =1.5mcg/kg, oral
Group B (n=6)	Doxycycline along with immune booster	Doxycycline = 20mg/kg, oral vitamin E=3mg/kg, oral vitamin C=2mcg/kg, oral selenium =1.5mcg/kg, oral

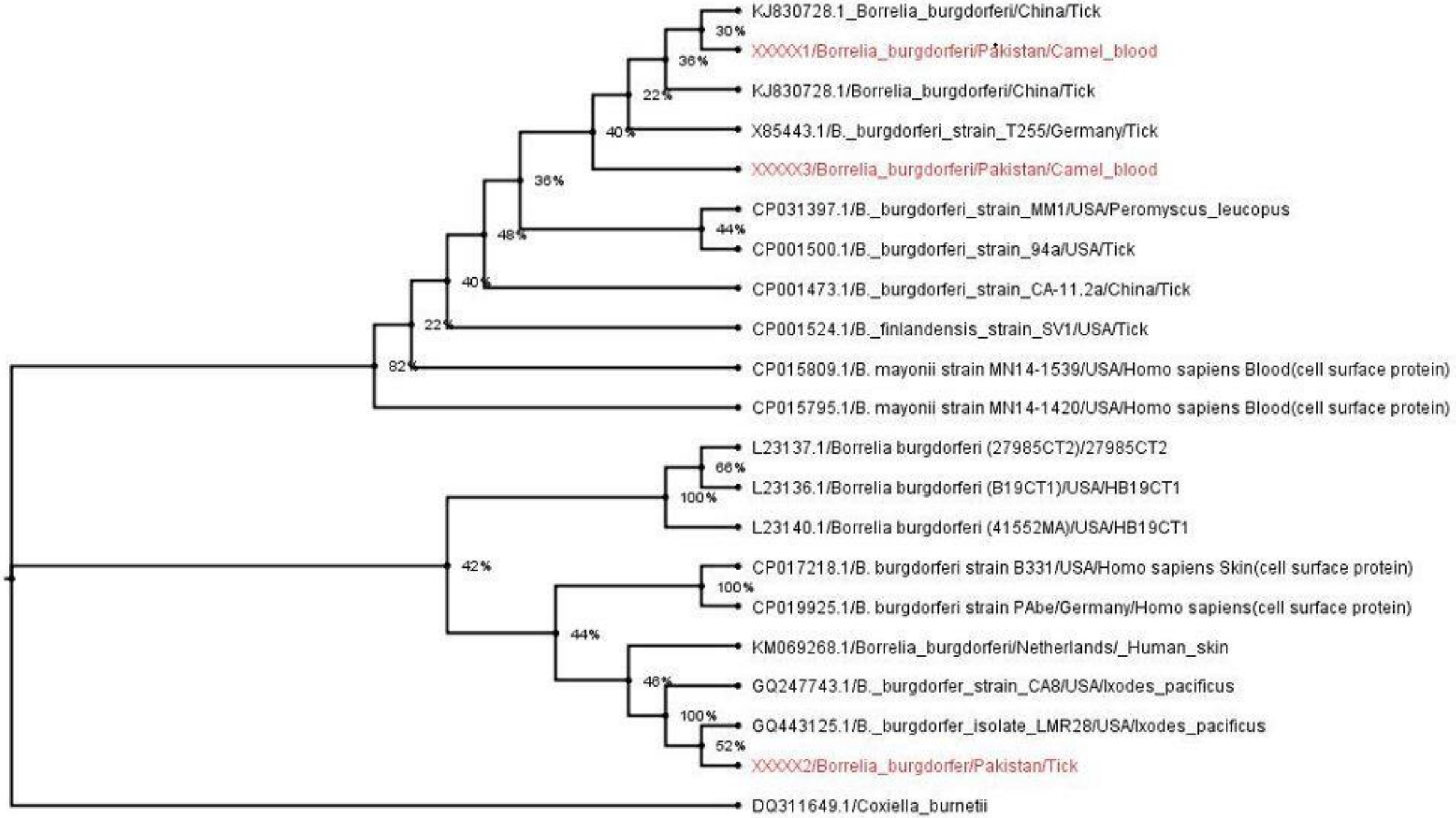
Group C (n=6)	**Doxycycline and turmeric along with immune booster**	**Turmeric= 3mg/kg, oral** **Doxycycline = 20mg/kg, oral** **vitamin E=3mg/kg, oral** **vitamin C=2mcg/kg, oral** **selenium =1.5mcg/kg, oral**
Group D (n=6)	Control positive (progression of disease signs)	(Gaffaar et al 2012) (Robless et al 2011)

For hematology and molecular study, 2ml blood from each rabbits was drawn from ear vein on 3rd, 7th, 12th, 15th and 17th days during treatment and processed in laboratory.

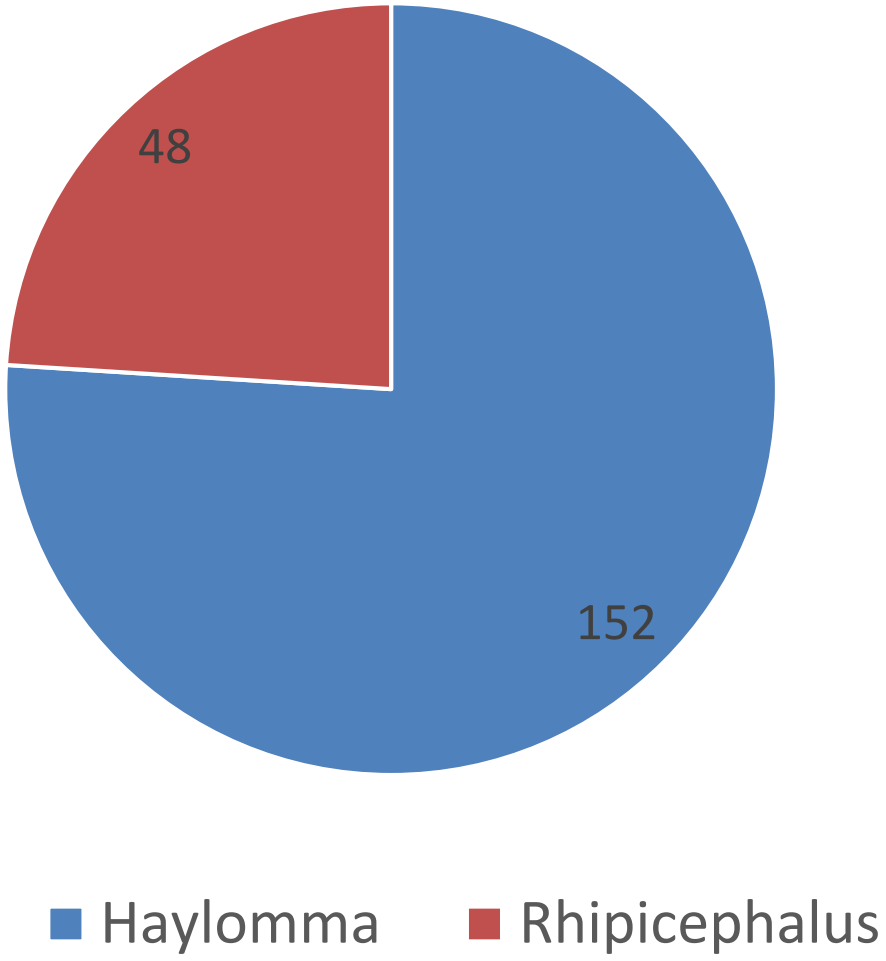

BORRELIA DETECTION IN BLOOD
3
5
8
135
2
5
7
270
Geimsa positive
ELISA positve
PCR positve
total
Bhakkar
Bahawalpur

PCR RESULT AND PHYLOGENTIC TREE

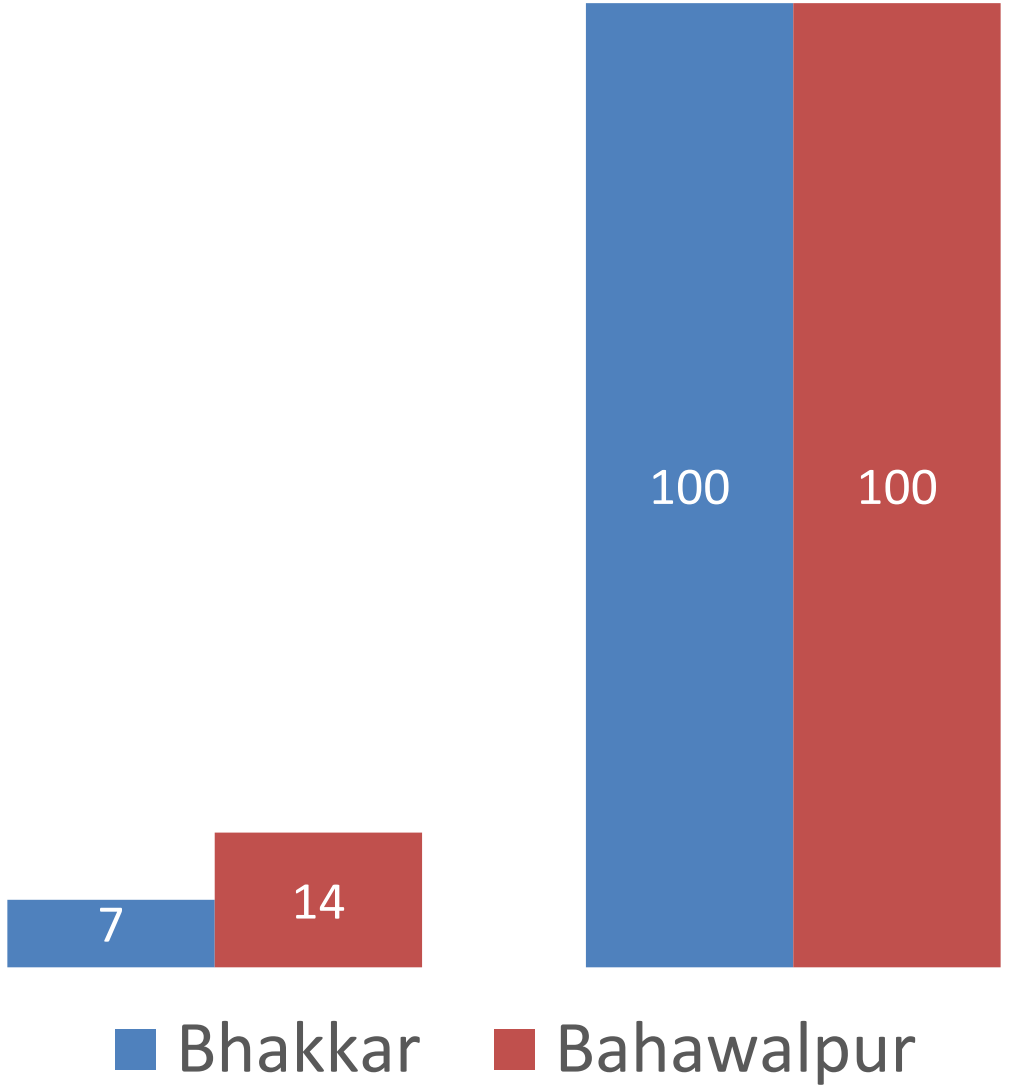



KJ830728.1_Borrelia_burgdorferi/China/Tick
30%
XXXXX1/Borrelia_burgdorferi/Pakistan/Camel_blood
36%
KJ830728.1/Borrelia_burgdorferi/China/Tick
22%
X85443.1/B._burgdorferi_strain_T255/Germany/Tick
40%
XXXXX3/Borrelia_burgdorferi/Pakistan/Camel_blood
36%
CP031397.1/B._burgdorferi_strain_MM1/USA/Peromyscus_leucopus
44%
48%
CP001500.1/B._burgdorferi_strain_94a/USA/Tick
40%
CP001473.1/B._burgdorferi_strain_CA-11.2a/China/Tick
22%
CP001524.1/B._finlandensis_strain_SV1/USA/Tick
82%
CP015809.1/B. mayonii strain MN14-1539/USA/Homo sapiens Blood(cell surface protein)
CP015795.1/B. mayonii strain MN14-1420/USA/Homo sapiens Blood(cell surface protein)
L23137.1/Borrelia burgdorferi (27985CT2)/27985CT2
66%
L23136.1/Borrelia burgdorferi (B19CT1)/USA/HB19CT1
100%
L23140.1/Borrelia burgdorferi (41552MA)/USA/HB19CT1
42%
CP017218.1/B. burgdorferi strain B331/USA/Homo sapiens Skin(cell surface protein)
100%
CP019925.1/B. burgdorferi strain PAbe/Germany/Homo sapiens(cell surface protein)
44%
KM069268.1/Borrelia_burgdorferi/Netherlands/_Human_skin
46%
GQ247743.1/B._burgdorfer_strain_CA8/USA/Ixodes_pacificus
100%
GQ443125.1/B._burgdorfer_isolate_LMR28/USA/Ixodes_pacificus
52%
XXXXX2/Borrelia_burgdorferi/Pakistan/Tick
DQ311649.1/Coxiella_burnetii

TICKS IDENTIFICATION

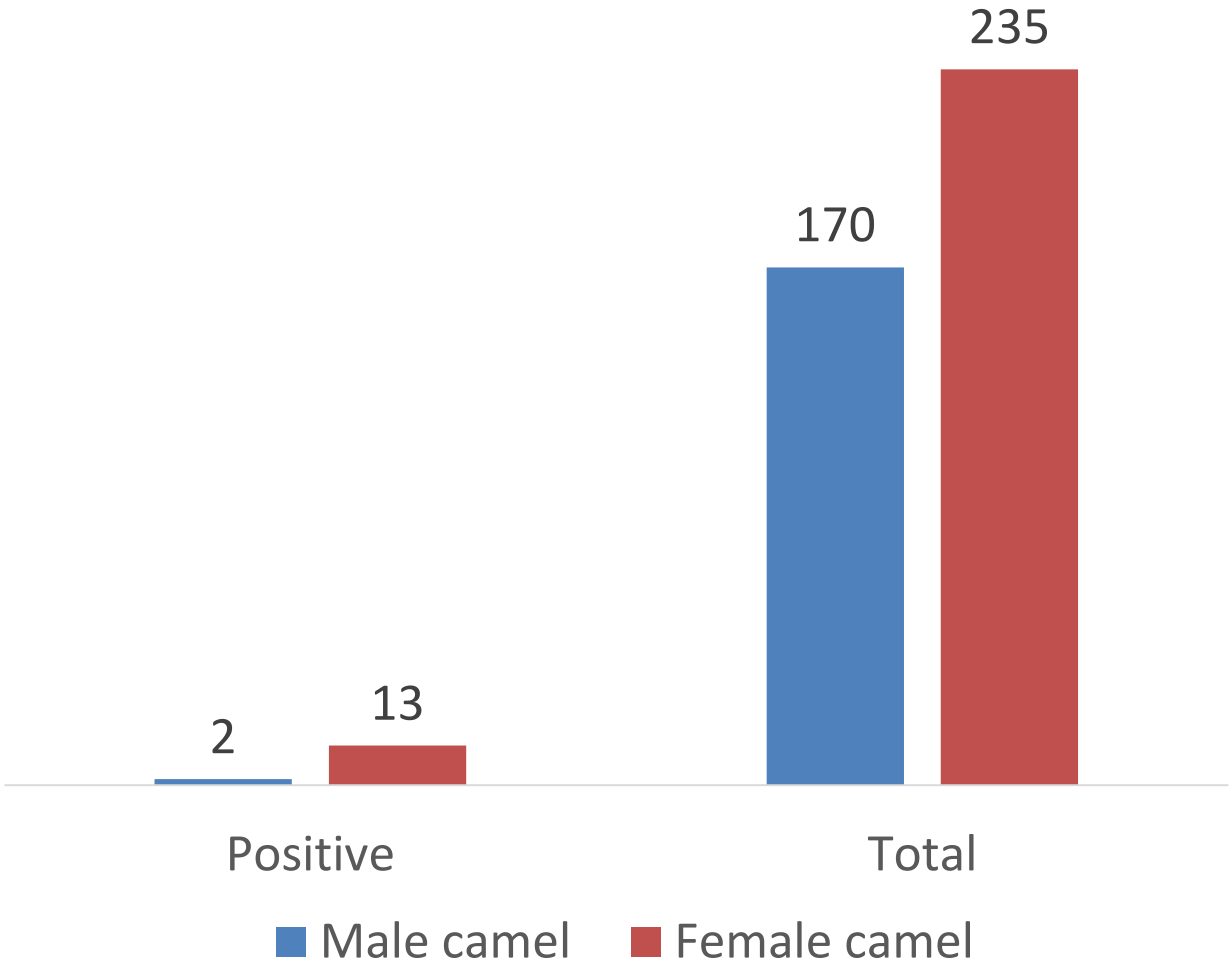


BORRELIA DETECTION IN TICKS

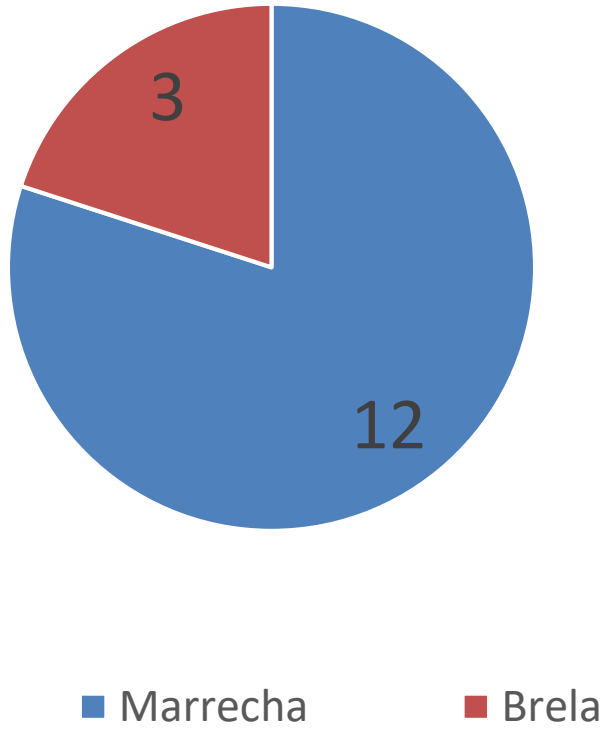


RISK FACTORS ANAYLSIS

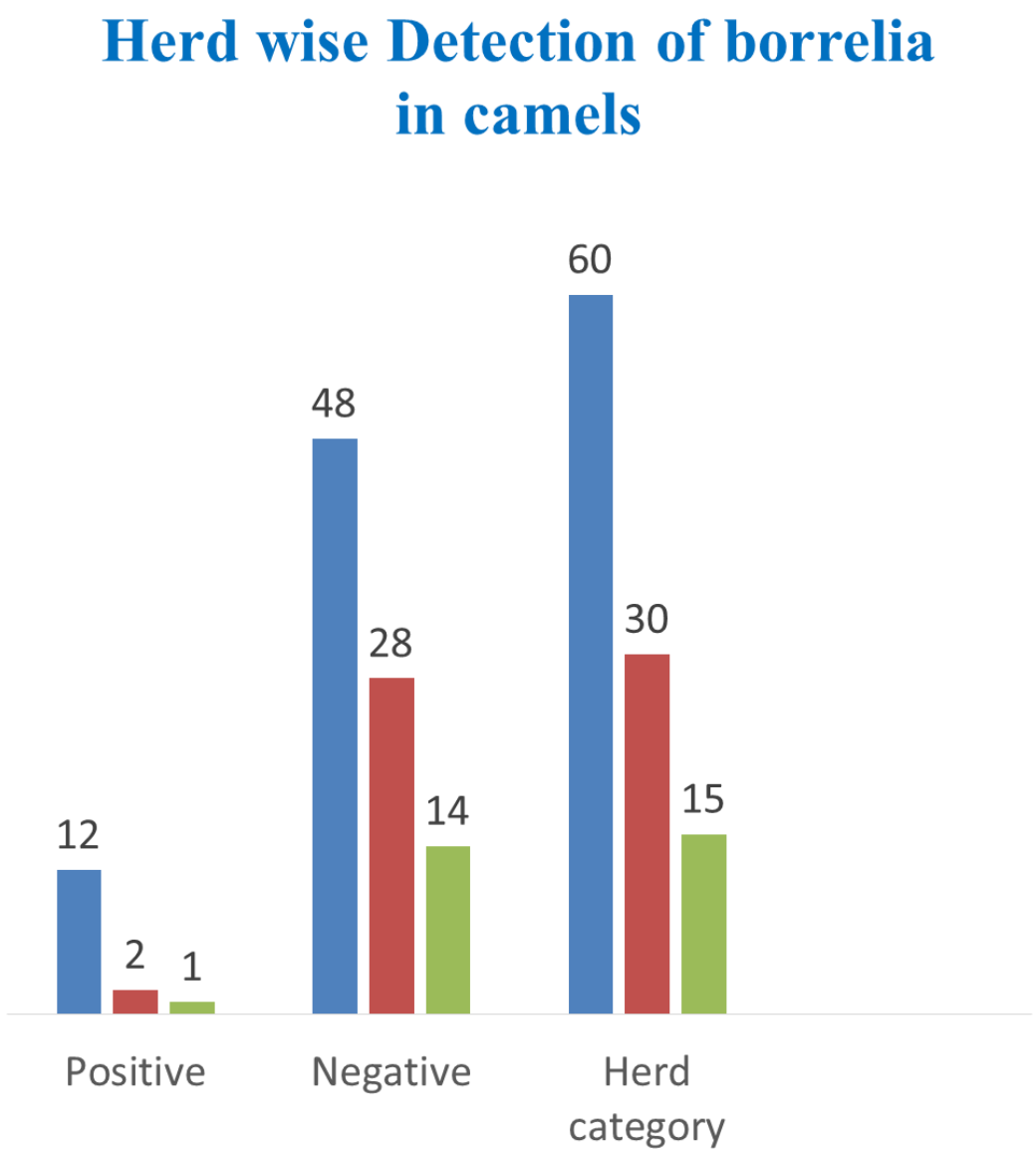
Gender wise Detection of borrelia in camels



Species wise detection of borrelia in camels

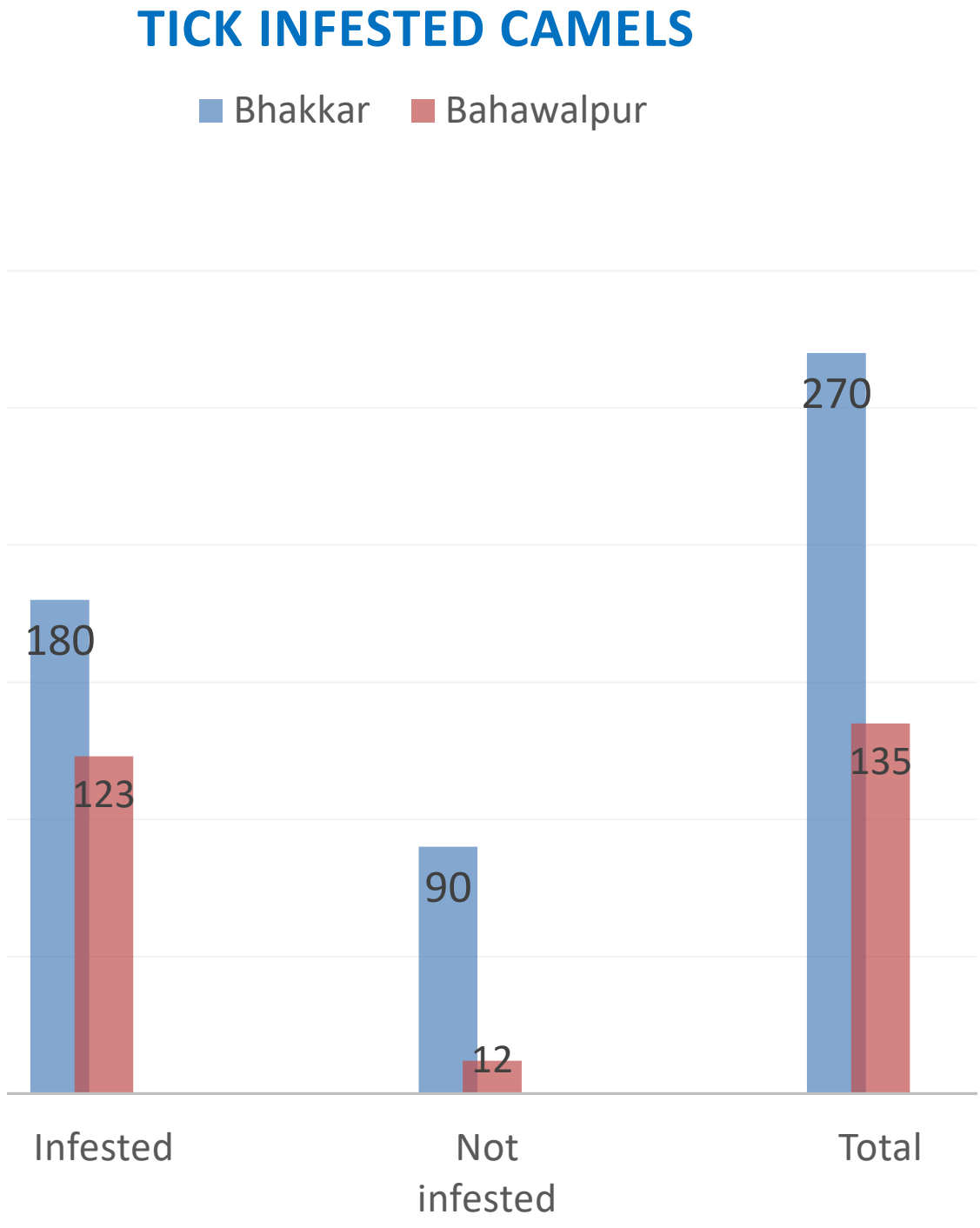


Herd Size	Positive	Negative	P value
60	12 (20%)	48 (80%)	**0.12**
30	2 (6.67 %)	28 (93.33 %)	
15	1 (6.67 %)	14 (93.33 %)	



Samples	Positive	Negative	Total
Tehsil Bhakkar	0 (0%)	30 (100%)	30
Tehsil Mankera	2 (1.67%)	118 (98.33%)	120
Tehsil darya khan	0 (0%)	60 (100%)	60
Tehsil kaloorkot	0 (0%)	60 (100%)	60

Samples	Positive	Negative	Total
Tehsil Bahawalpur	0 (0%)	27 (100%)	27
Tehsil Ahmedpur East	0 (0%)	27 (100%)	27
Tehsil Hasilpur	0 (0%)	27 (100%)	27
Tehsil Khairpur Tame ali	1 (3.70%)	26 (96.30%)	27
Tehsil Yazman	2 (7.41%)	25 (92.59%)	27



Samples Area (Districts)	Positive		Negative	Total
Bhakkar	5 (1.85%)		265 (98.15%)	270
Bahawalpur	5 (3.70%)		130 (96.30%)	135

Samples Area (Districts)	Positive	Negative	Total
Bhakkar	7 (2.59%)	263 (97.41%)	270
Bahawalpur	8 (5.93%)	127 (94.07%)	135

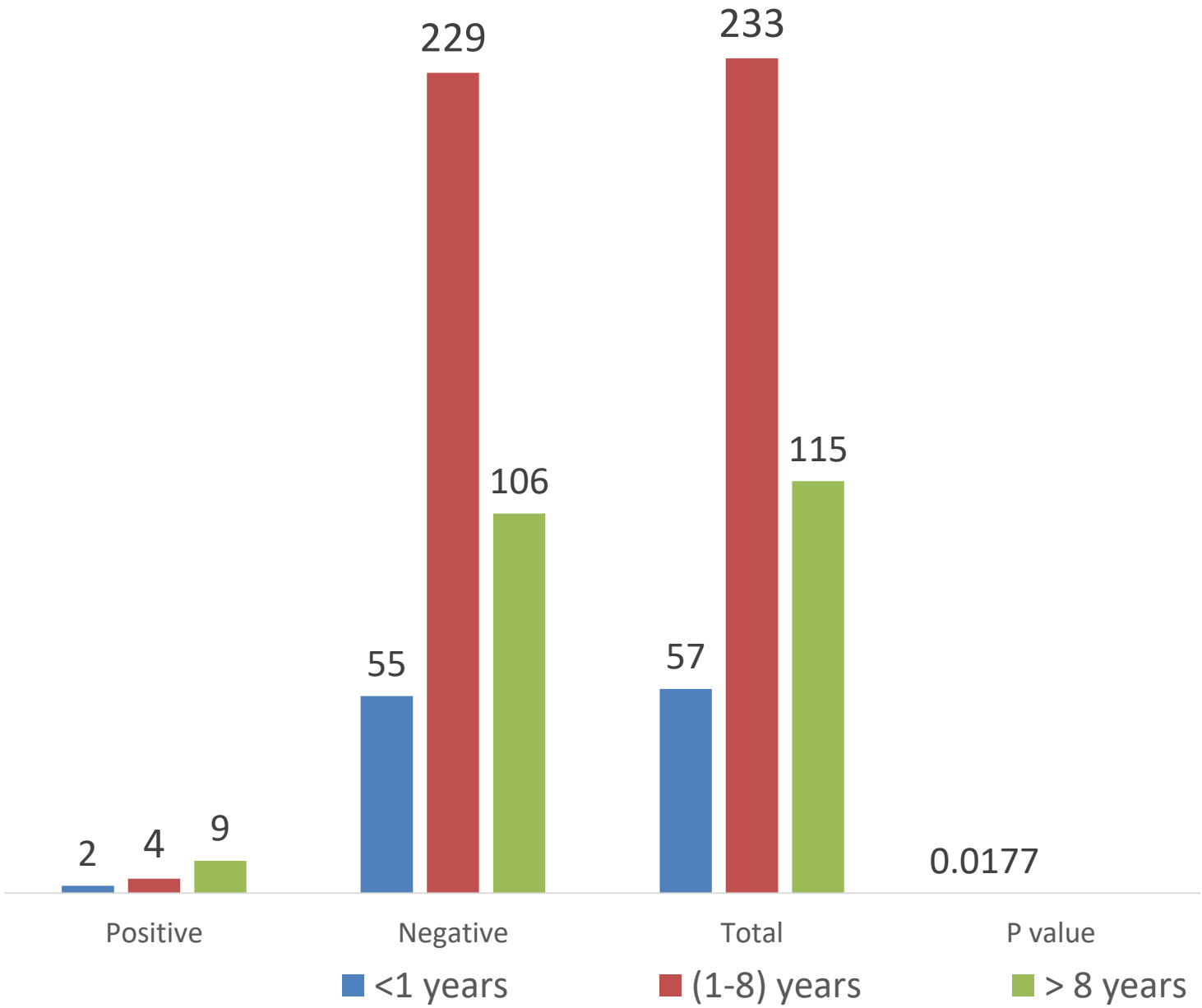
Samples	Positive	Negative	Total
Tehsil Bhakkar	0 (0%)	30 (100%)	30
Tehsil Mankera	3 (2.5%)	117 (97.5%)	120
Tehsil darya khan	0 (0%)	60 (100%)	60
Tehsil kaloorkot	2 (3.33%)	58 (96.67%)	60

Samples	Positive	Negative	Total
Tehsil Bhakkar	1 (3.33%)	29 (96.67%)	30
Tehsil Mankera	4 (3.33%)	116 (96.67%)	120
Tehsil darya khan	1 (1.67%)	59 (98.33%)	60
Tehsil kaloorkot	1 (1.67%)	59 (98.33%)	60

Samples	Positive	Negative	Total
Tehsil Bahawalpur	0 (0%)	27 (100%)	27
Tehsil Ahmedpur East	0 (0%)	27 (100%)	27
Tehsil Hasilpur	1 (3.70%)	26 (96.30%)	27
Tehsil Khairpur Tame ali	1 (3.70%)	26 (96.30%)	27
Tehsil Yazman	3 (11.11%)	24 (88.89%)	27

Samples	Positive	Negative	Total
Tehsil Bahawalpur	1 (3.70%)	26 (96.30%)	27
Tehsil Ahmedpur East	1 (3.70%)	26 (96.30%)	27
Tehsil Hasilpur	2 (7.41%)	25 (92.59%)	27
Tehsil Khairpur Tame ali	1 (3.70%)	26 (96.30%)	27
Tehsil Yazman	3 (11.11%)	24 (88.89%)	27

Age wise Detection of borrelia in camels



Clinical signs Reversal of infected Rabbits

Days	Group A	Group B	Group C	Group D	P-Value
3	6	6	3	6	0.02
7	6	5	3	6	0.01
11	4	3	0	6	0.002
15	3	0	0	6	0.002
17	0	0	0	6	0.004

OUTCOMES

- This study generated base line data for borrelia species in camel.
- These outcomes suggest that camels play a role in the transmission of borrelia pathogens.
- The study also helped in developing treatment protocol against borrelia infections in camel.
- It is anticipated that study was helped in providing insight to academia and researcher for study other aspect of this disease and pathogens

FUTURE RECOMMENDATION

- Diagnostic laboratories must be strengthen for the identification of borrelia spp. in camels.
- Ticks infestation control highly recommended to decrease the infection load with tick borne pathogens in camels.
- Govt. must include borreliosis along with other blood parasites pathogens in control measures.