Global Veterinaria 4 (1): 67-73, 2010
ISSN 1992-6197
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Impact of Experimental Infection with *Gyrodactylus* Species on the Density of Skin Mucus in Fries of Catfish (*Clarias gariepinus*) with Emphasis on the Pathological Changes

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Abstract: This study aimed to determine the effect of experimental infection of catfish (*Clarias gariepinus*) with *Gyrodactylus* sp. on the density of mucous cells "goblet cells" in the skin. Experimental infection of catfish fries was carried out with *Gyrodactylus* sp. , collected by dislodgment from heavy infected catfish and placed with 100 naïve catfish fries for 10 hours. One day post infection, fries were microscopically examined, prevalence was 100%. The infected fries were observed for 6 weeks for clinical signs, density of parasites, site of infection and density of mucous cells in the skin. The study reveled that, high density of infection with *Gyrodactylus* sp. increased the density of mucous cells in the skin of catfish (*Clarais gariepinus*) fries. Maximum infection was recorded at day 18, while the maximum density of goblet cells was recorded at 15-20 day. Caudal fins was the highest site of infection, while the abdomen was the lowest site of infection. It was concluded that the experimental infection of catfish with gyrodactylus led to an increase mucification by increase in mucous cell density and increased secretions of mucous.

Key words: Catfish · *Clarias gariepius* · Experimental infection · Gyrodactylus · Mucous cells · Caudal fins · Abdomen

INTRODUCTION

External parasitic infection for fish, especially scaleless fish like catfish (*Claris gariepinus)* has severe effect on skin intact and mucous cells density [1,2]. There are contrasting views about the effect of external parasitism, especially *Gyrodactylus* species on the density of mucus and mucous cells in the skin of fish, some researchers [3-5] have reported that mucification increases during ectoparasitic infection, others [2,6-10] have reported that external parasitic infections decrease mucous concentrations. Mucous cell secretions are generally labeled with protective functions that contribute to evasion of parasites and other infectious agents [11]. In this regard, it is not known specifically the relationship of mucous secretion to the survivality of ectoparasites, especially *Gyrodactylus* sp. Thus the present study aimed to investigate the impact of experimental infection with *Gyrodactylus* sp. in fries of catfish fry (*Clarias gariepinus*).

MATERIALS AND METHODS

Fish: Thousands of live *Gyrodactylus* sp. were collected by dislodgment method of Cusack [12] from naturally infected catfish (*Clarias gariepinus)* held at the laboratory aquaria and placed on physiological saline for experimental purposes according to Wells and Cone [8]. The collected parasites were kept for 12 hours in a plastic bag. Two plastic bags, each contains 4 liter de-chlorinated cooled oxygenated tap water were used. Each bag contained100 catfish fry (3-7g in weight and 4-5 cm in length). The collected parasites were placed for 12h. into one plastic bag while the other bag leaved free from parasites (Experimental catfish fries with no previous history of infection with gyrodactylids). aAfter one day of exposure, fries in two groups were microscopically examined to ensure that *Gyrodactylus* sp. had transferred successfully to all the fish.

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Experimental Design: Two groups, each of 100 fries were used, the first one was the infected group and the second was the uninfected group. Fry of catfish were held in 50 liter aquaria supplied with de-chlorinated tap water maintained at 20°C, fed on commercial pellet food twice daily (3% of their body weight). Two groups of infected and uninfected fry were used to monitor the development of infra populations over the course of 6weeks post infection using dissecting microscope. Every 7days (week), ten fries from each tank were anaesthetized individually with 125 mg/ml clove oil [13]. Intensity and sites of infection were recorded and returned to the respective tank. control and infected fish were handled in the same method including anaesthetizing. Three replicates of infected and control groups were anaesthetized and fixed in 10% buffered formalin on days 7, 14, 21, 28, 35 post infection, the parasites intensity and site of attachment were determined and recorded at the time of sample collection.

Preparation of Caudal Fins for Pathological Examination: Caudal fins from five fish in each group at the time of collection were removed, rinsed in distilled water and stained with haematoxline and eosin. Stained fins were rinsed in distilled water, dehydrated in ethanol and cleared over night with cedar oil and ethanol, cleared fins were mounted in Canada balsam and examined using low power microscope 40X. Number of mucous cells within mm^2 were counted and expressed. Number of mucous cells during various days post infection as well as the number of *Gyrodactylus* sp. at various sites of infection on the fish were compared using ANOVA at 95% confidence level [14].

RESULTS

Clinical Sings of Gyrodactylus Infection Infected in Catfish Fries: *Gyrodactylus* sp. affect catfish fries with abrasions Fig. (1.A) and hemorrhagic spots on the skin, eroded fins Fig. (1.B), hyper irritability with rubbing of bodies against sides of aquaria, gulping of air and surfacing, most of infected fries showed increased mucous secretion on the skin and gills.

Development of Gyrodactylus Infection: One day post infection, all fries showed the infection and the intensity of the parasites on the fries was 5-10 gyrodactylus during the 1st day post infection. Weekly counts revealed that the intensity increased to record the peak on the day 21 (PI), then the numbers of gyrodactylus parasites on individual fish declined until the end of the experiment. The prevalence of infection was 100% in all fries in infected groups. Parasitic identification of gyrodactylus sp. (Fig. 1.C) removed from 10 fries replicate samples at days 21and28 of the experiment post infection.

Intensity and Site of Infection with Gyrodactylus in Catfish (*Clarias gariepinus*): The intensity of infection reach to peak at about 21 day post infection, the *Gyrodactylus* sp. Were distributed among all fry body in all post infection samples. The most frequently occupied sites were the caudal fins, then the pectoral fin followed by anal fin then the dorsal fin and finally the abdomen in the fries. The peak intensity of infection was after 3weeks post infection.

Pathological Change of the Caudal Fins Post Infection: Infected fries showed excessive mucous secretions (Fig. 2 and 3). The normal skin structure in catfish was consist of 3 layers, The epidermis was several cell thick, made of stratified epithelium, a superficial epithelial layer, a middle Malpighian cells and a basal cuboidal to columnar cell layer that rests on a basement membrane. Large ovoid or cylinder cells with homogenously eosinophilic cytoplasm and one or more centrally located nuclei, corresponding to the so-called club cells were distributed at different levels in the epidermis. Mucus cells (goblet cells) can also be seen superficially. The epidermis was commonly infiltrated diffusely by an individual numbers of mononuclear cells.

Table 1: The distribution of *Gyrodactylus* sp. On different sites on catfish fries

Sit of infection	1st week	2nd week	3rd week	4th week	5th week	6th week	Total
Caudal fin	48	103	351	213	114	56	885
Pectoral fin	20	46	67	59	48	31	271
Pelvic fin	16	39	52	47	31	29	214
Anal fin	12	33	48	41	25	18	177
Dorsal fin	8	22	33	26	16	9	114
Abdomen	3	12	22	13	17	5	72
Total	107	255	573	399	251	148	1733

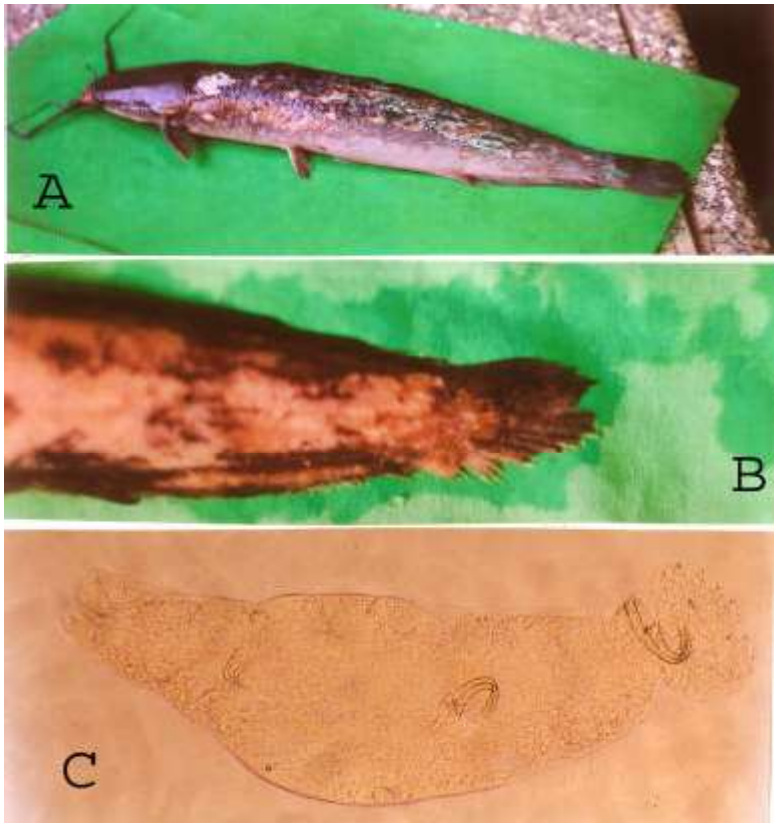

Fig. 1: Catfish *Clarias gariepinus* showing A. abrasions, B. eroded caudal fin C. Showing *Gyrodactylus* species

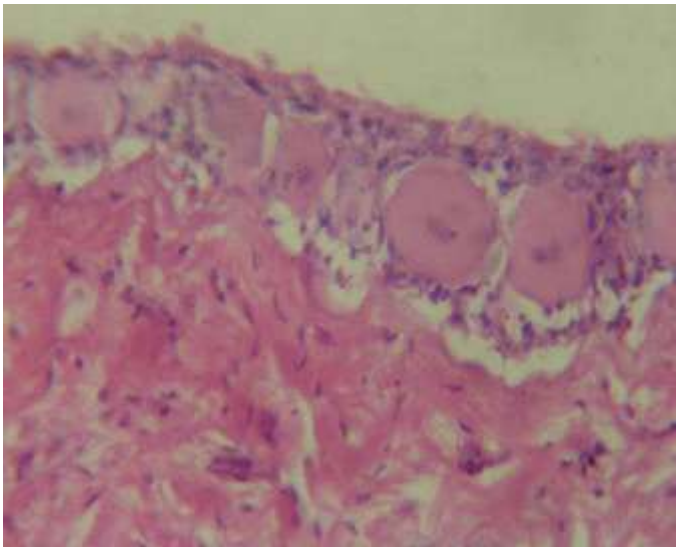

Fig. 2: The normal skin structure in Catfish (HandE X400).

Lipophores and melanophores may lie under the epidermis and at different levels of the dermis (Fig. 2). The dermis consisted of collagenous connective tissue fibers that can be differentiated into upper stratum sponginum and inner stratum compactum. The dermis is bounded internally by a single layer of cells which separates it from the deep hypodermis.

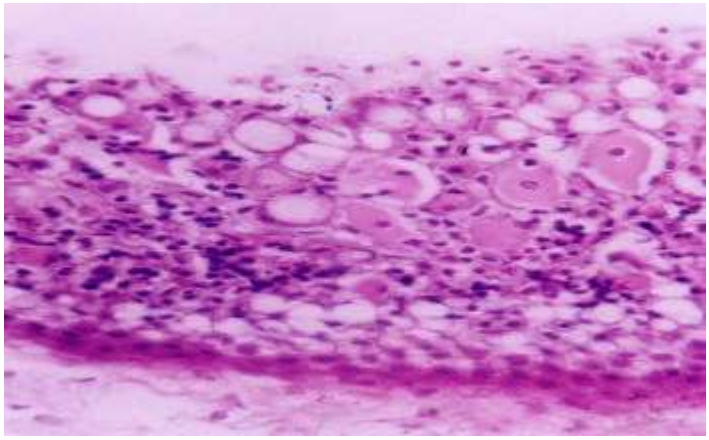

Fig. 3: Hyper activation and hypertrophy in the epidermal mucous cells of Catfish (HandE X 400)

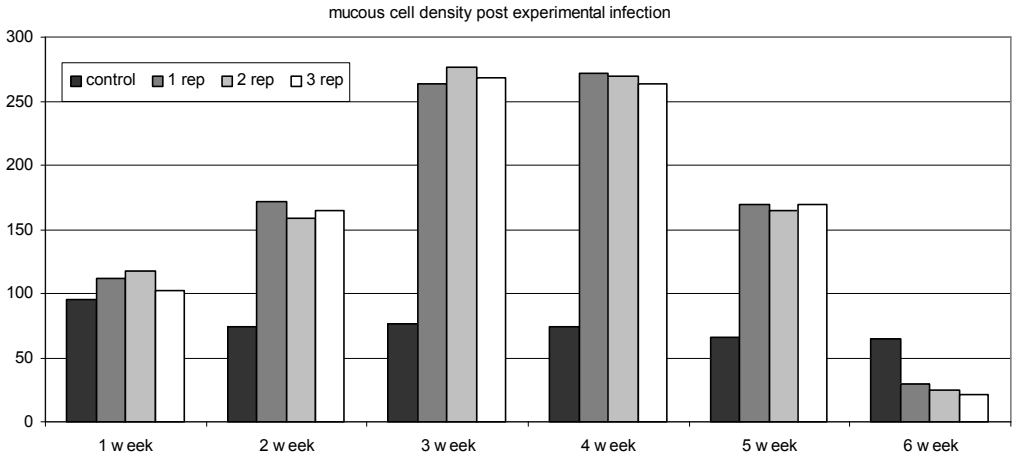

Fig. 4: Mucous cell density on the caudal fins of Catfish

In the present study, the skin of infested catfish with gyrodactylus revealed necrotic epidermal cells, with accumulated lymphocytes and leucocytes in area of necrosis, with hyper-activation and hyper-trophy of the goblet (mucus) cells (Fig. 3).

Mucous Cell Density on the Caudal Fins: The density of the mucous cells on the caudal fins on the day 24 in infected groups was higher while on day 42 post infection, it reduced to about 30% of the day 21 post infection. The increase of density of mucous cells was parallel to the increase of density of the parasitic infection on the caudal fins (Fig. 4).

DISCUSSION

The present study revealed that the intact skin of catfish (*Clarias gariepinus*) contains several hundred mucous cells mm^2 and indicates that these cells represent an important part of the structural make-up of the epidermal surface [8,15-17]. Fish epidermis is in direct contact with the environment and mucus is considered to be a first line of defense protecting the epidermis [18,19]. The protective mucus coat must be continuously secreted or it would likely be quickly removed by water friction to leave the skin exposed and become susceptible to infection. Handling stress were previously observed to increase the number of mucous cells in salomonids [20]. Handling stress is known to rapidly increase blood cortisol levels [21,22], while oral administration of physiological doses of cortsiol to brown trout markedly increased susceptibility to gyrodactylus infection [3]. Iger *et al*. demonstrated that increased levels of cortisol in rainbow trout *Oncorhynchus mykiss* induced mucous cells changes similar to that induced by infection of external parasites. Similarly, present study indicated that external parasites infection stimulates production of mucous cells as seen by the increase their densities in

histopathological examination. It can not be excluded that a pool of non- differentiated (rapid response cells) exists, these cells would then be capable of rapid differentiation and mucopolysaccharide synthesis. Mucous discharge was elevated indirectly (open mucous cells and lower mucous cell density) as a result of an accelerated stimulation of mucous release which cannot be compensated for by differentiation of new goblet cells. Gyrodactylids species in common ecto parasites on fish skin, fins and buccal epithelium [24], as these parasites are ovivivіparus (live bearing), egg development occurs in uterus, well-developed embryos are produced having completely formed genital system, immediately start feeding and act as parasite on the same host [5]. All parasites armed with two pairs of large anchors and marginal hooklets the parasite attach themselves to the skin with the long anchors penetrating the epithelial tissue of the epidermis, such skin flukes feed primarily on mucous epithelial tissues as well as blood [26]. The parasites regularly relocate around the body margins, frequently cross over the body surface [24,25]. The morphology of gyrodactylids elucidate the clinical sings appeared on the infected fish as hemorrhagic spots, hyper- irritability with rubbing, gulping of air and surfacing increasing mucous secretion on the skin and gills, this may be attributed to increased density of infestation (gyrodactylids) this go hand by hand with the results of Nigm El.-din and Saleh [27], Wellsand Cone [8], Sanchez *et al.,* [28] and Osman [26]. The experimentally infected *Claris gariepinus* with average 5 worms/fish appeared restless with signs of difficult respiration on 5th and6th days of infection, similar observations of rapid development of worms (gyrodactlids) noticed in *Macrogyrodactylus polypteri* parasitizing skin of *Polypterus senegalus* in Sudan [29] as 3605 worms per fish were produced within two weeks from initial infection which not exceeding 6 worms/fish. These results nearly agree with the results of the present study which record that the initial infection of gyrodactylus one day post infection was 5-10 worms/fish and weekly counts revealed that intensity increased to record the peak on the day 21post infection. infection resulted in significant increase in density of mucous cells on the caudal fins and rest of the body of the fries due to sever irritation attachment movements and feeding activity of *Gyrodactylus* species [26]. The change become evident until approximately 3 weeks, this results ,this results come with the results of Ekanem and Obiekezie [30], Urawa [31] anderson and Buchman [1] and Barker *et al.,* [5]. These results confirmed that the epidermal mucous cells are important as innate-Immune mechanism against gyrodactuls infection. The intensity of infection reach to the peak at day 18 also maximum increase in the mucous cells was recorded from 15 to 25 day. These result nearly agree with that obtained by Sterud *et al*., [2], Wells and Cone [8] and Cone and Cusack [32]. The mucus cells decreased in density when the parasite population was declined. The delay in appearance of significant alteration suggest it is linked directly with the development of parasite intensity to threshold level. The result nearly agree with Andersen and Buchmann [1] , Barker *et al.,* [5] and Heggberget and Johnsen [33]. Section from the caudal fins revealed evidence of inflammatory cells, increased mucous cells and thickening of the epidermis among infected fish skin [26]. Increasing of mucous cells generally accompanied with protective functions that contribute to evasion of *Gyrodactylus* sp. [11]. Regarding the distribution of gyrodactylids, the present study revealed that the increased intensity was in the caudal fins followed by pectoral fins and the lowest intensity was on the abdomen. These may be attributed to what is called the parasite intelligent for ,safety from water currents and external environment which affect the parasite survival on the infected host [1, 24,25].

Regarding the recorded pathological changes of infected fries with gyrodactylus, which were hyper-activation and hyper-trophy of the goblet (mucus) cells in the skin, in our opinion, these occurred as a results of infection with Gyrodactylus. These increases in the number and the size of goblet cells (mucous cells) to produce more amount of mucous which has a protective effect against any infection. These findings accentuates the findings of Olafsdottir and Buchmann [34] who pointed out that the epidermis consists of epithelial cells with mucous cells in between. These mucous cells excrete a slimy substance that covers the whole surface of the skin, with a protective layer. This mucous coating helps to protect the fish against infections. Moreover, they suggested that the mucous cell discharge elicited by infection is inhibited by the drug. The association with elevated parasite counts in these fish can be explained either by decreased anti-parasitic mucus action or by parasite predilection for intact mucous cells. These fingings accentuates the findings of Wells and Cone [17] who pointed out that the relation between experimentally infected fry of *Oncorliynclius mykiss* with the ectoparasites Gyroducrylus rolenirmensis and *Gyroducrylus salnionis* (Monogenea) and changes in mucous cell density in the epidermis covering the caudal fin during an ensuing 42 day epidemic. At 10°C,

infections of G. *colemanensis* rose to a recorded peak of 90 to 1 15 worms on day 27 post-infection and then declined toward extinction. Intensity of G. *salmonis* remained low throughout the experiment. Infection produced no clinical signs of disease and did not influence fry growth or survival. However, infected fish did have a 50% reduction in the number of mucous cells in the epidermis of the fin. The changes were first detected on day 24 post-infection and became increasingly pronounced during the subsequent 1 day period when parasite numbers declined drastically. The study hypothesizes that parasite activity on the surface indirectly leads to reduction in mucous cells through disruption of cell dynamics within the epidermis These findings were parallel with that described by Pottinger *et al.,* [7] in which the apparent effects of environmental factors on skin structure in hatchery-reared brown trout are shown to be mediated by the influence of these factors on the skin parasite population. The presence of ectoparasites promotes the concentration of epidermal mucus-secreting goblet cells, the magnitude of which is related to the intensity of infection. The range of roles which fish mucus is said to play very large proposed roles, respiration, ionic and osmotic regulation, reproduction, excretion, pathogens resistance, communications, feeding, nest building and protection potentially make mucus a highly multifunctionally material for the fish especially scale less fish as catfish *Clarias gariepinus* [35].

In a conclusion, the experimental infection of catfish with gyrodactylus led to an increase mucification by increase in mucous cell density and increased secretions of mucous (hyper activation) and these was in parallel with increased densities of gyrodactylids infections.

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