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Retrospective study of FMD serotypes and seasonal analysis of outbreaks using sero-typing strategy in Uttar Pradesh (India)

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Abstract

A laboratory evidence based study on FMD was conducted on the diagnosis of retrospective serotyping and seasonal analysis of outbreaks in cattle, sheep, pigs, goats and buffalo. The sample size was tongue epithelium, feet epithelium and blood serum (n= 40) and the samples were screened for the evidence of outbreak of FMD in Mathura, Pratapgarh, Jaunpur, Bhadoi and Etawa district of this study location. Seasonal analysis of the FMDV outbreaks revealed that out of total nineteen outbreaks, the highest 42.10% occurred in April followed by 26.30% in January and 15.80% in both December and March. The maximum numbers of outbreaks are observed in cattle followed by sheep and buffalo. For serotyping study of virus from five districts of Uttar Pradesh all the forty samples were processed using sandwich ELISA and LAB-ELISA techniques and the two-virus type, serotype A FMDV (84.61%) and serotypes O FMDV (15.39%) were recorded. However, serotype C and Asia-1 FMDV was not recorded from any of the outbreaks during the study year. Further we conducted a retrospective diagnosis study of antibody titre of more than 1.8 $\log_{10}$ against FMDV samples of serotype A were recorded 32 sera samples, 7 sera samples were found showing high titre against serotype O FMDV, 4 sera samples were found showing higher titre against FMDV type Asia-1. The present research work of FMDV outbreaks, April (beginning of summer season) and September (end of long rainy season), two times per, year can be suggested as the most suitable time for vaccination against FMD in the study area and imperative that findings of this work might be helpful in to validate the knowledge on diagnosis of FMD.

Keywords: FMDV, serotyping, virus types O, A and asia-1, outbreaks, seasonal analysis and retrospective diagnostic studies.

Introduction

Foot and mouth disease is an acute and highly contagious febrile disease affecting cattle, sheep, pigs, goats, buffalo and many species of cloven-hoofed wild life. FMD caused by a single stranded RNA virus belonging to the genus *Aphthovirus* in the family *Picornaviridae*. Identification of the FMD virus (FMDV) the causative agent of the disease, posed problems because of the occurrence of many types and subtype of the virus. Foot and mouth disease cause restriction to the trade of live animals and livestock products internationally (11). It is characterized by fever, loss of appetite, salivation and vesicular eruptions on the feet, mouth and teats (23). FMD is a global disease that through the years has affected most of the countries. It occurs throughout the world, most commonly in

Asia, Africa, the Middle East, and parts of South America. North America, Central America, Australia, New Zealand, Chile, Japan, and most of European countries have been recognized as free, and Uruguay and Argentina have not had an outbreak since April 1994 (20). Due to poor reporting from the African continent, FMD is considered endemic in most of the African Countries with only Morocco (based on serological survey), Swaziland, Lesotho, Zimbabwe, Namibia, Botswana and the Republic of south Africa being considered free of the disease by the OIE in 1999 (12). Infections in humans are very rare and minor clinical significance (2, 18). The disease is characterized by the formation of vesicles (fluid-filled blisters) and erosions in the mouth, nose, teats and fee tare.

Foot and mouth disease is endemic in India since many centuries. It is present almost in all parts of the country and occur round the year. Approximately 470 million domestic livestock are susceptible to FMD apart from the free living and captive wild ungulates. During centuries of evolution of FMD in the field, repeated opportunities for variation have led to the viral diversification which is, now a days, reflected in the co-existence of seven serotypes: A, O, C, SAT-1, SAT-2, SAT-3 and Asia-1 in the world (20). However in India only O, A, C and Asia-1 have been reported. Serotype 'C' too has not been recorded in the country since 1995. Serotype O is the most prevalent of the seven serotype and occurs in many parts of the world. Within type O, genetic lineages fall into geographically distinct groups known as topotypes. The Middle East, South Asian (ME-SA) topotype comprises a grouping of genetically similar viruses that is endemic to the region, from which the Pan Asia strain appears to have emerged (21). Although the exact origin of the Pan Asia strain is uncertain, the virus was first identified in northern India around 1990. FMD is endemic on the Indian subcontinent and approximately 90% of outbreaks caused by type-O virus (9). Vaccination against FMD is grossly inadequate in the country. The annual loss due to FMD in India is roughly estimated US dollar 800 million.

Currently there are seven serotypes of foot and mouth disease virus (FMDV), namely O, A, C, Southern African Territories (SAT) 1, 2 and 3, and Asia 1, which infect cloven-hoofed animals. Within these serotypes, over 60 subtypes have also been described using biochemical and immunological tests; and new subtypes occasionally arise spontaneously. However, at a specific time, there are only a few subtypes causing disease throughout FMD endemic areas. The importance of subtypes is that a vaccine may have to be tailored to the subtype present in the area in which the vaccine is being used (OIE, 2004). At present, a sequencing of FMD virus is increasingly being used to establish intratypic variations of FMD viruses and classifying viruses in to genotypes and lineages (20).

FMD is probably one of the most important livestock diseases in the world in terms of economic impact. The economic importance of the disease is not only due to the ability of the disease to cause losses of production, but also related to the reaction of veterinary services to the presence of the disease and to the restrictions on the trade of animals both locally and internationally (11). Despite the wide spread and enormous economic importance of FMD in India, clinical and serological studies to characterize the disease, under local Indian conditions have never been exhaustive and the endemic level has not been established. The extent to which a disease is recognized as a problem is often dependent on the efficacy of the means for diagnosing it and observing its occurrence (15). Therefore the main objectives of this study to validate the knowledge on diagnosis of FMD, note the earliest signs of affected animals, identify the serotypes of FMD

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virus circulating in the study areas of Uttar Pradesh and their retrospective diagnostic studies using sandwich ELISA and LPB ELISA method.

Materials and Methods

Diagnosis of FMD infected animals

Clinical diagnosis based on lesion identification, in the early stage of infection, FMD virus or viral antigens can be detected using several techniques. However, different serological methods are used to detect antibody against FMD virus and is the main indication that infection has taken place. The diagnosis of FMD infected animals can be characterized into two categories.

Field Diagnosis

In cattle and buffalo, FMD should be considered whenever salivation and lameness occur simultaneously and a when a vesicular lesion is seen or suspected. Fever often precedes other clinical signs; therefore, febrile animals should be carefully examined. Early diagnostic lesions may be found before animals start to salivate, have a nasal discharge, or become lame. Field diagnosis can present many difficulties due to viral infections of the mucous membrane, which produce similar clinical signs. Differential diagnosis for FMD should include vesicular stomatitis, rinderpest, malignant catharal fever, the bovine herpes infections, swine vesicular disease, vesicular exanthema of swine and bluetongue (3).

Laboratory Diagnosis

Due to the highly contagious nature and economic importance of FMD, the laboratory diagnosis and serotype identification of the virus should be done in a virus-secure laboratory (17). Appropriate samples for FMD laboratory diagnosis are; Vesicular fluid usually contains the highest quantity of virus. Epitheliums from early vesicles and from recently ruptured vesicles are tissue of choice for virus isolation (17). When epithelium tissue is not available from ruminant animals e.g. in advance or convalescent cases and infection is suspected in the absence of clinical sign, samples of oesophageal-pharyngeal fluids (OP) is collected by means of a probang and used for virus isolation (1). Other samples such as, blood with anticoagulant, Serum, and lymph nodes, thyroid gland, adrenal gland, kidney, and heart are good sources of specimens from postmortem.

Serum sample collection

The sample size was tongue epithelium, feet epithelium and blood serum (n= 40) were collected from FMD affected cattle, buffalo, goat and sheep population in five districts of Uttar Pradesh namely: Mathura, Pratapgarh, Jaunpur, Bhadoi and Etawa. The whole blood was collected from a jugular vein of randomly selected animals into 10 ml sterile vacationer tubes and stored overnight at room temperature for serum separation. The serum was then transferred into a single sterile cryovial, bearing the names of the animal species with sample number and transported in an icebox, to laboratory under cold condition. Serum samples preserved in 50% phosphate buffer saline (PBS), glycerol, pH 7.4 and stored at -20^{0}C until laboratory investigation.

Experimental Procedures

Liquid phase blocking ELISA

Antibody detection by liquid phase blocking ELISA detects and quantifies FMDV antibodies in serum of both infected and vaccinated animals (8). Plates were coated with 50µl trapping rabbit antibody stock (Rabbit anti-FMDV serotypes O, A, C and Asia-1) diluted 1:1000 in coating buffer (carbonate/bicarbonate) into 96 wells of ELISA plate reader (Hindustan Electronics) microplate and incubated at 40^{0}C

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over night. Simultaneously, 50µl of test and control sera (C++, C+ and C-), diluted 1:16 in diluents buffer A (PBS and Tween 20) were added into wells of flat-bottomed microplates and 50µl of FMDV antigen (serotypes O, A, C and Asia-1) diluted at suggested working dilutions were added into all 96 wells of the perspective polypropylene flat-bottomed microplates. Sera and antigen were mixed and incubated at 40C over night, washed with dilution of PBS at pH 7.4, three times; then, 50µl serum-antigen mixture was transferred from flat-bottomed microplates to the appropriate wells of ELISA plate reader plate and incubated at 37^0C for one hour, with continuous shaking. After microplates were washed, 50µl detecting antibody (Guinea pig anti-FMDV serotypes O, A, C and Asia-1), diluted 1: 1000 in diluent buffer B (PBS, Tween 20 and skimmed milk powder) was added into all 96 wells of the respective microplates and incubated at 37^0C for one hour with continuous shaking. After washing the plates, 50µl of conjugate (Horseradish peroxides conjugated rabbit anti-guinea pig immunoglobulin) diluted 1:200 in diluent buffer B was added into 96 wells of each microplate and incubated at 37^0C, for one hour with continuous shaking. Finally, the plates were washed and 50µl of substrate/chromogen (hydrogen peroxide (H_2O_2)/Ortho-Phenylene-diamine (OPD) solution was added and incubated at ambient temperature for 15 minutes (briefly placed on the shaker to ensure even mixing) before 50µl of stopping solution sulphuric acid H_2SO_4 was added into all 96 wells of the microplates. The ELISA reader was connected to the computer loaded with ELISA Data Information (EDI) Software, which is used to automate the reading of OD value and calculate the percentage inhibition (PI).

Antigen titration

Antigen titration procedures were used (7) to check the working dilution of each FMD antigen used in serotyping. Plates were washed three times between each stapes except after substrate added.

Estimation of titres

The percent inhibition in each well was calculated in reaction to antigen control using the formula:

$$\text{Percent inhibition} = 100 - \frac{\text{OD of test well} - \text{background OD}}{\text{OD of Ag control well - background}}$$

The reciprocal of log $_{10}$ dilution corresponding to 50% inhibition was considered to be the titre of the serum.

Data collection and analysis

The participatory and laboratory investigation results were analyzed using Statistical Package for Social Sciences (22) and Statistical software (STASOFT version 6.0), respectively.

Results and Discussion

Retrospective study of FMD serotypes disease outbreak using sero-typing strategy the two approaches was used e.g. field analysis and laboratory analysis of FMD. Firstly, the field analysis to diagnoses the FMD affected animals and note their earliest signs, Secondly, in laboratory analysis the seasonal variations of FMDV outbreaks and sero-typing retrospective studies using diagnostic tools and techniques LAB-ELISA.

Field analysis of FMD

Diagnoses the earliest clinical signs

When susceptible animals are in contact with clinically infected animals, clinical signs usually develops in 3 to 5 days (13), although in natural infection, the incubation period may range from 2-14 days. The severity of clinical signs of the disease varies with the strain of the virus, the exposure dose, the age, and breed of the animal, the host species, and its degree of immunity. The signs can range from a mild or in apparent in

sheep and goats to a severe disease occurring in cattle and pigs (17). FMD should be suspected wherever; vesicles are seen in cloven-hoofed. Vesicles begin as small white fluid filled areas that quickly grow to a blister about 3cm in diameter. Two or more blisters may join to form a large one. The blisters usually burst leaving a raw surface. These heal over a few days (Fig. 1a and 1b).

In Cattle and Buffalo: The earliest sign observed in the FMD affected animals are fever of 103-105°F, dullness, poor appetite and fall in milk production. These signs are followed by excessive salivation, smacking of the lips, grading of the teeth, drooling, serous nasal discharge; shaking, kicking of the feet or lameness; and vesicle (blister) formation. The predilection sites for vesicles are areas where there is friction such as on the tongue, dental pad, gums, soft palate, nostrils, muzzle, interdigital space, coronary band, and teats (20, 24). After vesicle formation, drooling may be more marked, and nasal discharge, lameness, or both may increase. Pregnant cows may abort, and young calves may die suddenly without developing any vesicle because of inflammation of the heart (Myocarditis) (3). Morbidity can approach 100%, but Mortality in adult animals is rare, although in young animals death can occur due to myocarditis and mortality can exceed 50% (24). Pregnant cows may abort (3). The course of an FMD infection is 2 to 3 weeks although infection may delay recovery of mouth, feet and teat lesions, resulting in hoof deformation, mastitis, low milk production, failure to gain weight, and breeding problems. A lactating animal may not recover to pre infection production because of

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Fig. 1a. Thin ropey saliva (demarcating with circle) in cattle affected early with foot and mouth disease.

Fig.1b. Pictures showing the clinical signs of FMD Virus in Foot lesions (demarcating with circle) in Cattles and Buffalos.

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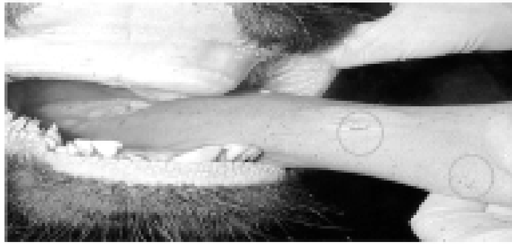
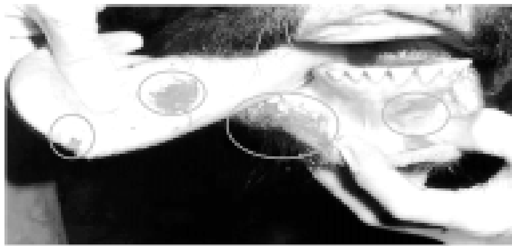
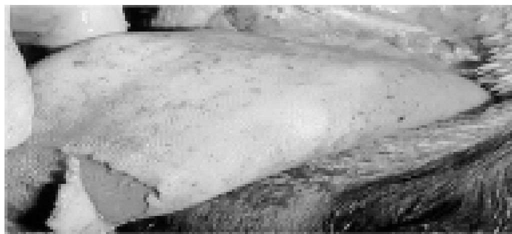


1. Tongue of a steer with 1-day-old vesicle. Which, ruptured when the tongue was drawn from the mouth.

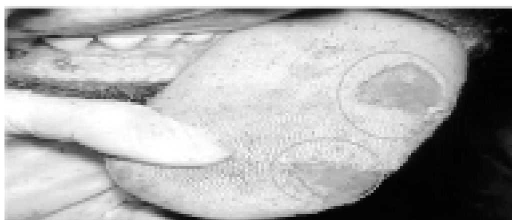
2. Steer with 2 days old ruptured vesicle along upper gum and several 1-day-old unruptured vesicles on the tongue.



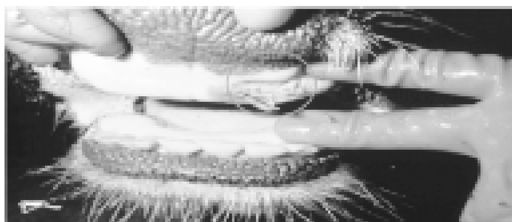
3. Two days old ruptured vesicles on the tongue, lower gum and lower lip of a steer.



4. A further examination of 2-days-old lesions in the mouth of a steer, sharp showing margins of lesions and red raw appearance of exposed dermis.



5. Tongue of a steer with 3 days old lesions. Sero-fibrinous exudation into the lesions has resulted in a loss of earlier red raw appearance and also sharpness of margination.



6. Mouth of a steer showing ruptured vesicles with exposed dermis on the upper gum.

Fig. 2. Pictures showing the clinical signs of FMD Virus(demarcating with circle) in Cattles and Buffalos.

damage to the secretory tissue. A chronic Panting syndrome characterized by dyspnoea, anaemia, hair overgrowth and heat intolerance has been reported as a sequel of cattle recovered from FMD associated with pituitary gland damage (4). If at pasture, the animal will be away from the rest of the heard and probably lying down. Loss of condition is marked because of the fever and because the mouth is so painful that the animal is afraid to eat Fig 2.

In Sheep and Goat

In sheep and goats, if the clinical signs occur, it tends to be very mild, and may include dullness, fever; and small vesicles or erosions on the dental pad, lips, gums, and tongue. Mild lameness may be the only sign Fig 3. . In lame animals, there may be vesicles or erosion on the coronary band or in the interdigital space. Infected animals may abort and nursing lambs may die without showing any clinical sign (10). Most of the signs listed for FMD were consistent which is indicated in veterinary literatures (5, 6, 19).

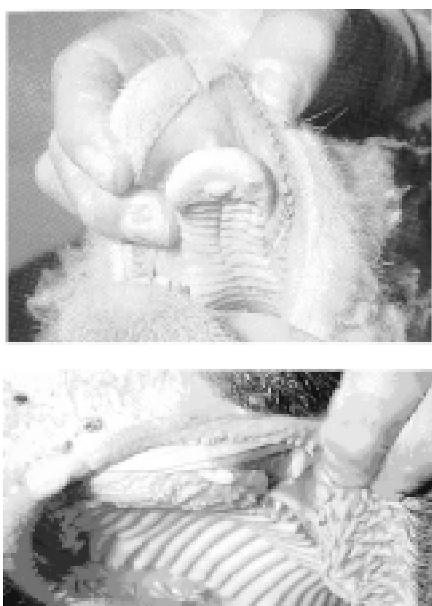


Fig. 3. Pictures showing the clinical sign of FMD Virus (demarcating with circle) in dental pad of Sheep.

Laboratory analysis of FMD

Serotyping retrospective studies based on ELISA and LAB-ELISA

The study of seasonal variations of FMDV outbreaks and sero-typing retrospective studies the techniques were used sandwich ELISA and LPB (Liquid Phase Blocking) ELISA. A total of n=40 samples were collected and investigated in nineteen outbreaks from five districts of Uttar Pradesh during the month of January to June, 2006. Seasonal analysis of the FMDV outbreaks (Fig. 4) revealed that out of total nineteen outbreaks, the highest 42.10% occurred in April (Eight outbreaks) followed by 26.30% in January (Five outbreaks) and 15.80% in both December and March (Three outbreaks). The maximum numbers of outbreaks are observed in cattle followed by sheep and buffalo (Table. 1). According to the annual report of Animal Health Division of Ministry of Agriculture in 2000, the incidence of FMD outbreaks has increased by 1.3-1.5 folds since 1990 (20). Extensive movement of livestock, the high rate of contact among animals at commercial markets, in communal grazing areas and at watering points, were among the reasons forwarded for the

Seasonal analysis of the FMDV outbreakes in %

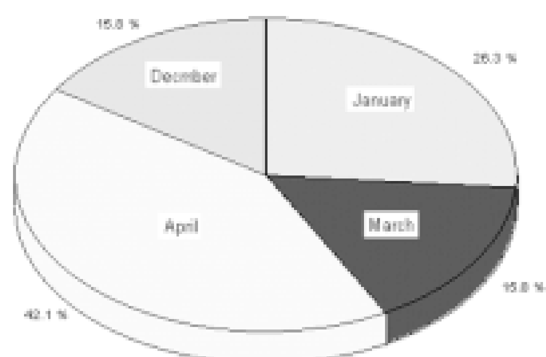


Fig. 4. Relationship between seasonal variations of FMDV and outbreaks percentage

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Table 1: Identification the virus type and distribution of FMDV in the affected ***Animals.***

Species	Number of Specimens	Virus types				
		"O"	"A"	"C"	Asia-1	*VNR*
Cattle	10	1	9	-	-	-
Buffalo	27	1	-	-	-	26
Goat	1	-	-	-	-	1
Sheep	2	-	2	-	-	-
Total	**40**	**2**	**11**	-	-	**27**

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increasing incidence of the disease in recent years (14). Disease was started from Mathura, Pratapgarh, Bhadoi, Etawa and Jaunpur districts of U.P. in the month of April (Fig. 4). For serotyping study of virus from five districts of Uttar Pradesh all the forty samples were processed using sandwich ELISA and LAB-ELISA techniques and the two virus type, serotype A FMDV (84.61%) and serotypes O FMDV (15.39%) were recorded. However, serotype C and Asia-1 FMDV was not recorded from any of the outbreaks during the year 2006 (Table. 1). The retrospective studies revealed the antibody titre of post infected animals (unvaccinated) against virus type O, A, C and Asia-1 (Table 2). In addition to 40 clinical samples from 19 outbreaks mentioned above 43 sera samples were also collected from affected / convalescing animals from another five FMD outbreaks and analyzed for antibody titre against FMD types O, A and Asia-1 for retrospective FMD diagnosis. Of these, antibody titres more than 1.8 $\log_{10}$ against FMDV serotype A were recorded 32 sera samples, 7 sera samples were found showing high titre against serotype O FMDV, 4 sera samples were found showing higher titre against FMDV type Asia-1 (Table 3). These retrospective studies could be used in addition to routine FMD typing as a definitive indication for FMD virus typing work. It is imperative that findings of this work might be helpful in formulating FMD emergency vaccination, within an infected area, has gained more preference in recent years, in an attempt to reduce the amount of virus circulating and spreading beyond the restricted area. The use of emergency FMD vaccines has two clear objectives. Firstly, to provide protective immunity, as rapidly as, possible to susceptible stock, and secondly, to reduce the amount of virus released and thereby limit the spread of disease and vaccination strategies in light of the concept of 'FMD free zone programme. Therefore, based on these findings, the following is recommended; the participatory epidemiological and conventional veterinary methods are complementary and they should be used side by side during animal health research, especially in FMD affected areas. An extensive regular serological survey, virus isolation, and characterizations of FMDV need to be conducted for a possible development of polyvalent vaccine. Based on the present study of FMDV outbreak, April (beginning of summer season) and September (end of long rainy season), two times per, year can be suggested as the most suitable time for vaccination against FMD in Utter Pradesh.

Table 2: Antibody titre (log $_{10}$) of sera samples tested for retrospective study

S.no.	Sample type	Sample no.	District	Species/ Breed	Sex	O	A	Asia-1
1	Serum	S-01/MTH/05	Mathura	B	F	1.5	1.5	1.2
2	Serum	S-02/PTG/05	Pratapgarh	B	F	>2.1	1.8	1.8
3	Serum	S-03PTG/05	Pratapgarh	B	F	1.2	1.3	1.5
4	Serum	S-04/PTG/05	Pratapgarh	B	F	1.2	1.5	1.2
5	Serum	S-05/JNP/05	Jaunpur	S	F	1.8	1.5	1.5
6	Serum	S-06/JNP/05	Jaunpur	S	F	>2.1	1.8	1.2
7	Serum	S-07/JNP/05	Jaunpur	S	F	1.8	1.8	1.8
8	Serum	S-08/JNP/05	Jaunpur	S	F	1.8	1.5	1.5
9	Serum	S-09/BHD/05	Bhadoi	C	F	>2.1	1.5	1.5
10	Serum	S-10/BHD/05	Bhadoi	B	F	>2.1	1.8	>2.1
11	Serum	S-71/ETW/05	Etawa	C	F	<1.5	>1.8	<1.5
12	Serum	S-72/ETW/05	Etawa	C	F	1.2	2.1	1.2
13	Serum	S-73/ETW/05	Etawa	C	F	<1.5	>1.8	1.5
14	Serum	S-74/ETW/05	Etawa	C	F	1.2	2.1	<1.5
15	Serum	S-75/ETW/05	Etawa	C	F	1.6	1.6	1.6
16	Serum	S-76/ETW/05	Etawa	C	F	<1.5	>2.1	<1.5
17	Serum	S-77/ETW/05	Etawa	C	F	1.2	2.1	1.2
18	Serum	S-78/ETW/05	Etawa	C	F	1.2	1.8	<1.5
19	Serum	S-79/ETW/05	Etawa	C	F	0.9	2.1	1.2
20	Serum	S-80/ETW/05	Etawa	C	F	0.9	>1.8	1.2
21	Serum	S-89/ETW/05	Etawa	C	F	1.5	1.5	<1.5
22	Serum	S-99/ETW/05	Etawa	C	F	<1.5	>1.8	1.5
23	Serum	S100-/ETW/05	Etawa	C	F	1.2	2.1	<1.5
24	Serum	S-101/ETW/05	Etawa	C	F	1.2	>1.8	<1.5
25	Serum	S-102/ETW/05	Etawa	C	F	<1.5	2.1	1.2
26	Serum	S-105/ETW/05	Etawa	C	F	1.4	2.1	<1.5
27	Serum	S-106/ETW/05	Etawa	C	F	<1.8	>1.8	1.5
28	Serum	S-107/ETW/05	Etawa	C	F	<1.5	2.1	1.8
29	Serum	S-109/ETW/05	Etawa	C	F	1.2	2.1	1.2
30	Serum	S-110/ETW/05	Etawa	C	F	1.2	2.1	<1.8
31	Serum	S-111/ETW/05	Etawa	C	F	1.2	>1.8	<1.5
32	Serum	S-112/ETW/05	Etawa	C	F	<1.5	>1.8	<1.5
33	Serum	S-113/ETW/05	Etawa	C	F	<1.5	2.1	<1.8
34	Serum	S-114/ETW/05	Etawa	C	F	1.2	2.1	<1.5
35	Serum	S-115/ETW/05	Etawa	C	F	0.9	2.1	1.2
36	Serum	S-116/ETW/05	Etawa	C	F	1.4	1.6	1.5
37	Serum	S-117/ETW/05	Etawa	C	F	1.6	1.2	1.2
38	Serum	S-118/ETW/05	Etawa	C	F	1.6	1.6	1.5
39	Serum	S-119/ETW/05	Etawa	C	F	1.2	1.8	1.2
40	Serum	S-120/ETW/05	Etawa	C	F	<1.5	2.1	<1.5
41	Serum	S-121/ETW/05	Etawa	C	F	1.2	>1.8	1.2
42	Serum	S-122/ETW/05	Etawa	C	F	1.2	<2.1	1.5
43	Serum	S-123/ETW/05	Etawa	C	F	1.5	<2.1	<1.5

B – Buffalo, C – Cattle, S – Sheep, F – Female

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Table 3: Serum antibody status of post infected animals (Unvaccinated) against type O, A and As-1 in sera collected during 2006.

S. No.	District	No. of sera sample tested	Anti body titre Log_{10}					
			Titre < 1.8			Titre >1.8		
			O	A	Asia-1	O	A	Asia-1
1.	Etawa	33	33	5	32	-	28	1
2.	Bhadoi	2	-	1	1	2	1	1
3.	Jaunpur	4	-	2	3	4	2	1
4.	Mathura	1	1	1	1	-	-	-
5.	Pratapgarh	3	2	2	2	1	1	1
Total		**43**	**36**	**11**	**39**	**7**	**32**	**4**

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Growth inhibition and induction of apoptosis in estrogen receptor-positive and negative human breast carcinoma cells by *Adenocalymma alliaceum* flowers

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Abstract

Adenocalymma alliaceum (*A. alliaceum*) is well known for its traditional medicinal uses and as a substitute for garlic. The methanol extract from *A. alliaceum* flowers (*AAF*) was investigated for its growth inhibitory activity on the estrogen receptor positive MCF-7 and estrogen receptor negative MDA-MB231 breast cancer cells by MTT assay. Treatment of breast cancer cells with different concentrations of *AAF* resulted in dose dependent growth inhibition with a growth inhibitory concentration (GI_{50}) of 53.1±4.1 µg/mL in MCF-7 and 23.9±3.7 µg/mL in MDA-MB-231 cells. Treatment of breast cancer cells with *AAF* resulted in time-dependent sequence of events marked by apoptosis, as shown by translocation of phosphatidylserine and activation of caspase-3. Analysis of data suggests that *AAF* exerts growth inhibition on both breast cancer cells through apoptosis induction, and that it may contain potent anticancer secondary metabolites valuable for application in drug products.

Keywords : *Adenocalymma alliaceum*, breast cancer cells, apoptosis, enrichment factor, sulphur compounds

1. Introduction

Breast cancer is the second leading cause of cancer-related deaths (1). The treatments include surgery, radiation, and in some cases, drugs that have a specific target such as tamoxifen in estrogen-dependent tumours (2). However, the majority of cases, especially those that result in metastasis, are still treated with conventional chemotherapy. The problem in drug resistance is a major obstacle in chemotherapeutic treatment, therefore, there is a great need for the development of new therapeutic drugs that will be more efficient or will synergise with existing ones.

There has been a growing interest in the use of herbs as a potent source of new therapeutic anticancer drugs. Plants contain a wide variety of secondary metabolites that have potent biological effects, including anticancer activity (3). In this research we focused on the growth inhibitory effect of the *Adenocalymma alliaceum* flowers on breast cancer cells and its mode of action.

Adenocalymma alliaceum Miers. (family: Bignoniaceae), commonly known as 'garlic creeper', is native to the Amazon rain forests of South America. The leaves and flowers are widely consumed by Brazilians as a substitute for garlic (4). The plant has a number of traditional medicinal properties such as antimycotic, analgesic, antiarthritic, anti-inflammatory, antipyretic, antirheumatic, antitussive, depurative, purgative, and vermifuge