World Journal of Clinical Pharmacology, Microbiology and Toxicology
World J. Clin.Pharmacol.Micrbiol.Toxicol Vol 1[3] September 2015: 53-57
Online ISSN 2454-1729
Journal's URL: http://wjcpmt.com
Impact factor [JIF] : 1.05

Original Article

Assessment of the Pathogenicity Property of *Fusarium graminearum* 1 in Balb/C Mice

Kedar B. Karki and Gemerlyn G. Garcia2
1 Institute of Graduate Studies, Central Luzon State University, Philippines
2 Assistant Professor Microbiology and Chairman Institute of Graduate Studies, Philippines

ABSTRACT
An experiment was conducted to investigate the immunologic property, pathogenicity and treatment of Fusarium graminearum infection. Several groups of mice were randomly selected for the following groups: (PC, T1 and T2 were groups of mice that respectively received a 1:1, 1:100 and 1:100,000 fungal dilution while T3, T4, and T5 were groups of mice that respectively received the same concentration but each were treated with Diethylamine Acetarsol (Acetylarsan). A group of mice was included as a negative control (NC).In vitro assays were used to examine the ability of F. graminearum to produce enzymes, which are thought as important virulence indicators. Results revealed the ability of the pathogen to produce collagenase and elastase. In addition, histopathological examination indicated vascular congestion and mild triaditis of the liver. Pulmonary congestion and lymphoid hyperplasia in the spleen were noted. The fungi were recovered from the liver, lungs, spleen and skin of the legs of some experimental animals. Likewise, increase in weight of the spleen doubled as early as the second week (from 49 mg to 80 mg) and progressed up to the fourth week (125 mg) where it tapered off in the untreated group. Similar increase in the weight of the spleen was observed in the treated group (40 mg to 64 mg) but not as great as that in the untreated group (105 mg). Hematological findings showed a lymphocytic count of 1.83 that increased to 3.356, monocyte count of 0.47 that increased to 0.981 and neutrophils increased from 0.399 to 1.698 in untreated groups. Lymphocyte count in the treated group was increased from 1.8 to 3.64, monocytes increased from 0.068 to 0.325 and neutrophils increased from 0.223 to 1.056. High incidence of death was observed in animals that did not receive treatment (PC, T1, and T2) while relatively lower death incidences were exhibited by groups that received diethylamine acetarsol (T3, T4 and T5).
Keywords: Fusarium graminearum, Balb/C Mice, T3, T4, T5

INTRODUCTION

There is insufficient information describing the pathogenicity of the fungus Fusarium in livestock in different parts of the globe. This fungus had been associated with Deg Nala disease. This affects largely buffaloes and cattle in India, Pakistan and Nepal.

The precise mechanisms underlying the observed symptoms of Deg Nala disease is not known. In this study, investigative efforts had been focused on the pathogenicity, ability to produce immune response and efficiency of diethylamine acetarsol as effective therapeutic agent.

Raising buffaloes and cattle in Pakistan, Nepal and India is one way of augmenting the financial resources of village people. Infections that may be debilitating in nature can cause significant economic losses as a result of decreased production confounded by reduced growth rate, mortality and poor animal performance. An effort to improve animal production in the village calls for suitable control or therapeutic measures of any disease. Experimental evaluation of the immunologic properties and treatment of *F. graminearum* infections should be considered.

The general objective of this study was to determine the pathogenicity, immunological characteristics and treatment of *F. graminearum* infection.

METHODOLOGY

Fusarium graminearum Test Strain.

The fungus *Fusarium graminearum* was obtained from the National Culture Collection of Microorganisms, Institute of Molecular Biology and Biotechnology, University of the Philippines at Los Baños, Laguna. The stock culture was inoculated in Sabouraud's Dextrose Agar and was kept at room temperature for two to five days.

Test for Pathogenicity

Elastase Production

Inoculation and Incubation of the Culture Medium. Sabouraud's dextrose agar was supplemented with 0.3% elastin (Sigma No.E 1625). Test strains (F. graminearum) and the positive control (P. aeruginosa) was inoculated as streaks in Sabouraud's dextrose agar supplemented with 0.3% elastin. Three plates were set as replicates for the test strain and the positive control. A negative control consisted of an uninoculated Sabouraud's dextrose agar supplemented with 0.3% elastin (also in three replicates) was run. Plates were incubated for two weeks at 37ºC.

Observation of Elastase Production. Clearing of zone around fungal colonies or bacterial colonies in the case for the positive control indicates elastase production. Designated scores were set forth to describe elastase production. These were presented as follows: 0.0, no clearing around colonies; 1.0, 20 – 30% of colonies on the surface of the medium are surrounded by clear zones; 3.0, 40 – 70% of colonies on the surface of the medium are surrounded by clear zones; 5.0, 80 - 100% of colonies on the surface of the medium are surrounded by clear zones. Observation of positive reaction was undertaken daily for two weeks. This procedure was conducted twice to confirm the result of the first experiment.

Collagenase Activity

Inoculation and Incubation of the Control Medium. Nutrient broth (5 ml) was supplemented with type I collagen from Bovine Achilles Tendon (5 mg). The media were dispensed in tubes and autoclaved at 115?C for 15 minutes. The tubes were inoculated with the test strain (F. graminearum) and Clostridium perfringens (positive control). Three tubes were set as replicates for the test strain and positive control. An uninoculated Nutrient broth supplemented with type I collagen from Bovine Achilles Tendon (also in three replicates) was included as a negative control. These were incubated at 37ºC for two weeks.

Observation of Collagenase Activity. The tubes were examined grossly for indication of collagen digestion during the entire incubation period. Designated scores were set forth to describe collagenase production. These were used as follows: 0.0, no indication of collagen digestion; 1.0, 20 – 30% of the collagen in the medium is digested; 3.0, 40 – 70% of collagen in the medium is digested; 5.0, 80 - 100% of collagen in the medium is digested. Observations of positive reaction were undertaken daily for two weeks.

RESULTS AND DISCUSSION

The colonies of *F. graminearum* were initially white in color and cottony at the surface of Sabouraud's dextrose agar. As the colonies matured, they became greenish to brown in color. This is in conformity with the morphological studies on *F. graminearum* conducted by Segal et al. in 1998.

Elastase and Collagenase Production

Table 1 shows the ability of *F. graminearum* to produce elastase and collagenase. The ability of *F. graminearum* to produce elastase was observed to be the same as that of the positive control (Pseudomonas aeruginosa). *F. graminearum* produced collagenase at a lower level when compared with the positive control (Clostridium perfringens). The degree of collagenase production manifested by both test strain and positive control was not significantly different. The effect of the above test suggests that enzymes like collagenase and elastase produced by *F. graminearum* may enhance its damaging effect on tissues. The characteristic lesions of the Deg Nala disease like drying and sloughing of tail tips (Irfan, 1971) and injuries of the extremities could be possibly related to the damaging effect of these enzymes on tissues in chronic cases of infection.

Table 1. Elastase and collagenase production of F. graminearum

CRITERIA	F. graminearum	POSITIVE	CONTROL	NEGATIVE	CONTROL
Elastase	5.00	(0.000)ns	5.00	(0.00)	0.00

P. aeruginosa

Collagenase	4.30	(0.942)ns	5.00	(0.00)	0.00	(0.00)

C. perfringes

Data presented as mean (? standard deviation) response of three replicates. Designated scores were set as follows to categorize responses: 0.0 – no indication of positive responses as described in the materials and methods; 1.0 – 20-30% of the colonies on the surface of the medium show positive response; 3.0 – 40-70% of the colonies show positive response; and 5.0 – 80-100% of the colonies on the surface of the medium show positive response. The criterion for elastase production was the presence of clear zones around the colonies white positive while collagenase production was indicated by collagen digestion. Ns, no significant differences compared with the positive control.

Mortality

Data on the mortality profile shown in Table 2 shows that there was high mortality rate (20%) in the group that received the undiluted.

Table 2. Weekly mortality profile (M/Total) of mice infected with F. graminearum

Week	PC	T1	T2	T3	T4	T5	NC
1	4/20 20%	2/20 10%	1/20 5%	1/20 5%	1/20 5%	1/20 5%	O/20 0%
2	3/16 18.75%	3/18 16.6%	1/19 5.26%	1/19 5.26%	0/19 0%	0/19 0%	0/20 0%
3	1/13 7.69%	1/15 6%	1/18 5.5%	0/18 0%	0/19 0%	0/19 0%	0/20 0%
4	0/12 0%	0/14 0%	0/17 0%	0/18 0%	0/19 0%	0/19 0%	0/20 0%
5	0/12 0%	0/14 0%	0/17 0%	0/18 0%	0/19 0%	0/19 0%	0/20 0%
6	0/12 0%	0/14 0%	0/17 0%	0/18 0%	0/19 0%	0/19 0%	0/20 0%

Data represent the ratio of the number of dead animals (M) over the total of animals (T) in a given treatment group at indicated time intervals. Values in parenthesis are mortality rates (%). Concentrated fungal inoculum (PC). This trend tapered down to 18.75% on the second week and reached a lower level (7.69%) on the third week. Relatively lower mortality rate (10%, 16.6%, and 6%) was recorded in T1 while T2 exhibited the lowest rate of mortality percentage from the first week to the third week of observation. These results were different from treatment groups that received the diethylamine acetarsol. Treatment 3 had mortality rates of 5.0% to 5.2% on the first and second week and no death was noticed thereafter. Treatments 4 and 5 manifested 5% mortalities only on the first week. No mortality was recorded in the negative control. Data on mortality profile show that infection with *F. graminearum* induced immediate death of experimental animals observed within the first four days post-challenge. No existing literature reveals elicited responses similar to this. The death recorded in the first few weeks post-challenge with *F. graminearum* indicated that the defense mechanism of the animals was inadequate to mount immune protection.

Histopathological Examination

Based on histopathological findings, various changes in the liver, lungs and spleen were observed. In the liver, vascular congestion and portal triaditis were noted. There were reactive nuclear changes; slight enlargement of the nuclei but no alteration of chromatin distribution was noted.

The nuclear membrane contains ample amount of cytoplasm. Some livers exhibited mild to moderate dysplastic changes which included prominent nuclear enlargement, hyperchromasia, pleomorphism and chromatin clumpings (Plates 1c to g). The amount of dysplastic changes in some hepatocytes gives the possibility of some of the cells that develop hepatocellular carcinoma. No indication of malignant cells was seen.

In the lungs, most of the changes seen were non-specific vascular congestion. Some emphysematous changes were seen but were sporadic and non consistently present.

In the spleen, lymphoid hyperplasia and vascular congestion were observed. These were being considered as non-specific changes.

This study offers a more detailed histopathological finding related to F. graminearum. Previous literatures revealed gross lesions that involved the skin, lungs, sinuses, spleen, kidney, muscles, CNS, and liver, heart, eyes, joints, and toe nails of infected animals (Boutati and Anaisse, 1997).

Fungal Recovery

Data in Table 3 show the recovery scores of *F. graminearum* from the different organs indicated. This is an initial attempt to recover *F. graminearum* from the infected animals. Data confirm the ability of the pathogen to colonize and establish itself in tissues of vital organ and cause impairment of function.

Splenomegaly

Splenomegaly (Table 3) was observed in the posittve control. The initial weight of 49 mg increased to 125 mg in the fifth week. The group that was given lower concentration of pathogen had the lowest spleen weight (43 mg) and reached weight of 115 mg in the fifth week. On those groups that were treated with antifungal drug, the lowest weight of the spleen was 60 mg and highest weight was of 200 mg in the fourth week.

This pattern indicated that in the control group and even in mice that received lower concentration of fungi, the body system took a longer time to develop the capacity to build up the body immune systems. These results are similar to the findings of other authors relating to splenomegaly in human beings diagnosed with systemic infection due to Fusarium (Guarro and Gene, 1995).

Table 3. Weekly weight of spleen in experimentally infected *F. graminearum* mice

Week	PC	T1	T2	T3	T4	T5	NC
1	0.0499 (0.009)	0.0435 (0.085)	0.0660 (0.006)	0.0395 (0.005)	0.0405 (0.0025)	0.0405 (0.0045)	0.0380 (0.003)
2	0.073 (0.0015)	0.073 (0.005)	0.0775 (0.005)	0.0675 (0.005)	0.126 (0.014)	0.063 (0.0015)	0.064 (0.002)
3	0.075 (0.005)	0.165 (0.055)	0.085 (0.005)	0.11 (0.01)	0.165 (0.055)	0.125 (0.005)	0.0415 (0.035)
4	0.08 (0.002)	0.0825 (0.0025)	0.055 (0.005)	0.105 (0.005)	0.2 (0.01)	0.1275 (0.0075)	0.045 (0.003)
5	0.125 (0.005)	0.115 (0.005)	0.0365 (0.0015)	0.079 (0.001)	0.175 (0.005)	0.1225 (0.005)	0.0455 (0.005)
6	0.0985 (0.005)	0.099 (0.005)	0.035 (0.005)	0.062 (0.005)	0.105 (0.005)	0.099 (0.005)	0.0485 (0.005)

Data are mean± weights of spleen (g) from animals experimentally infected with the fungal pathogen determined at the indicated time intervals. Data are mean± weights of spleen (g) from animals experimentally infected with the fungal pathogen determined at the indicated time intervals.

SUMMARY, CONCLUSION AND RECOMMENDATION

Based on the findings gathered in this study, *F. graminearum* is a pathological agent, which has the ability to infect vital organs of the body, which would cause impairment of organ functions. This pathogen possesses the ability to digest elastin and collagen that may be seen in body tissue which could be attributed to the manifestation of the disease. This study also indicated that *F. graminearum* caused substantial pathological damage to liver, lungs, and spleen. The pathogen was found to induce leukocytosis and marked increase of lymphocytes, neutrophils and macrophage. It was found that when infection was induced, the mortality ranged from 20% in first week and declined to 7.69% in the third week in positive control group and untreated group. While in treated group, mortality was only 5% from the first to third week.

Taking the result of this study, it is recommended that effort should be directed toward the prevention of *F. graminearum* infection in animals. Moreover, further studies should be conducted to confirm the direct involvement of other species of Fusarium on Deg Nala disease which would require application of recent fungal isolation and identification procedures. Finally the application of diethylamine acetarsol or its derivatives as a treatment for *F. graminearum* infection in domestic animals should be looked into.

REFERENCES

1. Afzal, B., R. Ahmad, T. Hussain And G. Jilani. 1994. Development For Em-Technology To Replace Chemical Fertilizers In Pakistan. In "Third Conference On Effective Microorganisms (Em).
2. Kyusei Nature Farming Center. Saraburi, Thailand. Pp. 45-66.
3. Anaissie, E., H. Kantarjian, P. Jones, B. Barlogie, M. Luna, G. Lopez-Berestein And G. Bodey. 1986. Fusarium: A Newly Recognized Fungal Pathogen In Immunosuppressed Patients. Cancer. 57:2141-2145.
4. Andrews, S. And J. I. Pitt. 1986. Selective Medium For Isolation Of Fusarium Species Dematiaceous Hyphomycetes From Cereals. Appl. Environ. Microbiol. 51:1235- 1238.
5. Arora S. P. , Kaur, P., Khirwar S.S. Chopra, R.C. And Ludri, R.S. (1975. Selinium Levels In Fodders And Its Relationship With Degnala Disease, Indian Vet. J. 50:482-84.
6. Bhatia, K.C. (1979). Studies On Degnala Disease With Particular Reference To Its Etipathology. Ph.D. Thesis, Haryana Agricultural University, Hisar.
7. Booth, C. 1971. The Genus Fusarium. Commonwealth Mycological Institute.
8. Boutati, E. I. And E. J. Anaissie. 1997. Fusarium, A Significant Emerging Pathogen In Patients With Hematologic Malignancy: Ten Year's Experience At A Cancer Center And Implications For Management. Blood. 90:999-1008.
9. Caplin, I. And D. L. Unger. 1983. Molds In The Southern California Deserts. Ann. Allergy. 50:260-263.
10. Casadevall, A. And L.A. Pirofski. 1999. Host-Pathogen Interactions: Redefining The Basic Concepts Of Virulence And Pathogenicity. Infect. Immun. 67:3703-3713.

11. Cho, C. T., T. S. Vats, J. T. Lowman, J. W. Brandsberg And F. E. Tosh. 1973. Fusarium Solani Infection During Treatment For Acute Leukemia. J. Pediatrics. 83:1028-1031.
12. Dhillon, K. S. 1973. Preliminary Observation On The Treatment Of Deg Nala Disease In Buffaloes. Indian Vet. J. 50: 5, 482-484.
13. Doyle, M.E. 1997. Fusarium Mycotoxins. Food Research Institute. Uw-Madison.
14. Guarro, J. And J. Gene. 1992. Fusarium Infections. Criteria For The Identification Of The Responsible Species. Mycoses. 35:109-114.
15. Guarro, J. And J. Gene. 1995. Opportunistic Fusarial Infections In Humans. Eur. J. Clin. Microbiol. Infect. Dis. 14:741-754.
16. Http://Www.Criver.Com/03cat/M/Mice/Balb/Cmice.Html, Copyright 2003, Charles Laboratories England
17. Http://Www.Nlacmolidol.Ac.Th/Page_Eng/Characteristic_Of_Inbred_Mouse.Html
Hue, F. X., M. Huerre, M. A. Rouffault And C. De Bievre. 1999. Specific Detection Of Fusarium Species In Blood And Tissues By Pcr Technique. 37:2434-2438.
18. Irfan, M. 1971. The Clinical Picture And Pathology Of Deg Nala Disease In Buffaloes And Cattle In West Pakistan. Vet. Rec. 88:422-424.
19. Irfan, M. And A. Maqbool. 1986. Studies On Deg Nala Diseases In Cattle And Buffaloes. Pakistan Vet. J. 6:87-93. Joffe, A. Z. 1978. Fusarium Poae And F. Sporotrichioides As Principal Causal Agents Of Alimentary Toxic Aleukia. In "Mycotoxic Fungi, Mycotoxins, Mycotoxicoses: An Encylopaedic Handbook. Vol. 3." T. D. Wyllie And L. G. Morehouse (Eds.). New York: Marcel Dekker. Pp. 21-86.
20. Kalara, D.S. Bhatia , K.C. Gautam, O.P. And Chuahan, H.V.S. (1972). An Obscures Disease (Possibly Degnala Disease) In Buffaloes And Cattle Studies On Its Epizootiology, Pathology And Etiology. Haryana Agric. Univ. J.Res. 2:256-64.
21. Karki, K. 1986. Deg Nala Disease In Nepal. District Livestock Services, Banke Nepal. Annual Report. P. 56. (Unpublished)
22. Kwatra, M.S. And Singh, Ajit (1972). Epidemiology And Etiological Observations On Gangrenous Syndrome In Buffaloes. J. Res. Punjab Agric.Univ. 8:518-25.Rajan, A., Krishnan Nair, M., Alikutty,
23. K.M., Maryama, K.I. And Valsala, K.V. (1977). Pathology Of Necrosis Extremities In Bovine – A Disease Resembling Degnala Disease. Kerala J. Vet. Sci. 8:77-86.Romans, G.B. (Personal Communication, Pathologist)
24. Segal, B. H., T. J. Walsh. J. D. Wilson And K. J. Kwon-Chung. 1998. Invasive Infection With Fusarium Chlamydosporum In A Patient With Aplastic Anemia. 36: 1772-1776.
25. Shirlaw, J. E. 1939. Deg Nala Disease Of Buffaloes: An Acccount Of The Lesions And Essential Pathology. Indian Vet. Sci. Anim. Husb. 9: 853-864.

CONFLICT OF INTEREST : Nil
Received : 01.06.2015
Accepted : 17.08.2015

CITATION OF THIS ARTICLE

Kedar B. Karki and Gemerlyn G. Garcia. Assessment of the Pathogenicity Property of *Fusarium graminearum* 1 in Balb/C Mice. World J. Clin. Pharmacol. Microbiol.Toxicol. Vol 1 [3] September 2015. 53-57