CHAPTER IV

RESULTS

<u>Experiment I : Growth Curve of P.multocida : CU strain</u>

Fig. 2 : The starting bacteria of each flask was almost equal. The log cells/ml. at 0 hr. was 6.18, 6.10 and 6.05 in BHI broth, tryptose broth with thiamine and flesh-liver-duck broth or about 10^{6} CFU/ml. In flesh-liver duck broth the bacteria died quickly, the log cells/ml at 24 hr was only 1.70 and the OS was nearly 0 furough out the incubation period. In another two flasks, the bacteria grew exponentially during 0 - 9 hr . the log cell/ml peaked in 9 hr was about 9.24 in BHI broth and 8.95 in broth tryptose broth with thiamine then it was coustant. At the end of incubation (24 hr) the log cells/ml in two flasks were 8.76 and 8.48 respectively. The turbidity during 0 - 3 hr was nearly unmeasured then increased sharply during 3 - 9 hr In BHI broth it peaked in 12 hr then it was constant through 24 hr . In tryptose broth with thiamine it was slighly increased in 9 to 24 hr .

Fig. 3 : Comparison of the growth curves of <u>P.</u> <u>multocida</u> CU strain in BHI broth incubated in various conditions suggestted that the growth at 37°C. shaking 200 rpm was the best and the growth decrease at 37°C static, 41.5°C static and 41.5°C shaking 200 rpm respectively.

At 37°C in both conditions, the bacteria grew exponentially during the first period of incubation. The log phase of growth was not observed. The viable cells peaked in 6 - 9 hr, the O.D. at 540 nm of 5 times diluted broth culture was about 0.24 and 0.80 respectively then it was continuously constant through the end of incubation (48 hr).

At 41.5 °C in static condition, the growth increased slowly and the log cells/ml peaked 9.15 in 18 hr then decreased rapidly whereas the O.D. of 5 times diluted broth culture peaked about 0.32 in 24 - 30 hr then slighly decreased. In shaken condition, the bateria grew a little bit during 0 - 3 hr, the log cells/ml. was only about 6.72 then the bacteria died gradually. For 48 hr incubation the log cells/ml. was uncounted. The O.D. of 5 times diluted broth culture was nearly 0 thoroughout the incubation period.

Table 5 showed the viable cell count per ml in 6 and 9 hr of BHI broth cultures incubated at 37 °C in static and shaking condition. In shaking condition the different viable cell on 6 and 9 hr was higher than that in static condition. Asian experiment 1 ; starting with the same amount of bacteria (1.05×10^6 CFU/ml.), on 6 hr the viable cells of 1.52×10^9 CFU/ml. would increase slightly to 2.85×10^9 CFU/ml. on 9 hr in static condition where as in shaking condition the viable cell of 3.1×10^3 CFU/ml. on 6 hr would reach highly to 9.91×10^9 CFU/ml. on 9 hr

<u>Experiment II : Virulence of the CU strain in Various Ages of Ducks</u>

Old ducks were more resistant to the CU strain of <u>P.multocida</u>. All one- week old ducks S/C inoculated with 1.0 ml of the stock culture of 1.60×10^3 CFU/ml. died at all. For 2-wk old , 3-wk old and 4-wk old ducks,16 of 20 ducks, 12 of 20 ducks and 1 of 20 ducks died following the 1.0 ml S/C inoculation with the stock cultures of 3.77×10^9 CFU/ml 1.18×10^9 and 1.67×10^9 CFU/ml respectively (Table 6).

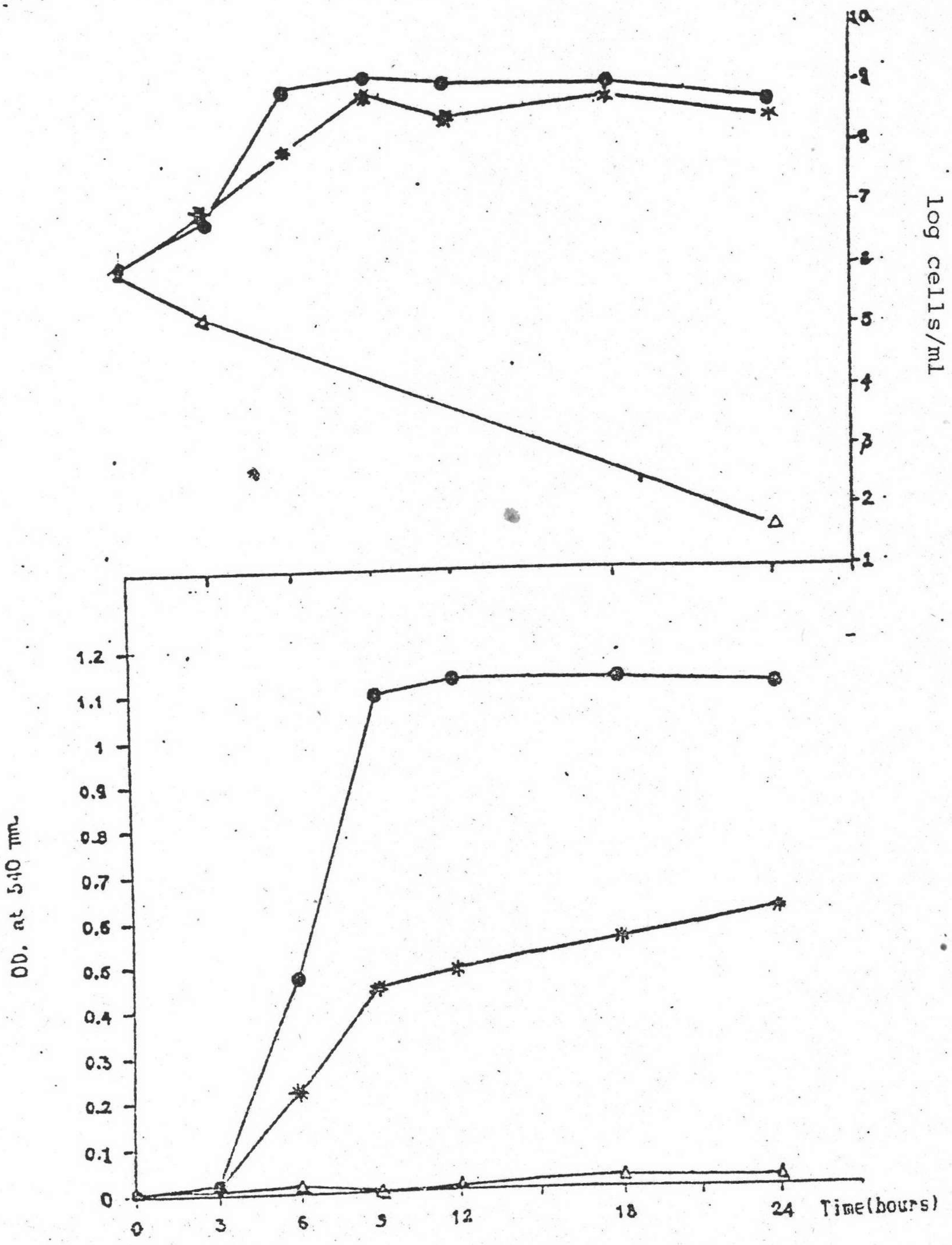

Fig.2 Turbidity and log number of viable cells per ml of Pasteurella multocida : CU strain incubated static at 37 °c in

(●) Brain heart infusion broth

(*) Tryptose broth with thiamine

(Δ) Flesh-liver-duck broth

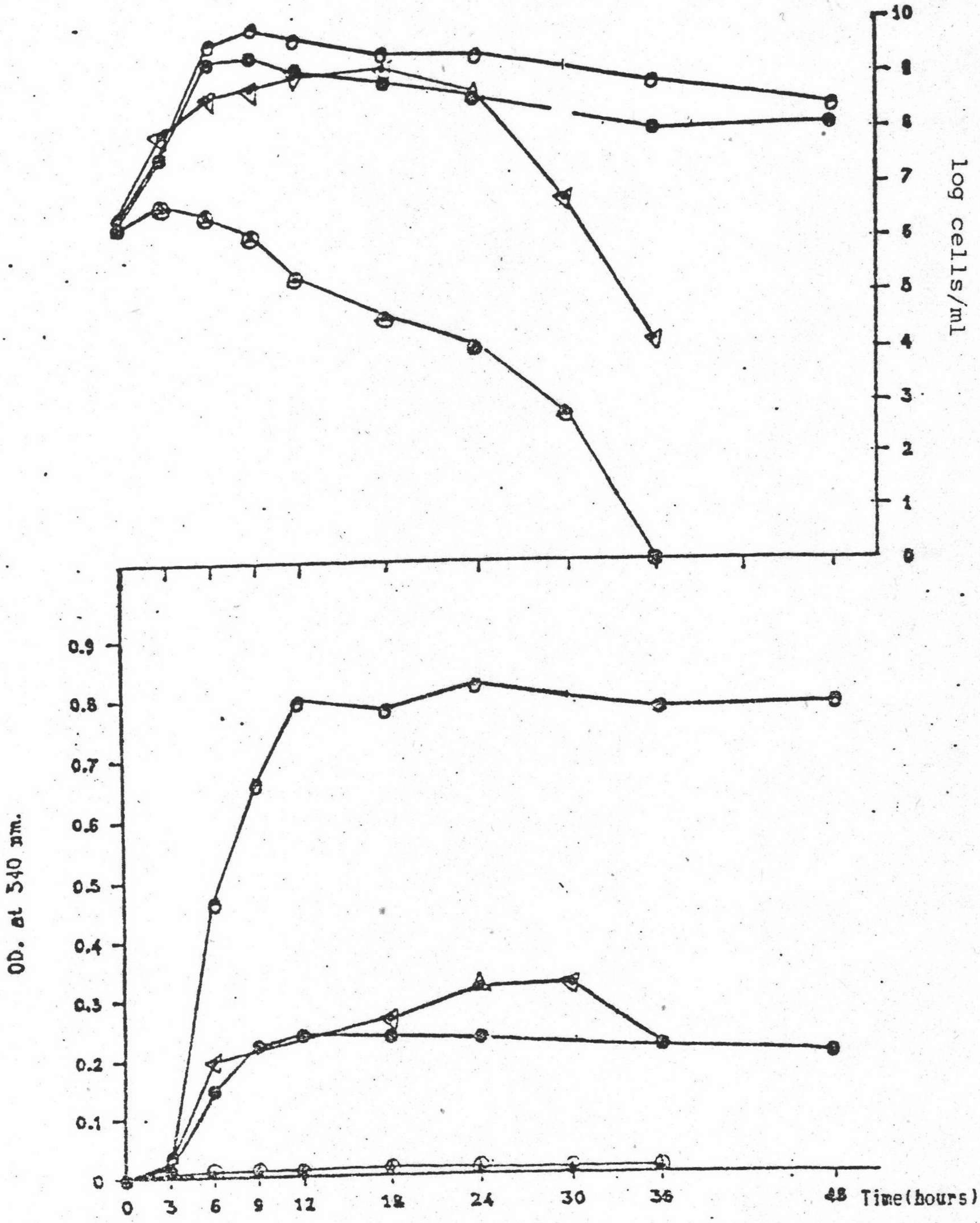

Fig.3 Turbidity(of 5 times diluted broth culture) and log number of viable cells per ml of *Pasteurella multocida* : CU strain incubated in brain heart infusion broth.

(●) Static at 37 °c (⊖) Shaking 200 rpm. at 37 °c

(▽) Static at 41.5 °c (⊖) Shaking 200 rpm. at 41.5 °c

Table 5 The viable counts (CFU/ml) of Pasteurella multocida : CU strain at 6 and 9 hour of brain heart infusion broth cultures incubated in static and shaking condition at 37°C

Experiment	static			Shaking 200 rpm.		
	o hr.	6 hr.	9 hr.	o hr.	6 hr.	9 hr.
1	1.05×10^6	1.52×10^9	2.85×10^9	1.05×10^6	3.1×10^9	9.91×10^9
2	1.1×10^6	1.70×10^9	2.70×10^9	1.8×10^6	1.71×10^9	6.8×10^9
3	1.6×10^6	9.3×10^8	1.54×10^9	2.0×10^6	3.2×10^9	1.81×10^{10}
4	2.22×10^6	1.27×10^9	2.34×10^9	3.61×10^6	2.15×10^9	4.8×10^9

Table 6 The virulence of Pasteurella multocida : CU strain in various ages of ducks

Age inoculated (wk)	Stock culture (CFU/ml)	Dilution					Control
		0	1:10	1:100	1:1000	1:10000	
1	1.60×10^9	21/21*	8/20	3/20	0/20	0/20	0/20
2	3.77×10^9	16/20	5/20	0/20	0/20	0/20	0/20
3	1.18×10^9	12/20	1/20	0/20	0/20	0/20	0/20
4	1.67×10^9	1/20	0/20	0/20	0/20	0/20	0/20

*No. died / No. inoculated

Experiment III Immunological Respouses

Protective Immunity

After the first vaccination of 1 x 10^9 bacteria per duck, 30% and 2% death occured in 100 ducks of each group of S/C vaccination and oral vaccination respectively. Following the second vaccination of 4.4 x 10^8 bacteria per duck, S/C exposure died 20% in 30 ducks.

S/C vaccinated group The level of protection expressed in term of survival rate was 30 , 100, 100, and 60% and in term of protection was -15, 56, 88, and 60% respectively in 1,2,4 and 8 wk after the first vaccination. Being noted, the apparently low immunity level occured in 1 wk after vaccination (Table 7, Fig 4). The higher percentage of survival and protection (more than 80%) was obtained through 8 wk following the second vaccination in 1 month interval (Table 8,Fig.5) and 88% survival rate or 71% protection was obtained in 4 wk after the second vaccination in 2 months interval (Table 9, Fig. 6).

Oral vaccinated group The percentage of survival was 80 , 90 , 44 and 10% respectively in 1, 2, 4 and 8 wk after the first vaccination. However, unsatisfied degree of protection of 10 - 46% was obtained (Table 7, Fig.4). Similarly to the S/C vaccination, the higher percentage of survival and protection (not less than 80%) was developed through 8 wk following the second vaccination in 1 month interval (Table 8, Fig.5) and 82% survival rate or 65% protectiin was developed in 4 wk after the secind vaccinatiin in 2 month interval (Table 9, Fig.6).

Drinking water vaccinated group The survival rate was 70,70, Ø, and 20% and the degree of protectiin was 25, 26, -12,and 20%, respectively in 1, 2, 4 and 8 wk after the first vaccination (Table 7, Fig.4). Double vaccination via this route induced higer immunity. In 1, 2, 4 and 8 wk after the second vaccination in 1 month interval, the survival rate was 89, 88, 50 and 65% and protectiin was 89,68,50 and 48% respectively (Table 8 Fig 5). in 4 wk post the second vaccination in 2 month interval, 100% survival rate or 83% protection was obtained (Table 9, Fig. 6).

Unvaccinated cintrol

The percentage of survival aming the cintrol group was 44 , 45, 12 and 20% respectively in 1, 2, 4 and 8 wk after the first vaccination and Ø , 20 , Ø, and 17% respectively, in 1, 2, 4 and 8 wk after the second vaccination in 2 months interval. and 17% in 4 wk after the second vaccination in 2 month interval.

Antibody Respinse

S/C vaccinated group The GMT of TA antibody against the 8:A stain autoclaved Ag ranged 10.77 - 25.40, 22.63 - 35.92 and 19.50 - 28.98 and those against the CU stain autoclaved Ag ranged 5.04 - 16.00 ,8.83 - 22.63 and 13.12 - 28.51 respectively for single vaccination double vaccination in 1 month interval and double vaccination in 2 months interval. There was a fluctuatiin of the TA titers. The GMT of PHA antibody against the 8:A sinicated Ag was 147.03 in 1 wk and precipitously dropped to 5.44, 8.00 and 6.43 in 2, 4 and 8 wk after the first vaccination while those against the CU sonicated Ag was highly as 588.13 and 512.00 in 1 and 2 wk then dropped sharply to lower than 2.00 in 4 and 8 wk after the first vaccination, Similar results in GMT as measured by PHA test was observed in the two programme of vaccination. The GMT against the 8:A stain sonicated Ag were 118.38, 17.96,5.66 and 9.75 and those against the CU strain sonicated Ag were 588.13, 362.04 and lower than 2.00 respectively in 1, 2, 4 and 8 wk after the second vaccination in 1 month interval. The GMT against the 8:A strain sonicated Ag were 114.04 ,25.40 and 14.49 and those against the CU strain sonicated Ag were 724.08,322.54 and lower than 2 respectively in 1,2,and after the secind vaccination in 2 minths interval. There was no apparent correlatiin between the TA and PHA titers and the degree of protectiin (Table 7, 8, 9, Fig 4 5,6,7).

Oral vaccinated group The GMT of TA antibody against the 8:A strain autoclaved Ag ranged 476 - 3200, 7.41 - 16.00 and 11.31 - 16.00 and those against the CU strain auto claved Ag ranged 4.76 - 11.31, 8.00 - 16.00 and 7.34 - 14.67 respectively for single vaccination, double vaccination in 1 month interval and double vaccination in 2 months interval. There was a fluctuatiin of the TA titer. The GMT of the PHA antibody against the 8:A strain sonicated Ag ranged 4.88 - 16.00, 9.75 - 16.00, and 11.31 - 32.00 for single vaccination, double vaccination in 1 month and 2 month interval and those against the CU strain sonicated Ag were mostly lower than 2.00 in all vaccination programme. There was no apparent correlatiin between the TA and PHA titers and the degree of protectiin (Table 7, 8, 9 Fig 4,5,6).

Drinking water vaccinated group The GMT of TA antibody against the 8:A strain autoclaved Ag ranged 6.17 - 17.45, 8.00 - 14.67, and 7.13 - 10.08 and those against the CU strain autoclaved Ag ranged 2.83

- 11.31, 6.50 - 11.89, and 8.98 - 10.08 respectively for single vaccination, double vaccination in 1 month interval and double vaccination in 2 month interval. There was a fluctuation of the TA titers . The GMT of the PHA antibody against the 8:A strain sonicated Ag ranged 1.74 - 8.72, 8.00 - 9.93 and 7.34 - 14.49 for single vaccination, double vaccination in 1 month and 2 month interval and those against the CU strain sonicated Ag were mostly lower than 2.00 in all vaccination programmer. There was no apparent correlation between the TA and PHA titers and the degree of protection. (Table 7, 8, 9, Fig.4,5,6).

It seems likely that the TA titers against both 8:A and CU strain autoclaved Ag of the S/C vaccinated group was slightly higher than the either two groups. Unexpectedly the 8:A strain tended to give higher in determinatiin of TA and PHA titers than the CU strain. The PHA titers detected by both strain were very low except the S/C vaccination provided a maximum titer at 1 wk after each vaccination then decreased precipitously.

Astinishing that the TA titers of each unvaccinated control were significantly ($P < 0.05$) higher than those of ducks before vaccination except a pair of values of 1.85 and 3.18 (GMT of cintrol in 1 wk after the first vaccination). Moreover the TA titer of the unvaccinated control were significantly ($P < 0.05$) higher than that of some vaccinated groups (Fig. 4, 5, 6).

As measuring the antibody against capsule Ag determined by the PHA test showed ninspecific positive reactiin that could not be explained.

Table 7 Antibody titers and protective immunity of ducks after the first vaccination

Route of Vaccination	Post Vaccination (wk)	TA.titer[a] (No.serum tested)		PHA titer[a] (No.serum tested)		No.survived/No.challenged (% survival)	% protection
		8:A autoclaved Ag	CU autoclaved Ag	8:A sonicated Ag	CU sonicated Ag.		
S/C	0[b]	8.00±0.14 (10)	1.85±0.28 (10)	2.38±0.23 (12)	3.83±0.26 (16)		
	1	25.4±0.17 (3)	5.04±0.14 (3)	147.03±0.25(5)	588.13±0.25(5)	3/10 (30)	-15
	2	10.77±0.16(7)	16.00±0.25(7)	5.44±0.27(5)	512.00±0.25(9)[c]	10/10 (100)	56
	4	21.11±0.27(5)	16.00±0.21(5)	8.00±0.27(6)	<2.00 (6)	7/7 (100)	88
	8	13.93±0.25(3)	9.19±0.14(3)	6.73±0.29(4)	<2.00 (4)	3/5 (60)	60
Oral	0	8.00±0.14(10)	1.85±0.28(10)	2.38±0.23(12)	3.83±0.26(16)		
	1	4.76±0.15(4)	4.76±0.15(4)	4.88±0.15 (7)	<2.00(7)	8/10 (80)	35
	2	7.13±0.35(6)	11.31±0.32(6)	16.00±0.35(7)	3.28±0.38(7)	9/10 (90)	46
	4	14.67±0.19(8)	11.31±0.16(8)	4.00±0.30(7)	<2.00 (7)	4/9 (44)	32
	8	32.0±0.00(6)	8.00±0.27(6)	13.45±0.14(8)	<2.00 (8)	1/10 (10)	10
Drinking Water	0	8.00±0.14(10)	1.85±0.28(10)	2.38±0.23(12)	3.83±0.26(16)		
	1	8.00±0.65(4)	2.83±0.30(4)	<2.00 (5)	<2.00(5)	7/10 (70)	25
	2	6.17±0.16(8)	4.76±0.35(8)	3.03±0.35(10)	<2.00(10)	7/10 (70)	26
	4	12.70±0.25(6)	4.00±0.19(6)	6.56±0.15(6)	<2.00(6)	0/8 (0)	-12
	8	17.45±0.25(8)	11.31±0.16(8)	8.72±0.31(9)	<2.00(9)	2/10 (20)	20
Unvaccinated Control	0	8.00±0.14(10)	1.85±0.28(10)	2.38±0.23(12)	3.83±0.26(16)		
	1	16.00±0.30(4)	3.18±0.35(4)	3.67±0.44(8)	<2.00(8)	5/11 (45)	
	2	16.00±0.35(7)	6.56±0.23(7)	5.19±0.16(8)	<2.00(8)	4/9 (44)	
	4	28.51±0.23(6)	16.00±0.19(6)	11.88±0.29(7)	<2.00(7)	1/8 (12)	
	8	35.92±0.30(6)	11.30±0.16(6)	8.83±0.32(7)	<2.00(7)	0/10 (0)	

a = GMT ± SD
b = Before vaccination at the age of 6 wk
c = GMT ± SD of 4 sera , another 5 sera showed GMT <2.00

Table 8 Antibody titers and protective immunity of ducks after the second vaccination on one month interval

Route of Vaccination	Post Vaccination (wk)	TA Titer[a] (No.serum tested)		PHA Titer[a] (No.serum tested)		No.survived/No.challenged (% survival)	% protection
		8:A autoclaved Ag	CU autoclaved Ag	8:A sonicated Ag	CU sonicated Ag		
S/C	0[b]	21.11±0.27(5)	16.00±0.21(5)	8.00±0.27(6)	<2.00 (6)		
	1	22.63±0.21(6)	14.25±0.30(6)	118.38±0.39(5)	588.13±0.14(5)	5/6 (83)	83
	2	35.92±0.23(6)	22.63±0.16(6)	17.56±0.12(6)	362.04±0.17(6)[c]	6/6 (100)	80
	4	26.91±0.29(4)	9.51±0.45(4)	5.66±0.30(4)	<2.00(4)	4/5 (80)	80
	8	35.33±0.21(7)	8.83±0.27(7)	9.75±0.15(7)	< 2.00(7)	7/7 (100)	83
Oral	0	14.67±0.19(8)	11.31±0.16(8)	4.00±0.30(7)	<2.00(7)		
	1	9.75±0.29(7)	11.89±0.16(7)	9.75±0.29(7)	2.21±0.37(7)	9/10 (90)	90
	2	16.00±0.25(7)	11.89±0.16(7)	10.77±0.24(7)	3.62±0.44(7)	10/10 (100)	80
	4	14.67±0.25(8)	16.00±0.16(8)	16.00±0.39(7)	3.28±0.38(7)	8/10 (80)	80
	8	7.41±0.24(9)	8.00±0.21(9)	14.93±0.36(10)	<2.00(10)	22/22 (100)	83
Drinking Water	0	12.70±0.25(6)	4.00±0.19(6)	6.56±0.15(6)	<2.00(6)		
	1	8.00±0.19(6)	9.19±0.25(6)	9.51±0.35(8)	<2.00 (8)	8/9 (89)	89
	2	14.49±0.21(7)	11.89±0.19(7)	5.94±0.57(7)	<2.00 (7)	7/8 (88)	68
	4	14.67±0.19(8)	6.73±0.21(8)	8.00±0.28(8)	2.00±0.00(8)	4/8 (50)	50
	8	12.13±0.21(10)	6.50±0.14(10)	9.93±0.32(16)	< 2.00 (16)	11/17 (65)	48
Unvaccinated Control	0	28.51±0.23(6)	16.00±0.19(6)	11.88±0.29(7)	<2.00(7)		
	1	32.0±0.00(6)	13.12±0.23(6)	5.19±0.28(8)	<2.00(8)	0/10 (0)	
	2	27.43±0.13(9)	7.41±0.28(9)	6.35±0.30(9)	<2.00 (9)	2/10 (20)	
	4	35.92±0.30(6)	11.31±0.16(6)	8.83±0.32(7)	<2.00 (7)	0/10 (0)	
	8	34.90±0.19(8)	8.00±0.23(8)	7.55±0.27(12)	<2.00 (12)	2/12 (17)	

a = GMT ± SD

b = 4 wk after the first vaccination

c = GMT ± SD of 4 sera , another 2 sera showed GMT <2.00

Table 9 Antibody titers and protective immunity of ducks after the second vaccination on two months interval

Route of Vaccination	Post Vaccination (wk)	TA titer[a] (No.serum tested): 8:A autoclaved Ag	TA titer[a] (No.serum tested): CU autoclaved Ag	PHA titer[a] (No.serum tested): 8:A sonicated Ag	PHA titer[a] (No.serum tested): CU sonicated Ag	No.survived/No.challenged (% survival)	% protection
S/C	0[b]	13.93±0.25(3)	9.19±0.14(3)	6.73±0.29(4)	<2.00 (4)		
	1	28.98±0.19(7)	26.25±0.21(7)	114.04±0.44(6)	724.08±0.32(6)	ND	ND
	2	28.51±0.12(6)	28.51±0.12(6)	25.40±0.31(6)	322.54±0.17[c](6)	ND	ND
	4	19.50±0.15(7)	13.12±0.29(7)	14.49±0.32(7)	<2.00 (7)	7/8(88)	71
Oral	0	32.0±0.00(6)	8.00±0.27(6)	13.45±0.14(8)	<2.00(8)		
	1	14.25±0.23(6)	10.08±0.25(6)	14.67±0.11(8)	<2.00(9)	ND	ND
	2	16.00±0.23(8)	14.67±0.19(8)	32.00±0.25(8)	<2.00(9)	ND	ND
	4	11.31±0.28(8)	7.34±0.11(8)	11.31±0.23(8)	<2.00(9)	9/11(82)	65
Drinking water	0	17.45±0.25(8)	11.31±0.16(8)	8.72±0.34(9)	<2.00(9)		
	1	ND	ND	ND	ND	ND	ND
	2	7.13±0.23(6)	8.98±0.23(6)	7.34±0.38(8)	2.83±0.32(8)	ND	ND
	4	10.08±0.25(6)	10.08±0.15(6)	14.49±0.21(7)	<2.00(7)	7/7(100)	83
Unvaccinated Control	0	35.92±0.30(6)	11.30±0.16(6)	8.83±0.32(7)	<2.00(7)		
	1	45.26±0.16(6)	10.08±0.25(6)	2.72±0.37(9)	<2.00	ND	
	2	32.0±0.23(8)	14.67±0.34(8)	4.92±0.45(10)	<2.00	ND	
	4	34.90±0.19(8)	8.00±0.23(8)	7.55±0.27(12)	<2.00	2/12(17)	

a = GMT ± SD
b = 8 wk after the first vaccination
c = GMT ± SD of 3 sera., another 3 sera showed GMT <2.00
ND = Not done

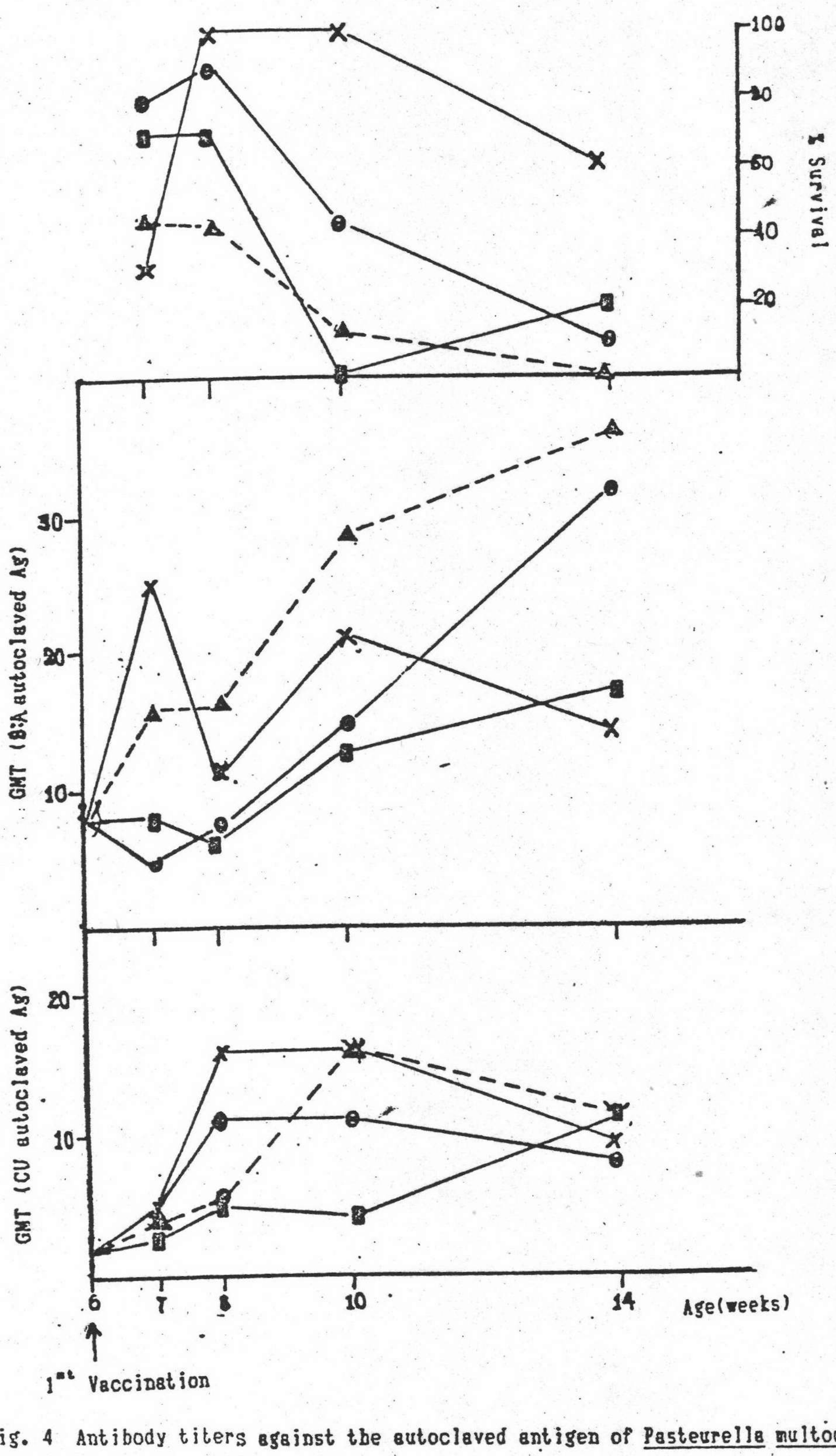

Fig. 4 Antibody titers against the autoclaved antigen of Pasteurella multocida strain 8:A and CU as measured by tube agglutination test and the percentage of survival of ducks after the first vaccination.

(X—X) S/C vaccinated group (θ—θ) Oral vaccinated group

(■—■) Drinking water vaccinated group (▲--▲) Unvaccinated control

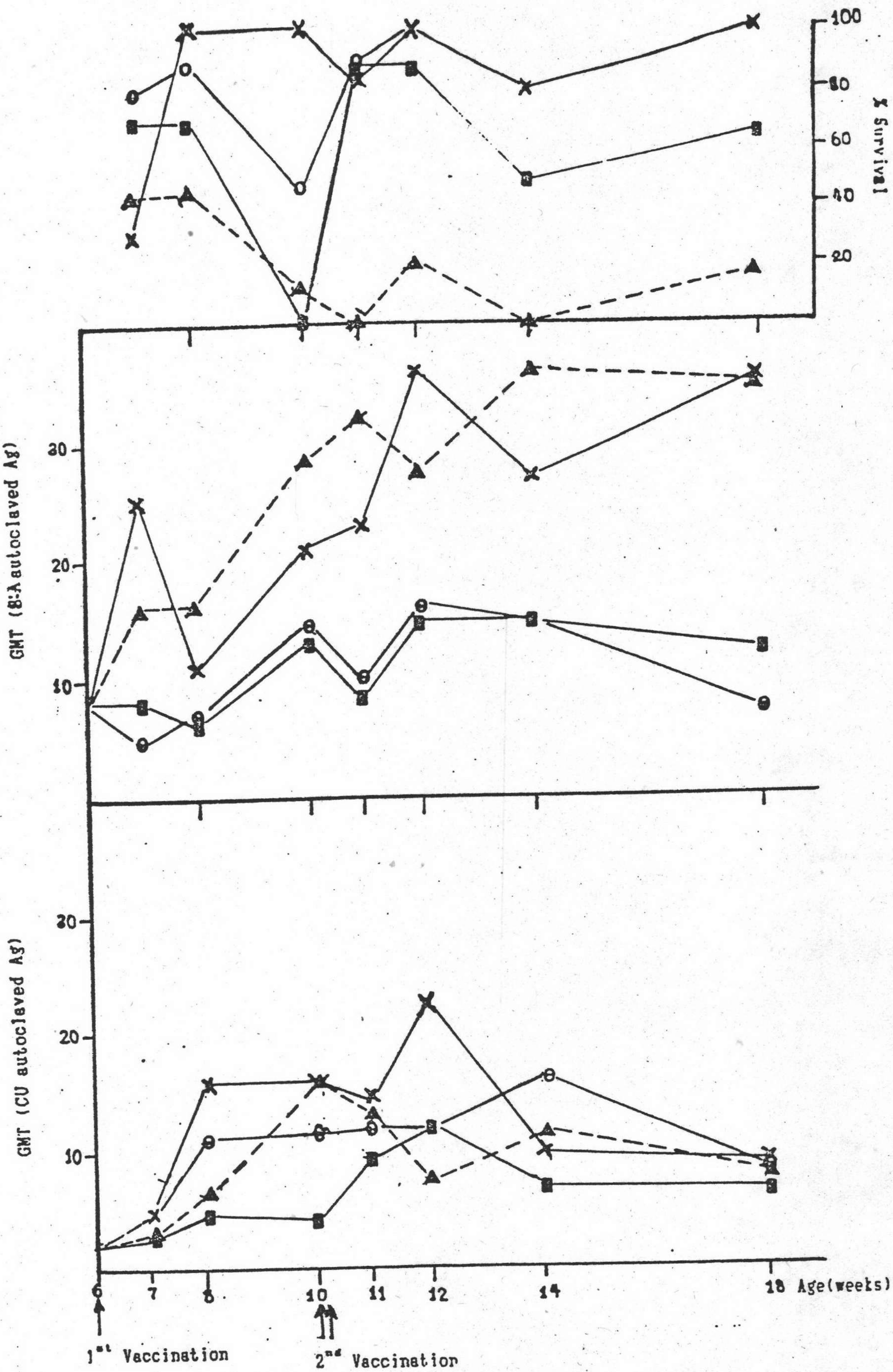

Fig. 5 Antibody titers against the autoclaved antigen of Pasteurella multocida strain 8:A and CU as measured by tube agglutination test and the percentage of survival of ducks vaccinated twice in one month interval.

(X——X) S/C vaccinated group (θ——θ) Oral vaccinated group

(■——■) Drinking water vaccinated group (▲--▲) Unvaccinated control

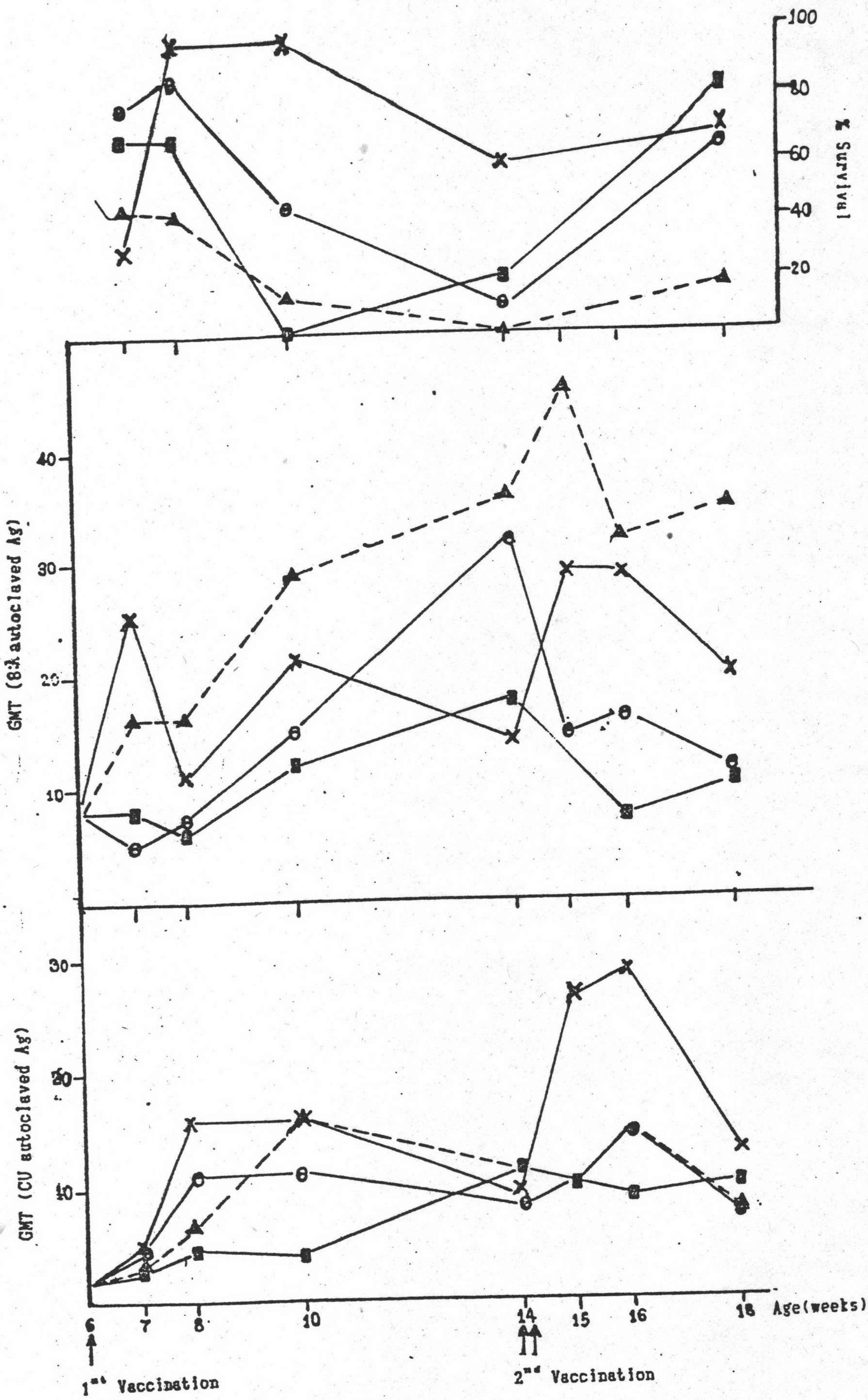

Fig. 6 Antibody titers against the autoclaved antigen of Pasteurella multocida strain 8:A and CU as measured by tube agglutination test and the percentage of survival of ducks vaccinated twice in two months interval.

(X——X) S/C vaccinated group (θ——θ) Oral vaccinated group

(■——■) Drinking water vaccinated group (▲- - -▲) Unvaccinated control

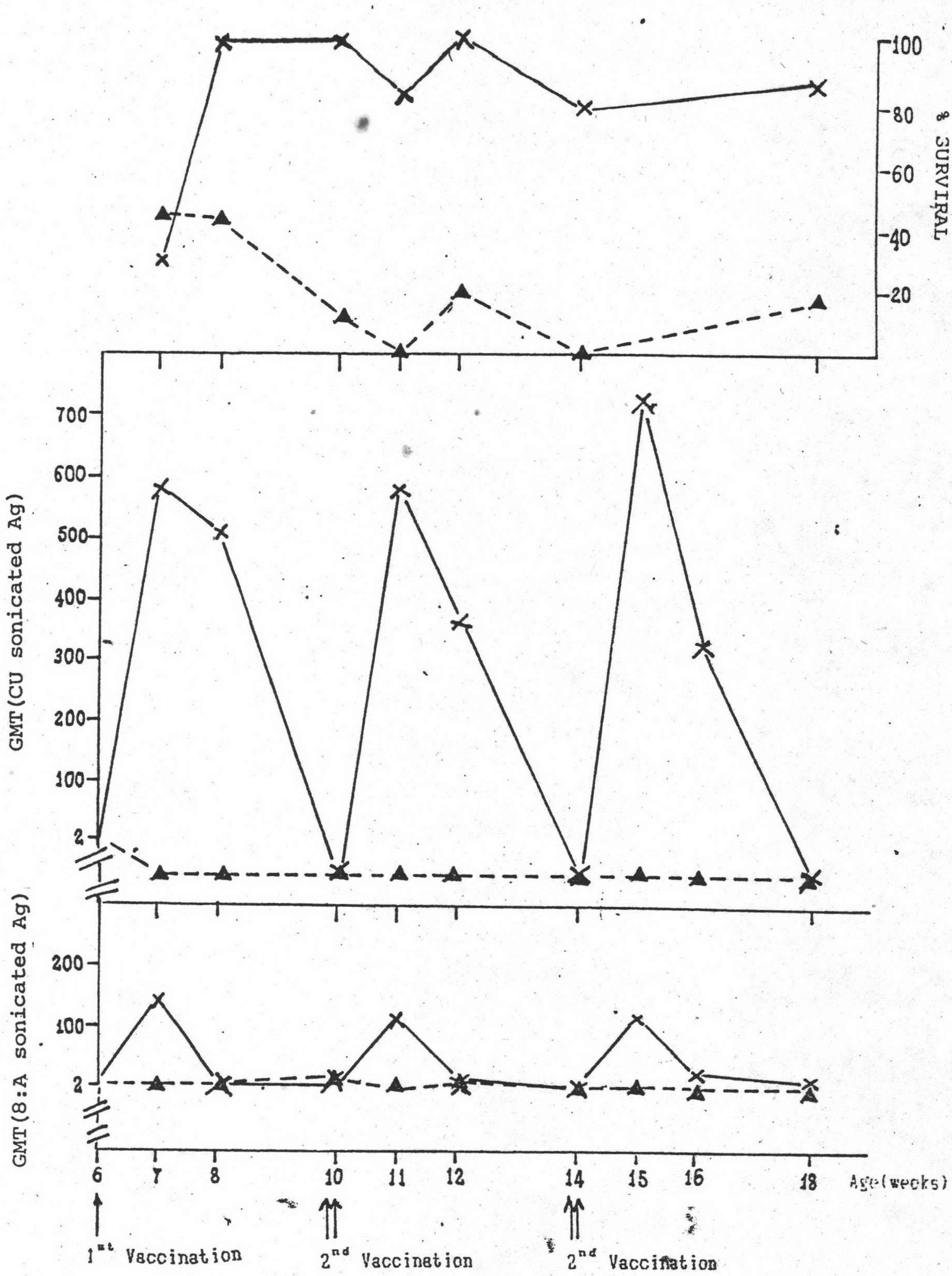

Fig. 7 Antibody titers against the sonicated antigen of Pasteurella multocida strain 8:A and CU as measured by passive hemagglutination test and the percentage of survival of Unvaccinated control and S/C vaccinated ducks
(X) S/C vaccinated group (▲) Unvaccinated control