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PREVALENCE AND DISTRIBUTION OF CUCUMBER MOSAIC VIRUS (CMV) IN MAJOR CHILLI GROWING AREAS OF PAKISTAN

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Abstract

In order to monitor and determine the incidence of *Cucumber mosaic virus* chilli pepper. Surveys were conducted in 73 fields throughout the Pakistan during 2006-07. Total 706 samples (Sindh 191, Punjab 257, KPK 51 and Balouchistan 207) were collected and tested through Direct Antigen Coating Enzyme Linked Immuno-sorbent Assay (DAC-ELISA). ELISA results indicated that CMV prevails throughout the Pakistan with a relative incidence of 44.7%. Maximum incidence of CMV was recorded in Sindh (51.8%) and Balochistan (17.47%) followed by Punjab (8.5%) and KPK (7.8%). No district was found free from CMV infection in Sindh and Balochistan. Among the weed flora of chilli pepper field, *Trianthema pentandra* (itsit), *Cyprus rotundus* (Deela) and *Portulaca olercea* (Kulfa) were found to be infected among the samples collected from the Sindh province.

Introduction

Chilli pepper (*Capsicum* spp.), member of family *Solanaceae*, is both a vegetable and a spice crop of significant economic value in Pakistan (Khan *et al.*, 2006). Chillies are the most important vegetable ranked after potato and tomato. It is a significant cash crop of Pakistan among the vegetable grown. *Capsicum annuum* L., and *Capsicum frutescens* L., are commonly grown species in Pakistan. The crop occupies 20 % of the total area under vegetable cultivation and is mainly concentrated in Sindh and Punjab province and on small scale in NWFP and Balochistan. Chillies are very sensitive to cool and wet weather, as the climatic conditions of Pakistan are very diverse and are grown in different ecological zones in each province. It covers an area of 57.44 thousand hectares, yielding an annual production of 186.5 thousand tonnes with an average of three tonnes per hectare. Sindh province produces 80% of chilli crop followed by Punjab with 10%. The crop is mainly concentrated in Layyia in Punjab and Hyderabad, Kunri and Tharparkar areas in Sindh province (Anon., 2009-10). Viral diseases of plants caused economic losses of 15 billion dollars per annum on worldwide basis (Van Fanbing, 1999), particularly in tropic and semi tropics regions, which provide ideal conditions for the perpetuation of viruses and their vectors. Losses due to plant viruses have also been reported in temperate regions of the world (Hull & Davies, 1992). Natural occurrence of several viruses have been reported on chilli by various workers (Martelli & Quacquarelli, 1983) and among them *Chilli leaf curl virus* (CLCV), *Cucumber mosaic virus* (CMV) and *Chilli veinal mottle virus* (ChiVMV) have been reported as most destructive viruses affecting chilli cultivation in term of incidence and yield losses (Green, 1992). Viruses disease complex produced various types of disease syndromes like mosaic, leaf distortion, yellowing, vein etching, stunting and narrowing of leaves (Green, 1991). CMV reduces the yield by 60% (Joshi & Dubey, 1973) and losses up to 97% have been reported in tropical area (Anon., 1993) under normal circumstances. CMV incidence is always lower than ChiVMV under experimental condition (Idris *et al.*, 2001). Weed hosts function as a reservoir for the virus and serve as primary source of inoculum for the development of disease epidemics. To date, there is one study of CMV infecting chilli pepper following by ChiVMV in Pakistan and this study showed that chilli pepper crop was also infecting with CMV (Shah *et al.*, 2001). Therefore the present paper study was conducted to determine incidence, prevalence and distribution of CMV in chilli pepper using enzyme linked immuno-sorbent assay (ELISA).

Material and Methods

Surveys and sample collection: Fields were selected randomly from various locations in four province of Pakistan in 2006-07 (Fig. 1). Surveys were performed in 73 fields of chilli throughout the Pakistan. Random and non-random sampling was done in such a way that *Capsicum* showing stunted plants with mottled, malformed and puckered leaves bearing small fruits deformed and marked by off coloured sunken areas were collected (Guldur *et al*., 2006). The sampling method described by Cochran (1977) was applied in this study, weed flora was also collected from chilli field where available and subjected to ELISA to determine as an alternate host of CMV in the fields. However they were not used in calculating the virus incidence data. All collected samples were placed in polythene bags, stored at 4°C and tested for the virus infection through DAC-ELISA.

Serological test (DAC-ELISA): The direct antigen coating (DAC- ELISA) method was performed according to Hobbs *et al.,* (1987). Chilli samples were ground in pestle and mortar by using coating buffer (Na_2CO_3 and $NaHCO_3$) pH 9.6 and 100 µL was loaded in each well of ELISA plate. Plate was then incubated at 4°C overnight in a refrigerator and washed with 3-4 times with PBST buffer. Polyclonal antiserum was added to healthy plant sap @ 1:1000µl and incubated for 1-2 hour at 30°C with repeated washing. 100µL of cross absorbed material was added to each well of the ELISA plate and the plate cover and with moist filter paper and incubated at 37°C for 4

hour or overnight at 4°C. Repeated washing was observed. Whole molecule goat anti rabbit conjugated (Sigma brand) was diluted in a conjugated buffer @ 1:30,000µL. 100µL of the conjugated mixture was added to each well of the ELISA plate and covered the plate with moist filter paper and incubated at 37°C for 4 hour or over night at 4°C followed by washing. Substrate solution was prepared by dissolving p-nitro-phenyl–phosphate tablet @ 0.5mg/mL immediately before use. 150µL of substrate was added to each well of the ELISA plate and incubated at room temperature in dark for 30 minutes or as needed for colour to develop. The colour development was observed visually and reaction was stopped by adding 50 µL of 3*M* NaOH. Absorbance value were read at 405nm using a micro-plate reader (company name) and also visually evaluated. Virus free chilli plants grown in insect proof growth chamber were used as negative control. Samples were considered to be positive when the absorbance at 405nm value exceeded 2 times from negative control.

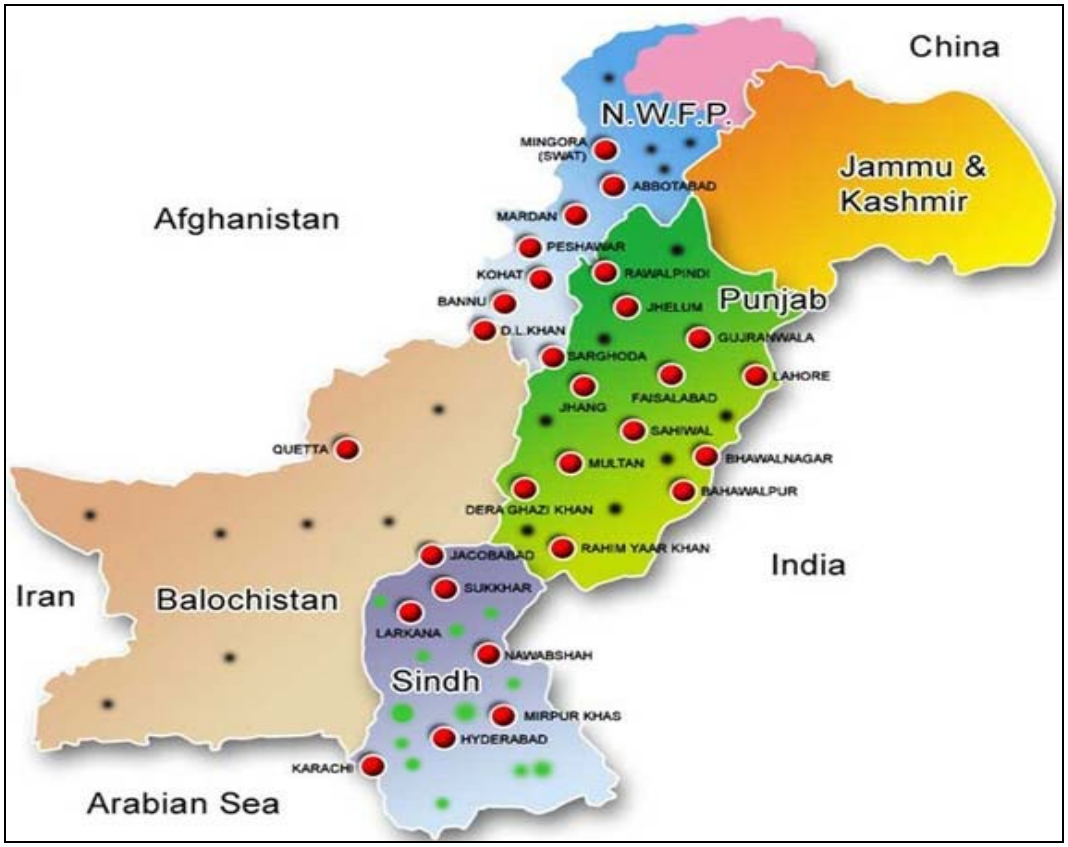

Fig. 1. Map of Pakistan showing the distribution and prevalence of CMV in Chilli growing areas of Pakistan.

Results and Discussion

A total of 73 fields were surveyed and 706 plant samples were randomly and non-randomly collected during survey conducted in the year 2006-2007. All the collected samples were subjected to DAC- ELISA and results of serological test showed that no district was found free from CMV infection. The relative incidence of CMV in Sindh, Punjab, NWFP (KPK) and Balochistan was recorded as 51.8%, 8.5%, 7.8% and 17.47% respectively (Fig. 2). CMV was found with higher incidence in Sindh province than other provinces of Pakistan. Shah *et al.,* (2001) and Shah *et al.,* (2009) also reported higher incidence of ChiVMV followed by CMV in Sindh.

CMV prevalence in Sindh: Sindh is the major chilli pepper growing area of Pakistan. Hot pepper is mostly cultivated throughout the year due to its diverse ecological conditions. During the survey year 2006-2007, the relative incidence was 40, 20, 70, 40, 40, 50, 80, 10, 40, 10, 70, 70, 60, 50, 70, 70, 50, 50, 100% recorded in Halla Road, Hala Bypass, Kissana Mori, Hyderabad, Dhe Gujju, Nisar Pur, Odakh Lal, Tando Adam, Dhe Rustam Leghari, Omer Kot, Kunri, Kunri District, Water Course 15, Inayat Abad, Thesil Thari, Thatta, Faqir Ghot, Makli, Maleer, Memon Ghot, respectively as shown in Fig. 3. No district was found free from CMV virus infection. Similar observations have been reported by Shah & Khalid (2001) and Shah (2007).CMV persists all over the country in different ecological zones. Main reason of its persistence is because of its inoculum which remains in the field and shift from one place to another due to the transportation of infested materials by human activities whereas for the long area dispersal of inocula is due to viruliferous aphids

through wind to a new cultivating area. Similar types of observations have also been found by Palukaitis *et al.*, (1992). Unfortunately, growers in the region are not aware of how viruses spread from plant to plant and do not know about precaution measures to control virus transmission. Weeds have an important role on virus epidemiology and a common problem in chilli growing areas of Sindh. The samples of weed flora from the chilli crop in the province included kulfa (*Portulaca oleracea*), itsit (*Trianthema pentandra*), foxtail, *Cynodan dactylon*, *Cyprus rotundus*, *Demostachya bipinnata*, S*alvadora oleoides*, *Echinochioa* sp and *Withania somifera* were tested through ELISA. In all samples only *Cyprus rotundus*, *Trianthema pentandra* and *Portulaca oleracea* were found as an alternate host of CMV (Table 1) and may be its carrier under field conditions. Virus infected weeds within a crop are a serious threat because these act as a primary source of inoculum for the healthy plant in their vicinity and similar type of results has been obtained by Ormeno *et al*. (2005) and Iqbal *et al*. (2011). CMV naturally infects many plants including cereals, forage, woody and harbaceacious ornamental, vegetables and fruit crops (Kaper & Waterworth, 1981). In addition to this CMV transmission through seeds of annual weed S*tellaria media* (Chickweed) has also been reported by Stevens (1983). Recent reports showed that aphid population is increasing tremendously due to favorable environmental conditions. The aphid has broad host range and being insect vector of CMV, *Aphis gossypii* has already been reported by Palukaitis *et al*., (1992).

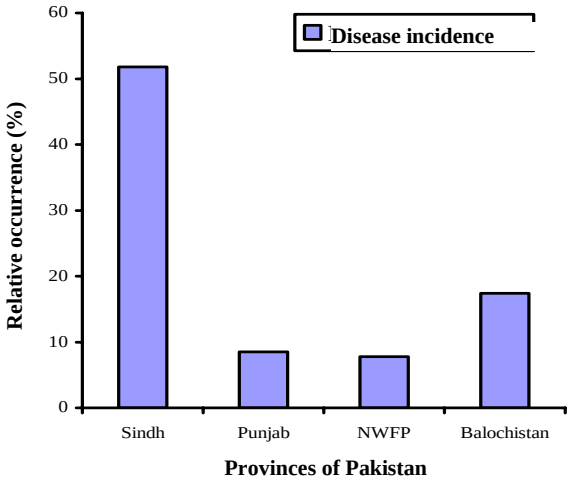

Fig. 2. Province wise relative occurrence of CMV during 2007.

Table 1. CMV prevalence in weeds flora of chilli crop.

S. No.	Weeds scientific name	ELISA ++ weed
1.	*Trianthema pentandra*	+
2.	*Cyprus rotundus*	+
3.	*Portulaca oleracea*	+
4.	Foxtail	-
5.	*Withania somnifera*	-
6.	*Echinochloa sp*	-
7.	*Salvadora oleoides*	-

+ : Samples were infected with virus

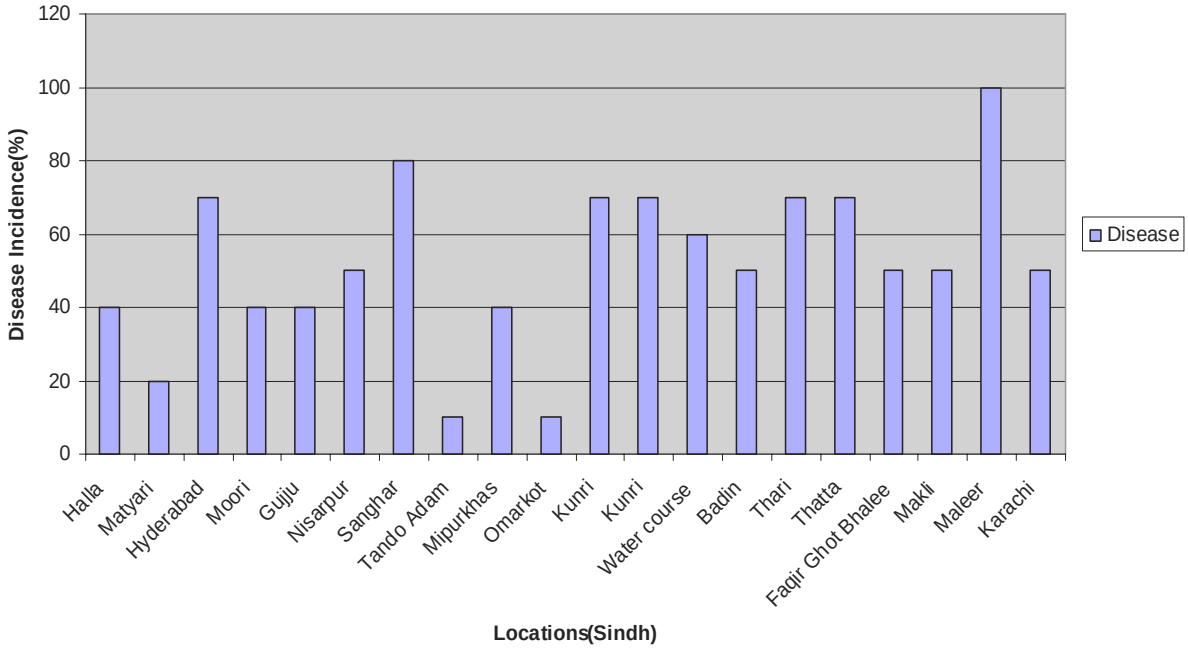

Fig. 3. Prevalence, distribution and relative incidence of CMV in Sindh.

CMV prevalence in Punjab: In Punjab during the year 2006-2007, a total of 257 chilli pepper samples were collected from different locations and were tested through ELISA against CMV. The relative incidences were found to 10, 10, 0, 0, 10, 10, 0, 4.4, 10, 10, 9.1, 0, 25, 10, 10, 12.6, 15, 20, 20, 10% in Dohk Fateh Shah, Nika Kahut, Dhudhial, Pidi Gheb, Leeti, Dhrabi, Wadi-E-Sonsakaser, Chengi, Timan, Mianwali, Lowa, Piplan, Fateh jang, Jhatla, Talagang, Chakwal, Multan Faisalbad, Bahawalpur and Lodhran, respectively. The highest

relative occurrence was found in Fateh Jang Thesil of Punjab. No incidence of virus from survey areas of Punjab is shown in Fig. 4. The relative incidence of CMV in Punjab province was 8.5% and these observations are in agreement with other scientists (Shah 2007).

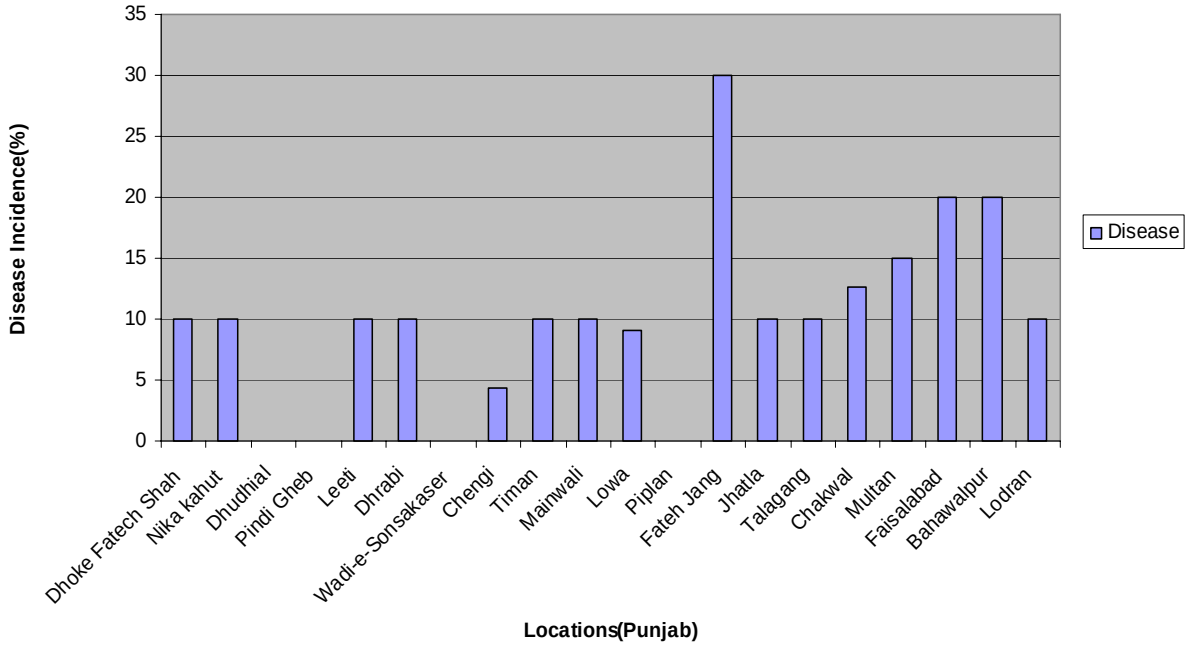

Fig. 4. Prevalence, distribution and relative incidence of CMV in Punjab.

CMV prevalence in NWFP (Now KPK): During the year 2006-07, total of 51 samples were collected from different locations of NWFP and tested through DAS-ELISA. The relative incidence of CMV recorded was 0, 25, 50, 0% in Malakand agency, Birkot, Swat and in Lower Dir, respectively. The relative incidence of CMV in the samples collected from the areas of NWFP is shown in Fig. 5. The data showed that overall relative occurrence of CMV was 7.8% in surveyed areas. However, CMV was not detected in the location from Lower dir. No CMV was detected in weeds and parasitic higher plants (*Cuscuta* sp.) collected from pepper fields of Mulkidam in Berikot area of district Swat.

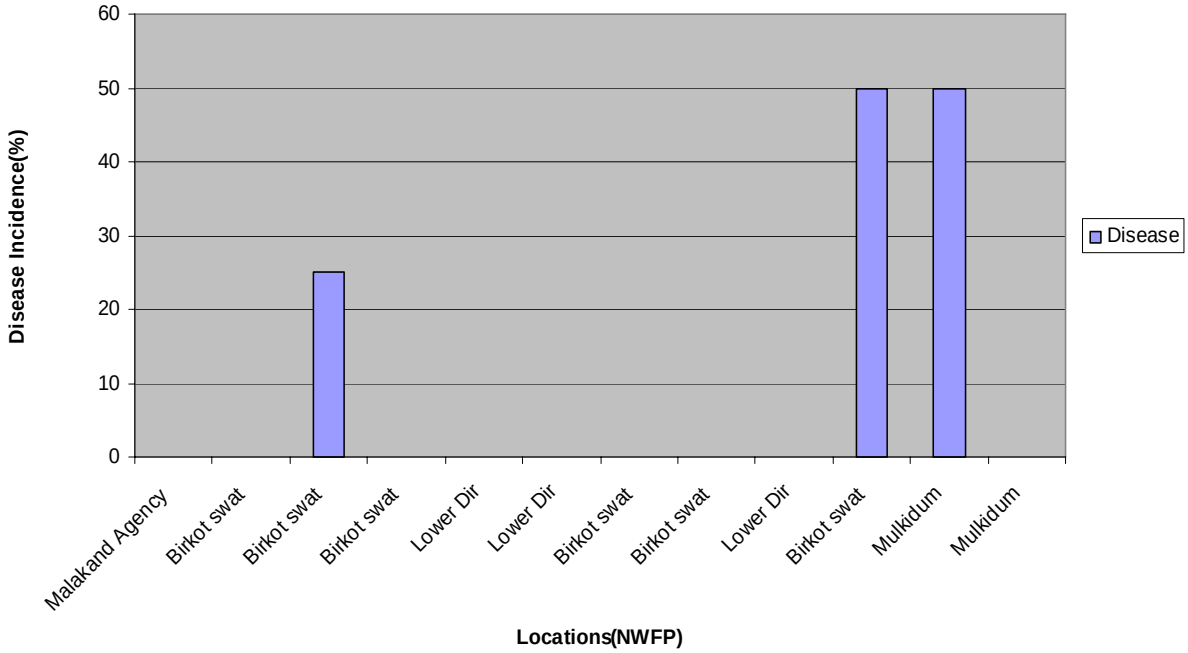

Fig. 5. Prevalence, distribution and relative occurrence of CMV in NWFP (KPK).

CMV prevalence in Balochistan: Chilli samples were collected from five different districts viz., Quetta, Pishin, Mastung, Kallat and Loralai of Balochistan. The relative incidence in different locations of these districts was recorded 0, 10, 0, 0, 0, 5, 12.5, 30, 20, 20, 80, 10, 30, 9, 18, 10, 33.3, 9, 20 and 0% in Nigana, Seriab, Malik Yar, Batayzai, Qila Amirjan, Ata Muhammad Kuchlak, Kalli Yahya Pur, Dum Kahi, Gugng Dori, Sharen Kuch, Dargi, Mahlari, Darwaish Abad, Hunna Orak, Surguz Azbakti, Shah Murada, Murtad, Jarmav and Khad Mucha, respectively. Maximum infection of CMV was recorded in village Dargai of district Loralai i.e., 80% as shown in Fig. 6.

The survey data showed that *Capsicum* grown in all over the Pakistan seems to be of narrow genetic base and thus susceptible to CMV infection. Such type of findings have also been reported earlier by Shah & Kahlid (1999); Shah *et al*., (2001).

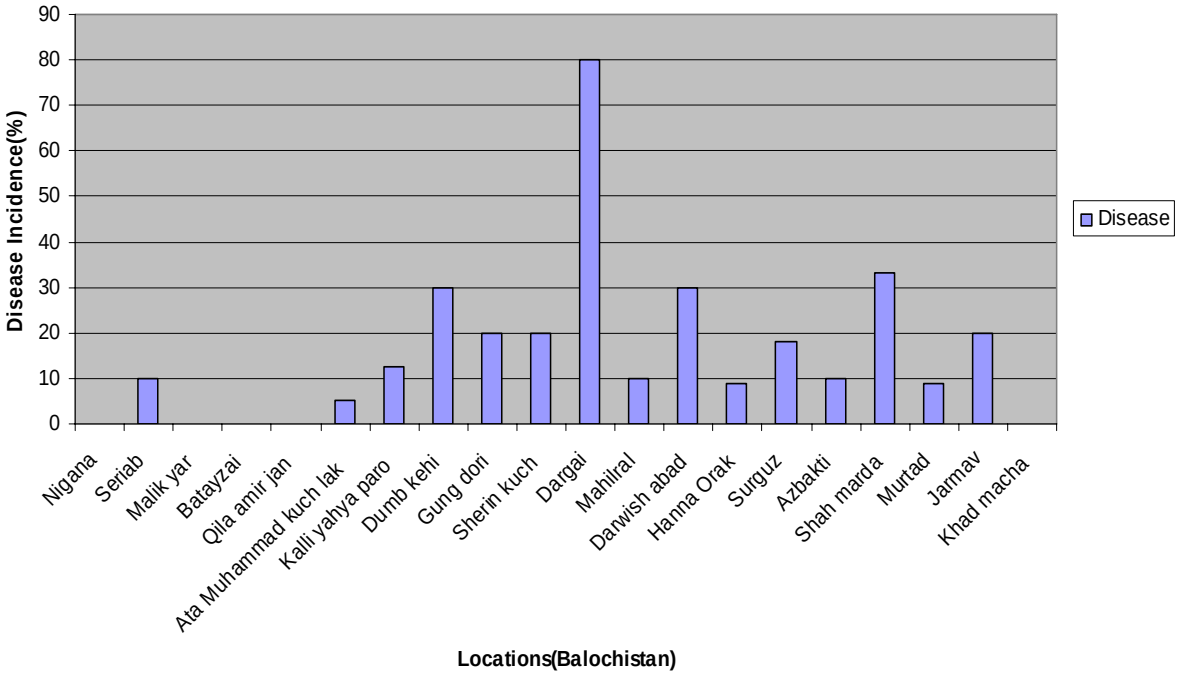

Fig. 6. Prevalence and distribution and relative occurrence of CMV in Balochistan.

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