Study on the Prevalence of Trypanosomes Affecting Bovine in Tsetse Infested Asossa District of Benishangul Gumuz Regional State, Western Ethiopia

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Abstract: A cross sectional study was carried out in Asosa district of Benishangul Gumuz Regional State, Western Ethiopia between October to December, 2015 to determine the prevalence of trypanosomosis, prevailing species of trypanosomes, associated risks and its vector density. Blood samples collected from (n = 458) randomly sampled cattle (Bos indicus) was examined using parasitological (buffy coat technique) and hematological (measurement of packed cell volume) procedures. An overall, 21 (4.58%) prevalence was recorded. The infection was caused by Trypanosoma congolense 17/21 (80.95%) and Trypanosoma vivax 4/21(19.04%). The infection rate difference amongst trypanosomes was statistically significant (P<0.000). Mean packed cell volume (PCV) value of the parasitaemic animals was lower (21.76% + 1.7) than aparasitaemic animals (24.71% + 1.04) and the variation was statistically significant (P<0.04). Overall, anemia prevalence of 39.52 % (181/458 was recorded and it was significantly higher (66.66%) in infected cattle than in non-infected (38.21%). Higher prevalence (5.16%) was registered in medium body conditioned animals. Significant association was not recorded within study sites, sex groups, age categories and body conditions (P > 0.05). Glossina morsitan sub morsitans was the only tsetse fly caught and its mean apparent density measured as f/t/d was 2.84. In addition, other mechanical vectors such as 0.071 f/t/d tabanids, 0.56 f/t/d Stomoxys and 0.11 f/t/d Haematopota were recorded. In conclusion, the result of the current study showed the economical importance of trypanosomosis in the area calling for devising strategic control efforts. [Asmamaw Aki Jano. Study on the Prevalence of Trypanosomes Affecting Bovine in Tsetse Infested Asossa District of Benishangul Gumuz Regional State, Western Ethiopia, World Rural Observ 2016:8(3):19-27]. ISSN: 1944-6543 (Print): ISSN: 1944-6551 (Online). http://www.sciencepub.net/rural. 3. doi:10.7537/marswr080316.03.

Key words: Asosa district, PCV, Risk factor, Trypanosome, Trypanosomosis, tsetse fly

1. Introduction

Trypanosomosis is a disease complex caused by several species of blood and tissue dwelling protozoan parasites of the genus Trypanosoma (Taylor KA, 1998; Uilenberg G, 1998; Singla LD, *et al.*, 2004). It is a disease of domestic livestock that causes a significant negative impact on food and economic growth in many tropical and subtropical countries of the world including sub-Saharan Africa (d'Ieteren GD *et al.*, 1998). The course of the disease may run from an acute and rapidly fatal to a chronic long lasting one depending on the vector-parasitehost interactions. It is characterized mainly by intermittent fever, progressive anaemia and loss of condition of susceptible hosts which if untreated leads to high mortality rates (Bourn D *et al.*, 2001; Aulakh GS *et al.*, 2005).

Anaemia is a major classical sign following infection with pathogenic Trypanosomes in cattle and other domestic animals (Murray M et al., 1988). Packed cell volume (PCV) usually gives an indication of the anaemia and disease status of a Trypanosome infected animal and is correlated with animal production and reproduction performance (Trail J *et al.*, 1991; Trail J.C.M. *et al.* 1993,].

Similar reports indicated that the prevalence of trypanosomal infections and herd mean PCV has relationship. PCV is expected to decrease with

increasing prevalence of trypanosomosis (Van den Bossche P. et al., 2001). Hence, the relationship between the prevalence of trypanosomal infections and herd mean PCV could be a useful tool in the management of trypanosomosis and planning of its control. However, this relationship has not been quantified in Ethiopia particularly in western Benishangul Gumuz Regional State despite Ethiopia is infected with five tsetse fly species (Glossina pallidipes, G. tachinoides, G. morsitans submorsitans, G. fusixipes fuscipes and G. longipennis) that vector 5 Trypanosome species (Trypano-soma vivax, T. congolense, T. brucei brucei, T. evansi and T. rhodendiense) out of six Trypanosome species existing in Ethiopia (Abebe G, 2005; Fuller G, 1978: Langride W.P., 1976).

African trypanosomosis is heamoparasitic disease considered as the main obstacle to animal production development (Getachew *et al.*, 1996). It is the wasting disease; affected animals are chronically unproductive in terms of milk, meat, manure, traction. The number of trypanocidal drugs available for treating and preventing infections in endemic areas is limited, and about 6 million doses are administered yearly in Africa. The drugs have been in the market for over 30 years, their range of therapeutic safety is small. The disease in Africa costs livestock producers

and consumers an estimated US \$ 1340 million each year (Radostits, 2006).

The influence of tsetse on African agriculture through the transmission of trypanosomosis continues to be a major constraint to the development of national economies and their achievement of self sufficiency in basic food production. The general distribution of tsetse flies is determined principally by climate and influenced by altitude, vegetation, and presence of suitable host animals (Leak SG., 1999).

In Ethiopia, the western and southern river basins are the severely trypanosomosis affected regions in the country. In the region specifically in the western part a wide diversity of tsetse and Trypanosome species and strains co-exist (Abebe G., 2005). The various Glossina species and trypanosomosis via invading about 220,000km2 of fertile land in southern and western parts of the country between longitude 33⁰ and 38⁰ E and latitude 5⁰ and 12⁰ N constrained the livestock production (Fuller G.K.,1978). Out of this 31,000 km² or (62%) Regional land area of Benishangul-Gumuz is infested with tsetse fly (NTTICC, 1996).

Tsetse flies are hard to control and the tsetse fly infestation is becoming more and more serious in Africa. The clearing of large forest tracks some time cause the flies to spread to more populated areas and the deforest land covered with savannah grass consequently newly invade by morsitans group (Jordan, 1986). Tsetse flies are enormous health risks in part of Africa they can transmit a disease trypanosomosis.

In Asossa district trypanosomosis was found to be one of the factors that hampered livestock rearing in most peasant associations. Therefore, a study on the status of the disease and investigating the vectors and their relative abundance is crucial for a successful control in the area. Therefore, the present work aimed at determining the prevalence of bovine trypanosomosis, associated risk factors, anemic status and apparent density of tsetse and other biting flies ascribed in the transmission of trypanosomosis.

2. Materials And Methods

Study Area: The study was conducted between October to December, 2015 in Asosa district of Benishangul Gumuz Regional State. It was conducted in six kebeles here after called sites namely: Tsetse adurnunu, Amba-8, Komeshga-26, and Komeshga-27. The district has 74 kebeles covering an area of 2317 km2 with human population of 47666. Asosa is located between $8^030^{\circ\circ}$ and $40^\circ27^{\circ}$ N and $34^\circ21^{\circ}$ and $39^\circ1^{\circ}$ E. It has an altitude range of 1000-1570 meter above sea level. Its annual temperature ranges between 16° c- 34° c and its annual rainfall range is 900-1300 mm and it extends from May to October with peak

rainy periods from June to August (NMSA, 2007). The livelihood of the society largely depends on mixed livestock and crop production having livestock population of 21,216 Cattle, 24,560 Goat, 4,073 Sheep, 4265 Equines, 4,372 donkeys, 66 horses, 50,315 Poultry & 26336 beehives (CSA, 2015).

Study Design and Study Animals: Cross sectional study design was used. A local zebu cattle (*Bos indicus*), which are usually kept under an extensive husbandry system grazing the communally owned pasture land throughout the year were randomly sampled. They grazed together during the day time and returned to their individual owner's farmstead each evening. The body condition of each of the study cattle was scored as good, medium and poor (Nicholson and Butterworth, 1986). Concurrently, their age was determined based on De-Lahunta and Habel (1986) principles as young (< 2 years old), 2-5 years old (matured) and adult (> 5 years old).

Sampling Techniques and Sample Size Determination: The study sites (Tsetse adurnunu, Amba-8, Komeshga-26 and Komeshga-27) was selected purposively as convenient. The animals were sampled randomly involving both sexes, all age groups, and all types of body conditions. The desired sampling size was calculated according to the formula given by Thrusfield, (2007). The sample size was determined based on the expected prevalence of 50%. confidence level of 95%, and 5% desired absolute precision. As result a total of 384 cattle were calculated but increased to (n=458) to increase precision and these cattle were sampled at their communal grazing area using simple random sampling.

Study methodology and Procedures

Packed cell volume (PCV) determination: Blood samples were obtained by puncturing the marginal ear vein with a lancet and collected directly into a pair of heparinised capillary tubes. The tubes were then sealed at one end with crystal seal. PCV was measured in a micro-haematocrit centrifuge (Hermmle Labortechnik, type Z, Germany). The capillary tubes were placed in microhaematocrit centrifuge with sealed end outermost. Then the tube was loaded symmetrically to ensure good balance. After screwing the rotary cover and closing the centrifuge lid, the specimens were allowed to centrifuge at 12,000 rpm for 5 minutes. After centrifugation, the capillary tubes were placed in a haematocrit reader. The length of the packed red blood cells column is expressed as a percentage of the total volume of blood. Animals with PCV less than 24% were considered to be anemic (OIE, 2008).

Buffy coat technique: Heparinized microhaematocrit capillary tubes, containing blood samples were centrifuged for 5 minutes at 12,000 rpm. After the

centrifugation, trypanosomes were usually found in or just above the buffy coat layer. The capillary tube was cut using a diamond tipped pen 1 mm below the buffy coat to include the upper most layers of the red blood cells and 3 mm above to include the plasma. The content of the capillary tube was expressed onto a glass slide, and covered with cover slip. The slide was examined under x40 objective and x10 eye piece for movement of parasite (Murray, 1991). Trypanosome species were identified according to their morphological descriptions as well as movement in wet film preparations (OIE, 2008).

Entomological data

A total of 78 monopyramidal traps including 16, 20, 15, 6 and 21 were deployed in the riverside and wooded grassland areas of Kitili, Burji, Gublak, Ipupuwa and Beles 2 kebeles, respectively. The density and species of tsetse flies were assessed using odour-baited monopyramidal traps deployed at 200-250 m intervals. The odour baits used contained acetone, octanol and cow urine with appropriate apertures in order to release the necessary amounts of attractants. After 48 hours of trapping, the trap cage was collected [17]. The species and sex of the captured flies were identified based on morphological characteristics [18]. The apparent density of tsetse flies was determined based on the daily mean number of flies captured in baited traps and recorded as fly per trap per day (F/T/D) [19].

Entomological survey: During the study four types of traps were deployed: 18 Monopyramidal, 15 monoconical, 12 biconical, and 4 NGU traps. The density and species of tsetse flies were assessed using odour-baited traps deployed at 200-250 m intervals. Every trap was odor baited with acetone and cow urine. The underneath of each trap pole was smeared with grease in order to prevent the ants climbing up the pole towards the collecting cage that could damage the tsetse flies. The trap deployment time was 48 hours. After the flies captured in the collecting cage they were then sorted by sex and species and recorded. The species of tsetse was identified based on the characteristic morphology. Other biting flies were also according separated to their morphological characteristics such as size, color, proboscis and wing venation structures at the genus level (Langridge, 1976; Fischer and Say, 1989). Sexing was done for tsetse fly just by observing the posterior end of the ventral aspect of abdomen by hand lens, as a result male flies easily identified by enlarged hypophageum. The apparent density of tsetse flies was determined based on the daily mean number of flies captured in baited traps and recorded as fly per trap per day (F/T/D) (LeakSGA et al., 2009).

Data management and Analysis: Raw data were entered into a Microsoft Excel spreadsheet and descriptive statistics were used to summarize the data. STATA® version 11.0 statistical software programs were used to analyze the data. The point prevalence was calculated for all data as the number of infected individuals divided by the number of individuals examined and multiplied by 100. The association between the prevalence of trypanosome infection and risk factors were assessed by chi-square test (χ 2), whereas the two sample student's t-test was used to assess the difference in mean PCV between trypanosome positive and negative animals. The test result was considered significant when the calculated p-value was less than 0.05 at 95% confidence interval (Thrusfield, 2005).

3. Result

Trypanosomes infection prevalence: Out of total animals examined(n=458), 21/58(4.58%) were infected with trypanosomes. The prevalence in terms of trypanosome species was 3.71% *T.congolense* and 0.87% *T.vivax*. The proportion of trypanosome species was 17/21(80.95%) *T. congolense* and 4/21(19.04%) *T. vivax* (Table 1). During study period mixed infection was not detected. The infection rate difference between trypanosomes was statistically significant (P<0.000).

Cattle PCV Distribution and Anemia in Studied Area: The mean PCV value for whole examined animals was 24.53 ± 3.17 SE. However, the mean PCV value for un infected animals was 24.71 ± 1.04 SE and mean PCV value of the infected animals was 21.76 ± 1.7 SE. The mean PCV values of cattle were significantly (P = 0.04) influenced by trypanosome infection as 21.76 % and 24.71 % PCV values in trypanosome positive and trypanosome negative animals were registered, respectively (Table 2). The overall anemia prevalence in the studied district was 39.52 % (181/458). The anemia prevalence was significantly higher in trypanosome infected cattle (66.66%) than in non-infected cattle (38.21) (*P* < 0.04). Of 39.52 % anemia prevalence, 3.05 % (14/458) was trypanosome infected animals. However, large number of animals 36.46% (167/458) had anemia (PCV < 24) without having trypanosome infection. Some animals 1.52% (7/458) were infected by trypanosome but their PCV was found normal (Table 3).

Prevalence of Trypanosomosis according to Age, Sex, sites and Body Condition: The highest trypanosomosis prevalence 6.12% was recorded in 2-5 years old animals whilst the lowest prevalence 0(0 %)were < 2years old. Slightly higher prevalence was registered in females 16 (5.22 %) than in males 5 (3.29%), which was statistically non-significant between age groups (p>0.05). Trypanosomosis was recorded across the study sites with the highest (6.66%) prevalence in Komoshega-27 PA and the lowest 2.08 % in Tsetse adurnunu PA. Trypanosomosis prevalence was statistically nonsignificant in sex groups and across study sites. The highest prevalence (5.16%) was found in medium body condition animals while the least (3.52%) in poor body conditions. This difference was statistically nonsignificant. The effect of age, sex, sites and body trypanosomosis condition on prevalence is summarized in table *Entomological Findings* : A total of 351 Tsetse and biting were caught during the study period from different site. Out of the total, 278 (79.20 %) were belonging to tsetse of the genus glossina, followed by 55 (15.66%) Stomoxy, 11 (3.13%) Haematopota and 7(1.99%) tabanus. Only G. sub. morsitans were identified in the survey site with the overall apparent density of 2.84 F/T/D (fly/trap/day). The highest fly density were observed in Komoshega-27 peasant association 123 (4.1 F/T/D) and the lowest recorded in Amba-8 54 (2.7 F/T/D) (Table 5).

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Trypanosomes	No. positive	Prevalence (%)	X ²	p-value
T. congolense	17	80.95		
T. vivax	4	19.04	367.39	0.000
Total	21	100		

Table 2: Mean PCV comparison between parasitaemic and aparasitaemic animals

Status	Frequency	Mean PCV (%)	SE	Overall PCV	\mathbf{X}^2	p-value
Parasitaemic	21	21.76	1.7	457		
Aparasitaemic	437	24.71	1.04	10,799	3.92	0.04
Total	458	24.53	3.17	11,236		

Table 3: Proportion of anemia in parasitaemic and aparasitaemic cattle population

Status	Anemia	Frequency	Percent	Percent Share Per Strata
infected	anemic	14	3.05	66.66
	non anemic	7	1.52	33.33
Non infected	anemic	167	36.46	38.21
	Non anemic	270	58.95	61.78

Table 4: prevalence of bovine trypanosomosis and its association with various risk factors in Asosa district

Risk factors	No. examined	No. positive	Prevalence (%)	χ^2	p-value		
Sites							
Tsetse adurnunu	96	2	2.08				
Amba -8	122	4	3.27	2 16	0.32		
Komeshga-26	120	7	5.83	5.40	0.52		
Komeshga-27	120	8	6.66				
Total	458	21	4.58				
sex							
Male	152	5	3.29	0.87	0.25		
Female	306	16	5.22	0.87	0.55		
Total	458	21	4.58				
Age(years)							
< 2	29	0	0				
2 - 5	310	19	6.12		0.06		
> 5	119	2	1.68				
Total	458	21	4.58				
Body conditions	Body conditions						
Good	160	7	4.37				
Medium	213	11	5.16	0.39	0.82		
Poor	85	3	3.52				
Total	458	21	4.58				

Tuble 5. Thes eaught in anterent areas of survey sites in Alsobsa abarter										
Sites	Total flies	No. of	Tsetse flies caught					Biting flies		
	caught	traps	No.	species	М	F	*F/T/D	Stomoxys	tabanid	Haematopota
Tsetse adurnunu	78	10	61		25	36	3.05	12	2	3
Amba-8	54	10	43		13	30	2.15	8	1	2
Komoshga-26	96	14	83	Gm	22	61	2.96	9	2	2
Komoshga-27	123	15	91		39	52	3.03	26	2	4
Total	351	49	278		99	179	2.84	55	7	11

Table 5: Flies caught in different areas of survey sites in Asossa district

F/T/D=fly per trap per day, Gm=Glossina morsitans submorsitans, M=male, F=female

4. Discussions

The current study revealed an overall prevalence of 21/458 (4.58%) in the study area. This finding was in agreement with earlier works of (Lelisa, K., 2015) who reported 5.43% prevalence in the neighboring Mandura district; (Tilahun *et al.*, 2014) studied the prevalence of cattle trypanosomosis, its vector density and distribution in Dale Sadi District, Kellem Wollega Zone and reported a prevalence of 6.34%. This result was agree with previous works of (Asmamaw A *et al.*, 2016) who reported 5.58% bovine trypanosomosis prevalence in the near by Pawe district.

The study showed that the infection was predominantly caused by T. congolense 17/21 (80.95%) and T. vivax 4/21 (19.04%). This result is in consonance with the reported proportions of T.congolense (77.6%) followed by T.vivax (14.9%) from Metekel and Awi zones (Mekuria, S et al., 2011). This result was also in consistent with prior reports of (Mulaw et al., 2011) who studied prevalence of major trypanosomes affecting cattle in the neighboring Asosa district of Benishangul Gumuz Regional State, Western Ethiopia and found T. congolense proportional prevalence of 66. 7%; (Abraham Z et al., 2012) worked on the prevalence of bovine trypanosomosis in selected district of Arba Minch, Sothern Ethiopia and reported T. congolense proportional prevalence of 61.4%; (Biyazen H et al., 2014) reported T. congolense proportional prevalence 63.64% during his work on trypanosomosis and anemia in cattle population of Dale Wabera district of Kellem Wollega Zone, Western Ethiopia; (Asmamaw A et al., 2016) demonstrated T. congolense proportional prevalence of 75.86% and proportional prevalence trypanosome vivax of 24.14% during his research on cattle trypanosomosis prevalence in Pawe district, Benishangul Gumuz Regional State, Western Ethiopia.

The high proportion infection rate of *T*. congolense in cattle might be attributable to the high number of serodems of *T*. congolense relative to *T*. vivax. It could also be due to the possible development of better immune response to *T*. vivax by the infected animals as demonstrated by (Leak S.G.A et al., 1993) Further, it might be attributed to the efficient transmission of *T.congolense* by cyclical vectors than *T.vivax* in tsetse-infested areas. Previous reports indicated that *T. congolense* and *T.vivax* are the most prevalent trypanosomes that infect cattle in tsetse infested and tsetse free areas of Ethiopia respectively (Langridage WP. 1976; Leak S.G.A 1999). Different studies (Leak S.G.A *et al.*, 1993; Rowland, W *et al.*, 1995) have indicated that *T. vivax* is highly susceptible to treatment while the problems of drug resistance are higher in *T. congolense*, and *T. congolense* is mainly confirmed in the blood, while *T. vivax* and *T. brucei* also invade the tissues (Stephen, 1986).

The prevalence of bovine trypanosomosis was studied between sex groups, age categories, a cross study sites and body conditions, significant association was not observed (P > 0.05). This might be because of equal chance of exposure to the parasite. This result is in agreement with previous reports (Mihreteab, B *et al.*, 2011, Teka, W *et al.*, 2012; Lelisa, K *et al.*, 2015).

The overall anemia prevalence in the studied district was 39.51 % (181/458). The anemia prevalence was significantly higher in trypanosome infected cattle (66.66%) than in non-infected cattle (38.21%) (P < 0.04). This is in concordance with previous results from different researchers (Mihret *et al.*, 2007, S. Tasew *et al.*,2012, M. Bekele *et al.*, 2011; Biyazen, H., 2014). Out of 39.51 % anemia prevalence, 3.05% (14/458) was trypanosome infected animals. Nonetheless, 36.46% (167/458) of non-infected animals were found to be anemic (PCV < 24). This indicates the fact that other factors such as gastrointestinal parasitism, nutritional deficiencies, fasciolosis and vector-borne diseases could affect the PCV value of cattle (Van den Bossche *et al.*, 2001).

This study revealed that 1.52% (7/458) of the cattle were infected by trypanosome; however, their PCV was laid in the normal range. This might be attributed to the capability of infected cattle to maintain their PCV within the normal range for a certain period of time. It could also be possibly due to inadequacy of the detection method used (Murray, M *et al.*, 1988), other anemia causing diseases (Van den

Bossche *et al.*, 2001), or delayed recovery of the anemic situation after current treatment with trypanocidal drugs. Furthermore, the occurrence of positive animals with PCV of greater than 24% might be thought of as recent infections of the animals (Van den Bossche *et al.*, 2001).

The overall mean PCV value for examined animals was 24.53 ± 3.17 SE. The mean PCV value of the infected animals was significantly lower (21.76 ± 1.7 SE) than that of uninfected animals (24.71 ± 1.04 SE). This result is in alignment with previous works (Ali, D *et al.*, 2011, Mulaw S., 2011; Asmamaw A. *et al.*, 2016).

Glossina moristans sub moristans was the only tsetse fly caught and its mean apparent density measured as f/t/d was 2.84. It accounts for 278 (79.20%) out of the total flies caught. In addition, other mechanical transmitters of trypanosomosis such as stomoxys 55 (15.66%), 11(3.13%) Haematopota and 7(1.99%) tabanid were recorded. The current findings were in consistent with previous works of (Solomon, M *et al.*, 2010) at Metekel Awi zones of Northwest Ethiopia, who reported 6.49 f/t/d and 0.65 f/t/d for tsetse and biting flies, respectively. It was also in agreement with findings of (NTTICC, 2004) at Bure Iluababor zone of Western Ethiopia which was reported to be 7.23 f/t/d, 3.13 f/t/d and 0.06 f/t/d for tsetse fly, *Stomoxys* and *Tabanus*, respectively.

And also the result agree with the previous works of (Asmamaw A *et al.*, 2016) at Metekel zone, pawe district of western Ethiopia, who reported 1.62 f/t/d Stomoxys, 0.41 f/t/d tabanus and 0.22 f/t/d hematopota.

This result was also consistent with the previous findings of (NTTICC, 2012-2014) at neigbouring Mandura districts of western Ethiopia which was reported to be 3.59 & 1.16 f/t/d; 0.15, 0.20 & 4.5 f/t/d; 0.02, 0.05 & 0.33 f/t/d; 0.014, 1.38 & 4.5 f/t/d) for tsetse fly, *stomoxys, tabanus* and *haematopota*, respectively. Similarly, It was also in consistent with the previous findings of (NTTICC, 2012 & 2014) at neigbouring Dangur districts of western Ethiopia which was reported to be 1.14 f/t/d; 4.04 & 0.09f/t/d; 3.84 & 0.04 f/t/d; 0.4 & 0.6 f/t/d) for tsetse fly, *stomoxys, tabanus* and *haematopota*, respectively.

5. Conclusions

Animal trypanosomosis is a major problem to livestock production and productivity in Asossa Districts. Since the Districts lies within the tsetse belt area, the expected prevalence of trypanosomosis prevailing in the area should be high. The present, overall trypanosomosis prevalence was 21 (4.58 %). Significant association was not recorded with study sites, sex groups, age categories and body conditions (P> 0.05) And also the study result of trypanosomosis and other factors such as (nutritional, seasonal; concurrent disease) was found to be negatively affects the PCV values of affected animals. The most widely distributed and dominant species is *T. congolense* 80.95% followed by *T.vivax* 19.04%, which was mainly transmitted by tsetse fly, Glossina morsitan sub morsitans (2.84 f/t/d/) and biting flies (stomoxys 0.56 f/t/d, tabanid 0.071 f/t/d and haematopota 0.11 f/t/d) respectively. In conclusion, the result of the current study showed the economical importance of trypanosomosis in the area calling for devising strategic control efforts.

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7/22/2016

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