CONTENTS

						Page
1 -		INTR(DUCTIO	N		
2 -	-	REVI	EW OF L	ITRATURE.		1
		2.1.	Introdu	action		1
		2.2.	Vaccin	ation		3
			2.2.1.		abortus smooth strain 19	3
				2.2.1.1.	Use of strain 19 vaccine in guinea pigs	3
				2.2.1.2.	Use of strain 19 vaccine in farm animals	6
			2.2.2.		abortus rough strain 45/20	18
				2.2.2.1.	Use of strain 45/20 vaccine in guinea pigs	18
				2.2.2.2.	Use of strain 45/20 vaccine in form animals	21
			2.2.3.		ransfer factor in transferring immune response	27
		2.3.	Evalua	tion of the	he immune response	38
			2.3.1.	Humoral	immune response	38
				2.3.1.1.	Humoral response to strain 19 vaccine	38
					a. In guinea pigs	38
					b. Humoral response to strain 19 in farm animals	39
				2.3.1.2.	Humoral response to rough strain 45/20 vaccine	50
					a. In guinea pigs	50
				1	b. Humoral response to strain 45/20 in farm animals	52
		2.4.	Cellul	er immune	response skin reaction	65

3.	MATE	RIAL AN	D ME	THODS	72
	3.1.	Materi	al		72
		3.1.1.	Bac	terial strains	72
			i)	Brucella abortus strain 544	72
			ii)	Rough living Brucella abortus strain 45/20	72
		3.1.2.	Vac	ines	72
			i)	Brucella abortus strain 19 (Aborsec) vaccine	72
			ii)	Rough Killed Brucella abortus strain 45/20 (Abortox) vaccine	72
		3.1.3.	Bruc	ella antigen	73
			i)	Smooth antigen	73
			ii)	Rough antigen	73
			iii)	Rose bengal stained antigen	73
			iv)	Rivanol antigen	73
			v)	Brucella antigen for C.F.T	74
		3.1.4.	Bruc	ellin	74
		3.1.5.	Med	e	74
			i)	Brucella Albimi agar	74
			ii)	Potato-infusion Agar	74
			iii)	Sheep Blood Agar	74
			iv)	Ficol hypaque medium	75
			v)	RPMI 1640 medium	75
			vi)	Phosphate buffer medium	76

3.1.6.	Chem	icals and biological reagents	75
	i)	Antibiotics	75
		a- Actidion	75
		b- Polymyxin B sulphate	76
		c- Bacitracin	76
	ii)	Byes	76
		a- Ethyl violet	76
		b- Trypan blue	76
	iii)	Phenol saline	76
	iv)	Peptone saline	76
	v)	Calcium Magnesium Veronal buffer	77
	vi)	2- Mecraptoethanol solution	77
	vii)	Rivanol solution 1.0%	77
	viii)Sodium bicarbonate solution 4.4%	77
	ix)	Hank's balanced salt solution	77
	x)	HEPES buffer solution	78
	xi)	Alsever's solution	78
	xii)	Heparin injection B.P	78
	xiii)Haemolysin	79
	xiv)	Complement	79
	xv)	Brucella control positive serum	79
	xvi)	Sheep blood corpuscles	79
3,1.7.	Expe:	rimental animal	80
	i)	Laboratory animals (guinea pigs)	80

		ii) Farm animals	80
		a- Cattle	80
		b- Buffaloes	80
3.2.	Methods	S	81
	3.2.1.	Preparation of Brucella abortus challenge strain 544	81
	3.2.2.	Preparation of Brucella Rough antigen	81
	3.2.3.	Preparation of the vaccine dose	83
		i) Aborsec vaccine	83
		ii) Abortox vaccine	83
	3.2.4.	Preparation and separation of sensitized lymphocytes from guinea pigs vaccinated with either Aborsec of Abortox vaccines	84
	3.2.5.	Preparation of extracts of sensitized	
		lymphocytes	84
	3.2.6.	Animal inoculation	85
		3.2.6.1. Infection with virulent smooth Brucella abortus	0.5
		strain 544.	85
		3.2.6.2. Vaccination of guinea pigs.	85
		3.2.6.2.1. Vaccination with smooth living attenuated Brucella abortus strain 19 (Aborsec) vaccine.	85
		3.2.6.2.2. Vaccination with rough killed Brucella abortus strain 45/20 (Abortox) vaccine.	86

	3.2.6.3.	Challenge infection	86
		a) Guinea pigs vaccinated with S.19.	86
		b) Guinea pigs vaccinated with Killed 45/20	87
	3.2.6.4.	Isolation of lymphocytes	87
		a) Sensitization of guinea pigs with brucella vaccine	87
		b) Animal inoculation with lymphocytes extracts	88
3.2.7.	adjuvant	plication of Killed 45/20 vaccine in cattle and	88
3.2.8.	Determina	ation of immune response	89
	3.2.8.1.	Skin test	89
	3.2.8.2.	Serological tests	90
		a) Preparation of sera	90
		b) Preparation of antigen	91
	3.2.8.3.	Tube agglutination test	91
	3.2.8.4.	Mercaptoethanol test M.E.T.	93
	3.2.8.5.	Rose Bengal test (R.B.T.)	93
	3.2.7.6.	Rivanol test(Riv.T.)	93
	3.2.7.7.	Complement fixation test	95
		a) Titration of haemolysin.	95
		b) Titration of complement.	96
		a) Shoop onwthme outes	98

d) Antigen titration	98
e) Test proper	101
i) Inactivation of serum.	101
ii) Technique of the test.	101
iii) Interpretation of the result	104
3.2.8. Bacteriological examination	104
4. RESULTS	106
4.1. Result of laboratory experiments	106
4.1.1. Studies on the immune response of guinea pigs to living <u>Brucella abortus</u> .	106
4.1.1.1. Results of the immune response of guinea pigs to virulent <u>Brucella abortus</u> strain 544 (10 ⁴ Org/ml.)	106
4.1.1.2. Results of the immune response of guinea pigs to living Brucella abortus strain 19 (Aborsec) vaccine	110
4.1.1.3. Studies on the immune response and bacteriological finding in guinea pigs vaccinated with S.19(Aborsec) and challenged with strain 544	114
4.1.1.4. Studies on the immune response in guinea pigs injected with extracts of strain 19 sensitized lymphocytes	
(transfer factor)	116

		4.1.1.5.	Studies on the immune response in guinea pigs infected with transfer factor and challenged with the virulent <u>Br.abortus</u> strain 544	118
	4.1.2.		on the immune response of guinea killed Brucella abortus	120
		4.1.2.1.	Results of the immune response to K 45/20 (Abortox) vaccine	120
Dr. y -		4.1.2.2.	Studies the immune response in guinea pigs vaccinated with K 45/20 A (Abortox) vaccine and challenged with strain 544	123
		4.1.2.3.	Studies on the immune response in guinea pigs injected with extracts of strain 45/20 sensitized lymphocytes (Transfer factor)	125
		4.1.2.4.	Studies on the immune response in guinea pigs injected with K 45/20 A sensitized lymphocytes and challenged with strain 544	127
	4.1.3.	Studies	on the immune response in control guinea pigs	129
4.2.		_	in farm animals vaccinated vaccine (Abortox)	131

5.	DISCUSSION	141
6.	SUMMARY	156
7.	REFERENCES	162
	ARABIC SUMMARY	

6- SUMMARY

A total of 525 guinea pigs was used for studying the immune response and efficiency of the Abor sec (5.19) and Abortox (S. 45/20) vaccines, the animals were divided into 9 groups.

Group I: (35 guinea pigs) inoculated with Abor sec vaccine (living attenuated) showed serological response detected by tube agglutination test (T.A.T.), mercaptoe-thanol test (ME.T.), Rose bengal test (R.B.T.), Rivanol test (Riv.T.) and complement fixation test (C.F.T.) from the seconed week after vaccination at various titres. The skin test using brucellin revealed positive results from the 2nd week and strain 19 organisms were recovered only at the 2nd and 3rd week.

Group II: (30 guinea pigs) was challenged with the virulent Br. abortus strain 544 (10⁴ Org/ml) 8 week after vaccination with Abor-sec vaccine and Killed 8 weeks later revealed negative serological results and no brucella organisms could be recovered from the spleen of all animals.

Group III: (35 guinea pigs) vaccinated with Abortox vaccine (Killed 45/20 adjuvaut) showed negative serological reaction to T.A.T, ME.T., R.B.T., Riv.T. and C.F.T.

when using smooth antigen, while all animals in the group gave 1/20 agglutination titre in T.A.T. when using rough antigen at the 2nd week which increased to 1/40 till the 5th week then declined again to 1/20 from the 6th week. ME.T. and C.F.T. titres were detectable when using rough antigen at 1/20 from the 3rd week till the 8th week. The skin test was negative during the various weeks of experiments and no brucella organisms were recovered as the vaccine was killed.

Group VI: (60 guinea pigs) was vaccinated with abortox vaccine and challenged with S.544. One half of the animals received a dose of 5 x 10³ Org/ml and the other half with a dose of 10⁴ Org/ml at the 8th week. after vaccination and killed 8 week later All animals showed negative results to the serological tests and brucella organisms strain 544 were recovered only from the guinea pigs challenged with a dose of 10⁴ organisms/ml.

Groups V and VI: (30 guinea pigs each) were vaccinated with Abor sec and Abortox vaccine from each group 6 animals were killed respectively weekly from the second week till the 6th week. Blood samples were

collected on heparine, lymphocytes were separated and extracts were prepared from these lymphocytes by freezing and thawing:

The obtained lymphocyte extracts of group V were injected in 50 guinea pigs in a dose equal to 10⁶ cells animal to demonstrated the transfer of immunity by the sensitized lymphocytes.

The guinea pigs showed negative serological results in all serological tests used but gave positive skin reaction when done 5 days after injection of the extracts. These animals showed a positive serological reactions after being challenged with strain 544 (10⁴ organisms/ml) but no brucella organisms could be recovered after challenge. The skin test was still positive, however the diameter of erythema increased. The obtained lymphocyte extracts of group VI were likewise injected in 50 guinea pigs equal to 10⁶ cells/animal. These animals showed negative results both in serological tests and skin test. After challenge with S.544 (10⁴ Org/ml) positive serological and skin reactions were demonstrated in all animals, in addition to isolation of Brucella organisms from these animals.

3 other groups VII, VIII and IX were used as controls.

Group VII: (35 guinea pigs) was left untreated as a control for vaccination with Aborsec and Abortox vaccines.

ted challenged control and group IX (50 guinea pigs) was used as control for the transfer factor experiment. All animals were injected with lymphocytes extracts from non vaccinated animals, 25 animals were challenged thereafter with strain 544 and the other animals were left unchallenged. All control animals in the 3 groups were tested serologically and by skin test in paralled with the experiments in the above mentioned groups.

Field trails to study the immune response of Abortox vaccine in cattle and buffaloes were done in four governmental farms.

In farm -A- a total of 506 Friestan cattle was vaccinated with Abortox vaccine in two doses one month apart and received a booster dose after one year. They showed negative serological results at the time of the lst vaccination and after 6 months later, but 41.1%, 16.6% and 28.6% were positive to the T.A.T., ME.T. and R.B.T. respectively when examined one month after the

booster dose, The monthly successive examination till the 7th month after the booster dose revealed that only 4 animals were positive to T.A.T., ME.T. and R.B.T. which were considered latent carriers for brucella infection and were slaughtered.

In farm -B- a total of 1927 Friesian cows was vaccinated with Abortox vaccine in two doses two months apart 35.8% of the animals were positive to T.A.T. and 4.3% suspecious at the time of lst vaccination. The positive animals were slaughtered.

at the time of second vaccination 20% of the remaining animals were positive and 13.7% suspecious.

All positive animals were also slaughtered and the suspecious animals (169) were isolated and examined separately every month along with the negative Cows. The negative animals remained negative till the end of the experiment. Of the suspecious animals 92 showed positive results, they were also slaughtered the semaing 77 animals became negative and were added to the negative herd again.

In Farm -C- a total of 1000 Friesian cattle was vaccinated with a bortox vaccine in one dose and revaccinated 10 months later. They showed negative T.A.T. results at the time of lst vaccination, but when they were

examined 8 months later one animal was positive and 4 animals were suspecious. The 5 animals were slaughtered.

When the farm was examined again at the time of revaccination all animals were still negative but one month after revaccination there were 10 positive and 5 suspecious cows which were also slaughtered. At two months after revaccination 4 aminals revealed positive reactors to T.A.T. and were slaughtered. At 8th month after revaccination 26 positive animals were detected and were immediately slaughtered. In the following month a bortion sterm in pregnant vaccinated Cows occurred and 56 animals showed positive results to T.A.T. and 130 animals were suspecious. Brucella organisms were recovered from a borted foeti. This occurred subsequent to the introduction of infected sheep to the vaccinated herd.

In farm.-D- a total of 100 pregnant buffaloes was vaccinated with Abortox vaccine with one dose. 57% of them showed positive agglutination titre that varied from 1/20 to 1/80 when examined 4 months after vaccination, this percentage showed gradual decline by the following examinations using T.A.T., M.E.T. and C.F.T. and all were negative 9 months after vaccination.