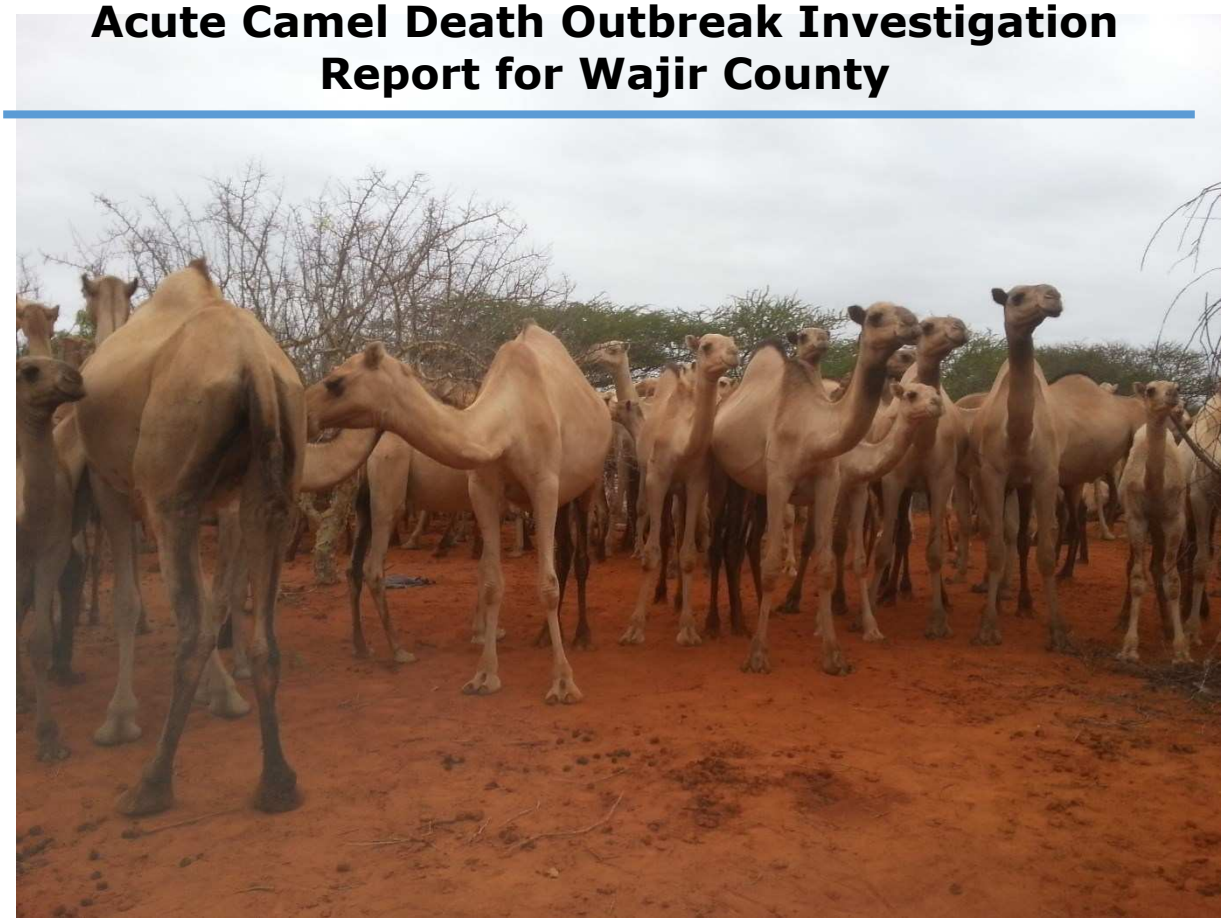
Technical Report

Acute Camel Death Outbreak Investigation Report for Wajir County



Submitted by:

University of Nairobi
African Drylands Institute for Sustainability (UoN-ADIS)

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Acknowledgment

We acknowledge the financial support received from Mercy corps and are grateful for the logistical and community mobilisation support by the Wajir County Veterinary Department staff. The support and good will from Yusuf Gedi, Wajir County, CEC of Agriculture, Livestock and Fisheries and Prof. Gakuya, Chairman of Clinical Studies Department (CSD), University of Nairobi, is also highly appreciated. Lastly, we thank Dr. Kiprono, Wajir County Director of Veterinary Services and Camel owners/herders in Riba for taking part in the interviews and allowing sampling of animals.

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1.0 Background information

Wajir County Department of Agriculture, Livestock and Fisheries (WDALF) in December 2015 reported to the Directorate of Veterinary Services (DVS) several cases of acute deaths of adult Camels in Wajir West, Wajir South and Eldas Sub-counties. The acute camel death syndrome begun immediately after the enhanced October to November 2015 rainy season. In response to this outbreak, a joint team comprising of Wajir County Veterinary and DVS staff conducted an outbreak investigation in January, 2016. The investigation was carried out at Griftu and Hadado in Wajir West sub-county; Kotulo in Tarbaj sub-county; Machesa and Banini in Wajir South sub-county. The probable diagnosis arrived at was Haemorrhagic Septicaemia (HS) which was based on clinical and post mortem findings as samples submitted for laboratory confirmation were inconclusive. In addition, white blood cell differential count analysis of whole blood collected from sick camels in January 2016, revealed an increase in lymphocyte count alluding to a possible viral involvement. In May 2016 a resurgence of acute camel death syndrome was reported in Wajir East sub-county. It is under this background that the WDALF sought partnership with University of Nairobi- African Drylands Institute for Sustainability (UoN-ADIS) and Mercy Corps to carry out a second outbreak investigation.

1.1 Objectives of the outbreak investigation assignment

(1) To determine the spatio-temporal trend of acute Camel death syndrome in Wajir County and

(2) To identify the causative agent of the acute camel death syndrome through field sampling and analysis in specific sites in Wajir East Sub-County.

1.2 Approach

A participatory approach using a previously described guideline for disease outbreak investigation was used[1]. The main strategy used in the current outbreak investigation that differed from the January 2016 investigation was the analysis of EDTA blood samples within 1 hour after collection. In addition, peripheral blood smears and lymph node aspirate smears were fixed in

[1] Kane A.J. and Morley P.S. (1999). How to investigate an outbreak. A paper presented during the 1999 AAEP scientific conference.

methanol and stained with Giemsa immediately after collection. This strategy ensured the integrity of blood and lymph aspirate cells was maintained. A recent study has indicated that blood smears made from EDTA tubes that have been stored for more than 4 days often have unacceptable artifacts of the red blood cells. In addition, the prolonged storage in EDTA (even when refrigerated) compromises the blood cells membrane integrity thus affecting the reliability of results[2]. Another strategy used was to have swabs intended for bacterial culture be first dipped in a pre-enrichment media (Buffered peptone) before being placed in a transport media (Stuart Transport Medium) this would increase the viability of any microbiological specimen collected[3].

2.0 Methodology

The field outbreak investigation was carried out on 13th to 17th June 2016 at Riba in Wajir East sub-county. The location visited was based on surveillance information of unusual death of adult Camels after exhibiting clinical signs consistent with acute camel death syndrome. The investigation team was composed of UoN-ADIS Animal Health consultant (Dr. Gitonga), UoN-Clinical Studies Department (CSD) Laboratory Technician (Jane Kamau) and staff from Wajir County Veterinary department (Dr. Abukar, Mr. Farah and Ms. Asli). Other team members included 2 drivers and 4 students who were on attachment. Primary disease outbreak information was obtained from Key Informant Interviews (KIIs) with staff from Wajir Veterinary department and Focus Group Discussions (FGDs) with camel herders in Riba. Secondary information was used to triangulate the primary information and was obtained from past outbreak investigation reports for Wajir and Marsabit, research publications, technical reports and laboratory results obtained from the UoN-CSD haematology laboratory. The outbreak investigation also included sample collection from sick camels exhibiting clinical signs associated with acute camel death syndrome. The samples collected included;

[2] Samuel Antwi-Baffour, Elizabeth Quao, Ransford Kyeremeh, Seidu Abdulai Mahmood. Prolong Storage of Blood in EDTA Has an Effect on the Morphology and Osmotic Fragility of Erythrocytes. International Journal of Biomedical Science and Engineering.Vol. 1, No. 2, 2013, pp. 20-23. doi: 10.11648/j.ijbse.20130102.11

[3] The necropsy book by John M. King, Lois Roth-Johnson, David C. Dodd and Marion E. Newsom

peripheral blood smears from the tip of the ear, lymph node aspirate smears (Figure 1) and whole blood in EDTA tubes.

Figure 1: Dr. Gitonga collecting a lymph node aspirate from an enlarged sub-mandibular gland.

Blood and lymph node aspirate smears were immediately fixed and stained after collection and later examined under a light microscope (Figure 2). Whole blood in EDTA tubes was analysed using the microheamatocrit centrifuge technique (Figure 3) so as to determine the packed cell Volume (PCV) and allow Buffy coat examination (BCE). PCV and BCE results were used to determine if Camels sampled had Trypanosomiasis infections using a methodology described previously[4].

[4] Nyang'o J M (1993). Clinical manifestation of experimental *Trypanosoma evansi* infection in the dromedary camel and the effect of treatment on haematological, biochemical and serological values. MSc. thesis from the Department of Clinical Studies, Faculty of Veterinary Medicine, University of Nairobi.

Figure 2: Laboratory technician Jane Kamau examining a methanol fixed and Giemsa stained blood smear under the light microscope. Inset, is Mr. Farah from Wajir County Veterinary Department

Figure 3: Blood in EDTA tubes was drawn into capillary tubes which were then centrifuged in a micro-haematocrit machine for 5 minutes at 10,000 revolutions per minute (rpm).

Figure 4: Dr. Abukar demonstrating to student on attachment how to read PCV values.

3.0 Findings

3.1 Spatio-temporal trend of acute Camel death disease syndrome in Wajir County

Secondary information indicated that Acute Camel death syndrome also known as Camel Sudden Death syndrome was first reported in Afar region in Ethiopia in May 2005. A disease with similar signs was later reported in Somalia between April/May 2006 and September/December 2006. The first outbreak report of Camel Sudden Death syndrome in Kenya occurred in Mandera and Wajir counties in February 2007. Throughout May to December 2007 outbreaks continued to be reported in Garissa, Marsabit, Isiolo, Samburu and Turkana Counties[5]. The 2007 outbreak of acute camel death syndrome in Wajir County occurred in Wajir North before spreading to Wajir East, Wajir West and Wajir South. Subsequent outbreaks occurred in 2014, 2015 and 2016. Acute camel death outbreaks in Wajir have a temporal pattern that indicates a seasonal risk factor as most outbreaks occur 1 month after the March/April and October/November rains when the camels are in good body condition and have sufficient browse fodder. This period is also associated with an increase in the population of biting flies and ticks specifically *Rhipicephalus and Hyalomma* species (identified by DVS staff during the January 2016 outbreak investigation).

3.2 Case definition

Primary and secondary information indicates that acute camel death syndrome in Wajir manifests in 2 forms the Sudden Death syndrome and the Acute death syndrome.

Sudden Death syndrome

This form mostly affects adult camels (> 5years) who are in good body condition and at their peak of productive and reproductive life that is bulls, recently calved, lactating and pregnant cows. In this form, there are no prior signs of illness in the animals. Most are reported to drop dead while feeding or are found dead in the morning.

[5] Gluecks Ilona and Marion Younan (2010). Enhanced Livelihood in the Mandera Triangle (ELMT) Technical Report on Camel Sudden Death Syndrome: Outbreak of unknown camel disease in the Horn of Africa.

One camel herder in Riba commented that during the January 2016 outbreak one of his recently calved adult female dropped dead as she was being milked. The camel herders have named this form of the disease 'Risasi' which is a Swahili word for bullet. The attack rate[6] of the current outbreak that begun in May 2016 was low (3%) in Riba area (6 animals died from a group of 200 adult camels at risk). Post-mortem (PM) examination reports indicate that there are no visible (gross) lesions in any organ. However, one elderly camel herder during the FGDs indicated that he once saw haemorrhages on the heart muscle (myocardium). This form may not be zoonotic as most animals that died in January 2016 were consumed with no adverse effects reported in humans. Due to the sudden occurrence of this form, blood and tissue sampling as well as treatment has not been done as most cases occur in remote locations far from animal health officials or community disease reporters. In addition, reports of death often reach county veterinary officials 1 or 2 weeks after incidences as most remote locations in Wajir have no mobile network connectivity.

Acute death syndrome

This is the most common form and it affects both sexes of camels that are above 3 years of age and are in good body condition with no prior history of illness. Acute Camel death form was described as having a short clinical course of 3 to 5 days. On the 1st day the animal is off-feed, dull, lethargic, has increased bilateral watery eye discharge (lacrimation), has signs of fever due to ruffled appearance of coat and has enlarged superficial lymph nodes (sub-mandibular and cervical/pre-scapular). By the 2nd day lacrimation becomes more and may change to mucoid discharge, there is respiratory distress manifested as laboured breathing, coughing and nasal discharge. By the 3rd day sick animals become recumbent (sternal) and are anorexic they also develop a foul smell from the mouth (halitosis) and sometimes bloat. Most animals die by the 3rd day and the few that survive to the 4th or 5th day may develop spontaneous bleeding from body orifices (nostrils, mouth and anal opening) with some having a rupture of a head swelling or one of the

[6] Attack rate = Number of new cases since onset of outbreak X100
Total number of animals at risk at onset of outbreak

superficial lymph nodes before death. Post mortem findings are based on FGDs, KIIs and DVS report of the first outbreak investigation in January 2016. PM lesions include petechial and echymotic haemorrhages in the visceral organs (heart, liver, kidney, lymph nodes and sub-cutis), enlarged and haemorrhagic superficial and internal (mesenteric) lymph nodes. There is also an increased accumulation of fluid in the abdominal, and thoracic cavities as well as fluid in the pericardial sac. The fluid may be serous or haemorrhagic. The liver appears cooked and Kidneys are enlarged and friable.

FGDs and KIIs information indicate that sick camels treated early (within the first 2 days of illness) recovered well. A combination of the following drugs was used for the January and May 2016 outbreaks in Wajir; 1. Antibiotics (either Penstrep®, Oxy-tetracycline or Tylosin) for 3 days at the correct weight dosage 2. Multivitamin and 3. Trypanocidal drugs (Triquin®). Prognosis of recovery after treatment decreased if the camel had been recumbent for more than 3 days. In addition, camel herders in Wajir West during the 2015 outbreak requested the county government in a cost-sharing agreement to purchase and vaccinate camel herds with Blanthrax® vaccine. The vaccine provides protection against Black quarter and Anthrax infections. It was noted that vaccinating in contact and at risk healthy animals markedly reduced the incidence of new cases. Based on this observation, the Wajir County veterinary department also vaccinates camel herds in areas that report acute camel death cases and reports indicate the vaccine offers protection for 6-12 months. In Riba area, where the current investigation was done, approximately 3,000 camels received the Blanthrax® vaccine.

3.2.1 Summary of Clinical findings

During the current field investigation conducted on 13th to 17th June 2016 at Riba in Wajir East sub-county, 6 camels were identified by herders as showing signs similar to acute death syndrome. The camels were from 2 herds that had experienced mortalities of adult camels the week prior to our visit. The camels were between 2 to 3 years of age and included 3 females and 3 males. All 6 animals had a history of being off-feed for 2 days, had increased

lacrimation, a cough and enlarged sub-mandibular and cervical/pre-scapular superficial lymph nodes (Figure 5). Clinical examination revealed tick infestation especially of the inner ear pinna (Figure 6), neck region, inner nostrils and underside of the tail. The rectal temperature of the 6 animals ranged from 36.9 to 37.6 ◦C and were considered to be within the normal range. In addition, all animals sampled had a normal pink colour of the conjunctival mucus membrane.

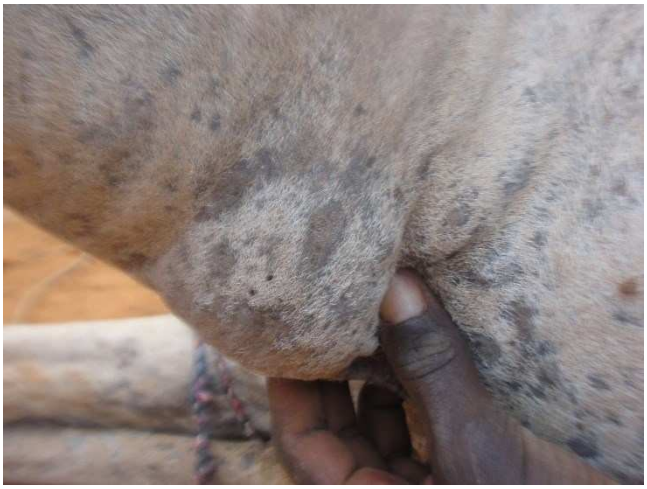

Figure 5: Enlarged cervical/pre-scapular lymph node in one of the 6 camels sampled

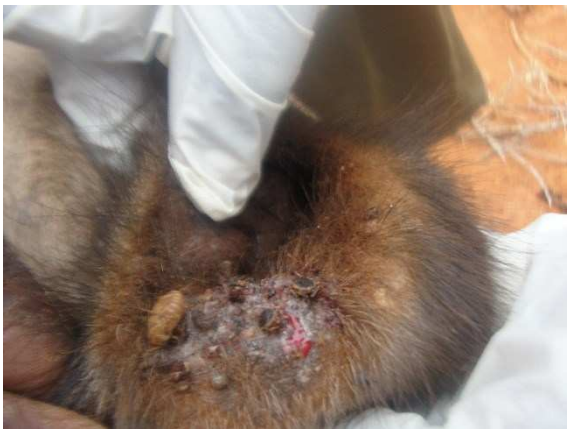

Figure 6: Tick infestation of the inner ear pinna in one of the 6 camels sampled

3.2.2 Summary of sample results

Samples collected were processed and examined while in the field. The average PCV was 31.0% for female and 31.3% for male animals with both having a range of 27-38%. These PCV values were considered normal based

on work from a previous study in Kenya[7] and in Sudan[8]. No trypanosome parasites were seen on buffy coat examination. Blood smears were used to differentiate the white blood cells (WBCs) count percentage (%) of the 3 main population of WBCs in camel blood that is; neutrophils, lymphocytes and eosinophils (Table 1). The results in table 1 indicate that 4 of the 6 camels had an increased neutrophil count indicting a possible bacterial involvement given that the total lymphocyte count was also low. Blood smears were also checked for presence of protozoan or rickettsia parasites, camel 6 had cytoplasmic inclusions of the lymphocytes, one of the white blood cells. These inclusions resembled a tick-borne parasitic infection with *Erhlichia* or *Theileria* species. Camel 4 had only one red blood cell infected with a *Babesia* organism also a tick-borne infection. However, the absence of clinical signs as seen in a recent natural outbreak in Saudi Arabia[9] meant that the *Babesia* finding was incidental. There were no abnormalities noted in the cells from the lymph node aspirate smears of all 6 animals.

Table 1: Blood smear results for the 6 sick camels sampled in Riba area in Wajir East

Blood smear Sample number	Lymphocyte count N (45-70%)	Mature Neutrophil count N (27-50%)	Eosinophil N (0-2%)	Parasite presence
Camel 1 - Female	30	68	2	Negative
Camel 2 - Male	41	62	0	Negative
Camel 3- Female	51	49	0	Negative
Camel 4- Female	41	59	0	Positive for *Babesia* parasite in one red blood cell
Camel 5 – Male	46	49	0	Negative
Camel 6- Male	31	54 total (52 mature: 2 immature)	1	*Positive for Parasitic morulae in the lymphocyte cytoplasm*

*N= Normal values of WBC differentiation count adopted from Nyang'o (1993)[15]

[7] Nyang'o J M (1993). Clinical manifestation of experimental *Trypanosoma evansi* infection in the dromedary camel and the effect of treatment on haematological, biochemical and serological values. MSc. thesis from the Department of Clinical Studies, Faculty of Veterinary Medicine, University of Nairobi.

[8] Babeker E A and Suleem E A (2013). Observation of certain hematological and biochemical parameters in nomadic camels in the Sudan. University of Bakht Alruda Scientific Journal Issue No.6.

[9] Swelum A.A., Ismael A.B., Khalaf A.F. and Abouheif M.A. (2014). Clinical and laboratory findings associated with naturally occurring babesiosis in dromedary camels. Bull Vet Inst Pulawy: 58: 229-233.

Following the acute camel death outbreak investigation carried out in Wajir[10] and Marsabit[11] Counties by DVS staff in January 2016. A total of 73 whole blood samples in EDTA tubes were submitted to the UoN-CSD haematology laboratory on 11th, 16th and 18th February. Most samples showed an extensive lysis/ rupture of red blood cell membranes as they were submitted past the recommended period[12], this meant that PCV analysis could not be done. However, the WBCs membranes were intact and a differentiation count was done. The results as shown in table 2 below indicates that the average WBCs differentiation count was normal, however 36 animals had an elevated lymphocyte count (lymphocytosis) that is, a count above the normal 70%.

Table 2: Average differential WBC count for whole blood submitted after the January 2016 outbreak in Wajir and Marsabit Counties

WBC type		Count (%)	CI: Upper limit	CI: Lower limit
Lymphocyte	N	73	73	73
	Minimum	43		
	Maximum	91		
	Mean	**68.85**	**66.40**	**71.14**
	Std. Deviation	10.506	8.817	12.070
Neutrophil	N	73	73	73
	Minimum	9		
	Maximum	56		
	Mean	**25.30**	**23.00**	**27.45**
	Std. Deviation	9.543	7.219	11.504

The normal WBCs differentiation count of camels unlike in other ruminants' is characterised by a higher lymphocytes to neutrophil count ratio and interpretation of haematology results should take this into consideration. Disease conditions that result in lymphocytosis in camels include viral

[10] Final Report into the Investigation of Sudden Deaths in Camels in Wajir County carried out on 2nd to 7th January 2016.
[11] Report on Camel Death Investigation in Marsabit County carried out on 25th to 31st January 2016.
[12] Samuel Antwi-Baffour, Elizabeth Quao, Ransford Kyeremeh, Seidu Abdulai Mahmood. Prolong Storage of Blood in EDTA Has an Effect on the Morphology and Osmotic Fragility of Erythrocytes. International Journal of Biomedical Science and Engineering.Vol. 1, No. 2, 2013, pp. 20-23. doi: 10.11648/j.ijbse.20130102.11

infections[13] such as para-influenza [14]and trypanosomiasis infections[15]. Reduced lymphocyte count has been reported in *Theileria camelensis* infections[16]. A parasite check of the 73 blood smears from the January 2016 outbreak revealed that 36 animals had cytoplasmic inclusions in the lymphocyte and neutrophil cells that resembled parasitic morulae (*Erhlichia* or *Theileria* species). This finding was also noted in our investigation and will require further investigation to link the presence of infected ticks with animals manifesting with clinical disease and have parasitic morulae in their blood smear cells. The January 2016 outbreak in Marsabit indicates that there were isolated cases that manifested with nervous clinical signs consistent with Heart Water these include aimless walking and head pressing. The presence of the *Amblyomma* tick specie the common vector agent for Heart water disease in Marsabit may also support this observation and may indicate that in some cases Heart water may occur concurrently with acute camel death syndrome.

4.0 Conclusion

- A tentative diagnosis of bacterial infection as the cause of illness for the 6 camels sampled in Riba was arrived at. The main challenge faced in isolating the causative agent was that the 6 cases did not present with the classical signs of acute camel death syndrome hence bacterial swabs could not be collected as there were no nasal secretions or mortalities during the investigation period. This may indicate that the outbreak that began in May 2016 may have been reducing in intensity.
- We hypothesise that the causative agent for the acute camel death syndrome may be due to multifactorial agents. Secondary information

[13] A joint VSF-Germany, VSF-Suisse, Clinic of Cardiology Marburg -Germany and DVS laboratory report from a formalin-fixed heart sample submitted to Germany for virus isolation.
[14] Kebede F and Gelaye E (2010). Studies on major respiratory diseases of camel in North-eastern Ethiopia. African Journal of Microbiology Research. Vol.4 (14): 1560-1564.
[15] Nyang'o J M (1993). Clinical manifestation of experimental *Trypanosoma evansi* infection in the dromedary camel and the effect of treatment on haematological, biochemical and serological values. MSc. thesis from the Department of Clinical Studies, Faculty of Veterinary Medicine, University of Nairobi.
[16] Hamed I.M., Zaitoun M.A.A., El-Allawy T.A.A. and Mourad I.M. (2011). Investigation of *Theileria camelensis* in camels infested by Hyalomma dromedary ticks in Upper Egypt. Journal of Advanced Veterinary Research. Vol.1:4-7.

point to the primary infecting agent to be of viral origin possibly Para-influenza virus. This initial infection then results in a reduced immunity of affected camels thus allowing secondary bacterial infections to set in, either *Pasteurella* organism that causes Haemorrhagic Septicaemia or *Clostridium* organisms that cause Enterotoxaemia.

- To be able to come up with a definitive causative agent there is need to have a joint working group composed of Wajir county veterinary staff, DVS staff and UoN faculty of veterinary staff so as to be able to harmonise field sampling procedures during outbreaks and leverage on diagnostic expertise. Furthermore, a collaborative research investigation will need to be conducted to establish if the heavy tick infestation of camels is resulting in transmission of tick-borne infections such as Heart water, Babesiosis or Theileriosis.

5.0 Way Forward

- Wajir County should set aside resources for regular (monthly) livestock disease surveillance as this will allow early detection of diseases and control. This will in turn reduce the high mortality rates of camels currently being experienced during outbreaks.
- There is need to build the capacity of Wajir County veterinary department through training of staff on proper field sampling and basic field diagnostic techniques. The University of Nairobi through its outreach mandate is willing to partner with the County on the trainings.
- Wajir County should also consider building a simple diagnostic laboratory to allow for haematology and bacteriology testing. As requested by Yusuf Gedi- CEC Agriculture, Livestock and Fisheries. The consultants have attached as an annex to this report the list of equipments and reagents that can be procured.

Annex 1: List of Laboratory Equipments

Haematology laboratory

1. Compound light microscope with x4,x10,x40 and x100 (oil immersion) objectives
2. Microscope glass slides
3. Capillary tubes with anticoagulant
4. Blood collection vacuum tubes (EDTA and Serum)
5. Plastic Pasture pippete for harvesting serum from clotted blood.
6. Cryo-vials for serum storage
7. Coplin jars for staining
8. Methanol for fixing
9. Giemsa stain
10. Staining rods or racks or tiles
11. Refrigerator with freezer and refrigerator compartments
12. Whatman Filter paper (large pore)
13. Automatic Micro-haematocrit and ordinary centrifuge
14. Haematocrit reader.

Bacteriology laboratory

1. Sinks should be made of porcelain
2. Incubator that can be set at 37 and 44 ◦C.
3. Hot air oven (optional)
4. Bunsen burner
5. Nichrome Wire Inoculating Loop, 9 inch
6. Gram stain
7. Refrigerator with freezer and refrigerator compartments
8. PH meters and water distiller as Wajir water is saline
9. Various basic media; Blood Agar, MacConkey Agar, Nutrient Broth, Nutrient Agar and Peptone Water.
10. Transport media- Stuart Media and Buffered Peptone
11. Water baths
12. Anaerobic jar
13. Compound light microscope with x4, x10, x40 and x100 (oil immersion) objectives.