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PREVALENCE AND COINCIDENCE OF HELMINTH AND *SALMONELLA* INFECTION IN A COHORT OF HOSPITAL PATIENTS IN CAMEROON

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ABSTRACT

Salmonellosis is a bacterial infection caused by *Salmonella* species. It is often the consequence of host sensitivity, which is influenced by helminthic infections. The aim of this study was to assess the biological relationship between *Salmonella* and gastrointestinal helminths. Three hundred patients coming for consultation at Dschang District Hospital, Saint Vincent De Paul, and Ad-Lucem Hospitals and presenting with salmonellosis symptoms and signs, between October 2015 and January 2016 gave blood and stool samples for serological and coprological (stool culture and flotation technique) analysis respectively. Vidal test revealed a *Salmonella* prevalence of 68.7% while stool culture made on *Salmonella-Shigella* agar gave a prevalence of 16.33%; Vidal false positives was 23.79%. While prevalence of helminthic infections was 4.33%; three gastrointestinal helminths were identified: *Ascaris* spp., *Trichuris* spp., and Hookworms with prevalence and intensity of 3(322,50), 1.33(76.67) and 0.66% (50,00). *Salmonella*-helminth association graded to 0.38 showed an odds ratio (OR) of 7,54 and denote that gastrointestinal helminths favor the installation and rise *Salmonella* proportion in the host. Moreover, the association link between these pathogens was parasitism. Finally, the consumption of raw or underdone food (salad, fruit, eggs and meat), drinking spring water, and bad body hygiene were identified as epidemiological risk factors for *Salmonella* and helminthes contamination.

KEYWORDS: Co-infection, *Salmonella*, helminths, Vidal, stool culture.

INTRODUCTION

Salmonella species are gram-negative enterobacteria. They are non-capsulated, non-sporulated, and anaerobic bacilli with have characteristic flagella, somatic, and outer coat antigen.[1] Salmonella infection is a major human food-borne infection worldwide[2] causing an estimated 21.7 million patients and 217,000 deaths annually of typhoid and paratyphoid fevers[3-4], with an estimated 3.4 million cases and a case fatality rate of 20% nontyphoidal salmonellosis.[19] However, data specific to the African region are limited.[5] Clinical under detection of salmonellosis is common in Africa.[4] The current gold standard diagnostic is stool and blood culture to isolate the bacteria. Practically, in the city of Dschang and generally in Cameroun salmonellosis is diagnosed by Vidal test. Patients sometime received typhoid treatment when they are not infected by salmonella. The economic impact of this wrong diagnosis and treatment will be dealt with another paper.

Despite a clear understanding of the disease mechanisms and treatment, lack of diagnostic capabilities and surveillance systems, among other factors, has made it difficult to accurately describe the burden of typhoid and paratyphoid fever, and also invasive nontyphoidal salmonellosis disease in Africa.[5] There are numerous limitations with notification data but it still provides some information of relevance to etiological considerations. The case comparisons using notification data on risk factors are also only a crude guide to possible etiology of salmonellosis.[6] Nevertheless, the results are consistent with foodborne transmission being relatively important. Some data also provide evidence for person-to-person transmission. Nevertheless, the analyses by rurality suggest that rural factors may be collectively important in salmonellosis transmission overall.[7] This suggests major roles for all of the following transmission mechanisms: helminth infections, foodborne, and hygiene.[7,8,9]

The present study was undertaken to assess the biological relationship between *Salmonella* and gastrointestinal helminths. The first research question was to determine the comparability between stool culture and Vidal diagnosis. The second question was to examine if *Salmonella* infection is linked to helminthic infection. The third question was to determine the etiologic factors involved in salmonellosis.

MATERIAL AND METHODS

Sample Collection: Three hundred (300) samples were randomly collected using based on and the Lorenz statistical formula. The samples included blood and stool from patients. Prior to the enrolment, voluntary and informed consents were obtained from ok patients. Ethical approval was also obtained from the Cameroon Bioethics Initiative (CAMBIN). Stool and blood samples were collected aseptically into sterile flask and dry tube, respectively. The samples were transported in cold packs to the Laboratory of Microbiology and Antimicrobial Substances for serological testing and culture. The samples were then transferred to the Laboratory of Applied Animal Biology and Ecology for parasitological testing.

Salmonella Diagnosis

Stool culture and Vidal test were used for the detection of *Salmonella* detection. The conventional tube agglutination Vidal test was performed using the Vidal Pasteur kit, containing O and H antigens of *Salmonella typhi* and *S. paratyphi* A, B and C antigens. A negative saline control was introduced in each batch of tests. The sera were initially tested at a dilution of 1/100 and further at serial dilutions of 1/200, 1/400, 1/800 and 1/1600 in 0.9% normal saline when a 1/100 dilution gave a positive result. The sera were centrifuged at 3000 r.p.m. for 5 min and the results read immediately. Stool was inoculated on *Salmonella-Shigella* Agar using the quadrant methods of.[18] Inoculates were incubated at 37 °C for 24 to 48 h and the cultures were observed daily.

Helminths Eggs Detection

Helminth eggs detection was performed using a modified Willis technique.[10]

The Willis technique is based on the principle that 2 g of stool are mixed with 60 ml of saturated saline solution (400 g of NaCl in l of distilled). Due to the fact that stool was preserved in 10 ml of formalin, it was difficult to extract 2 g of stool from the mixture, so we had to measure the total weight (**MT**) of every flask containing stool, the weight of 10ml of formalin (**m1)** and empty flask weight (**mt**). The exact stool weight (**mmf)** within every flask was then deducted by the following formula: **mmf = MT – (m1 + mt)**. the proportion of stool percentage within every flask (% mmf) was obtained by using the following formula: %**mmf = mmf x 100/ (mmf + m1).**

To deduct Yg of the stool-formalin mixture, and to calculate Xg with the formula
Xg = %mmf x Yg;

introduce the Yg of the mixture into a Becher of 100 ml, adjust (60x X/2) ml of saturated saline solution in the Becher. The mixture was triturated and the homogeneous solution was filtered using a tea sifter and conjointly introduced into test tubes until an upper meniscus formed and McMaster cell. A slide was settled on the tubes. Parasite ova migrated to the slide and 5 minutes later, the slide was observed on a microscope at10 and 40X respectively.[11]

Risk Factors: All potential risk factors were assessed through questionnaires which were distributed to all participants in the study.

Data management and analysis.

Data management, entry and analysis were done using Excel (Microsoft® Office Excel 2016) and SPSS software (version 22.0). Prevalence's were compared using X2 test. The concordance between Vidal and culture were conjointly evaluated with Mc Nemar's Chi-square test with continuity correction.[12] Fisher's exact test for count data and odds ratio were used to evaluate the *Salmonella* and helminths relationship. Multiple Correspondence Analysis (MCA)[13] was used for grouping of epidemiological risk factors.

Ethics Statement

Voluntary and informed consents were obtained from ok patients. Ethical approval was also obtained from the Cameroon Bioethics Initiative (CAMBIN). Moreover all adult subjects provided informed consent, and a parent or guardian of any child participant provided informed consent on the child's behalf. Informed consent given was written.

RESULTS

As shown in Table 1, 68.7% of the blood samples were positive for serological test while 16.33% were positive for stool culture. The equivalence of the two diagnostic methods was tested with a table of contingency table (Table 2). The two tests were not concordant with a threshold of probability equal to 0.000, Mc Nemar's chi-squared was 153.0566 with a p-value < 2.2e-16. Thus, despite the fact that Vidal test is more sensitive, it is less specific than culture.

The ova of three nematodes (*Ascaris*. *Trichuris*. and hookworm) (Table 3) were observed in the stools. In an attempt to elucidate the relationship between *Salmonella* and gastrointestinal helminths, another table of contingency was done (Table 4). The odds ratio was 3.451 with an asymptotic standard error of 0.593, and a lower and upper confidence interval of 1.079 and 11.039 respectively. Odds ratio was higher than 1, suggesting that *Salmonella* infection is associated to helminth

infections with 5% threshold of probability. The p-value = 0.04361 with 95% confidence interval.

To determine the etiologic factors, all the participants were provided with questionnaires in which some behaviors were listed. A total of fourteen risk factors were identified (Fig 1). Every participant was considered as a vector. The analysis of the repartition of these factors indicate that they were grouped and projected on two perpendicular axes. Furthermore, they constituted of a linear combination of the 300 patients, represented in cloud not. From the central axis to the right, there is an improved hygiene with the consumption of mineral water and the use of bleach water. Above the central axis, there is consumption of raw food.

Moreover, co-infection is present where the hygiene level decreases. The position of every patient is shown on Fig 2. These participants occupied the central axis. The above behaviors are discriminated in Fig 3. There is a positive correlation between drinking water and fruit washing, and between the type of latrine and hand washing. Thus, drinking water, salad consumption, type of hand washing latrine and fruit washing are important etiologic factors for *Salmonella* infections.

Table. 1: Presence/absence of *Salmonella* in relation to test type.

Test type		
Salmonella	**Widal**	**Culture**
Negative	94	251
Positive	206 (68.7)	49(16.33)
Total	300	300

Table. 2: Culture and Widal contingency table.

Culture		
Widal	**Negative**	**Positive**
positive	93	1
negative	158	48

Table. 3: prevalence of gastro intestinal helminthes.

Gender	**Parasites**					**Total Examinated**
	Infected n(%)	***Ascaris***	***Trichuris***	**Ankylostoma**	***Ascaris* & *Trichuris***	
Female	7(3,80)	3(1,63)	1(0,54)	2(1,09)	1(0,54)	184
Male	6 (5,17)	4(3,45)	1(0,86)	0	1(0,86)	116
Total	13(4,33)	7(2,33)	2(0,66)	2(0,66)	2(0,66)	300

Table 4: Helminths absence/presence in relation to the result of culture.

Culture		
Helminths	**Negative**	**Positive**
Positive	243	44
Negative	8	5

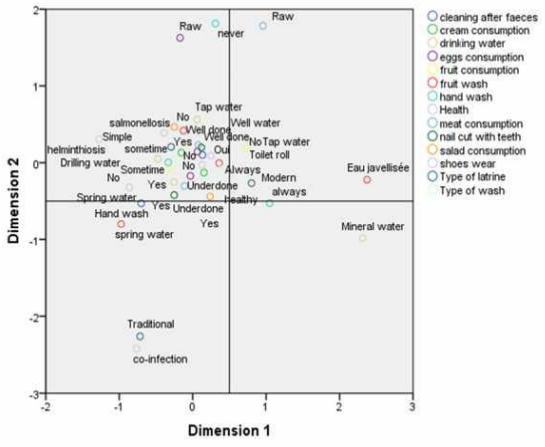

Figure. 1: Joint plot of behavioral risk and infection type.

To determine the etiologic factors, all the participants were provided with questionnaires in which some behaviors were listed. A total of fourteen risk factors were identified. Every risk factors are represented by a color. These factors were projected on two perpendicular axes (Dimension 1 and 2).

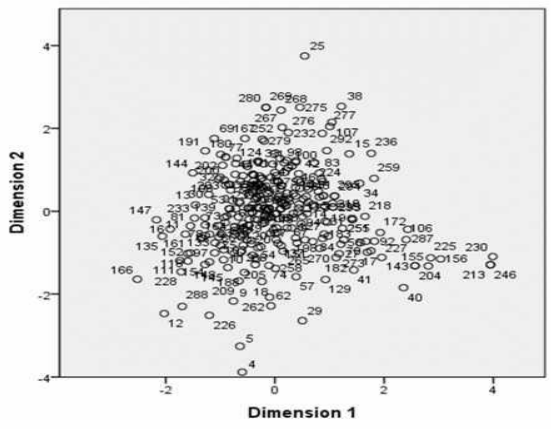

Figure. 2: Projection of sampled subject on first two axes of Multiple Correspondence Analysis (MCA).

The 300 participants were listed from 1-300 and projected on two perpendicular axes (Dimension 1 and 2).

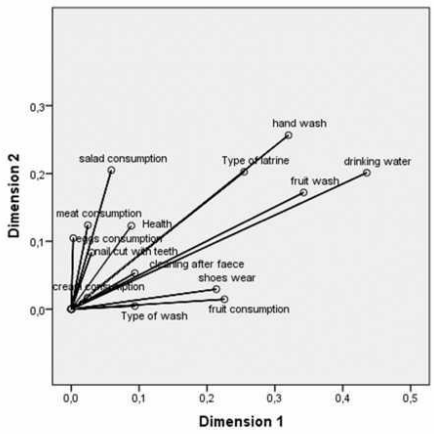

Figure. 3: Discrimination of epidemiological risk factors.

Important etiologic factors for *Salmonella* infection are projected on two perpendicular axes (Dimension 1 and 2). The length of an axis reflects the importance of the etiologic factor.

DISCUSSION

Salmonella is a major cause of food-borne gastroenteritis worldwide. To diagnose the infection generally in Africa and particularly in Cameroon, Vidal and stool culture are usually used in practice. Vidal test is more sensitive than stool culture, but non-specific.[14] Moreover, Vidal test is associated with false positives. This situation can be due to the non-specific antibodies cross reactions of *Salmonella*. These antibodies may be due to the presence of other pathogens such as *Plasmodium*, bacteria belonging to other genera like *Escherichia, Morganella, Proteus, Enterobacter* and other enterobacteria.[15] The three helminths identified in the study are wide-spread. Their low prevalence can be due to the deparasitation campaign initiated by the Neglected Tropical Diseases (NTDs) through the National Program Against Schistosomiasis and Intestinal Helminthiasis (NPASIH). Our result show that *Salmonella* infection is associated to helminth infections. This can be explain by the fact that helminth parasite can mechanically transmit the bacteria to the host.[16] The worm also modulates the host immune system, which alter the response of other antigens specifically, it stimulates T cells (Th2 and Treg) that can alter host protection against bacterial infection.[17] In many parts of the world, enteric bacterial infections often occur in individuals who are also infected with parasitic worms.[2]

Based on the questionnaires responses, our results show that drinking water, salad, type of latrine, hand, and fruit washes are important etiologic factors for *Salmonella* infection. This situation may be due to the fact that *Salmonella* and helminths share similar socials circumstances such as poverty and lack of hygiene. Moreover, these factors occupied the first rank in the transmission of these pathogens.

CONCLUSION

The Vidal test and bacterial cultures provides very different assessment of whether a patient is Salmonella-infected. Vidal test has low specificity and positive predictive values, but It has good negative predictive values which indicate that negative Vidal test result has a good indication for the absence of the disease. The prevalence of helminth infection is higher in salmonella-positive patients compare to patients that are deemed Salmonella-negative.

Nevertheless, using Vidal test as the only laboratory test for the diagnosis of typhoid fever will result in misleading diagnosis. Therefore, it is very essential to use culture technique to diagnose enteric fever. We have shown that, *Salmonella* and gastro-intestinal helminths share social circumstances such as poverty and lack of hygiene. Although the results from our study do not fully represent the real phenomenon occurring during the presence of the two pathogens in the host, a better understanding of this interaction will aid in finding ways to solve these public health problems.

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