



Investigation of sporadic mortalities in a flock of experimental guinea fowls (*Numida meleagris*) in Jos, Plateau State, Nigeria

NO Ameji^{1,2*}, GI Kuzayed^{3,4}, DO Oshadu⁵, OO Oladele^{1,4}, JR Ponzin⁴, PU La'ankwap⁴, D Gadzama⁴ & MO Akinsola³

1. Department of Veterinary Medicine, Faculty of Veterinary Medicine, University of Jos, Plateau State, Nigeria
2. TETFund Centre of Excellence in Food Security, University of Jos, Plateau State, Nigeria
3. Department of Theriogenology, Faculty of Veterinary Medicine, University of Jos, Plateau State, Nigeria
4. Veterinary Teaching Hospital, University of Jos, Plateau State, Nigeria
5. Department of Veterinary Parasitology & Entomology, Faculty of Veterinary Medicine, University of Jos, Plateau State, Nigeria

*Correspondence: Tel.: +2348035907570; E-mail: ameji@unijos.edu.ng

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Abstract

Intensive guinea fowl farming is expanding in Africa, but poor management practices and infectious diseases can threaten their survival and productivity. This study investigated the causes of sporadic mortalities in a flock of experimental guinea fowls at the Veterinary Teaching Hospital, University of Jos, Nigeria. The birds were acquired from a free-range system with a sudden onset of mortality in the flock three days after being housed together for acclimatisation. Clinical and postmortem examinations were conducted on live and dead birds. Tentative diagnoses of aspergillosis, helminthosis, and colibacillosis were made while samples were sent to the Microbiology and Parasitology laboratories for confirmatory diagnoses. Laboratory results identified *Ascaridia numidae*, *Capillaria* spp., *Heterakis gallinarum*, and *Raillietina tetragona*, a tapeworm measuring 35 cm, as well as *Aspergillus* spp. and *Escherichia coli*, as the causes of comorbidities and mortalities. High worm burdens were recorded in egg counts per gram (EPG) of faeces using the modified McMaster method, with a mean bulk egg count of 5,533 EPG (range 4,800-6,400; standard deviation 255.5 EPG). Bacterial and fungal isolates showed sensitivity to gentamicin and copper sulphate, respectively, but resistance to enrofloxacin, tylosin, and fluconazole. It was concluded from this investigation that helminthosis, bacterial, and mycotic diseases may be prevalent in guinea fowl under free-range conditions, posing significant threats to their survival when birds are acquired from a free-range system for intensive rearing. It is recommended that health screening be carried out before birds under free-range are housed under intensive management, which can lead to the congregation of a large number of birds in the same environment.

Keywords: Colibacillosis, Diseases, Guinea fowls, Helminthosis, Husbandry, Mortalities, Mycoses

Introduction

Intensive guinea fowl farming is increasingly gaining importance in Nigeria and across most of Africa because guinea fowls are considered vigorous, hardy, and largely disease-resistant, especially in adulthood (Ebegbulem, 2018). The known breeds of the guinea fowl are the helmeted guinea fowl, the crested guinea fowl, the black guinea fowl, and the vulturine guinea fowl. Among these breeds, the helmeted guinea fowl, with three main varieties, is the commonest domesticated breed worldwide (Shoyombo *et al.*, 2021).

In Nigeria, other parts of Africa, and Asia, guinea fowls are kept on free-range as scavenging birds in rural areas with limited or no healthcare and low productivity (Shoyombo *et al.*, 2021). However, mycotic and parasitic diseases, which are often neglected, are among the major causes of death and decreased productivity in free-ranging guinea fowls (Saratu *et al.*, 2019).

The high susceptibility of guinea fowls to mycotic and parasitic diseases may be attributed to the husbandry practice of keeping birds on free-range, where they scavenge for feeds made of beetles, cockroaches, and earthworms, which are intermediate hosts of some internal parasites, thereby increasing their risk of parasitic infections (Ebegbulem, 2018; Shoyombo *et al.*, 2021).

There have been few research studies on the guinea fowl in developing countries, with limited documented prevalence data and management information, especially on its intensive farming in the past. However, the indigenous guinea fowl is thought by many to be hardy, resistant to a few diseases, and does not require elaborate, expensive housing when raised on free-range using a wide range of non-conventional feeds not used for chickens (Ebegbulem, 2018). These make guinea fowl the choice of the rural populace, which can be integrated into a crop-livestock farming system (Shoyombo *et al.*, 2021).

Previous studies have shown roundworms such as *Ascaridia* spp., *Capillaria* spp., *Subulura* spp., *Heterakis* spp., as well as cestodes such as *Raillietina* spp. and *Hymenolepis carioca* to be the predominant helminths recovered from guinea fowls, causing morbidity and mortality (Saratu *et al.*, 2019).

Previously, the effects of helminthosis on the survival of adult guinea fowls were thought to be negligible, with limited documentation of co-morbid infections in intensively managed guinea fowl following transition from a free-range system. This study was carried out to investigate the causes of sporadic mortalities in a flock of experimental guinea fowls in

the Veterinary Teaching Hospital, University of Jos, Nigeria.

Case Management

Case history

A case of sporadic mortalities in a flock of 120 guinea fowls of over 10 months of age was reported to the Poultry and Fish Clinic of the Veterinary Teaching Hospital, University of Jos, Plateau State, Nigeria. On the first day, 25 carcasses of the guinea fowls were presented, while another 10 birds were lost over the next five days, with mortalities totalling 35 birds before recovery.

It was revealed that the birds were acquired for experimental purposes from farmers who kept them on free-range in different locations in Bebeji Local Government Area of Kano State, Nigeria, and were transported three days earlier to Jos, Plateau State, Nigeria.

On arrival, the birds were housed together in an experimental pen, fed with commercial chicken growers' pellets (Vital feed[®], Grand Cereal and Oil Mills, Jos, Plateau State, Nigeria) and served water with multivitamins (MiavitR, Stefan Niemeyer GmbH, Germany) to acclimatize them to the environment before commencement of any routine clinical screening and experiments.

Clinical and postmortem examinations

Clinical examination of the birds was carried out during farm visits, with notable clinical signs on most flock members observed being depression, somnolence, inappetence, sneezing, rales, greenish/yellowish diarrhoea, weight loss, and ruffled feathers. All the dead birds were examined at necropsy; gross lesions observed were dehydrated and pale carcasses, ascites, hydropericardium, slight congestion of liver with streaks of pallor, slightly pale, consolidated, pneumonic and frothy lungs with marginal necrosis (Plate I), yellowish/greenish mucoid intestinal contents with occlusion of the intestinal lumen by both roundworms and tapeworms (Plate II), severe haemorrhages and necrosis in the intestine with sloughing of the intestinal mucosae (Plates I & II).

Based on the history of sudden and rising daily mortalities, clinical signs, and post-mortem lesions observed, four diseases, namely aspergillosis, colibacillosis, helminthosis, and necrotic enteritis, were drawn out as differential diagnoses. However, tentative diagnoses of aspergillosis, helminthosis, and colibacillosis were made with samples collected and

sent to the Microbiology and Parasitology laboratories for confirmatory diagnosis.

Laboratory investigations

The roundworms and tapeworms were sent to the Parasitology Laboratory of the Veterinary Teaching Hospital, University of Jos, for identification. Also, the lungs, liver, and spleen from all the carcasses examined were sent to the Microbiology Laboratory for bacterial and fungal isolations and identifications. Also, fresh faecal samples collected during farm visits were sent to the Parasitology Laboratory to aid in determining worm burdens by egg counts.

The lung samples were processed and cultured on Sabouraud's Dextrose Agar (SDA) at 37°C for 3-5 days. The fungal growths on SDA were identified by examining the growth plate on the surface and the reverse side for fungal morphology, staining with lactophenol cotton blue, and then examining with a light microscope at x40 objective lens, and compared with the molds identification chart (Plate III).

The remaining portion of the tissues was processed and cultured on Nutrient and MacConkey Agar, and incubated at 37°C for 24 hours. The suspected lactose-fermenting bacterial colonies on MacConkey agar were further sub-cultured on eosin methylene blue (EMB), which showed greenish metallic sheen, typical of *Escherichia coli* (Plate III). The suspected pure culture of the *E. coli* isolate was examined for colonial morphology, stained with Gram's stain, and subjected to further biochemical tests, including catalase, oxidase, indole, triple sugar iron (TSI), and urease for identification.

The worms were examined using a magnifying lens and a dissecting

microscope. The entire length, external, and other morphological features of the helminths, including

the cranial and caudal portions, were studied to identify the worms.



Plate I: Guinea fowl carcasses (A); emaciated carcasses with lean and pale breast muscles (B); long tapeworms occluding the intestinal lumen (C & D) of dead guinea fowl seen at necropsy



Plate II: Mucoïd and necro-haemorrhagic materials mixed with worms in the intestinal content (A); entwined and tortuous tapeworms and roundworms within the intestinal lumen (B); ballooned caeca filled with numerous roundworms visible on the intestinal serosa (C); consolidated, pneumonic/necrotic and partially congested lungs from carcasses of guinea fowls at necropsy (D)

Fresh faecal samples were collected from live birds during farm visits on 3 different days for examination of worm eggs.

Faecal flotation was carried out by dissolving 4g of fresh faeces in 56 ml of saturated sodium chloride solution of specific gravity of 1.20, as described by Tagesu (2018) and revealed eggs of Ascarid and capillariid worms. The bulk egg per gram of faeces (EPG), which is the total helminth egg load in faeces, and that of each nematode species, was determined using the modified McMaster technique (Tagesu, 2018) by applying the formula:

$$\text{EPG} = \frac{\text{Number of worm eggs counted in the McMaster chambers} \times \text{Dilution factor}}{\text{Volume of fluid in the chambers}}$$

Antimicrobial susceptibility testing

The antimicrobial susceptibility testing was performed using the modified Kirby-Bauer disk diffusion method (CLSI, 2020). The discs were made in-house from sterile Whatman filter paper impregnated with between 10 and 30 µg of antibiotics and 10 and 20 µg of antifungal agents. The antifungal agents used were Fluconazole (20 µg), Nystatin (20 µg), Itraconazole (10 µg), and CuSO₄ (10 µg), while the antibacterial agents used were gentamicin (10 µg), penicillin (10 µg), streptomycin (15 µg), Oxytetracycline (30 µg), Tylosin (15 µg), Florfenicol (20 µg), and Enrofloxacin (10 µg).

The bacterial colonies in the growth phase to be tested were diluted in 2 mL of sterile normal saline to obtain 0.5 McFarland standards, and the diluted cultures were compared using a Wickerham card. The inocula were streaked evenly with sterile swabs onto Mueller-Hinton agar. The antimicrobial discs were dispensed with sterile forceps onto the inoculated agar, which was then incubated at 37°C for 18-72 hours (CLSI, 2020). Antimicrobial susceptibility was

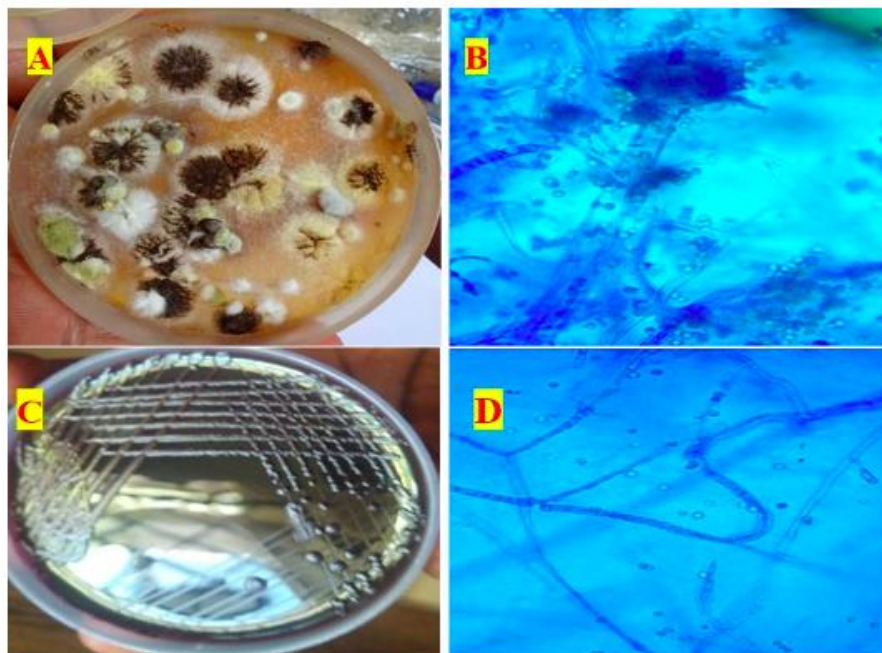


Plate III: Lung tissues cultured on SDA yielded fungal colonies of *Aspergillus niger* and *Aspergillus flavus* after 72 hours (A); the fungal isolates stained with lactophenol cotton blue, showing *Aspergillus* filaments with branching hyphae identified through colonial morphology and conidial structures (B & D), and colonies of *Escherichia coli* on EMB agar (C)

read by measuring the zones of inhibition in millimetres using a metre rule and interpreted according to standard laboratory procedures using a chart (CLSI, 2020).

Laboratory results and management

Based on the morphology of the helminths, the egg type, their sizes, and the predilection sites in the host, the worms were identified. The ascarid worms (Plate IV b) were identified as *Ascaridia numidae* (intestinal worms) and *Heterakis gallinarum* (caecal worm) using the features of the cranial and caudal ends (Plate IV a & d). The ascarid eggs, which are oval in shape, are quite indistinguishable from one another. However, both the adult worms and the eggs of *A. numidae* are slightly larger than those of *H. gallinarum* if they are placed side by side (Plates IV c). *Capillaria* spp. were identified basically through their size, predilection site in the proventriculus, and ovoid eggs, with a thick shell, two polar plugs, and a single cell (Plate IV b). The tapeworm was identified as *Raillietina tetragona*, the longest tapeworm of poultry known, measuring 35 cm, with an oval scolex and suckers at the anterior end, attached to a thin, elongated neck, and a larger posterior strobila (Plate V). The egg count per gram of faeces (EPG) for bulk and specific worm burdens

showed high worm infestations (Table 1). The culture of lung tissues from the carcasses on SDA yielded *Aspergillus niger*, *Aspergillus flavus*, and a few *Aspergillus fumigatus* (Plate III). Also, the bacterial isolate obtained was identified and biochemically characterized as *E. coli* (Plate III and Table 2).

Management was instituted immediately using caredex® (Mebendazole 500mg/g U.S.P.; Animal Care Konsult Ltd, Ogere, Ogun State, Nigeria) at 1g/L for 2 days to treat the helminthosis. The bacterial and fungal diseases were managed based on antimicrobial susceptibility testing using Doxygen® (Gentamicin 200 mg and Doxycycline 200 mg; Kepro B.V., Holland) at 1g/2L in combination with CuSO₄ (Merck KGaA, Germany) at 3g/10 litres of drinking water for 10 days (Table 3).

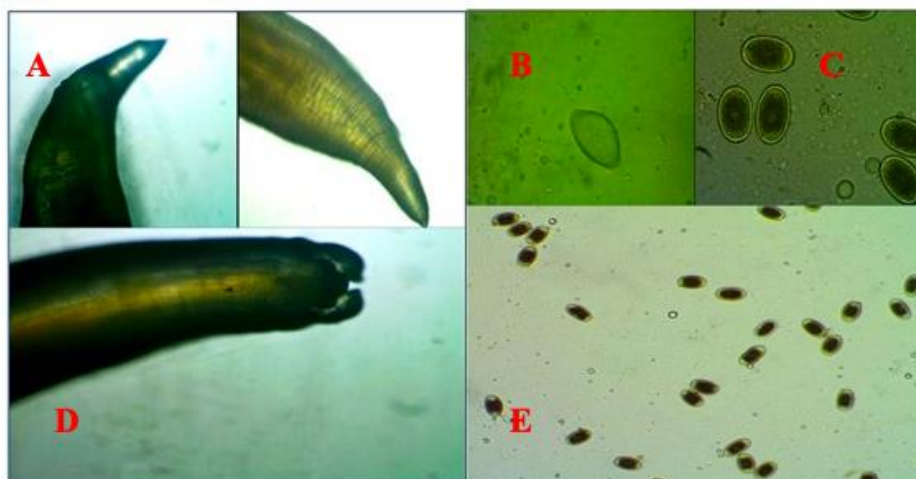


Plate IV: Caudal and cranial ends of Ascarids (*Ascaridia numidae* and *Heterakis gallinarum*) identified using a dissecting microscope, x 400 (A & D), as well as eggs of *Capillaria* spp., x 100 (B); and eggs of Ascarid worms, x 400 & x100 (C & E) seen from the float of intestinal content of guinea fowls after necropsy



Plate V: Ascarid worms isolated in the gut (A) and a few thoroughly rinsed for laboratory examination (B), and an adult tapeworm, *Raillietina tetragona* (C & D) of about 35 cm extracted from the intestinal lumen of guinea fowl at necropsy

Table 1: Relative Worm Burdens Indicated by Worm Egg Count per Gram of Fresh Faeces Obtained from the Poultry Pen Housing the Guinea Fowls.

Sample	Bulk EPG	Deviation	<i>Ascarid</i> spp.	Deviation	<i>Capillaria</i> spp.	Deviation
A	5,400	133	4,200	133	1,200	267
B	6,400	867	4,600	533	1,800	333
C	4,800	733	3,400	667	1,400	67
Mean EPG	5,533	-	4,067	-	1,467	-
STDEV		255.6		611		305.5

Table 2: Morphological and biochemical identification of an isolate of *Escherichia coli* from tissue samples of dead guinea fowl sent to the Laboratory.

S/N	Test	Identification Features
1	Morphology on EMB	Circular, convex, non-spore forming short rods, smooth & shiny colonies with metallic sheen
2	Gram's Reaction	Negative
3	Catalase	Positive
4	Lactose	Positive
5	Oxidase	Negative
6	Indole	Positive
7	TSI	Acid/Acid with gas
8	Urease	Negative

Table 3: Antimicrobial Susceptibility Testing of Bacterial and Fungal Organisms isolated from carcasses of Guinea Fowls after Necropsy.

Test Type	Antimicrobials	Sensitive	Intermediate	Resistant
Antifungal Susceptibility Testing	Enrofloxacin	--	--	14 mm
	Florfenicol	--	15 mm	--
	Gentamicin	17 mm	--	--
	Penicillin	--	20 mm	--
	Streptomycin	16 mm	--	--
	Tylosin	--	--	12 mm
	Copper sulfate	17 mm	--	--
	Fluconazole	--	--	13 mm
	Itraconazole	23 mm	--	--
	Nystatin	--	16 mm	--

Discussion

Helminthosis and fungal diseases, though often neglected, pose major threats to the productivity and survival of guinea fowls under an extensive farming system. Helminthosis has been reported by Saratu *et al.* (2019) to be a major cause of losses and sudden deaths, especially in free-range guinea fowl.

Birds kept under a free-range system are prone to parasite infection through ingestion of contaminated soil, feed, or intermediate hosts such as earthworms, insects, or snails, according to a report by Ebegbulem (2018). This study lent credence to this assertion as the experimental guinea fowls were acquired from local farmers who kept them under a free-range system and transported them over a long distance from Kano to Jos, Plateau State. Stress associated with the long journey might have impacted the birds' immune system, thereby exacerbating latent aspergillosis, as evidenced by the clinical signs and mortalities recorded.

The major helminth groups seen in this study were basically nematodes (*Ascaridia numidae*, *Heterakis gallinarum*, and *Capillaria* spp.) and a cestode (*Raillietina tetragona*). These findings are similar to

those reported in Nigeria and other parts of Africa (Ebegbulem, 2018; Saratu *et al.*, 2019). Although it is very difficult to diagnose certain helminth parasites to the species level in routine faecal analysis, the *Capillaria* spp. encountered in this study is suspected to be *C. annulata* (syn. *Eucoleus annulatus*), a common parasite of gallinaceous birds that has a predilection for the crop and causes significant damage to the mucosal lining of the ingluvis and the oesophagus. The inflammation and swelling might have made it difficult for the affected birds to swallow food, leading to severe weight loss and emaciated carcasses observed in this study.

Transmission of *Heterakis gallinarum* and most endoparasites occurs through the ingestion of intermediate hosts such as earthworms, snails, beetles, and other insects, a common occurrence in birds under a free-range system, as might be the case with the guinea fowls in this study, which were initially kept under free-range conditions.

Ascaridia numidae, other roundworms, and tapeworms are known to cause intestinal obstruction, anorexia, diarrhoea, and reduced weight gain, as well as secondary bacterial infection due to

mucosal damage, as seen in the present study, which is similar to previous reports of the effects of these helminths in poultry (Saif *et al.*, 2013; Ebegbulem, 2018; Saratu *et al.*, 2019). In addition, *Heterakis gallinarum* is the intermediate host of *Histomonas meleagridis*, the cause of blackhead, which can cause sporadic mortalities in turkeys and even guinea fowls according to previous reports (Saif *et al.*, 2013; Ebegbulem, 2018).

Helminthosis causes considerable economic losses to poultry farmers worldwide by reducing productivity through the competition of the parasites with infected hosts for essential dietary nutrients, leading to lower weight gain and stunted growth, as reported by Ebegbulem (2018). In severe cases or co-infection, it may even cause death, especially when the worm burden is high, as seen in this study, which is similar to reports by other investigators (Saif *et al.*, 2013; Ebegbulem, 2018; Saratu *et al.*, 2019).

However, Ebegbulem (2018) reported that guinea fowl is tolerant to most viral and bacterial diseases of poultry, such as colibacillosis. However, weakened immunity may arise from the stress of transportation to a new environment, and other comorbidities, as seen in this study, can result in outbreaks of such diseases.

Colibacillosis is a complex syndrome of diseases caused by *Escherichia coli* and may arise as a primary or secondary infection in the host animal. A common and often fatal form of the disease is colisepticemia, known to cause blood poisoning and septic shock in both young and adult guinea fowls that are reared intensively, often leading to sporadic mortalities as seen in the present study, which is similar to the report of Srinivasan *et al.* (2025). Hence, the sporadic mortalities recorded in this study might be due to colisepticemia and other accompanying comorbidities.

Fungal diseases are primarily reported by Saif *et al.* (2013) as opportunistic infections that thrive under poor environmental, husbandry, and stress conditions. Aspergillosis caused by *Aspergillus* spp. was reported by Saif *et al.* (2013) to be the major fungal mycosis in guinea fowls and other birds. The sudden occurrence of mortalities in the flock might be due to the presence of comorbidities and activation of latent infections in the guinea fowls by environmental stressors such as overcrowding, transportation, and poor ventilation, as previously reported (Saif *et al.*, 2013).

Aspergillosis is reported by Saif *et al.* (2013) to occur either as an acute form, characterised by high morbidity and mortality in young birds, or as a chronic

form, which is sporadic and occurs in older birds, with often low mortality. In the present study, co-infections might have accounted for the high and persistent mortality.

Joffe *et al.* (2017) reported that Mebendazole, a benzimidazole, is active against a wide range of helminths, including cestodes, and is effective against ova, larvae, immature, and adult stages. Mebendazole is also reported by the same author to be of value in the treatment of fungal mycoses and tumour cells (Joffe *et al.*, 2017). This might have contributed synergistically to the recovery of the treated birds from the mycotic co-infections in this study.

Copper sulfate has been known for a long time as a biocide and an antimicrobial used in treating fungal and some bacterial infections with considerable efficacy. The antimicrobial susceptibility testing result was what necessitated the use of copper sulfate in the present investigation, with proven recovery. Ameji *et al.* (2020) reported the success of copper sulphate, a biocide and an antimicrobial, in the treatment of fungal and some bacterial infections. In addition, the mixed bacterial infection was treated concurrently with a gentamicin-based antibiotic to which the isolate was susceptible.

In another vein, the resistance of bacterial and fungal isolates to enrofloxacin and fluconazole, respectively, which are common prescription drugs for treating human infections, is of significant concern. These resistant isolates may perpetuate resistance genes in the environment and eventually cross to humans, driving antimicrobial resistance (AMR), which is of global concern, according to the report by Perry *et al.* (2016).

This investigation concludes that helminthosis, bacterial, and mycotic diseases are prevalent in guinea fowl farming under extensive or poor management systems, posing significant threats to their survival and productivity. The impacts of these co-infections may be greater under an intensive or improved management system, especially when rearing stock is obtained from birds under a free-range system, as witnessed in this study.

It is recommended that quarantine and health screening tests be conducted before birds raised under free-range conditions are housed in intensive care, as this may lead to a large number of birds congregating in the same environment with sudden outbreaks of diseases from latent infections.

Conflict of Interest

The authors declare that there is no conflict of interest.

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