ASSOCIATED BACTERIAL INFECTIONS AND SOME HAEMATOLOGICAL CHANGES IN *CORDYLOBIA ANTHROPOPHAGA* INFESTED RABBITS

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ABSTRACT

This study was carried out to determine the associated bacterial infection and some haematological changes in *Cordylobia anthropophaga* infested rabbits. The research was carried out in National Veterinary Research Institute, Vom, Jos South Local Government Area of Plateau State, Nigeria. 11 New Zealand rabbits were used for this experiment, 2 of them served as controls while 9 served as the tests. The rabbits were raised in a simulated habitat and monitored for four weeks. The test rabbits (9) rabbits yielded 100% infestation with cutaneous myiasis. The larvae when developed and harvested were identified to be those of *Cordylobia anthropophaga* using their posterior spiracle mounting. Cultures of exudates from the cavities of larvae also yielded 100% infection, with *Staphylococcus aureus* being the only bacteria isolate. There were increases in the white blood cell (WBC) count and decreases in the packed cell volume (PCV) in all the test rabbits with increasing days of infestation. Rabbit I had 88.8mg/dl WBC count and a PCV value of 42.0g/l before infestation and 142.7mg/dl with a PCV value of 35.4g/l when infested. This gave a differential increase value of 53.9mg/dl (60.7%) as the highest. The attraction of *Staphylococcus aureus* to the cavity of larva is inimical; hence the need for adequate hygiene and proper management practices for both animal and human infestations.

Key words: Cordylobia, Bacteria, Myiasis, Infection, White Blood Cell.

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INTRODUCTION

Cordylobia anthropophaga which belong to the family of Calliphoridae is a dipterous fly responsible for cutaneous myiatic infection in human and animal during its larva stage. This fly is commonly known as the "*African tumbu fly*", and is commonly found in tropical Africa around forest areas though can be seen world wild because of air travel, as human movement caries it outside endemic areas. The second and the third stage larvae can only be seen in the infested host tissue (Tamir *et al*, 2003)

Ojemudia *et al*, (2011) reported that secondary bacterial infection at the site of infestation can be observed. A larva in the subcutaneous tissue of a host inflicts injury which attracts the response of the host immune system. The development of the larva within the cavity and the release of pus-like exudates through the exposed spiracle from boil-like tissue attract bacterial infection, which in turn is enhanced particularly when the environment surrounding the site of larval infestation is contaminated (Ockenhouse *et al*, 1990)

At the site of penetration, a red papule is formed and gradually enlarges. At first the host may experience only intermittent slight itching, but pain develops and increases in frequency and intensity as the lesions develops into a furuncle which attract a constant touch-scratching and rubbing of the furuncles (Adisa and Mbanaso, 2006). This phenomenon enhances bacteria contamination. The furuncle's aperture opens, permitting fluid containing blood and waste products of the maggot to drain, hence creating a conducive environment for the bacteria to grow (John and William, 2006).

Secondary bacterial infection is rare in *C. anthropophaga* infestations. The lesion heals rapidly after the larva is removed or it's spontaneous exit. Complications include cellulitis, abscess formation, osteomyelitis and tetanus (Millikan, 1999). The development of a wound infection depends on the complex interplay of many factors. If the integrity and protective

function of the skin is breached, large quantity of difference cell types will enter the wound and initiate inflammatory response. This may be characterized by the classic signs of redness, pain, swelling, raised temperature and fever (John and William, 2006).

Wound infection is not a modern phenomenon. As early as 14-37AD there is documentary evidence that Cornelius Calsus (a roman physician) descried the four principal sign of inflammation and used 'antiseptic' solutions (Kidshealth 1995). Another Roman Physician, Claudius Galan (130-200 AD) had such an influence onthe management of wounds that he is still thought of by many today as the 'father of surgery'. It should also be remembered that he and some of his followers instigated the 'laudable pus' theory, which incorrectly considered the development of pus in a wound as a positive part of the healing process. Furthermore, as a result of the increasing volumes and depth of research on this pathogen, some findings have been obtained in *Staphylococcus epidermidis* that have paradigmatic character for many Staphylococci and Gram-positive pathogens colonizing the wounds (ShuYeong and Michael, 2008).

Several Staphylococci species are notorious as human pathogens and are the focus of worldwide intensive research efforts (Ball, 2005). However, Staphylococci are also associated with a large number of animal species and cause several infections of major economic importance. In addition, the zoonotic transmission of Staphylococci to human, especially, those which are antibiotic resistant, is a growing to Public Health (Fitzgerald and Penades, 2008).

In an infection, the immune system of the infected host develops antibodies to combat the pathogens that have just entered the host system. The effect, thus, depend on the specific infection. For instance, Sickle cell anemia distorts the normal circular shape of the red blood cells. In general, however, every infection causes an increase in white blood cells whose job is to fight off infection/intruders in the body infection lead to full blown disease which often attacks,

mutates, or destroys healthy red blood cells as a way to continue living inside the host (Chisholm and Nancy, 2011).

The immune system is a complicated one, living multiple mechanisms and cascade system. White blood cells play a large part in the initial reaction of the body to a pathogenic or body. Neutrophils travel through the blood stream constantly, which are released at the site of infection or injury. Neutrophils are phagocytic, which means they can ingest pathogens. When this occurs, they form a phagosome into which reactive oxygen species such as superoxide and hydrolytic enzymes are released. This, in turn, should kill the offending bacteria (Daniela *et al*, 1997).

Neutrophils are a type of leukocytes, or white blood cells. They play an important role in the immune system of the body. They are one of the first blood cells to be sent to the site of infection and are largely responsible for the whitish colour of pus, as they make up most of it. The immune system uses them as part of front line attack to fight any infection or foreign body that enters the body. Those cells will continue to mature while in circulation. Depending on the type of infection (acute or chronic) certain types (neutrophils, monocytes, eosinophil, macrophages, or lymphocytes) will be present in lesser or greater number and reflect the blood test result (CBC with Differential) (Daniela *et al*, 1997)

Laboratory measurement of white blood cells is used by Doctor to diagnose infection and measure the functioning of the immune system. The measurement is expressed as absolute neutrophil count. A significant rise in white cells, especially Neutrophils, may indicate an infection in the body (ShuYeong, 2008).

Eosinophil has a bi-lobed nucleus and is increased in many types of parasitic infections against larvae of parasitic worms and unicellular organisms seem to be one of their primary function. The granules contain major basic protein which is toxic to many parasitic larvae. They

have receptor for the antibody immunoglobulin E (IgE) which is thought to reflect their role in parasitic infection (Varani *et at,* 19997).

MATERIALS AND METHODS

Experimental setup: This experiment was carried out in Federal College of Veterinary and Medical Laboratory Technology, National veterinary Research Institute, Vom, Plateau State.

A total number of eleven (11) rabbits were used in this experiment. Two (2) were controls; while nine (9) were tests. The experiment was carried out within five weeks in an open and cemented ground where the rabbits were allowed free movement. All the rabbits were marked with a number from one (1) to nine (9) and the two control marked A and B for proper identification.

Collection of larvae for examination: The rabbits were examined each day for myiasis. The site of larva infestation was covered with oil. As the larva pulled out of the cavity to obtain air it was harvested (Ojemudia *et al*, 2011). The larvae collected were taken to the parasitology laboratory, Federal College Veterinary Medical Laboratory Technology for examination and identified using the method of Service, (1980).

Collection of swabs from infected sites: Swabs were aseptically collected from the cavities where larvae were harvested and taken to the bacteriology laboratory, FCVMLT, for bacteriological analysis. A direct smear was made from the swab after it has been cultured. The Gram staining procedure was carried out according to that stated in Cheesbrough, (2006).

Bacteriological analysis: Swabs were inoculated into blood agar medium and incubated for 24 hours at 37º C. Emerging colonies were identified using cultural, morphological and biochemical characteristics.

Characterization and Identification of Isolates: The phenotypic and biochemical characteristics used in identifying isolates include Gram staining, catalase and coagulase tests. These tests were performed using the methods of Cheesbrough, (2006).

Haematological Analysis

Collection of blood for analysis: The blood was collected from the ear-lobes of the rabbits into an EDTA plastic container after dilation with xylene. Thin films were made immediately on a clean grease-free glass slide and allowed to dry.

The whole blood was immediately taken to the laboratory to determine the total white blood cells using the Mindray BC-2800 Vet Auto-analyzer. The thin Film prepared from the fresh blood were stained with leishman stain for differential count, according to the method by Cheesebrough, (2006)

RESULTS

The result from this study showed that at week one (1) the rabbits adapted to the environment while their waste (faeces and urine) accumulated and attracted the flies to the area. Only one rabbit, “rabbit G” was infested in the second week. This increased to five at the 3rd week and all nine (9) became infested by the fourth week. The larvae harvested from the infested site were identified to be those of *Cordylobia anthropophaga.* Table 1 shows the infestation rate of *Cordylobia anthropophaga* among susceptible rabbits. Only *Staphylococcus aureus* was isolated

from the cavities where larvae were harvested in all test rabbits. This is shown in Table 2. Table 3 showed the pre-infestation haematological analysis. The haematological analysis at the onset and when the infestation became severe is shown in Table 4 and 5 respectively. There were increases in white blood cell count values and decreases in pcv values with increasing severity of infestation. For instance, Rabbit G with early infestation showed a rise in white blood cell values from 90.1mg/dl (pre-infestation) to 124.0mg/dl (when infestation was severe). There was also a corresponding decrease in pcv values from 37.9% to 24.0%. The percentage increase in total white blood cell count is shown in Table 6 while Figure 1 is a bar chart showing the survival rate of experimental rabbits. It showed that all experimental animals were infested by the 4th week of study, 5 had complications, 3 died while 6 survived.

Table 1: Infection rate of *Cordylobia anthropophaga* among susceptible Rabbits

Rabbit	Week 1 (adaptation)	Week 2	Week 3	Week4
Control 1	-	-	-	-
Control 2	-	-	-	-
Test A	-	-	+	+
Test B	-	-	-	+
Test C	-	-	-	+
Test D	-	-	+	+
Test E	-	-	+	+
Test F	-		-	+
Test G	-	+	+	+
Test H	-	-	-	+
Test I	-	-	+	+

Table 2: Prevalence of bacteria associated with furuncle of *Cordylobia anthrophaga* in infested rabbits

Rabbits	A	B	C	D	E	F	G	H	I
Staphylococcus aureus	+	+	+	+	+	+	+	+	+

Table 3: Pre-infection hematological analysis

Rabbit	Wbc 10^9/L	Lymph 10^9/L	Mono 10^9/L	Gran 10^9/L	Rbc 10^{12}/L	Pcv %	Hb g/L	Plt 10^9/L
Control 1	80.3	8.2	2.1	70.0	5.31	41.9	421	105
Control 2	95.7	10.2	2.7	82.8	5.57	34.7	348	212
Test A	91.2	9.3	2.7	79.0	3.69	44.2	445	253
Test B	87.6	6.7	2.1	78.8	5.45	38.1	384	674
Test C	90.4	8.9	2.6	78.9	5.73	37.0	369	779
Test D	85.8	6.1	2.3	77.2	5.61	38.2	388	542
Test E	82.5	7.9	2.2	72.4	4.51	32.2	324	421
Test F	80.6	7.6	2.1	70.9	7.33	46.7	451	235
Test G	90.1	8.7	2.4	77.0	5.35	37.9	378	357
Test H	79.8	7.2	2.1	70.5	5.57	37.7	382	188
Test I	88.8	6.9	2.0	71.9	5.74	42.0	413	801

Key:

Wbc = white blood cells; Lymph = lymphocyte; Mono = monocytes;

Gran = granulocyte; Rbc = red blood cell; Pcv = packed cell volume;

Hb = haemoglobin; Plt = platelete; L = Litre

Table 4: Haematological analysis at the onset of infection

Rabbit	Wbc 10^9/L	Lymph 10^9/L	Mono 10^9/L	Gran 10^9/L	Rbc 10^{12}/L	Pcv %	Hb g/L	Plt 10^9/L
Control 1	80.1	7.9	3.2	73.3	5.38	41.2	419	121
Control 2	95.3	10.5	2.6	82.4	5.52	34.9	349	225
Test A	93.4	9.8	2.9	80.7	3.70	43.7	436	215
Test B	87.6	6.7	2.1	78.8	5.44	38.2	385	452
Test C	90.7	9.2	2.7	78.8	5.74	37.1	374	526
Test D	86.3	6.4	2.3	77.6	5.65	38.9	386	441
Test E	95.7	9.6	2.8	83.3	4.48	30.1	300	318
Test F	82.3	7.9	2.2	77.9	6.53	38.5	383	289
Test G	102.2	14.2	3.6	84.4	4.16	29.2	294	327
Test H	82.3	9.2	2.4	68.7	5.21	33.2	333	216
Test I	98.2	13.3	3.6	81.3	5.52	40.3	411	752

Key: Wbc = white blood cells; Lymph = lymphocyte; Mono = monocytes; Gran = granulocyte; Rbc = red blood cell; Pcv = packed cell volume; Hb = haemoglobin; Plt = platelete; L = Litre

Table 5: Hematological analysis when the infection was severe

Rabbit	Wbc 10^9/L	Lymph 10^9/L	Mono 10^9/L	Gran 10^9/L	Rbc 10^{12}/L	Pcv %	Hb g/L	Plt 10^9/L
Control 1	80.6	7.5	7.1	76.5	5.29	41.8	407	87
Control 2	95.5	10.7	2.8	82.0	5.58	35.3	347	126
Test A	126.6	17.3	4.3	105.0	3.49	23.3	258	110
Test B	99.1	11.5	3.0	84.6	5.44	35.4	345	23
Test C	95.8	11.0	2.9	81.9	5.96	37.1	365	114
Test D	89.3	8.5	2.4	78.4	5.78	40.2	402	70
Test E	118.7	14.5	3.7	100.5	4.25	27.2	268	68
Test F	100.8	10.8	2.8	87.2	5.00	32.7	329	58
Test G	124.0	17.4	4.1	102.5	3.71	24.0	254	257
Test H	110.9	13.3	3.4	94.2	4.36	28.9	276	245
Test I	142.7	20.7	5.6	116.4	5.46	35.4	345	39

Key: Wbc = white blood cells; Lymph = lymphocyte; Mono = monocytes; Gran = granulocyte; Rbc = red blood cell; Pcv = packed cell volume; Hb = haemoglobin; Plt = platelete; L = Litre

Table 6: Percentage increase in total white blood cell count

Rabbit	Pre-infection WBC count (10^9/L)	Active infection WBC count (10^9/L)	Differential value (10^9/L)	Percentage value
Test A	91.2	126.6	35.4	38.8
Test B	87.6	99.1	11.5	13.1
Test C	90.4	95.8	5.4	6.1
Test D	85.8	89.3	3.5	4.1
Test E	82.5	118.7	36.2	42.9
Test F	80.6	100.8	20.2	25.1
Test G	90.1	124.0	33.9	37.6
Test H	79.8	110.9	31.1	39.1
Test I	88.8	142.7	53.9	60.7

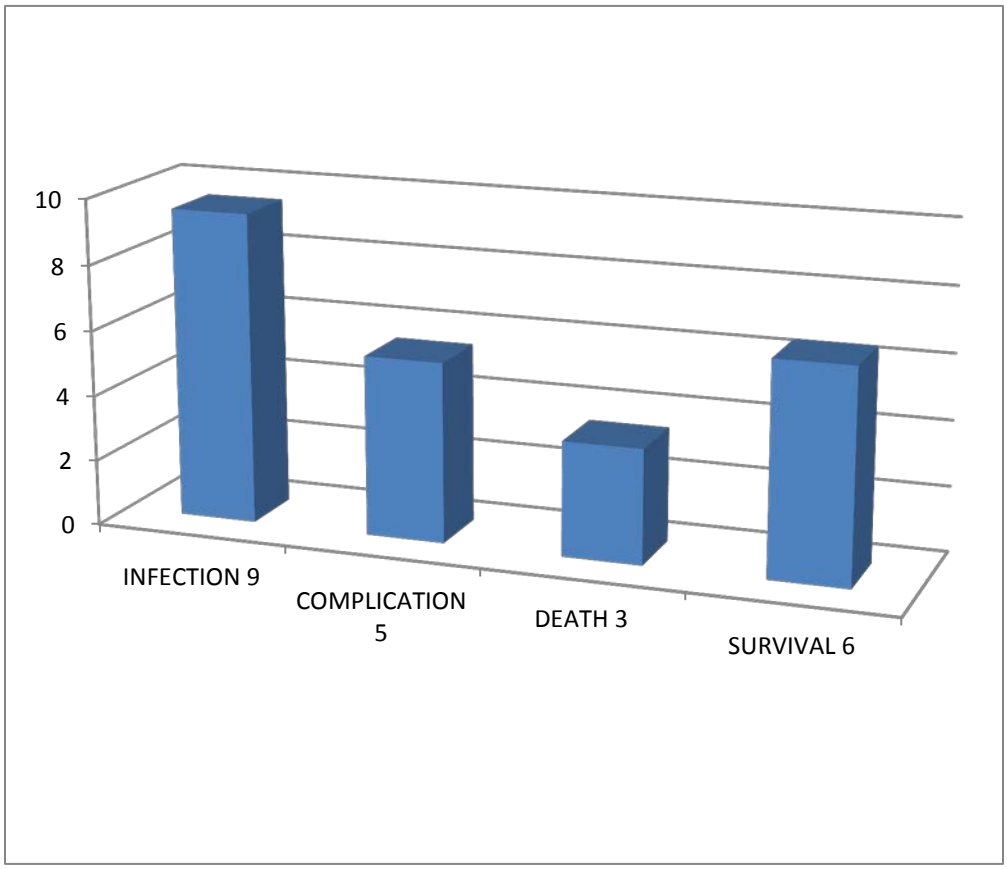

Figure 1: Survival rate of the experimental rabbits.

DISCUSSION

This research showed a high level of susceptibility of rabbits to *Cordylobia anthropophaga* with a 100% *Staphylococcus aureus* infection in the cavities where larvae were extracted. This agrees with the research carried out by Ojemudia *et al,* (2011), who reported the presence of *Staphylococcus aureus* in the cavities of *C. anthropophaga* larvae extracted from humans and dogs.

Though some researchers have reported the rarity of secondary bacterial infections in *C. anthropophaga* infestations, it is imperative as revealed by this current study that cutaneous / furunclar myiasis caused by *Cordylobia anthropophaga* can be incriminated with bacterial infection. Hence, all of the rabbits experimented on were infected with *Staphylococcus aureus* as the opportunistic bacteria. The two organisms, *Staphylococcus aureus* and *Cordylobia anthropophaga* live in a seemly symbiotic association which attracts further research.

This experiment revealed that the cavities from which the parasites were harvested in all the rabbits were infected with *Staphylococcus aureus.* The prevalence of staphylococcal infection in this study confirmed the study of Lederman *et al*, (2008) who stated that bacterial skin infections may occur more frequently after bites and other wounds in the tropics, particularly when good hygiene cannot be maintained. Organisms responsible are commonly *Staphylococcus aureus* or *Streptococcus pyogenes*.

Animals may be more susceptible to bacterial infection due to their unhygienic disposition in the environment. The exudate secreted from larval infested sites enhanced bacterial infection, thus, agreeing with Amanda (2011), that any skin disease or injury on the skin surface increases *Staphylococcus* infection except when it is kept in hygienic condition with any agent that is inimical to the growth of such bacteria.

The death of the three (3) rabbits with complications may be due to; the ectopic invasion by the larvae which caused injuries and destruction of tissues along the routes of migration. Also, their death could be due to co-infection of the two organisms (*Cordylobia* larva and *Staphylococcus*) which could have led to complication and attendant health problems. The later agrees with the argument of (ITHAKA, 2011) that the interaction of toxin or enzyme released by the larvae-bacteria can cause the erosion of bones and teeth.

The different levels of susceptibility of rabbits to *C. anthropopha* infestation depended on various factors such as; availability of the parasites to the susceptible rabbit, contact between the rabbit and the larva which encouraged penetration, the active nature of the rabbit for the parasite to have assess and of course, the lack of cleanliness of the pens were necessary tools which attracted the flies.

Heamatological analysis showed a marked increase in the WBC count (particularly the granulocytes) of the different rabbits in response to the larva infestation as compared to the count before infestation. This agrees with the previous research carried out by Anna (2001) who iterated that there is eosinophilia due to myiatic infection in human. On the other hand, there is a significant decrease in the levels of Packed Cell Volume (pcv) and haemoglobin showing an effective and progressive attack by the larvae. The rise in the WBC levels may not only be due to the presence of the *Cordylobia* but also the bacterial infection due to *Staphylococcus aureus.*

The difference in response of antibodies was a complex interplay of the levels of parasite and bacterial infections, though the first and major activation of the antibody response was due to larva infestation, whereas the opportunistic bacterial infection may have had less influence on the antibody response. Hence, the onward research on this specific deferential response of antibodies to bacteria and *Cordylobia* infestation will be advancement to this research.

Importantly, the determination of WBC and differential count as indicators for ascertaining antibody response to specific infection should be critical and specific as to avoid error of miss diagnosis, since the parasite/bacterial infection complex in this experiment may have resulted to the mix antibody response. Therefore, other specific diagnosis should be conducted for quality and dependable result while the WBC deferential count should serve as a confirmatory test.

The symptoms observed from the infestations of the rabbits in this research include furuncles/swellings which result to limping of the infested rabbits, lack of appetite for food, emaciation, sluggishness and emission of pus from the infected sites particularly those with complications.

RECOMMENDATION AND CONCLUTION

From this research, it is revealed that all the *Cordylobia* infested rabbits were co-infected with *Staphylococcus aureus*. Therefore it is certain that *Staphylococcus aureus* is associated with cutaneous myiasis caused by *Cordylobia anthropophaga*. The abundant presence of bacteria (normal floral) on the skin of humans as well as animals is a risk factor for opportunistic Staphylococcal infection if there is an abrasion, or other injuries such as furuncles caused by infecting agents like *Cordylobia anthropophaga*. Therefore, the reduction or regulation of the number of these bacteria on the skin through proper hygiene will limit the infection rate of skin injuries. With the major increase in the WBC count during larva infestation of rabbits in this experiment, it is obvious that a right shift in white blood cell in patients may be taken as an indicator for parasitic infection.

Once an infestation is underway as may be discovered in livestock and human, treatment needs to be applied immediately. First the larvae must be eliminated or removed through

pressure around the lesion with the use of forceps after covering with oil or Vaseline. Secondly the wound must be cleaned and disinfected to avoid bacterial contamination. Infestation already involving well developed larva and furuncle should be treated with the necessary antibiotics to eliminate the possible opportunistic bacterial infection. Further control is necessary to avoid re-infestation.

In the case of susceptible animals, the pens should be regularly cleaned and the animals observed at regular interval. It is also possible to treat livestock with the use of slow release boluses containing ivermectin which can provide long term protection against the larvae development. Small animals may be dipped, which involves drenching the animal in insecticide to prevent the growth of the larvae.

To prevent human infestation with these larvae, particularly, the infants, washed cloths should always be ironed. This should be a regular application particularly during raining season when there is not enough sun shine to dry cloths.

However, the consciousness of unpopular diseases such as this by researchers, physicians, families and the community will go a long way to avoid preventable diseases that could be endemic.

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