

Animal Health Research Institute
Assiut Regional Laboratory

**ZOONOTIC IMPORTANCE OF BRUCELLOSIS
AMONG FARM ANIMALS AND VETERINARY FIELD
EMPLOYEES AT ASSIUT GOVERNORATE**
(With 4 Tables and 2 Figures)

By

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أهمية البروسيلا كمرض مشترك بين حيوانات المزرعة
والعاملين في المجال البيطري في محافظة أسيوط

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من خلال البرنامج القومي لمكافحة البروسيلا خلال الفترة من يوليو ١٩٩٨ حتى سبتمبر ٢٠٠٠ تم فحص ١٧٤٥٦ بقار ، ١٩٨١٨ جاموس ، ٣٢٩٣٩ غنم ، ٢٠٢٤١ ماعز سيرولوجيا للبروسيلا باختباري الـروزينجال و اختبار المحمض المتوازن الشريجي. وقد خضعت العينات الإيجابية لهما لاختباري النثرن الأنوبي البطي و الريفانول. وقد تمت الدراسة في ثمانية مراكز بمحافظة أسيوط. وأسفرت النتائج عن تسجيل مركز أسيوط أعلى نسبة إصابة في الأبقار يليها منفلوط بنسبة ١.٢ ، ٠.٨ % على الترتيب. أما في الجاموس سجلت منفلوط أعلى نسبة إصابة يليها صدفا بنسبة ٠.٤٨ ، ٠.٤٣ % على الترتيب. وسجلت منفلوط أعلى نسبة إصابة بروسيللا في الغنم يليها صدفا بنسبة ٤.١٣ ، ٢.٠١ % على الترتيب وسجلت منفلوط أيضا أعلى نسبة بروسيللا في الماعز يليها أسيوط بنسبة ١.٣٢ ، ٠.٥٨ % على الترتيب. وأسفرت مناقشة النتائج عن ظهور منفلوط تليها أسيوط ثم صدفا أعلى مراكز في محافظة أسيوط كبؤر عدوى للبروسيلا. وعلى الجانب الآخر تم فحص عدد ١٨٨ عينة دم من العاملين في المجال البيطري بـثلاث (أطباء بيطريون ، إداريون ، عمال) لكلي الجنسين. وتم الفحص سيرولوجيا للبروسيلا بالاختبارات الأربعة على النحو السابق ذكره وكانت النسبة الكلية في المحافظة هي ١٢.٢٣% وكانت الإصابة في الفئات الثلاث كالآتي ١٦.٧ ، صفر ، ٩.٨ % على الترتيب. وتمت دراسة نسبة الإصابة في المراكز المختلفة طبقا لمواقع عملهم فجاقت أعلى نسبة في منفلوط ٢٣.٢٥% تليها أسيوط ٢٠.٤٥% ثم صدفا ٨.٦٩%. فضلا عن أن المصابين في أكثر البؤر إصابة في الحيوان كان لديهم أعلى نسبة للأجسام المضادة وقد نوقشت هذه النتائج التي وضحت أن أكثر بؤر الإصابة في الحيوان كانت أكثرها إصابة للعاملين في المجال البيطري خاصة الأطباء البيطريين.

SUMMARY

Through the brucella eradication programme during July 1998 up to september 2000, 17456 cattle, 19818 buffaloe, 32939 sheep and 20241 goat were investigated serologically for brucella. Screening tests had been done using both Rose Bengal and Buffered Acidified plate antigen tests. Positive samples were subjected to both tube agglutination and rivanol tests. The study was applied in eight localities at Assiut Governorate. The study resulted that Assiut localiy recorded the highest incidence in cattle brucellosis followed by Manflout as 1.2& 0.8% respectively. About buffaloe brucellosis, Manflout recorded the highest incidence followed by Sedfa as 0.48 & 0.43% respectively. Manflout scoured 4.13% as ovine brucellosis followed by Sedfa recording 2.01%. Also, Manflout scoured 1.32% as caprine brucellosis followed by Assiut recording 0.58%. Data discussion revealed that Manflout, Assiut then Sedfa were the highly infected foci of brucella. On the other hand, 188 human sera (veterinary services employees) were serologically investigated for brucella under three categories (veterinarians, officials and workers) of both sexes. Serotesting was carried out as mentioned above. Seroreactor prevalence allover the work was 12.23% and for the three categories, it was 16.7, 0, 9.8% respectively. Locality prevalences were studied and revealed that Manflout recorded the highest prevalence (23.25%), Assiut recorded 20.45% then Sedfa (8.69%). Rather than the victims in the highest infected focus had the highest antibody titer. These findings were discussed and declared that the most infected focus in animal brucellosis was the most infected focus in human brucellosis among occupational veterinary field employees especially veterinarians.

INTRODUCTION

Human brucellosis is primarily an occupational hazard in USA, Middle East and Africa affecting people who work with infected animals and their tissues or those laboratory employees exposed to clonical isolates of attenuated vaccines. (Ruben *et al.* 1991). Information concerning human seroprevalence and incidence of brucellosis in Middle East is rare as reported by Idris *et al.* (1993), but when brucellosis is suspected or reported among animals, the medical services should be altered to the possibility of human infection (Scroka & Seroka, 1993). In

such areas, the occupational exposure to the infection is much more important than drinking infected raw milk or milk products and the disease become a great occupational hazard for veterinarians. (Christie 1987).

In developing countries where animal brucellosis is endemic, the serological tests are difficult to interpret as the individual (occupationally exposed to infection) is identified as belonging to a high risk group (Cooper 1992). There the problem is compounded by an absence of national surveillance programmes, diagnostic facilities or reliable data of human brucellosis (Cooper 1991) owing to poor reporting and lack of co-operation between veterinarians and public health officials (Nicoletti, 1992). The persistence of the animal reservoir of infection, low physician awareness, poor availability of diagnostic facilities as well as non existence of regional data bases contribute towards the perpetuation of this zoonosis (Handa *et al.*, 1998).

According to the World Health Organization, about half a million cases of human brucellosis occurs each year (FAO/WHO, 1986), the matter which magnitudes the importance of incidence determining in endemic areas. Mismanagement on animal quarantine, eradication of infected animals or vaccination in the poor areas account for the major reasons of human brucellosis occurrence (Wang *et al.*, 1998).

In Egypt, brucellosis is still an endemic disease had reported among domestic animals in spite of the persistence of national surveillance and control programme through test and slaughter, but human brucellosis is under estimated (Nawar *et al.* 1992).

The present work aimed to illustrate the brucella infection among domestic animals through the national surveillance and control programme corresponding to serological investigation for the disease among veterinary service employees (high risk group) in several selected locations at Assiut Governorate in order to declare the necessity of large scale seroprevalence performance among those high risk group.

MATERIAL AND METHODS

Animal sampling:

Through the national brucella eradication programme in Assiut Governorate and during the period from July 1998 up to September 2000; 17456 cattle, 19818 buffaloes, 32939 sheep and 20241 goats were serologically investigated at eight localities (Dairout, Alqussia,

Manflout, Assiut, Abo-teeg, Abnob, Sedfa and El-Ghanayem). Investigated samples were screened by both Rose Bengal Plate Test (RBPT) and Buffered Acidified Antigen Test (BAPAT) at field laboratories of each locality where seropositive for each or both tests were sent to the central laboratory (Assiut Vet. Laboratory, Animal Health Research Institute) for confirmation. Four serological tests (both screening tests, tube agglutination test [TAT] as well as Rivanol test) were performed accurately where, those seropositive for either one or both screening tests only without confirmatory tests were excluded. The seroreactors samples at least with the lowest dilution of Rivanol test were considered seropositive animals and they would be slaughtered under eradication programme rules (test and slaughter).

Human sera sampling:

At the available six of the previously mentioned localities (Manflout, Assiut, Abo-teeg, Abnob, Sedfa and El-Ghanayem) 188 sera samples of veterinary field employees (veterinarians, officials and workers) of both sexes were collected. Samples were screened by both RBPT and BAPAT and confirmed by TAT and Rivanol test.

Antigens:

All used four antigens were obtained from Veterinary Serum and Vaccine research Institute-Abbasia, Cairo-Egypt. RBPT and TAT were carried out according to Alton *et al.* (1975). BAPAT was performed as described by Angus and Barton (1984), while Rivanol test was applied according to Anon (1984).

RESULTS

All data obtained through the investigation were illustrated and manifested in Tables 1-4 and Figures (1 & 2).

DISCUSSION

Brucellosis is a major zoonotic disease and virtually all human infections drive directly or indirectly from animal exposure. The disease exists worldwide especially in Medeterranian (Corbel 1997). Despite vaccination campagins, *Brucella melitensis* remains the most important cause of human brucellosis. The interchange of information and surviellence data between Health and Veterinary Services is mostly essential for prevention of human brucellosis (Seroka and Seroka 1993).

Nicoletti (1992) recommended the cooperation between Public Health and Veterinary Officials while Maichomo *et al.* (1998) recommended better training for judging the agglutination results as they compared between the serolaboratory results for human brucellosis where had been done in 5 laboratories, four were belonging to Health Services and the last one was the central Veterinary Laboratory. Poor agreement between RBPT results in the four health laboratories but good agreement between RBPT, TAT and Complement fixation test which performed in Veterinary laboratory concluding that all these tests were performed well there.

As an attempt to illustrate a real prevalence and inclusive concept about brucella infection among domestic animals in Assiut Governorate, the present study was designated upon some foundations: Firstly, the long duration recording to avoid false prevalences such as those which had been done at the time of an outbreak with false higher incidences as well as, the long lapse between the peak of infection in animal and man was attributed by Wassif *et al.* (1992) to the prolonged incubation period. Secondly, all districts all over the governorate were manifested to avoid the biased incidences. Thirdly, the prevalence of the disease in a district for a species was counted as the proportions of already seropositive cases which were slaughtered according to the law of eradication programme. Fourthly, all farm animal species were included to realize the actual occupational hazard where the victims contact with all species alike. Finally, human locality was determined upon work district regardless to the residence address because of the infection contraction might be achieved during working in contact with infected animals.

For animal brucella diagnosis, screening by RBPT and BAPAT is recommended (Huber & Nicoletti, 1986 and Samira El-Gibaly, 1993) due to their high sensitivity where no missed positive cases. The confirmation with Rivanol test is also recommended due to its high specificity and reliability in detecting the infected cases without serious number of false positive (Huber and Nicoletti, 1986). The present investigation revealed brucella incidences in Assiut Governorate among cattle, buffalo, sheep and goats as 0.53, 0.26, 1.35 and 0.46% respectively (Table 1 and Fig. 1).

In the present work, the highest brucellosis incidence among cattle in Assiut followed by Manflout localities representing 1.2 & 0.8% respectively where El-Ghanayem and Abo-Teeg showed the lowest incidences representing 0.1 & 0.16 % respectively (Table 1 and Fig. 1.a).

The highest incidence appeared in the investigation among buffalo was related to Manflout followed by Sedfa localities representing 0.48 & 0.43% respectively while no cases were detected as a seropositive in Assiut locality (Table 1 and Fig. 1,b). These findings indicated that the infection among large ruminant-where the veterinary aid and interference is needed- was more focussed mainly in Assiut (the highest in cattle brucellosis) and Manflout (the highest in buffalo brucellosis). Manflout recorded the highest incidence in ovine brucellosis followed by Sedfa locality representing 4.13 & 2.01% respectively and also it recorded the highest incidence in caprine brucellosis followed by Assiut representing 1.32 & 0.58% respectively (Table 1 and Fig. 1). From these obtained data, it is concluded that Manflout followed by Assiut then Sedfa localities were the highest foci of animal brucellosis because these localities still persistent foci of brucellosis for long time and require more efforts to minimize these high incidences. These present findings give a high risk of infection among humans in these localities.

Where brucellosis exists in stock animals, the disease resembles an occupational hazard for veterinarians, farm workers, abattoir workers as well as laboratory workers (Madkour, 1992). Its diagnosis is made when brucellae be isolated from patient's blood, bone marrow or other tissue fluids, but the rate of isolation varies from 15 to 70% depending on methods used and length of incubation (Young, 1995) as well as the early tissue localization of the organism which lowers the probability of isolation from blood to 10-30% only (Cooper, 1991). Owing to above mentioned difficulties, serological tests appear to be the reliable and dependable tools in diagnosis of human brucellosis.

In the present study, human surveillance for brucellosis was carried out by screening tests (RBPT & BAPAT) and confirmed by both TAT and Rivanol tests. Rose Bengal test is highly sensitive and can be proposed as a screening test for medical diagnosis or human epidemiological survey (Christic, 1990 and Ludat *et al.*, 1995). BAPAT is recommended by Lucero and Bolpe (1998) as it is an inexpensive practical screening test for human brucellosis. It reduces the non specific reactors detected by standard agglutination test and it is now used to screen for human brucellosis where it detected 100% of patient sera which were culture-confirmed brucellosis. The present results confirmed these recommendations as all seroreactors (23) were detected by BAPAT

due to higher sensitivity than RBPT which detect only 21 samples (Table 4).

In endemic area, the interpretation of laboratory seroresults is associated with uncertain especially among population of high risk who may be recurrently exposed to infection. Then the diagnosis of active disease should be made only by the presence of symptoms and signs compatible with brucellosis, but those who are occupationally exposed to infection frequently develop high antibody titers in the absence of active disease (Christie, 1987). These high antibody titers are noted in asymptomatic population due to either past infection or current active subclinical disease or even due to antigen exposure without active disease (Cooper, 1992). Amal (1994) detected 21% of farm workers in 3 farms in Assiut as seropositive when their antibody titers were not correlate with symptomatic findings.

Human brucellosis incidence through the present investigation all over the governorate among the three investigated categories (veterinarians, officials and workers) showed 12.23% seroreactors (Table 3) who all of them were asymptomatic well individual.

In Middle East, the available literatures showed human brucella incidences as 0.08% in Kuwait (Mousa *et al.* 1987), 0.04% in Jordan (Dajani, *et al.* 1989), 11.1% among antenatal women and 4.9% among asymptomatic recruit in Saudi Arabia (Cooper 1991 and 1992) and 3.24% in Oman by Idris *et al.*, (1993) who recorded that the number of reported human cases of brucellosis showed an overall increase during past years in countries of the Middle East and the animals continue to carry the infection, therefore the possibility of future in the number of human cases cannot be dismissed.

In Egypt, brucellosis is a neglected and uncontrolled disease (Nawar *et al.* 1992) and the annual reported cases can be considered low in comparison to Spain and USA inspite of some Governorates in which human brucellosis is an endemic such as Cairo, El-Monofia and Alexandria (Wassif *et al.* 1992). The authors attributed the state of other governorates where no cases of human brucellosis either they had no cases actually or there was lack of notification or absence of accurate methods of diagnosis or even inadequate hospital records.

The present investigation revealed that number of brucellosis seroreactors belonged to Manflout, Assiut and Sedfa were 10, 9 & 4 with the percentages of 23.25, 20.45 and 8.69% respectively (Table 3 & Fig. 2), while no case record was obtained belonged to the other three

localities; Abo-Teeg, Abnab and El-Ghanayem (Table 3 & Fig. 2). The ten seroreactors of Manflout showed ten rivanol positive reactions (the highly specific) giving the most highly titers of TAT, but the nine seroreactors of Assiut showed only six rivanol positive results of low titers as well as lower titers in TAT reaction (Table 4). The findings indicated that Manflout as a focus of infection is of great hazard than Assiut which appeared to be more than Sedfa. It is obvious that, the most highly endemic locations of animal brucellosis is the most occupational hazard for human disease as in a certain location, the highly animal incidence correlate with highly occupational incidence. The incidence of the disease among veterinarians and workers were 16.7 and 9.8% respectively while no a single case was detected belonged to official category which indicated that the latter (officials) are away from contracting the infection (Table 3 and Fig.2). In Manflout and Sedfa, all seroreactors were veterinarians while in Assiut, five from the nine seroreactors were veterinarians with the incidences of 31.2, 14.3 & 14.8% respectively (Table 3 and Fig. 2). The obtained results showed that among the high risk group, veterinarians are the most population probably contract the infection giving rise to high titers in the absence of active infection and being asymptotically well individuals because of recurrent exposure. These obtained findings declared the finding of Xue *et al.* (1998) as there were more infected cases among veterinarians and herdsmen than among other jobs and young adults seemed to be at most risk and also coincided with Salata (1988) who stated that in endemic areas, the subclinical asymptomatic form occurs in high risk group affecting up to 33% of veterinarians.

Through the present study, no any female human serum sample could be detected as seroreactor and all nine sera samples were negative (Table 2). Christie (1987) recorded a higher incidence of brucellosis in men than women hypothesizing that it may reflect greater susceptibility of men to the infection while Cooper (1991) detected a higher incidence among women than men. The author attributed the condition to an increased exposure to infected livestock or to increased susceptibility of women to the disease in the bedouin pastoralist society. Sultanov and Saidov (1998) detected 3.3% positive of pregnant women working on live-stock farms and poultry farms through urban and rural consultation clinics in Dagestan.

Sex factor study requires more investigations, more and large scale surveillances. Rural and urban societies, different ages and job categories must be included to study the sex susceptibility.

As Nawar *et al.* (1992) recommended a national programme for the control of the disease, the present work recommends the performance of this programme at least for Veterinarians- the most affected population of high risk group.

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Table 1: Numbers of examined farm animals and the proportion of seropositives of different species at several localities in Assiut Governorate.

Locality	Examined animals								Proportion of seropositive animals							
	Cattle		Buffaloes		Sheep		Goat		Cattle		Buffaloes		Sheep		Goat	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Dairout	2550	0.47	3694	1	4613	0.03	3102	0.06	17	0.47	1	0.03	49	1.06	13	0.42
Alqasria	2606	0.46	2238	8	5708	0.35	2865	0.38	12	0.46	8	0.35	22	0.38	15	0.57
Manfour	2112	0.80	2079	10	4126	0.48	2495	0.48	17	0.80	10	0.48	170	4.13	35	1.22
Assini	3640	1.02	2014	0	6218	0.00	3098	0.01	37	1.02	0	0.00	86	0.01	18	0.58
Abou-terg	1812	0.16	4715	13	5072	0.27	2148	0.27	3	0.16	13	0.27	29	0.57	0	0.00
Abouab	0	0	0	0	1338	0	0	0	0	0	0	0.00	21	0.82	0	0.00
soda	2324	0.18	2529	11	3080	0.43	3554	0.43	5	0.18	11	0.43	62	2.01	12	0.34
Eghasayem	1932	0.10	2851	9	2980	0.25	2980	0.25	2	0.10	9	0.25	16	0.57	3	0.1
Total	17456	6.53	19813	5	32959	6.26	20247	6.26	93	6.53	5	6.26	445	1.25	84	0.46

Table 2: The investigated human sera of high risk occupational individuals with different jobs at several localities in Assiut Governorate.

Residence	Total	Sex		Job category			
		Male	Female	Veterinarians	Officials	Workers	Workers
Manflout	43	42	1	32	7	4	4
Assiut	44	41	3	27	3	14	14
Abou-teeg	14	14	0	5	3	6	6
Abnoub	34	33	1	16	10	8	8
Sedfa	46	42	4	28	3	15	15
El-Gahanayem	7	7	0	0	3	4	4
Total	188	179	9	108	29	51	51

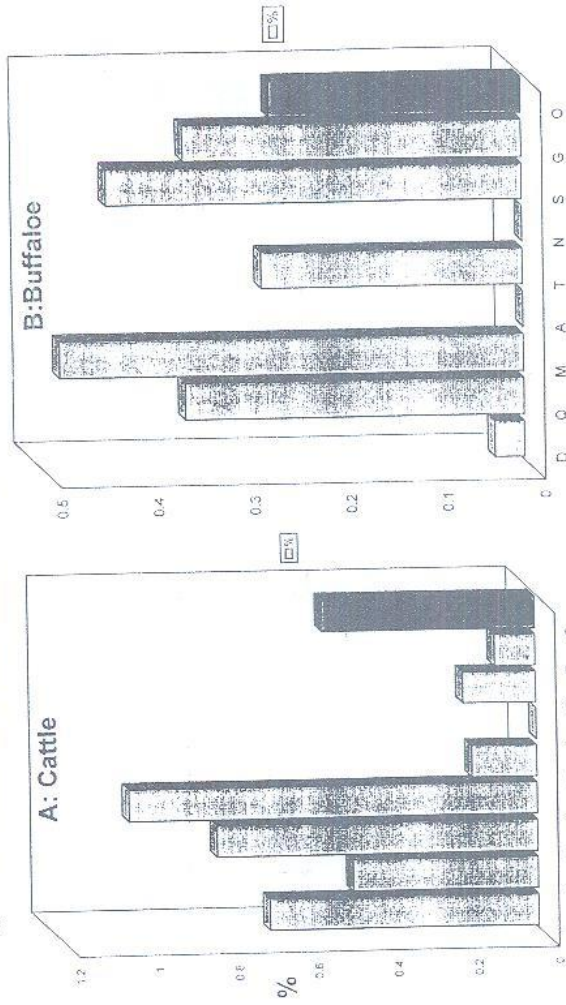
Table 3: Percentage proportions of sero-reactor individuals with different job categories at several localities.

Locality	seroreactors			Job categories					
	No.	%	No.	Veterinarians		Officials		Workers	
				No.	%	No.	%	No.	%
Manflout	10	23.25	10	31.2	0	0	0	0	0
Assiut	9	20.45	4	14.8	0	0	0	5	35.7
Abou-teeg	0	0	0	0	0	0	0	0	0
Abnoub	0	0	0	0	0	0	0	0	0
Sedfa	4	8.69	4	14.3	0	0	0	0	0
El-Gahanayem	0	0	0	0	0	0	0	0	0
Total	23	12.23	18	16.7	0	0.00	5	9.8	9.8

Table 4: Different serological reactions for seroreactor samples of humans

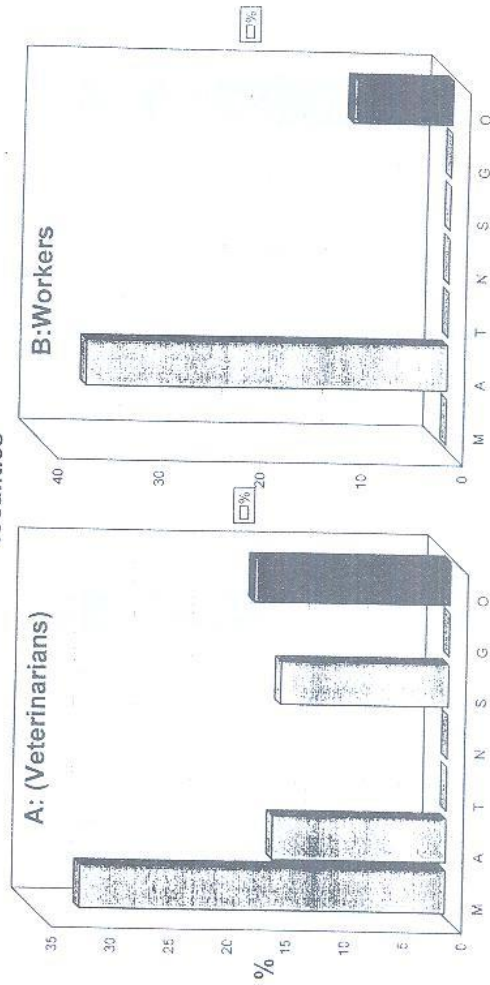
Locality	Total sero-reactors	Serological tests																	
		R					TAT					RivanoI							
		B	A	P	T	A	T	1/10	1/20	1/40	1/80	1/160	1/320	Total	1/75	1/50	1/100	1/200	1/400
Manflout	10	10	10	0	0	1	2	2	2	2	2	3	10	0	2	1	5	2	10
Assiut	9	8	9	0	3	2	1	0	0	0	0	6	6	2	3	0	0	1	6
Abo-teeg	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Abnoub	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Sedfa	4	3	4	1	3	0	0	0	0	0	0	4	4	0	3	0	0	0	3
El-Gahaayem	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total	23	21	23	1	7	4	3	2	3	2	3	20	2	8	1	5	3	19	

Fig.1: Incidence of brucellosis among farm animals in different localities

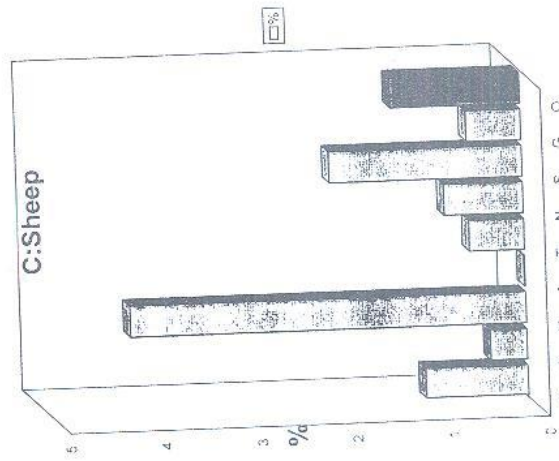
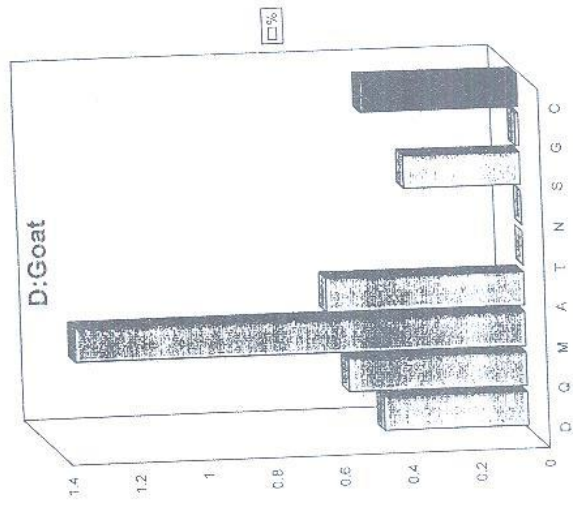


D: Dirout Q: Alqusia M: Manflout A: Assiut T: Abo-Teeg
 N: Abnoub S: Sedfa G: El-Ghanayem O: Overall

Fig. 2: The seroreactor proportions among investigated individuals at different localities



M: Manflout A: Assiut T: Abo-Teeg N: Abnoub
 S: Sedfa G: El-Ghanayem O: Overall



D: Dirout Q: Alqusia M: Manflout A: Assiut T: Abo-Teeg
 N: Abnoub S: Sedfa G: El-Ghanayem C: Overall