4.0 RESULTS

The study on the prevalence of *Trypanosoma evansi* infection in bovines in three districts each of Karnataka and Andhra Pradesh based on wet blood film examination, blood smear staining, buffy coat technique, Indirect Enzyme Linked ImmunoSorbent Assay and Enzyme Immuno Transfer Blot during different seasons with relation to their age and sex, was conducted.

The protein profiles of the whole cell lysate antigen of *T. evansi* was analysed by SDS-PAGE and the comparison of different diagnostic tests was studied including Indirect ELISA and EITB.

In Karnataka, a total of 231 and 215 blood samples were collected from cattle and buffaloes, respectively from sick/suspected and apparently healthy animals (Table 1) and a total of 210 and 150 sera samples were collected from cattle and buffaloes respectively. The places covered under Karnataka state included Halalur, Honnattiholur, Nidhige of Shimoga district; Madakaripura, Gollarahatty, Lambanahalli of Chitradurga district; Karignur, Arehalli and Muktenahalli of Davanagere district (Table 2).

### 4.1.1 Prevalence of *T. evansi* infection in bovines in three districts of Karnataka based on blood smear staining and wet blood film examination

In Shimoga district, 45 and 57 samples were screened, of which one cattle (2.22%) and two (2.50%) buffaloes were found positive respectively in Halalur. In Honnattiholur 46 and 42 cattle and buffaloes were examined respectively and one (2.38%) buffaloe was found positive where as in Nidhige 38 cattle and 49 buffaloes were examined, but were not positive for *T. evansi* (Table 2)(Fig.6). The organisms were was identified based on the descriptions of Stephen, 1986.

In Chitradurga district, 53 and 68 samples of cattle and buffaloes were screened and there was one (1.88%) positive case in cattle in Madakaripura. In Gollarahatty 44 and 55 cattle and buffaloes respectively were screened and none were positive, whereas in Lambanihalli, out of 73 and 67 cattle and buffaloes screened one (1.36%) cattle and one (1.49%) buffaloe were found positive (Table 2)(Fig.6).
In Davanagere, out of 74 and 65 samples that were screened of cattle and buffaloes respectively, one (1.35%) cattle and two (3.07%) buffaloes revealed organisms. In Arehalli, of the 38 and 54 samples screened from cattle and buffaloes, one (2.63%) of the cattle was positive, whereas in Muktenahalli, out of the 35 and 33 cattle and buffaloes screened none revealed infection (Table 2)(Fig.6).

The overall prevalence of *T. evansi* infection in Shimoga district was 0.77 per cent in cattle and 2.02 per cent in buffaloes, whereas in Chitradurga district, 0.58 and 1.05 per cent infection rate was observed in cattle and buffaloes respectively and in Davanagere district, 1.36 and 1.30 per cent prevalence of *T. evansi* was recorded in cattle and buffaloes, respectively (Table 2).

### 4.1.2 Season-wise prevalence of *T. evansi* in bovines in three districts of Karnataka

The season-wise prevalence of *T. evansi* in bovines in Karnataka is tabulated in Table 3, Fig.7. In the cold weather, (January and February), the number of blood samples screened were 117 and 124, and only one each of cattle & buffaloes comprising (0.85%) (0.80%) of cattle and buffaloes, respectively were positive. In hot weather (March-May) the prevalence of *T. evansi* in cattle and buffaloes was one (1.07%) and one (0.83%), respectively out of 93 and 120 samples screened.

In the south-west monsoon (June-September) period 135 and 125 cattle and buffaloes were screened which manifested two (1.48%) and two (1.60%) respectively for *T. evansi*, whereas in north-east monsoon (October-December) the prevalence of *T. evansi* recorded was one (0.99%) and two (1.65%), respectively against 101 and 131 cattle and buffaloes screened.

### 4.1.3 Sex-wise prevalence of *T. evansi* in bovines in three districts of Karnataka

The males screened were 168 cattle and 234 buffaloes in different districts of Karnataka (Table 4).
Among 168 male cattle examined, two (1.19%) were found positive for *T. evansi* and one (0.42%) was positive for *T. evansi* among 234 male buffaloes. The female cattle examined were 278 and three (1.17%) were found positive for *T. evansi*, whereas from 256 female buffaloes examined five (1.95%) were found positive for *T. evansi* (Table 4, Fig. 8).

4.2. **Age-wise prevalence of *T. evansi* in bovines in three districts of Karnataka**

The results of age-wise prevalence of *T. evansi* in bovines has been depicted in (Table 5, Fig. 9). Out of the 124 cattle screened in the age group of 6 to 8 years two (1.61%) were found positive and the highest prevalence of *T. evansi* was recorded in the age group of 3 to 6 years (2.56%). (one out of 78 screened). A prevalence rate of 1.76 per cent was found in the age group of 1 to 3 years, (positive for *T. evansi* one out of 68 examined). In the age group of 8 years and above a prevalence rate of 1.33 per cent was recorded (one out of 75 animals screened). No prevalence was recorded in the age group of less than one year old cattle and buffaloes. In buffaloes, highest prevalence was recorded in the age group of 6 to 8 years (2.27%) (two out of 88 screened). The age group of 8 years and above showed a prevalence of 1.92 % out of 104 screened. Between the age group of 3 to 6 years 1.19% prevalence of *T. evansi* was recorded against 125 screened.

4.2.1 **Quantitative Buffy Coat (QBC) method for detection of *T. evansi* organisms**

In the present investigation, *T. evansi* organisms were detected in all the 25 blood samples out of 25 collected from mice experimentally infected with *T. evansi*. In all the cases, the organisms were found to be motile ranging from sluggish to active and revealed fluoroscene as the QBC-V tubes were pre coated with acridine orange dye. The motility of organisms represented very peak stages of parasitaemia in experimental infection.

4.2.2 **Results of parasitological diagnostic tests for the detection of *T. evansi* in bovines in three districts of Karnataka**
Among the 159 cattle sick/suspected for *T. evansi*, the blood smear staining method detected five (3.15%) positive cases, but for the cattle which are apparently healthy, either the wet blood film or the blood smear staining could detected the *T. evansi*. In the buffaloes which are of sick/suspected category, six of the animals (3.46%) were found positive by blood smear staining method (Table 6).

4.3. Detection of *T. evansi* by buffy coat technique (BCT) in bovines in three districts of Karnataka

The results of the detection of *T. evansi* by buffy coat technique (BCT) in bovines in Karnataka has been represented in Table 7. Out of the 34 cattle sick/suspected for *T. evansi*, one was (2.94%) found positive. In the buffaloes, which are sick/suspected for *T. evansi*, the BCT detected one (4.00%) out of 25 examined and in the apparently healthy buffaloes none were positive for *T. evansi*.

4.4.1 Sero diagnosis to detect the prevalence of *T. evansi* infection in bovines in three districts of Karnataka

4.4.2 Preparation of whole cell lysate antigen of *T. evansi*

The whole cell lysate antigen of *T. evansi* was prepared and used for the standardization of ELISA, SDS-PAGE and EITB. The protein concentration of whole cell lysate antigen was 870 μg/ml.

4.4.3 Enzyme Linked Immuno Sorbent Assay (ELISA)

4.4.4 Assay standardization

The working dilutions of conjugate, antigen and serum were determined to be 1:10,000, 5 μg and 1:100, respectively by checker board assay method.

4.4.4.1 Determination of cut off value

The results of the preliminary assays performed on twenty negative foetal calf serum samples were detected and the mean OD value was 0.369 with standard deviation of 0.009 and the cut off value was 0.396. The cutoff value in ELISA was 0.396 and the absorbance values ranged from 0.403 to 0.993 and are depicted in Fig. (5).
4.5 Season-wise prevalence of *T. evansi* in bovines in three districts of Karnataka by ELISA

The season-wise prevalence of *T. evansi* infection bovines in based on the ELISA is depicted in Table 8, Fig. 10. The number of cattle which were sick/suspected examined in cold weather (January and February) was 20 and out of which two (10.00%) were found positive. In apparently healthy cattle 12 serum samples were screened but could not detect the *T. evansi* antibodies. In the hot weather (March-May) out of 13 sick/suspected cattle examined 2 animals were found positive for *T. evansi* antibodies whereas in apparently healthy cattle no infection could be detected. During the South-West monsoon (June-September) out of 68 cattle which were in the sick/ suspected category three (4.41%) showed presence of antibodies against *T. evansi*. Among the apparently healthy cattle one (3.33%) was positive against 29 screened. The buffaloes which were sick suspected for *T. evansi* and examined in the cold weather was 15, but one (6.6%) was positive for *T. evansi* antibodies.

In hot weather 16 buffaloes which were sick/suspected were screened and two (12.5%) were positive. Out of the 47 sick/suspected buffaloes screened four (8.51%) manifested antibodies against *T. evansi*. During the north-east monsoon, 32 buffaloes were screened and three (9.37%) showed antibodies against *T. evansi* (Table 8). Out of the five apparently healthy buffaloes examined during cold weather none was positive and during hot weather also none was positive in the 12 apparently healthy buffaloes examined. During south-west monsoon out of the 10 apparently healthy buffaloes screened, one (10.00%) was positive, whereas in north-east monsoon out of 13 examined one (7.68%) was positive for *T. evansi*.

The overall seasonal prevalence of *T. evansi* based on ELISA in different seasons were 6.25, 7.40, 4.08 and 3.77 per cent respectively, in cold, hot, south-west monsoon and north-west monsoon in cattle, similarly in buffaloes it was 5.00, 7.14, 8.77 and 7.27 per cent. (Table 8, Fig.10).

4.5.1 Sex-wise prevalence of *T. evansi* in bovines in three districts of Karnataka by ELISA
Out of the 32 male sick/suspected cattle examined one (3.12%) was positive for antibodies against *T. evansi* and in the 28 male buffaloes which were sick/suspected one (3.57%) was positive. In 42 apparently healthy male cattle, none was positive, but in 18 apparently healthy male buffaloes one (5.55%) was positive for *T. evansi*. In the 93 sick/suspected female cattle eight (8.60%) were positive and in case of 63 sick/suspected female buffaloes nine (14.28%) were positive. The apparently healthy female cattle examined were 43 and one (2.32%) was positive, whereas against 41 apparently healthy female buffaloes examined one (2.43%) manifested antibodies against *T. evansi* (Table 9).

Based on ELISA, in male cattle, the overall prevalence recorded was 1.35 and 4.34 per cent in male buffaloes. In case of female, 6.61 and 9.61 per cent of prevalence of *T. evansi* was recorded in cattle and buffaloes, respectively (Table 9, Fig.11).

### 4.5.2 Age-wise prevalence of *T. evansi* in bovines in three districts of Karnataka by ELISA

The results of age-wise prevalence of *T. evansi* in bovines in Karnataka by ELISA is depicted in Table 10, Fig.12.

In cattle and buffaloes of age group below one year, in the sick/suspected cattle and buffaloes, none were positive for *T. evansi*. In the age group of one to three years in the sick/suspected cattle screened, 16 and 2 (12.5%) were positive, whereas in apparently healthy cattle one was positive (4.76%) out of 21 screened. In the buffaloes in the one to three year age group out of 15 screened one (6.67%) was positive and one (5.88%) out of 17 apparently healthy buffaloes indicated *T. evansi* antibodies. Between the age group of three to six years in the sick/suspected cattle examined, 27 and 2 (7.40%) revealed antibodies against *T. evansi*. Out of 25 apparently healthy cattle one (4.00%) indicated positive result and in the similar age group of 21 sick/suspected buffaloes three (14.28%) were positive whereas one (7.14%) out of 14 apparently healthy buffaloes indicated positive reaction. In the age group of six to eight years, 31 sick/suspected cattle were examined, none were positive. In the 17 apparently healthy cattle, one (5.88%) was positive. In the buffaloes of age group six to eight years, 18 sick/suspected were
examined and out of which one (5.56%) was positive whereas in 16 apparently healthy buffaloes one (6.25%) indicated positive reaction. In the age group of above eight years, 27 sick/suspected cattle were screened two (7.40%) had antibodies against *T. evansi* and out of 14 apparently healthy cattle one (7.14%) was positive for *T. evansi* and out of 12 apparently healthy buffaloes one (8.34%) had antibodies against *T. evansi* (Table 10).

Based on ELISA, between the age group of one to three years, prevalence rate of 8.1 and 6.25 per cent was recorded in cattle and buffaloes, respectively, whereas 5.76 and 1.42 per cent was observed in cattle and buffaloes in the age group of three to six years. A prevalence of 2.08 and 5.88 per cent was recorded in cattle and buffaloes, respectively in the age group of six to eight years, wherein 7.31 and 18.18 per cent prevalence of *T. evansi* was recorded in the above eight years age group of cattle and buffaloes, respectively (Table 10, Fig 12).

### 4.5.3 Sero diagnosis of *Trypanosoma evansi* in bovines in three districts of Karnataka by EITB

The results of Enzyme Immuno Transfer Blot (EITB) in *Trypanosoma evansi* infection of bovines of Karnataka is indicated in Table 11. Out of the 80 cattle examined 7 (8.75%) were found positive, whereas 20 (22.22%) buffaloes were found positive out of 90 buffaloes screened.

### 4.6 Comparator of diagnostic tests for the detection of *Trypanosoma evansi* infection in bovines in three districts of Karnataka

The results of various diagnostic tests employed in the present study were summarized in Table 12, Fig.13. In the wet blood film examination method, out of 446 and 490 cattle and buffaloes screened, none revealed *T. evansi* organisms. In the blood smear staining method, out of the 446 screened 5 (1.12%) were positive whereas 6 (1.22%) buffaloes out of 490 screened by blood smear staining method were found infected.
The buffy coat technique (BCT) revealed one (2.00%) positive case in cattle out of 50 examined whereas one (1.81%) buffaloe was positive out of 55 screened. In ELISA, out of 210 cattle, 10 (4.76%) had antibodies against *T. evansi* and 12 (8.00%) buffaloes were positive out of 150 screened. Enzyme immunotransfer blot revealed 7 (8.75%) positive cases out of 80 cattle and 20 (22.22%) buffaloes were positive out of 90 screened (Table 12, Fig.13).

4.6.1 Sensitivity and specificity of ELISA and EITB in the diagnosis of *T. evansi* in bovines in three districts of Karnataka

The sensitivity and specificity of the ELISA observed was 55.00 and 81.81 %, respectively whereas EITB indicated 87.09 and 85.18 % sensitivity and specificity (Table 13, Fig.14).

4.7 Details of blood and serum samples collected for the diagnosis of *T. evansi* in bovines in three districts of Andhra Pradesh state are indicated in Table1

4.7.1 Prevalence of *T. evansi* in bovines in three districts of Andhra Pradesh state based on blood smear and wet blood film examination

In Andhra Pradesh, three districts were selected to study the prevalence of *T. evansi* viz., East Godavari, Krishna and Guntur district. From East Godavari, the places included were Kakinada, Mandapeta and Patavala. In Krishna district, Gannavaram, Buddavaram and Kaluvapamula were screened whereas from Guntur district, Rentachinthala and Mallavaram were included. Out of 74 cattle examined at Kakinada, 7 (9.45%) were found positive for *T. evansi* and in the buffaloes 11 (12.79%) were positive out of 86 screened. In Mandapeta, 2 (6.45%) cattle were positive out of 31 screened. The buffaloes screened were 27 out of which 4 (14.81%) was positive. In Patavala, out of 27 cattle examined, one (3.70%) was positive and out of 21 buffaloes screened 3 (14.28%) were found to be positive. In Krishna district, out of 21 cattle examined one (4.76%) was positive for *T. evansi* organisms whereas one (5.88%) buffalo out of 34 screened was positive. In Buddavaram out of 17 cattle screened none were positive but 3 (14.28%) buffaloes were positive out of 21 examined. At Kaluvapamula, one (7.14%) cattle and one (3.70%) buffalo revealed *T. evansi* organisms against 14 and 27 screened respectively. In Guntur district, out of 16 cattle screened at Rentachinthala one (6.25%)
was positive for *T. evansi* organisms and similarly one (3.57%) buffalo was positive out of 28 animals screened. In Mallavaram of Guntur district, out of 15 cattle examined none were positive whereas 2 (6.45%) buffaloes were positive out of 31 examined (Table 14).

The overall prevalence of *T. evansi* in East Godavari district recorded was 7.57 and 13.43 per cent in cattle and buffaloes, respectively. In Krishna district, 2.84 and 7.31 per cent of cattle and buffaloes respectively were positive whereas in Guntur district, 3.22 and 5.08 % prevalence of *T. evansi* was observed (Table 14, Fig.15).

### 4.7.2 Season-wise prevalence of *T. evansi* in bovines in three districts of Andhra Pradesh

The results of season-wise prevalence of *T. evansi* in bovines in Andhra Pradesh in bovines is indicated in Table 15, Fig.16. In the winter season (November-February), the number of cattle examined were 41 and two (4.25%) was positive, whereas 8 (10.81%) out of 74 buffaloes examined in winter season were positive. During the summer season (March-June) 2 (2.56%) cattle indicated positive for *T. evansi* organisms against 78 screened and 5 (5.37%) buffaloes were positive out of 93 animals examined. In the monsoon season (July-October) 9 (9.37%) cattle were positive out of 93 screened, whereas 14 (12.96%) buffaloes were positive out of 108 blood samples screened during the summer season.

### 4.7.3 Age-wise prevalence of *T. evansi* in bovines in three districts of Andhra Pradesh

The results of age-wise prevalence of *T. evansi* infection in bovines in Andhra Pradesh is depicted in Table 16 Fig.17. The age group of cattle less than one year examined were 45 and one (2.22%) was positive for *T. evansi* organisms whereas 4 (8.51%) buffaloes out of 47 buffaloes examined were positive. Between the age group of three to six years, 3 (5.88%) out of 51 cattle were positive, whereas 5 (10.20%) out of 49 buffaloes screened were found positive for *T. evansi* organisms.

Between the age group of six to eight years 4 (6.34%) cattle were positive out of 63 animals screened, whereas 8 (10.66%) buffaloes revealed *T. evansi* organisms out of 75 examined. The cattle above eight years of age examined revealed that 3 (11.53%) out
of 26 blood samples to be positive, whereas 9 (16.66%) buffaloes revealed that out of 54 screened were positive (Table 16).

4.8 Sex-wise prevalence of *T. evansi* in bovines in three districts of Andhra Pradesh

The results of sex-wise prevalence of *T. evansi* in bovines in Andhra Pradesh is indicated in Table 17 Fig.18. Out of 72 male cattle examined 4 (5.55%) were found positive and out of 112 male buffaloes screened 7 (6.25%) were positive. Out of the 143 female buffaloes examined 20 (6.29%) were positive, whereas 20 (12.26%) female buffaloes were positive out of 163 animals screened.

4.8.1 Results of the parasitological diagnostic tests for *T. evansi* in bovines in three districts of Andhra Pradesh

Out of the 135 sick/suspected cattle examined one (0.74%) was found positive by wet blood film examination and 9 (6.66%) were positive by staining method. Among the 80 apparently healthy cattle, 3 (3.75%) shown positive by staining method but none could be detected by wet blood film examination.

Out of the 150 sick/suspected buffaloes examined 2 (1.33%) were positive by wet blood film examination and 20 (13.33%) were positive by staining method. In the 125 apparently healthy buffaloes one (0.80%) was positive by wet blood film examination, whereas 4 (3.20%) were positive by the staining method (Table 18).

4.8.2 Detection of *T. evansi* by buffy coat technique (BCT) in bovines in three districts of Andhra Pradesh state

The results of detection of *T. evansi* buffy coat technique in bovines in Andhra Pradesh is tabulated in Table 19. Out of the 32 sick/suspected cattle examined 3 (9.37%) were positive and one (4.16%) cattle was positive out of the 24 apparently healthy cattle screened. Out of the 47 sick/suspected buffaloes screened 5 (10.63%) were positive, whereas one (4.54%) shown positive out of 22 apparently healthy buffaloes screened were detected as positive.
4.8.3 Season-wise prevalence of *T. evansi* in bovines in three districts of Andhra Pradesh by ELISA

The results of season-wise prevalence of *T. evansi* in bovines in Andhra Pradesh is indicated in (Table 20, Fig.19).

During the winter (November-February) 36 sick/suspected cattle were examined out of which 4 (11.11%) were positive and 6 (19.35%) were positive during summer (March-June) season, whereas 9 (16.96%) were positive out of 45 sick/suspected cattle screened during monsoon (July-October). The apparently healthy cattle screened in the three different seasons were 16, 20 and 27, out of which 1 (6.25%), 2 (10.00%) and 3 (11.11%) were positive in winter, summer and monsoon seasons respectively. The sick/suspected buffaloes screened during the winter season were 42, out of which 6 (14.28%) indicated positive and 4 (8.88%) sick/suspected buffaloes were positive against 45 screened during the summer season. In all, 11 (17.46%) sick/suspected buffaloes were positive out of 63 animals examined during the monsoon season. The apparently healthy buffaloes examined during winter season were 26, out of which 2 (7.69%) were positive and 3 (17.64%) apparently healthy buffaloes were positive out of 17 screened during the summer season, whereas 4 (18.18%) out of 22 apparently healthy buffaloes screened during monsoon season were positive (Table 20).

The ELISA revealed the prevalence of *T. evansi* infection of 9.61, 15.68 and 16.66 per cent in winter, summer and monsoon seasons, respectively in cattle, whereas 11.76, 11.29 and 17.64 per cent, infection rate was observed in buffaloes in the three seasons respectively. respectively was observed in buffaloes (Table 20, Fig.19).

4.9 Sex-wise prevalence of *T. evansi* infection in bovines in three districts of Andhra Pradesh by ELISA

The results of sex-wise prevalence of *T. evansi* in bovines in Andhra Pradesh is depicted in Table 21 Fig.20. Out of the 45 male sick/suspected cattle examined 7 (15.55%) were positive and 9 (16.36%) buffaloes out of 55 male sick/suspected animals examined were positive. Out of 36 apparently healthy male cattle screened, 5 (13.88%) were positive and one (16.39%) buffalo was positive out of 61 apparently healthy male
buffaloes screened. The sick/suspected female cattle examined were 63, out of which 10 (15.87%) were positive and 18 (26.47%) buffaloes were positive results among the 68 female sick/suspected buffaloes screened. The apparently healthy female cattle examined were 31 and of which 3 (9.67%) were positive whereas 2 (6.45%) buffaloes were positive for *T. evansi* organisms against 31 apparently healthy female buffaloes screened (Table 21).

Based on ELISA, the prevalence of *T. evansi* infection observed was 14.81 and 8.62 per cent respectively in male cattle and buffaloes whereas 13.82 and 20.20 per cent of the animals were found positive in female cattle and buffaloes respectively (Table 21, Fig.20).

4.9.1 **Age-wise prevalence of *T. evansi* infection in bovines in three districts of Andhra Pradesh state by ELISA**

The prevalence of *T. evansi* infection in bovines in different age groups in Andhra Pradesh screened by ELISA is shown in Table 22 Fig.21. The sick/ suspected cattle examined below one year of age was 19 and out of which one (5.26%) was positive and one (4.00%) buffalo was detected as positive for *T. evansi* infection out of 25 sick/suspected buffaloes screened, but in the apparently healthy cattle and buffaloes below one year of age the prevalence of *T. evansi* could not be recorded. The sick/ suspected cattle examined between one to three years of age were 26 and out of which 5 (19.23%) were positive and 4 (16.00%) sick/ suspected buffaloes were positive against 25 buffaloes screened in the age group between one to three years. The apparently healthy age group between one to three years of cattle examined were 18 and out of which one (5.55%) was positive results whereas one (3.12%) apparently healthy buffalo was also positive for *T. evansi* infection against 32 examined. In the age group between three to six years, the sick/ suspected cattle examined were 25 and out of which one (4.00%) animal was positive and 10 (24.39%) buffaloes were positive out of 41 sick/ suspected buffaloes screened between the age of three to six years.
The apparently healthy cattle including 34 animals were examined between age group of three to six years and only one animal (2.94%) was positive, similarly only one (4.34%) buffalo was positive out of 23 apparently healthy buffalo screened in the age group of three to six years. The sick/suspected cattle screened between the age group of six to eight years was 21 and out of which 7 (33.33%) were positive whereas 8 (19.51%) buffaloes were positive for *T. evansi* antibodies against 41 screened between the age group of 41 six to eight years in the sick/suspected category. The apparently healthy cattle examined in the age group of six to eight years was 19, but none revealed antibodies against *T. evansi*, whereas 2 (11.76%) apparently healthy buffaloes were positive out of 27 screened in the age group of six to eight years. The sick/suspected cattle examined in the age group of above eight years was 17, out of which 6 (35.29%) were positive, and 2 (11.76%) buffaloes were positive out of 17 examined in the age group of sick/suspected buffaloes above 8 years of age. Out of 15 apparently healthy cattle examined in the age group of above eight years 3 (20.00%) were positive and one (5.55%) buffalo was positive among 18 apparently healthy buffaloes in the age group above eight years of age (Table 22, Fig.21).

Based on ELISA, a prevalence of 2.43 and 1.46 % was recorded in cattle and buffaloes, respectively in the less than one year age group. Between one to three years age group 13.63 and 10.52 % of cattle and buffaloes respectively had infection whereas in the age group of three to six years, 3.38 and 17.18 per cent of infection could be detected in cattle and buffaloes respectively. In the age group of six to eight years 17.5 and 14.70 % prevalence was recorded, whereas it was 28.12 and 8.57 % in the above eight years of age in cattle and buffaloes, respectively.

4.9.2 Sero-diagnosis of *Trypanosoma evansi* infection in bovines in three districts of Andhra Pradesh by EITB

The results of *Trypanosoma evansi* infection in bovines in three districts of Andhra Pradesh state by EITB is presented in Table 23. Out of the 60 cattle examined 19 (31.66%) were positive and 43 (47.77%) buffaloes gave positive results by EITB out of 90 animals screened.
4.10 Comparison of different diagnostic tests for the detection of *T. evansi* infection in bovines in three districts of Andhra Pradesh

The results of different diagnostic tests adopted for the detection of *T. evansi* in bovines in Andhra Pradesh is presented in (Table 24, Fig. 22). Out of the 215 cattle screened by wet blood examination one (0.46%) was positive wherein 3 (1.09%) buffaloes were positive by wet blood film examination out of 275 examined. In the blood smear staining methods, 12 (5.58%) cattle were positive out of 215 animals screened whereas 24 (8.72%) buffaloes were positive out of 275 screened. The cattle screened by buffy coat technique were 56, out of which 4 (7.14%) were positive and 6 (8.68%) buffaloes were positive for *T. evansi* organisms out of 69 animals screened. The ELISA detected antibodies against *T. evansi* in 25 (14.28%) cattle out of 175 screened whereas 30 (13.95%) buffaloes were positive out of 215 screened. In the EITB 25 (41.66%) of the cattle cattle were positive out of 60 animals screened whereas 37 of buffaloes (41.11%) were positive out of 90 animals examined.

4.10.1 Sensitivity and specificity of ELISA and EITB in the diagnosis of *T. evansi* infection in bovines in each of the three districts of Andhra Pradesh and Karnataka

The sensitivity and specificity of ELISA and EITB in the diagnosis of *T. evansi* infection in bovines in Andhra Pradesh was 53.92 and 80.51 %, respectively (Table 25)(Fig. 23) whereas the sensitivity and specificity of ELISA and EITB in Karnataka observed was 81.81 and 85.18 % respectively in the diagnosis of *T. evansi* infection in bovines (Table 26).

4.11 Protein profile of whole cell lysate antigen of *T. evansi* using SDS-PAGE

In the present study, the protein profile of *T. evansi* was studied using 10 per cent resolving gel and 4.5 per cent stacking gel.

4.11.1 Protein profile of whole cell lysate antigen of *T. evansi*
The molecular weight of whole cell lysate antigen of _T. evansi_ organisms was calculated by comparing the results with standard curve. The standard curve was obtained by plotting of Rf values on Y-axis and molecular weight on X-axis using semilog graph paper. The whole cell lysate antigen of _T.evansi_ revealed a total of 10 polypeptides ranging between 69.18 to 15.85 kDa. The polypeptides of 69.18, 60.28, 54.95, 50.12, 43.65, 34.97, 31.62, 28.84, 22.91 and 15.85 kDa were observed (Table 27).

4.12 **Immunoreactive peptides detected using hyper immune serum by EITB**

The polypeptides detected on western blot using hyper immune serum raised against the whole cell lysate antigen of _T. evansi_ ranged between 50.12 to 15.85 kDa. A total of five polypeptides of size 50.12, 40.74, 25.70, 24.55 and 15.85 kDa were identified on blots (Table 28).

4.13 **Immunoreactive peptides detected using known positive serum for _T. evansi_**

The polypeptides observed on western blots using positive sera for _T. evansi_ ranged between 50.12 to 19.95 kDa. A total of three polypeptides of size 50.12, 25.70 and 19.95 kDa were identified on blots (Table 29).

4.14 **Statistical analysis**

The prevalence rate of _T.evansi_ infection based on ELISA was not significantly different between the species of animals, season, gender and age wise but significant difference was found between the diagnostic tests carried out.