Special Parasite Pathogens Journal (SPPJ)

Km 58 Mbarara Fort-Portal Rd, Box 324, **RC No: 144926.** **ISSN: 2415-0134.**
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Prevalence of Cryptosporidiosis among diarrhea patients attending clinics in Bushenyi district of Uganda

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Citation: Agwu E, Oming S, Moazzam ML. Prevalence of Cryptosporidiosis among diarrhea patients attending clinics in Bushenyi district of Uganda. *Spec parasite pathogens J.* 2015; 1 (1): 01-08.

Abstract

Background: Diagnosis of Cryptosporidiosis and associated diarrhea especially among HIV infected and AIDS patients remain a big challenge for health care providers in this region.

Objective: To determine the prevalence of cryptosporidiosis among the hospital attendees in the Bushenyi district of Uganda.

Materials and Methods: Standard parasitological methods were used in the identification of parasites including cryptosporidiosis from among the 105 diarrhea patients whose samples were analyzed.

Results. We report a 66.7% (70 out of 105) overall parasite prevalence and 16.2% (17 out of 105) prevalence of *Cryptosporidium* oocysts was found in the patients studied. Fifty-two percent (52.1%) of the patients were HIV positive and the majority of the study population presented with persistent diarrhea (67.4%). High percentages of HIV-positive cases were observed in the 25-34years (44.0%) and the 35- 45 years (28.0%) age groups. Other intestinal parasites found to be causing diarrhea alongside *cryptosporidium* are *hookworm* which was observed in 12 (11.4%) patients, *Ascaris lumbricoides* also in 12 (11.4%) patient, *Entamoeba histolytica* was found in 11 (10.5%) patients, *Giardia lamblia* in 5(4.8%) patients, *Isospora belli* in 5(4.8%), *Cyclospora cayetanensis* in 4 (3.8%) patients, *Strongyloides stercolaris* in 2 (1.9%), and *Trichuris trichiura* was found in 2 (1.9%) patients.

Conclusion: Poor hygiene both personal and environmental, poor diagnostic, clinical, prevention, and control skills facilities may have impacted on Cryptosporidiosis reported in this study. Opportunistic infections and comorbidities by other parasites were noted in HIV/AIDS among the studied population. Microscopic detection of *Cryptosporidium* species assists ineffective intervention.

Introduction:

Cryptosporidium pavum and *Cryptosporidium hominis* are protozoan parasites that have remained predominant both in immunocompetent and in immunocompromised HIV Infected and malnourished individuals (1). Other species, such as *C. felis* (2-3), *C. meleagridis* (3-4), *C. canis* (3, 5), *C. suis* (3), *C. muris* (6), and more rarely *C. baileyi* (7) can infect humans too, especially children under the age of 5 years and immunocompromised individuals especially those infected with HIV (8).

In immunocompetent individuals, infection with this parasite may be asymptomatic and self-limiting, but in immunocompromised patients such as those with HIV/AIDS *Cryptosporidium* spp. may cause severe, chronic, and possibly fatal diarrhea and wasting disease syndrome. In 2004, cryptosporidiosis was added to the WHO's 'Neglected Diseases Initiative' linked to poverty and lack of access to good health services (9).

Cryptosporidiosis prevalence in children varies extensively across sub-Saharan Africa and within certain subcategories of the population. Although rarely diagnosed in children, coinfection with other enteric pathogens occurs frequently, because of common exposure to the disease agent through poor sanitation and hygiene and because of immune predilection mostly induced by HIV infection.

In most sub-Saharan countries, cryptosporidiosis prevalence peaks among children aged 6–12 months and decreases afterward. Breast-feeding may afford some protection, either through conferment of immunoglobin or avoidance of contaminated water. This may explain why infection is delayed until after the age of 6 months, an age that is commonly marked by the introduction of complementary foods. Children likely experience infection throughout childhood and adolescence, although the clinical significance becomes less apparent with age. This is probably attributable to the development of immunity following frequent exposure to oocysts in the contaminated environment.

All Cryptosporidium species are transmitted in the various hosts by ingestion and inhalation of oocysts, irrespective of the species types. In young children, infections with *C. hominis* and, if symptomatic, *C. parvum,* are often heavily associated with fecal lactoferrin and growth shortfalls. *C. hominis* appears to stimulate inflammation irrespective of age.

This raises important questions on how it may precisely induce greater proinflammatory response (10). *C. meleagridis* can be confirmed as an emerging human pathogen, being responsible for 1% of all infections in England (11) and about 10% in Perù, where its prevalence is as high as for *C. parvum* (12). Geographical patterns for both parasite species were observed among the countries (Uganda, Serbia, Turkey, Israel, UK, USA, and New Zealand), possibly because of different prevailing ecological determinants of transmission.

The predominance of *C. hominis* was observed in developing countries, such as pediatric populations from (6), Malawi (13), Kenya (14), India (15), Haiti (16), and Brazil (17), children and elderly persons from South Africa (18), and hospitalized HIV-infected children from South Africa (19) and Uganda (20).

Poor diagnostic skills and technologies may lead to under-reporting while the increased regional prevalence was caused by the extensive diagnostic activity of reporting laboratories (21), suggesting that, even in high-income countries, routine diagnostic protocols should be thoroughly integrated with advanced identification workflows.

Cryptosporidiosis was one of the original AIDS-defining illnesses and as such was associated with an increased risk of death (22). The emergence of drug-resistant HIV variants and failure or discontinuation of highly active retroviral therapy (HAART) has been associated with the re-emergence of *Cryptosporidium* spp. Infection in these patients (23-24). In the absence of a universal treatment program and

with the lack of access to care, cryptosporidiosis continues to be a significant public health challenge in developing countries. There is no universally accepted effective specific treatment for cryptosporidiosis in immunocompromised patients, and continued efforts to develop strategies to prevent and treat this disease in vulnerable populations remain invaluable.

There is no surveillance on cryptosporidium-associated diarrhea and other related diseases in Bushenyi despite its association with HIV/AIDS which has continued to ravage local inhabitants especially those living in hard-to-reach areas where many still die unknown uncharacterized preventable opportunistic infections. Management of diarrhea in both HIV-infected and AIDS patients and the general population still depends on presumptive diagnosis with no database for precise confirmation of etiology. Efforts to improve the database may impact the diagnostic capacity of healthcare providers.

Objective

This study was therefore designed to outline the burden and prevalence of *Cryptosporidium spp* in the Bushenyi district with the ultimate goal of mapping out the disease epidemic among the most vulnerable population of HIV/AIDS patients.

Materials and Methods

This is a cross-sectional prospective study conducted at the Kampala International University-Teaching Hospital (KIUTH) located in Ishaka-Bushenyi district southwestern Uganda. The study populations were Hospital attendees aged 15 years and above, that presented with gastroenteritis and chronic debilitating diarrhea at KIUTH. Diarrhea in this study was defined as passing three or more watery stools within 24 hours. The study focused on and recruited all consenting men and women aged 15 years and above who were diagnosed with diarrhea for a minimum of one week. Informed consent was sought and obtained from participants and approval was obtained from appropriate official approving bodies. Confidentiality was ensured for all the views, results, and comments obtained.

The 105 stool samples collected between May and September 2009 and analyzed was guided by the sample size (n), calculated using standard formula $n = z^2 p (1-p^{19})/d^2$, where: d = margin of error of 0.05, p = prevalence of cryptosporidiosis in Uganda (9%), (25), z =level of significance (1.96) for a confidence interval of 95%.

Stool samples were analyzed using standard microbiological methods to identify and characterize etiological agents of cryptosporidiosis in this region. Demographic characteristics such as age, sex, educational level, and health status of the participants were also collected to help as a reference point to interpret the data generated. Statistical Package for the Social Sciences (SPSS) software version 22 was used to test the significance of the data (dependent and independent variables) generated from the investigation.

To be more specific: stool specimens were examined macroscopically for their consistency (formed, loose soft & watery), color, odor, and presence of blood and mucus, gross inspection was also carried out on stool for parasites that may be seen as in the case of tapeworm proglottides, roundworm and pinworm. Unstained wet films were made by emulsifying a small number of feces in a drop of physiological saline placed on a slide while stained smears were prepared with 1% aqueous solution of eosin and Lugol's iodine diluted 1 to 5 solution. The slide was placed on the microscope stage and examined using 10x and 40x objective lens and the findings were recorded. Decontamination of slides was achieved 5% Lysol.

Formol-ether concentration technique was performed by transferring half tea-spoonful of feces in 10 ml of water in a glass container and mixed thoroughly. Two layers of gauze were placed on a funnel and the content strained into a 15 ml centrifuge tube, and later centrifuged for 2 minutes at about 500g, and the entire process of washing and centrifuging repeated 2 times. The sediment was suspended in 7 ml of 10%

formaldehyde (1 part of 40% formalin in 3 parts of physiological saline) and three ml of ethyl acetate were added. The tubes were closed with stoppers and shacked (vortexed) vigorously to mix well. The stoppers were removed and centrifuged at 500g for 2 minutes. The tubes were allowed to rest in a stand for about 10 minutes. Four layers now became visible thus: the top layer consisted of ether, the second was a plug of debris, and third was a clear layer of formalin and the fourth was the sediment.

The plug of debris was detached from the side of the tube with the aid of a glass rod and the liquid poured off, leaving a small amount of formalin for the suspension of the sediment. With a pipette, the sediment was removed and mixed with a drop of iodine, and examined under the microscope with low (x10) and high power (x40). The use of modified Ziehl-Nielsen stain for fecal smears has already been established as a known standard parasitology method for coccidian protozoa detection. Fecal smears were, therefore, made both directly from stool samples and the concentration deposits.

The smear was allowed to air dry and fixed in methanol for 3 minutes. Smears were then stained with double strength strong carbon fuchsin for 20 minutes. The smears were decolorized using acid alcohol (1% HCl in methanol) for 20 seconds and rinsed in tap water. Counterstaining was carried out using 0.4% methylene blue for 60 seconds. The smears were then rinsed thoroughly in running tap water and the undersurface of the slides was wiped clean using cotton wool. The smears were left to air dry and examined using 40x and 100x objectives and the result was recorded.

Extensive measures were undertaken to ensure quality results. There was strict monitoring of the quality of specimens, turnaround time, sample preparation and examination, reagents preparation and equipment, reviewing test results and control using established aseptic laboratory standard operation procedures (SOPs). Quantitative data were analyzed using the statistical package for the social sciences (version 22.0). Pearson's two-tailed correlation coefficients (a=0.05) and linear logistic regression were the statistical tools used to determine the significance of data obtained. Limitations of this study included the unwillingness of some patients to participate in the study even after consenting earlier. However, after thoroughly explaining the purpose of the study, they were assured of confidentiality for all the information, views, and comments obtained from them and these improved the situation. Poor financial, human and material resources limited the study to phenotypic assessment effectively excluding molecular assay which could have assisted in answering deeper questions regarding cryptosporidiosis. Lack of enough facilities, sophisticated equipment, and time for research could not allow a more comprehensive study to be taken.

Results

We report a 66.7% (70 out of 105) overall parasite prevalence and 16.2% (17 out of 105) prevalence of *Cryptosporidium* oocysts was found in the patients studied. No parasite was detected in 37 (35.2%) patients investigated. The age range was between 15 years to 80 years. The majority 34(32.4%) of the patients were between 25-34 years. Females 67(63.8%) formed the largest part of the study population. Fifty-two percent (52.1%) of the patients were HIV positive and the majority of the study population presented with persistent diarrhea (67.4%). High percentages of HIV-positive cases were observed in the 25-34years (44.0%) and the 35- 45 years (28.0%) age groups.

Cryptosporidiosis was common among females, who also showed a higher HIV prevalence. Parasites were detected in 70 samples analyzed giving a total parasite prevalence of 66.7%, while no identifiable parasite was found in 37(35.2%) of the study population. *Cryptosporidium* species showed the highest prevalence of 16.2% of the parasites detected. Other intestinal parasites found to be causing diarrhea alongside *cryptosporidium* are *hookworm and Ascaris lumbricoides with 12* (11.4%) prevalence each.

Entamoeba histolytica was found in 11 (10.5%) patients, *Giardia lamblia* in 5(4.8%) patients, *Isospora belli* in 5(4.8%), *Cyclospora cayetanensis* in 4 (3.8%) patients, *Strongyloides stercolaris* in 2 (1.9%), and *Trichuris trichiura* was found in 2 (1.9%) patients.

The study also reveals that cryptosporidiosis is high in people of poor socio-economic status, out of 17 cases of Cryptosporidiosis study 12(70.6%) was found to be associated with poor living conditions, they use un-boiled, unfiltered drinking water some time direct from the swamp which may be contaminated with *cryptosporidium* oocysts and these increase the risk of infection. The level of education also contribute to *cryptosporidium* infection, people of lower educational levels were found to be associated with Cryptosporidiosis, out of 17 cases, 14 patients (82.4%) was found to be having a lower educational background as compared to only 3 patients (17.6%) which was found to be having at least some good level of educational background.

Table1. Age and sex distribution in the study population

Age grp	Percentage (%) N = 66%	Male n = 38	Female n = 67
15-24	19(18.1%)	7(18.4%)	12(17.9%)
25-34	34(32.4%)	11(28.9%)	23(34.3%)
35-44	23(21.9%)	9(23.7%)	14(20.9%)
45-54	15(14.3%)	6(15.8%)	9(13.4%)
55-64	10(9.5%)	4(10.5%)	6(9.0%)
65-74	4(3-8%)	1(2.8%)	3(7.9%)
75-84	1(0.95%)	0(0.0%)	1(1.5%)

Table 2. Cryptosporidiosis and HIV distribution in the study population according to age.

_Age group	Cryptosporidium		Health status	
	Positive n =17	Negative n = 88	HIV Positive n = 54	Unknown n = 51
15-24	1(5.9%)	9(10.2%)	3(10.3%)	13(17.1%)
25-34	9(52.9%)	32(36.4%)	13(44.8%)	26(34.2%)
35-44	4(23.5%)	23(26.1%)	8(27.6%)	17(22.4%)
45-54	2(11.8%)	14(15.9%)	3(10.3%)	11(14.5%)
55-64	0(0.0%)	6(6.8%)	1(3.4%)	3(3.9%)
65-74	0(0.0%)	3(3.4%)	1(3.4%)	4(5.3%)
75-80	0(0.0%)	1(1.1%)	0(0.0%)	2(2.6)

Key; %=percentage, n=number

Table 3 Distribution of cryptosporidium with other intestinal parasites according to sex

Parasites	Patients		
	Male n = 38	Female n = 67	Total n = 105
Cryptosporidium	6(5.7%)	11(10.5%)	17(16.2%)
Cyclospora cayetanensis	1(1.0%)	3(2.9%)	4(3.8%)
Giardia lamblia	2(1.9%)	3(2.9%)	5(4.8%)
Hookworm	5(4.8%)	7(6.7%)	12(11.4%)
Strongyloides stercolaris	0(0.0%)	2(1.9%)	2(1.9%)
Entamoeba histolytica	4(3.8%)	7(6.7%)	11(10.5%)
Isospora belli	2(1.9%)	3(2.9%)	5(4.8%)
Trichuris trichiura	0(0.0%)	2(1.9%)	2(1.9%)
Ascaris lumbricoides	4(3.8%)	8(7.6%)	12(11.4%)

Key; %=percentage, n=number

Discussion

The sustained increase in cases of diarrhea in resource-poor settings despite increasing research attention warrants investigation, and the emergence of new technologies to improve our parasite recovery potentials holds promise for success in diarrhea research and interventions. Successful epidemiological assessment of any disease type includes an effective description of its disease etiology. Effective management of diarrhea in HIV-associated disease will therefore include the ability to map out the microbial etiology of common disease.

In this study, 16.2% of cryptosporidiosis was reported among the study population of adults aged 15 years and above. This is higher than the report of 1% from England and 10% from Peru (11-12). Differences in prevalence reported in the studied sub-regions may be associated with poor diagnostic and human resources for health. This suggestion is based on the fact that good facilities are often associated with better prevention and control measures compared with poor or limited resources. Again different prevailing ecological determinants of parasite spread may explain the observed differences in geographical patterns of the parasites in developed (UK, USA, New Zealand, and Israel) and developing (Uganda, Turkey, and Serbia) countries.

Generally, any disease associated with poor hygiene is likely to be more prevalent in countries with limited resources. Cryptosporidiosis may be recognized as an emerging infection throughout the world especially in places with poor personal, water, and food hygiene. Factors that help in parasite transmission include transmission in daycare centers, swimming pools, public water supplies, and other water sources. The main risk factor for disease spread among the study population in Ishaka, Bushenyi district is poor personal water and food hygiene as the purity of the water cannot be guaranteed.

The water source used by the majority of the participants is stagnant ponds and crater lakes while pipe-borne water is available for the privileged few who can pay for the services. Previous epidemiological studies of *C. parvum* have typically utilized pathogen detection in feces as a marker of acute infection. In numerous studies, *C. parvum* was more commonly found in the feces of diarrheal patients. Therefore, all sources of drinking water since birth could be potential sources for the acquisition of *C. parvum.* Large outbreaks of cryptosporidiosis attributable to contaminated municipal water supplies have been documented.

Cryptosporidium was detected by modified acid-fast staining of fecal smears in only 2% of samples analyzed in Accra, Ghana (26) compared to 16.2% obtained in this study with the same detection protocol or modified acid-fast staining of fecal smears. This is different from 8.7% from Accra using real-time, state-of-the-art DNA technologies (27). The impact of study design

and/or protocol on disease burden estimates was demonstrated in a recent review of studies from sub-Saharan Africa that used healthcare-seeking behavior as a substitution for more severe illness and found higher rates of cryptosporidiosis among children recruited in hospital-based studies (14.6–22.2%) compared to community-based studies (7.5–12.5%) (28). Seropositive rates of 17–54% were found in the United States, reaching 70% among children living near the Mexican border. Rates were somewhat higher in Southern and Eastern Europe (33–88%) and some developing countries (64–94%) (29-31).

These high prevalences may be due to under-reporting, poor diagnostic skills caused indirectly by poor resources regarding management and control of the disease in developing countries. If the use of improved diagnostic technology impacted the burden of the disease reported in other regions that could not have been the case in this study since methods used were only improved Zeel Nelson techniques backed by experienced microscopists.

In this study, 52.9% of *Cryptosporidium species* were seen among 44.8% of HIV patients in the region and cryptosporidium disease transmission was significantly ($p<0.05$) dependent on HIV transmission. Most Uganda Cryptosporidiosis is due to *C. hominis* and *C. pavum* (6, 9) but similar studies in other African countries show that *C hominis* is the predominant pathogen for Cryptosporidiosis (20, 5). Another Uganda study in Mulago Hospital shows 8.5% prevalence in all participants whether they were HIV patients or not (32).

This may also be due to the sensitivity of the diagnostic methods used in the Mulago study, rather than an exceptionally high prevalence in these populations. HIV/AIDS status of participants may also be a factor since it has been reported that HIV coinfection with the sexually transmitted disease has a mutually beneficial effect whereby STD assists HIV disease to progress while virus also helps HIV to progress as well.

Diarrhea in HIV/AIDS is known to be caused by any of the following common pathogens, including viruses, fungi, bacteria, and helminths. There is no specific mix of pathogens and opportunistic agents present in HIV-associated diarrhea although the enteric pathogens vary from patient to patient and from country to country.

One pathogen, which in immunocompromised HIV-positive individuals can cause chronic diarrhea, is *Cryptosporidium*. In Immunocompetent individuals, cryptosporidiosis is usually self-limiting, but not in individuals who are HIV-positive and immunocompromised. Diarrhea is not life-threatening, but can severely hamper daily activities and lower quality of life.

Cryptosporidiosis is often linked to impaired immunity, particularly HIV-associated immunosuppression, hence many of the studies on *Cryptosporidium* prevalence have been performed on HIV-positive patients, most often including adults, but also children (33). In our study, we found an association between *Cryptosporidium* infection and HIV-status. Although the HIV-status was only known for approximately one-third of the children, HIV-positive patients were almost eight times more likely to have *Cryptosporidium* than those who were HIV-negative. The study among Kenyan children also found this association, but with an odds ratio of 3.1 (32).

A study of Ugandan children with persistent diarrhea found that HIV-positive children were 18 times more likely to have *Cryptosporidium* than those who were HIV-negative (32). There was no child examined in this study and therefore we cannot predict what the situation of children may be using this study. However, *Cryptosporidium* should not be ignored as a cause of diarrhea in small children not known to be HIV-positive, because it is reported as an important pathogen at all sites regardless of HIV-prevalence, and the second most common pathogen causing diarrhea in infants (34).

Overall parasites prevalence was 66.7% and other parasites identified from the samples analyzed excluding cryptosporidium was, included the following: 12 (11.4%) of hookworm and *Ascaris lumbricoides* patient*,* 2 (1.9%) of *Strongyloides stercolaris* and *Trichuris trichiura*. Hookworm lowers haemoglobin levels and triggers anemia. Studies in East Africa demonstrated a strong correlation between hookworm infection and anemia.

In a community in coastal Kenya, severe anemia was associated with hookworm infections (>200 eggs per gram) at all ages (range 6–76 months), in both sexes, (35). In Zanzibar, Tanzania, low haemoglobin concentrations were associated with malaria parasitaemia in children less than 30 months, and with hookworm intensity in children aged 30–71 months. About *T. trichiura*, moderate-heavy infections have similarly been associated with higher anaemia levels, particularly in malnourished children (36), and co-infection with *T. trichiura* and hookworms may exacerbate hookworm-mediated anaemia (37).

There is now a substantial body of research that demonstrates how Soil-Transmitted Helminths (STH) infections impair healthy nutrition (38-39). Growth in STH-infected children is compromised through a variety of mechanisms, including reduced food intake due to malabsorption and/or reduced appetite (39). As a result, infected children show higher levels of stunting (38). *Ascaris* live in the gut, where it interferes with the absorption of vitamin A.

In preschool children (PSAC) whose diet may already dangerously be low in vitamin A, *Ascaris* infection may trigger clinical vitamin A deficiency and thus may significantly contribute to increased morbidity (such as blindness) and mortality. There were no school children in this study but the study population who were recruited based on possession of chronic diarrhea and immune suppression induced probably by malnutrition or HIV/AIDS may also be exposed to such health risks as gross vitamin A deficiency.

From birth to the time child is ready to enter school, significant skills are acquired in different domains that pave the road for the rapid cognitive development that takes place at around 6 years. It is evident that soil-transmitted helminths (STH) infections negatively impact motor and language development. STH infections early in life may therefore negatively affect these cognitive indicators when they are measured (40).

This could be due to a variety of causes, both direct and indirect, including induced sleeplessness and micronutrient losses, mainly iron. Changes in cognitive performance may also be plausibly linked with inflammatory response and cytokines triggered by parasitic infections. Other parasites identified from the study included 11 (10.5%) *Entamoeba histolytica*, 5(4.8%) *Giardia lamblia*, 5(4.8%) *Isospora belli,* and 4 (3.8%) *Cyclospora cayetanensis* respectively in patients (41-42). That *E. bieneusi* and *Cryptosporidium* were also found is consistent with previous observations and in other populations (43-46).

The prevalence of Cryptosporidium in HIV-infected children is as high as *Cryptosporidium* (73.6%), *E. bieneusi* (76.9%), both (69.2%), or one or both (81.3%). These findings support the fact that factors contributing to parasites distribution are still very much common among the studied population and therefore warrant more detailed research attention for better health care.

Conclusion

The observed Cryptosporidiosis among the studied adult population may be due to poor hygiene both personal and environmental, poor diagnostic, clinical, prevention and control skills facilities may have impacted also the reported cases of Cryptosporidiosis in this study. Opportunistic infections and comorbidities by other parasites were noted in HIV/AIDS among the studied population. *Cryptosporidium parvum* may be diagnosed by either Ziehl-Neelsen stain or immunofluorescence tests in smears of unconcentrated stool specimens at a significant

amount to assist in effective intervention. Coinfection of Soil-transmitted helminths with HIV-infected and AIDS patients was also noted.

Cryptosporidium Training efforts to improve hygiene, risk assessment, and practices should be improved. Improved hygiene, education to the mass and provision of clean drinking water within the community may reduce the spread of *cryptosporidium* infections.

Since *Cryptosporidium* has the potential to infect many people from a common point-source outbreak, much research still needs to be done to clarify the relationship between low numbers of oocysts in drinking water and the frequency of cryptosporidium infection; determine the asymptomatic carrier rate for *Cryptosporidium* in immunocompromised persons and the chance that these individuals will develop cryptosporidiosis when their CD4 counts drop to a low level.

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