Chapter 5

Discussion

Comparison of various antigens for the detection of agglutinating antibody

The study of various antigens to detect antibody to P.multicida shows that autoclaved antigen, which is used in the tube agglutination test, is the most sensitive whereas formalinized , HCl-treated and Living antigens are unable to detect the level of antibody. Our experiment coincided with a report published by Alexander and Soltys (63) who also stated that autoclaved antigen is good for determining the present of antibody in turkeys vaccinated with formalin-killed whole-cell P. multocida whereas it is impossible to detect its presence when one uses formalinized antigen. The same conclusion was reached in a study by Namioka (41). According to Heddleston and Walko (74) it is possible to use formalinized antigen to determine the presence of antibody by using the plate agglutination method. Schlink and Olson (80) used HCl-extract somatic antigen to examine the antibody level in turkeys by means of the tube agglutination test and found that, by using this antigen serum diluted 1: 128 could be detected. In the same way,Coates et al.(81) and Nathanson (8) who also used the same antigen,found a higher titer than Schlink and Olson (1:256 and 1:320). There is no correlation therefore,between their three later experiments and our study.

Lipipun (88) was able to use living antigen to determine the level of antibody in rabbits' immune sera; howver, our study of ducks' sera reached the opposite conclusion. A possible explanation for this may be the difference of serotype of organism and the respond of different species of experimental animal to different antigens.

Comparison of antibody titer as measured by the tube agglutination and the microtitration methods.

The tube agglutination and microtitration methods can be used to evaluate the agglutinating antibody.The level obtained from these two measurements is comparable. The microtitration method utilized less equipments and reagent (0.05 ml) when a lot of serum needed to be tested. Schlink and Olson (80) observed that the microtitration method was more sensitive than tube agglutination approach.

Determination of antibody titer by tube agglutination (TA) and indirect hemagglutination (IHA) tests

From the antibody titer in ducks vaccinated with five experimental vaccines, mineral oil adjuvanted vaccine gives the highest titer (tested by TA method) at 15 days post-vaccination, Our study correlates with Heddleston and Hall (7), Saitanu et al. (8) and Laorpaksa et al. (9) who found that mineral oil will enhance the antibody level. The other two-adjuvanted-corn-oil and aluminium hydroxide gel yield a lower antibody titer than mineral-oil vaccine, with a statistical significance of $p<0.05$. Although the concentration of bacterin in FDC vaccine is twice as high as it is in MDC vaccine, the latter yields a higher titer than the FDC vaccine at 15 days postvaccination. This indicates that mineral oil is effective in enhancing the antibody level.

After the 2^{nd} vaccination, the antibody titers (detected by the TA test) of all groups of ducks were lower than after the first one. This unusual observation correlates with the study of Pongsopida et al.(84) but conflicts with the general principle of immune response (82,83). The difference between the antibody titer after the 1^{st} vaccination and that measured after the 2^{nd} one was significantly different ($p<0.05$) in all the groups of ducks.

From the antibody titer as detected by IHA test, it was also found that mineral-oil-adjuvanted vaccine group induced the antibody, the highest peak being reached at 15 days postvaccination. Aluminium hydroxide gel and corn oil produce the lower level of antibody. However, there is no significant difference between these 3 groups ($p>0.05$). The results do not seem to correlate when the antibody titer is detected by the TA test. There is a low level of antibody present in the aluminium hydroxide gel and corn oil groups as detected by the TA test. This indicatesthat the IHA test is a better method than the TA test to use with adjuvanted groups and that each type of adjuvant can increase the antibody level. Heddleston and Reisinger (4) observed that aluminium hydroxide gel is a good adjuvant for heightening the immunity level in turkeys. With regard to non-adjuvanted vaccines, as detected by the IHA test, the antibody titer was surprisingly lower than that detected by the TA test.

After the 2nd vaccination, the three adjuvanted vaccines prodece a high antibody level as measured by the IHA test but there is no statistical difference between that and the antibody titer after the first vaccination ($p>0.05$). Thus there seems to be a contrast between formalin-killed whole-cell and Livestock Department vaccine-non adjuvanted vaccines and these 3 adjuvanted vaccines. The two-nonadjuvant vaccines yield a high antibody titer after the 2^{nd} vaccination than the 1^{st} one, that is statistically significant as measured by the IHA test.

The antibody levels determined by the IHA test are higher than those evaluated by the TA test in adjuvanted groups. On the 15th day after the first vaccination, a titer of 1: 268 could be detected by IHA test, whereas a titer of 1:125 could be detected by means of the TA test, in the MDC vaccine group. It was noted that the IHA antibody titer was high at an interval of 120 days after the 1st vaccination for all vaccine groups with the exception of the DCL group. This abnormality can not, however, be explained.

Correlation between antibody and protection against challenge

According to the results, one injection of mineral-oil-adjuvanted vaccine and Formalin-killed whole-cell vaccine afforded 100 % survival (Table 13) throughout the entire (3 month) experiment. These results were similar to those of Heddleston and Hall (7), who studied chickens and Layton (3), who studied ducklings. They concluded that mineral oil induced strong immunity. Although the concentration of bacterin in FDC vaccine is twice as high as it is in MDC vaccine,the protection afforded by MDC vaccine is as good as that afforded by FDC vaccine. In practical terms, the preparation of FDC vaccine is easier than that of the MDC vaccine which is in the form of an emulsified vaccine.One injection of aluminium -hydroxide -absorbed vaccine showed a decline of 66.6 % survival which were similar to those of Heddleston and Reisinger their study in chickens. They found that the aluminium hydroxide-absorbed vaccine provided a lower level of protection than that produced by the water-in-oil emulsified vaccine. Although the concentration of bacterin in ADC vccine is higher than in the MDC vaccine, it provided a lower level of protection a 66.6% survival rate while with the MDC vaccines, the survival rate was 100 %. One injection of corn oil adjuvanted vaccine gives a level of protection of 90%,and 70 %, at 15 and 30 days, respectively postvaccination, but at 60 and 90 days postvaccination it afford 100 % survival. Because of this abnormal result, it is impossible to draw any valid conclusion with regard to the vaccine's efficacy. The same phenomenon occurred with one injection of Livestock Department vaccine which afforded a yielded 66.6 % level of survival at 60 days postvaccination, but yielded a 100% rate of survival at the end of the experiment.

As to the potency of the vaccines, used in this experiment. The calculated potency was <60% at 90 days postvaccination. Thus potency of the vaccine at that time was unsatisfactory. Since the control groups of ducks exhibited a high percentage of survival (66.6), this result can not, however, be explained.

One injection of MDC, ADC, FDC, and DCL vaccines as measured by the percentage of survival and the calculations relating to potency at 60 days postvaccination was 85.8%, 85.8%, 85.8%, and 52.4%, respectively.

Two injections of Livestock Department vaccine could protect all of the vaccinated ducks (100%) throughout the whole 5 1/2 month (Table 14). Ranked in order of the level of protection they afforded,the results were as follows: the vaccine from CDC and FDC 80%, the MDC vaccine 75% and the ADC 50%. This shows that aluminium hydroxide gel dose not provide a longer degree of immunity when compared with other types of vaccine. But Bhasin and Biberstein, who studied in Turkey (73), indicated that aluminium hydroxide gel was equal effective to,or even slightly more effective than mineral oil. Although the concentration of bacterin in ADC vaccine was higher (13.2×10^{7} CFU/dose) than the concentration of bacterin in MDC vaccine (7.7×10^{7} CFU/dose), the ADC vaccine afforded a lower level of protection (50%).

The percentage of potency afforded by MDC, CDC, FDC, and DCL vaccine was 60.8, 65.8, 65.8, and 85.8%, respectively at the and of the experiment.

The potency at 90 days after the second vaccination cannot be calculated due to the 100% survival rate in the control ducks. This result can not, however, be explained.

No correlation between the TA titer and protection was observed in the present experiment . This was similar to the findings of Marshall et al.(87) who reported that a poor degree of correlation between antibody titers measured by the microtiter agglutination test and the level of protection. These results were also similar to those obtained by Alexander and Soltys (63) and Heddleston and Watko (74). However, Coate et al. (81), Nathanson et al. (64), and Pongsopida et al.(84) found a correlation between serum antibody titer and resistance. This discrepancy may be due to the various types of antigens, the different methods used to detect the antibody titer and the different serotypes used in the experiments.

No correlation between the IHA titer and protection was observed in this experiment either. These findings were similar to those Bhasin and Biberstein (73), although Solano et al. (85) and Dua and Maheswaran (54) reported a significant degree of correration between antibody measured by means of the IHA test and the level of protection.

Immune response in male and female ducks.

According to the findings of this study, there is no difference, statistically, between the antibody titer of male and female ducks. Sex does not seem to play a part in immune response. Therefore in experiments inwhich a lot of ducks are needed, the male is preferable because of the lower cost.

Table 9 Comparison of various antigens for the detection of agglutinating antibody in ducks vaccinated with mineral-oil and corn-oil-adjuvanted vaccine.

Interval after vaccination (day)	Type of vaccine	Agglutinating titer											
		AA									FA	HA	LA
		N	0	4	8	16	32	64	128	256	0	0	0
0	MDC	84	32[a]	20	23	8	1	-	-	-	40	40	20
	CDC	53	20	15	14	2	2	-	-	-	40	40	20
15	MDC	39	-	-	3	1	3	3	6	23	25	25	20
	CDC	35	-	-	1	9	8	10	6	1	20	20	20
30	MDC	17	-	-	-	3	2	7	4	1	17	17	10
	CDC	17	2	-	5	4	6	-	-	-	17	17	17

AA = Autoclaved Antigen

FA = Formalinized Antigen

HA = HCl-treated Antigen

LA = Living Antigen

N = Number of Sample

a = Number of Ducks

Table 10 Antibody titers against P.multocida in vaccinated and unvaccinated ducks as measured by the tube agglutination method and microtitration method.

Type of vaccine	post vacci-nation (days)	No. of sample	Agglutination Test*		P
			Tube agglutination Method	Microtitration Method	
Control	0	134	4.24±0.319	3.05±0.217	p>0.05
DCL	15	18	45.25±0.404	26.39±0.489	>0.05
Control	15	36	3.34±0.290	2.88±0.206	>0.05
DCL	30	10	12.12±0.240	12.12±0.199	>0.05
Control	30	-	ND	ND	
DCL	45	15	18.37±0.442	9.18±0.399	>0.05
Control	45	18	4.70±0.379	4.00±0.332	>0.05
DCL	60	12	7.55±0.498	3.74±0.354	>0.05
Control	60	16	2.82±0.150	3.51±0.218	>0.05
DCL	90	14	6.56±0.432	5.65±0.316	>0.05
Control	90	12	3.36±0.278	3.23±0.182	>0.05
DCL	120	7	6.56±0.384	4.41±0.192	>0.05
Control	120	11	5.65±0.150	3.52±0.281	>0.05

ND = Not Done

* = Geometric Mean ± Standard Deviation

DCL = Livestock Department Vaccine

Table 11 Comparison of antibody titers of ducks, given one injection of vaccine with protection against challenge with virulent *P. multocida*

Type of vaccine	Post vaccination (day)	TA Titer (GM ± S.D)	IHA Titer (GM ± S.D)	%survival
Mineral il adjuvanted vaccine (MDC)	15	125.74±0.469 (n=39)	268.99±0.240 (n=14)	100
	30	58.98±0.340 (n=17)	164.69±0.411 (n=11)	100
	60	16.81±0.368 (n=14)	17.44±0.591 (n=8)	100
	90	5.65±0.451 (n=2)	22.60±1.05 (n=2)	100
Corn oil adjuvanted vaccine (CDC)	15	44.34±0.359 (n=34)	80.63±0.650 (n=9)	90
	30	15.99±0.265 (n=15)	36.75±0.517 (n=10)	70
	60	10.07±0.323 (n=15)	14.67±0.571 (n=8)	100
	90	5.65±0.336 (n=4)	7.99±0.538 (n=5)	100
Aluminium hydroxide absorbed vaccine (ADC)	15	48.74±0.315 (n=28)	161.26±0.375 (n=9)	100
	30	15.99±0.289 (n=13)	43.06±0.530 (n=7)	100
	60	9.51±0.278 (n=12)	17.14±0.578 (n=10)	100
	90	4.0 (n=2)	16.00±0.903 (n=4)	66.6
Formalin-killed whole cell Vaccine (FDC)	15	82.62±0.327 (n=19)	21.77±0.711 (n=9)	100
	30	18.56±0.232 (n=14)	19.50±0.474 (n=7)	100
	60	14.81±0.387 (n=9)	22.62±0.721 (n=8)	100
	90	8.0 ±0.368 (n=4)	11.31±0.620 (n=4)	100
Livestock Department vaccine (DCL)	15	51.98±0.331 (n=20)	16.95±0.569 (n=12)	100
	30	12.12±0.199 (n=10)	16.00±0.459 (n=6)	100
	60	10.55±0.361 (n=5)	2.36±0.240 (n=5)	66.6
	90	2.82±0.150 (n=2)	5.03±0.142 (n=3)	100
Control	15	3.84±0.321 (n=35)	5.75±0.538 (n=21)	10
	30	4.75±0.249 (n=16)	4.00±0.466 (n=10)	36.3
	60	4.30±0.291 (n=19)	3.31±0.446 (n=11)	14.2
	90	4.21±0.275 (n=13)	2.10±0.08 (n=13)	66.6

Table 12 Comparison of antibody titers of ducks, given two injections of vaccine with protection against challenge with virulent P.multocida

Type of vaccine	day after 2nd vaccination	TA Titer (GM ± S.D)	IHA Titer (GM ± S.D)	%survival
Mineral oil adjuvanted vaccine (MDC)	30	22.62±0.349 (n=20)	215.26±0.130 (n=8)	100
	90	11.88±0.505 (n=7)	256.0 (n=2)	100
	135	12.69±0.141 (n=3)	2.51±0.141 (n=3)	75
Corn oil adjuvanted vaccine (CDC)	30	5.35±0.501 (n=19)	25.99±0.522 (n=10)	100
	90	8.64±0.331 (n=9)	139.58±0.486 (n=8)	100
	135	6.34±0.281 (n=6)	8.00±0.425 (n=5)	80
Aluminium hydroxide absorbed vaccine (ADC)	30	14.81±0.387 (n=9)	63.99±0.499 (n=8)	100
	90	5.46±0.260 (n=20)	71.20±0.470 (n=13)	100
	135	5.27±0.385 (n=10)	26.25±0.657 (n=7)	50
Formalin-killed whole cell Vaccine (FDC)	30	16.75±0.204 (n=15)	9.33±0.545 (n=9)	100
	90	6.62±0.498 (n=11)	98.70±0.497 (n=8)	100
	135	6.72±0.391 (n=4)	25.39±0.141 (n=3)	80
Livestock Department vaccine (DCL)	30	15.16±0.520 (n=13)	7.99±0.448 (n=9)	100
	90	10.07±0.224 (n=6)	19.50±0.766 (n=7)	100
	135	6.06±0.306 (n=5)	2.0 (n=3)	100
Control	30	4.30±0.291 (n=19)	ND	40
	90	4.38±0.327 (n=15)	ND	100
	135	4.0 ±0.301 (n=4)	ND	14.2

ND = not done

Table 13 Comparison of the efficacy of one injection of vaccine in ducks when challenged with virulent P.multocida

Type of vaccine	Postvaccination (day)	No.of duck before challenge	No.of duck after challenge	Potency* %	survival %
Mineral oil adjuvanted vaccine (MDC)	15	11	11	90	100
	30	9	9	63.7	100
	60	9	9	85.8	100
	90	3	3	33.4	100
Corn oil adjuvanted vaccine (CDC)	15	10	9	80	90
	30	10	7	33.7	70
	60	10	10	85.8	100
	90	4	4	33.4	100
Aluminium hydroxide absorbed vaccine (ADC)	15	10	10	90	100
	30	10	10	63.7	100
	60	6	6	85.8	100
	90	3	2	0	66.6
Formalin-killed whole cell Vaccine (FDC)	15	7	7	90	100
	30	8	8	63.7	100
	60	7	7	85.8	100
	90	4	4	33.4	100
Livestock Department vaccine (DCL)	15	6	6	90	100
	30	6	6	63.7	100
	60	3	2	52.4	66.6
	90	2	2	33.4	100
Control	15	10	1		10
	30	4	4		36.3
	60	7	1		14.2
	90	6	4		66.6

ND = Not Done

Potency* = % survival of vaccinated bird -% survival of unvaccinated bird.

Table 14 Comparison of the efficacy of two injection of vaccines in ducks when challenged with virulent *P.multocida*

Type of vaccine	day after 2nd vacci-nation	No.of duck before challenge	No.of duck after challenge	Potency' %	survival %
Mineral oil adjuvanted vaccine (MDC)	30	8	8	60	100
	90	6	6	NT'''	100
	135	4	3	60.8	75
Corn oil adjuvanted vaccine (CDC)	30	9	9	60	100
	90	6	6	NT'''	100
	135	5	4	65.8	80
Aluminium hydroxide absorbed vaccine (ADC)	30	8	8	60	100
	90	10	10	NT'''	100
	135	10	5	35.8	50
Formalin-killed whole cell Vaccine (FDC)	30	5	5	60	100
	90	6	6	NT'''	100
	135	5	4	65.8	80
Livestock Department vaccine (DCL)	30	5	5	60	100
	90	6	6	NT'''	100
	135	4	4	85.8	100
Control	30	5	2		40
	90	4	4		100
	135	7	1		14.2

NT''' = Not tested because the no mortality was observed in the control ducks

ND = Not Done

Potency' = % survival of vaccinated bird - % survival of unvaccinated bird

Table 15 Antibody titer measured by tube agglutination test before and after challenge with P.multocida in ducks vaccinated once with various vaccines.

Type of vaccine	Postvaccination (day)	titer before challenge (G.M.±SD)	titer after challenge (G.M.±SD)	P
Mineral oil adjuvanted vaccine (MDC)	15	125.74±0.469	ND	-
	30	58.98±0.340	26.25±0.310	< 0.05
	60	16.81±0.368	17.66±0.105	> 0.05
	90	5.65±0.451	12.69±0.375	> 0.05
Corn oil adjuvanted vaccine (CDC)	15	44.34±0.359	ND	-
	30	15.99±0.269	6.34±0.375	< 0.05
	60	10.07±0.323	25.39±0.375	< 0.05
	90	5.65±0.336	8.00±0.491	> 0.05
Aluminium hydroxide absorbed vaccine (ADC)	15	48.74±0.315	ND	-
	30	15.99±0.289	13.71±0.341	> 0.05
	60	9.51±0.278	13.92±0.351	> 0.05
	90	4.0	22.6±0.752	> 0.05
Formalin-killed whole cell Vaccine (FDC)	15	82.62±0.327	ND	-
	30	18.56±0.232	11.31±0.301	> 0.05
	60	14.81±0.387	31.99±0.301	> 0.05
	90	8.00±0.368	20.15±0.567	> 0.05
Livestock Department vaccine (DCL)	15	51.98±0.331	ND	-
	30	12.12±0.199	16.00±0.212	> 0.05
	60	10.55±0.361	90.50±0.150	< 0.05
	90	2.82±0.150	16.00±0.301	> 0.05
Control	15	3.84±0.321	ND	-
	30	4.75±0.249	ND	-
	60	4.30±0.291	ND	-
	90	4.21±0.275	13.92±0.583	> 0.05

ND = Not Done

Table 16 Antibody titer measured by tube agglutination test before and after challenge with P.multocida in ducks vaccinated twice with various vaccines.

Type of vaccine	day after 2nd vacci-nation	titer before challenge (G.M.±SD)	titer after challenge (G.M.±SD)	P
Mineral oil adjuvanted vaccine (MDC)	30	22.62±0.349	21.53±0.316	> 0.05
	90	11.88±0.505	7.99±0.602	> 0.05
	135	12.69±0.141	203.18±0.141	< 0.05
Corn oil adjuvanted vaccine (CDC)	30	5.35±0.204	9.18±0.420	> 0.05
	90	8.64±0.315	12.69±0.210	> 0.05
	135	6.34±0.140	26.99±0.181	< 0.05
Aluminium hydroxide absorbed vaccine (ADC)	30	14.81±0.330	16.00±0.223	> 0.05
	90	5.41±0.480	3.56±0.114	> 0.05
	135	5.27±0.220	63.99±0.453	< 0.05
Formalin-killed whole cell Vaccine (FDC)	30	16.75±0.314	19.02±0.213	> 0.05
	90	6.62±0.420	8.00±0.334	> 0.05
	135	6.72±0.110	53.81±0.186	< 0.05
Livestock Department vaccine (DCL)	30	15.16±0.240	7.99±0.410	> 0.05
	90	10.07±0.171	2.29±0.200	> 0.05
	135	6.06±0.312	76.10±0.358	< 0.05
Control	30	4.30±0.121	ND	-
	90	4.38±0.327	8.00±0.245	> 0.05
	135	4.00±0.301	32.00	> 0.05

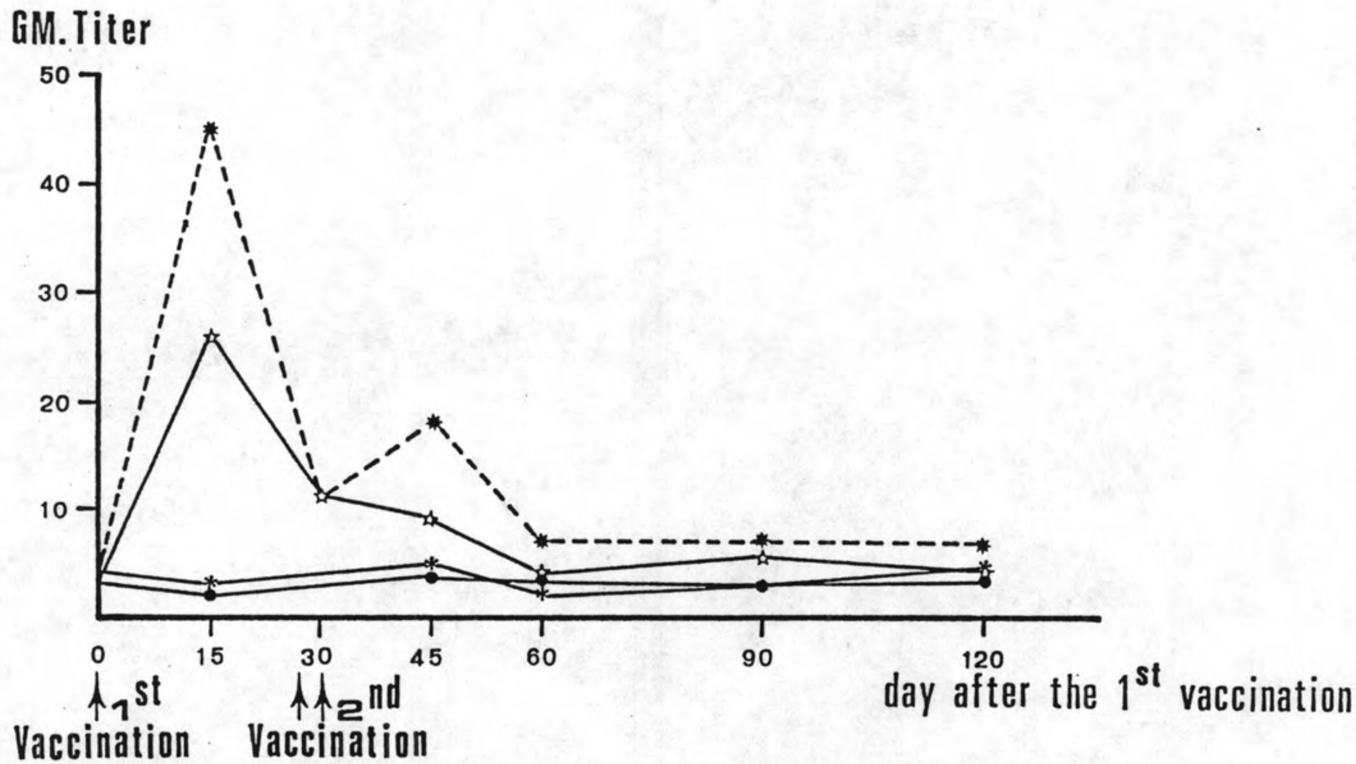


Fig. 1 The antibody level of ducks, vaccinated twice with Livestock Department vaccine as detect by tube agglutination and microtitration method. (✱) tube agglutination (TA) method, (✫) microtitration method (✱) control, TA method, (●) control, microtitration method, (↑) represented the date of vaccination.

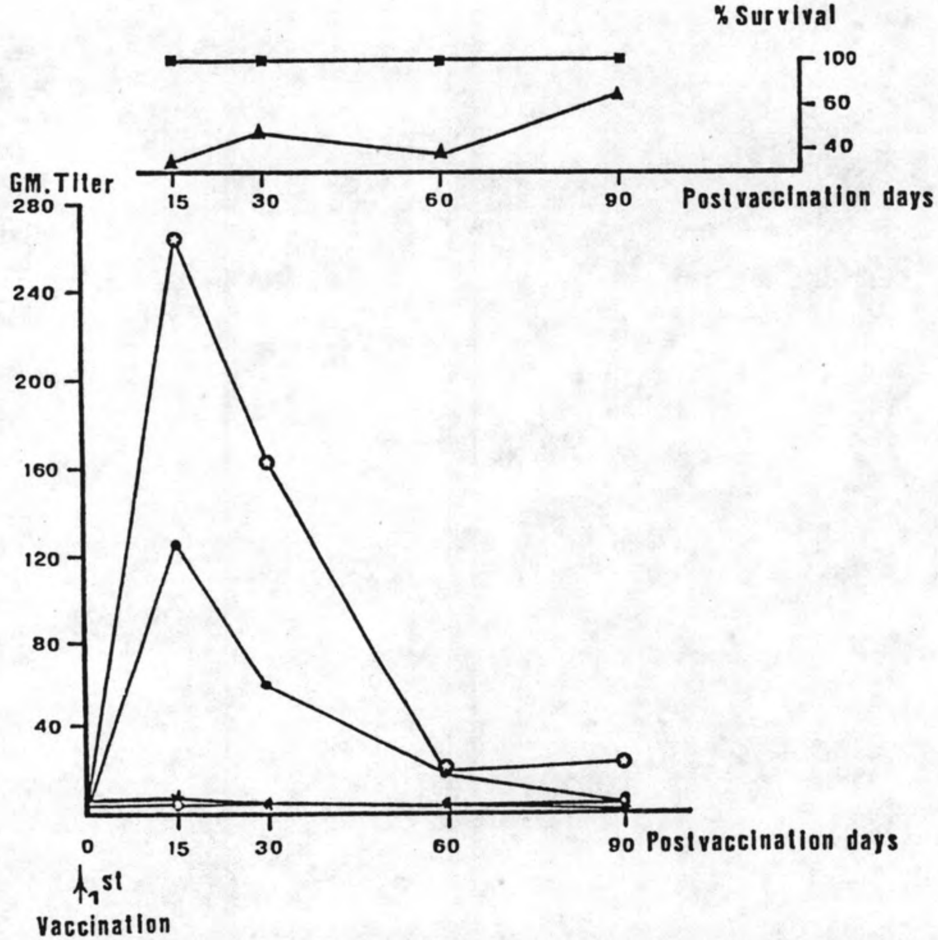


Fig. 2 The antibody titer and percentage of survival of ducks vaccinated once with mineral-oil-adjuvanted vaccine as measured by tube agglutination test and indirect hemagglutination test. (✪) IHA, (●) TA, (✶) IHA, control, (○) TA, control, (■) % survival of ducks vaccinated with MDC, (▲) % survival of control, (↑) represented the date of vaccination

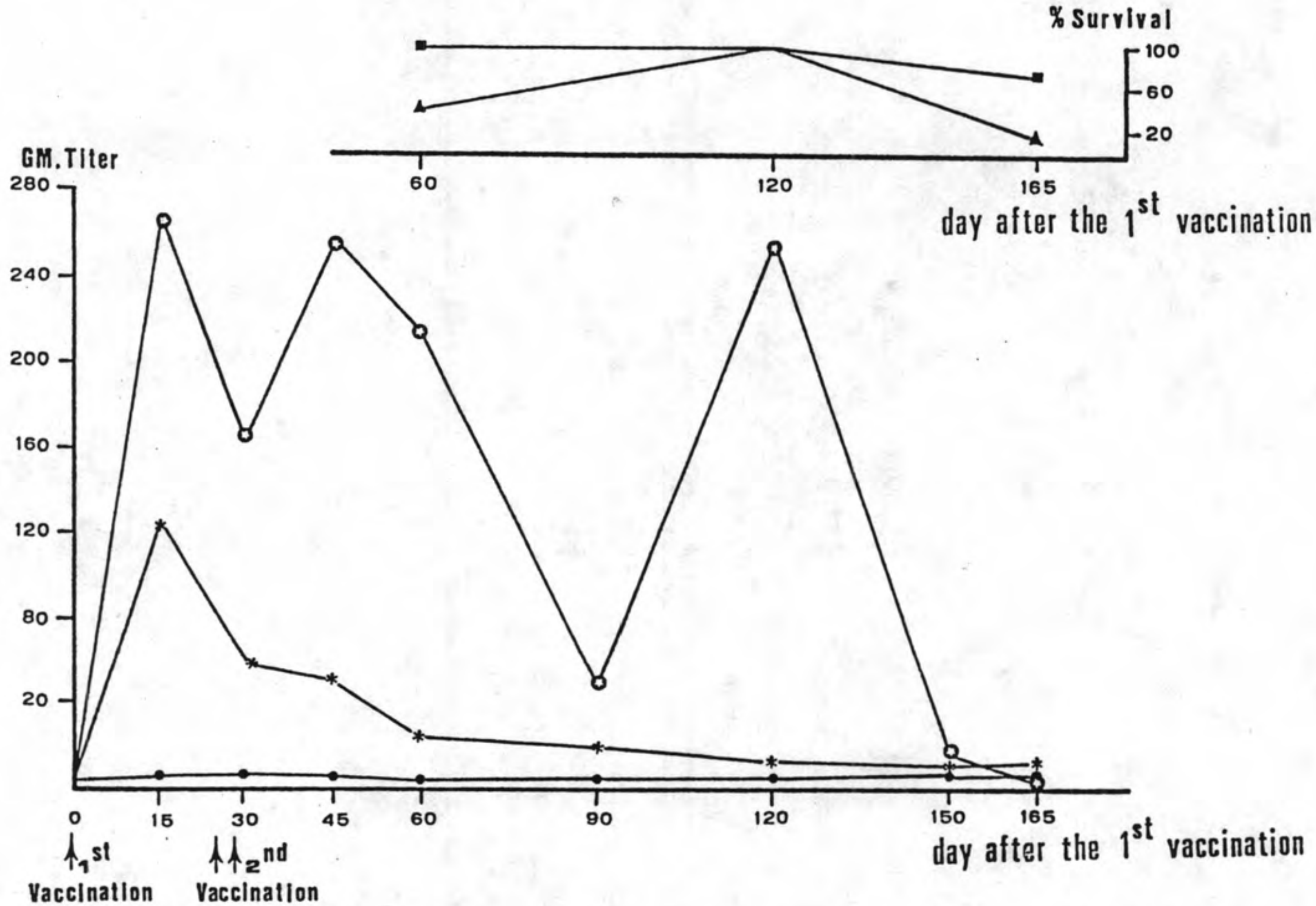


Fig. 3 The antibody titer and percentage of survival of ducks vaccinated twice with mineral-oil-adjuvanted vaccine as measured by tube agglutination test and indirect hemagglutination test. (✪) IHA, (*) TA, (●) TA, control, (■) % survival of ducks vaccinated with MDC, (▲) % survival of control, (↑) represented the date of vaccination

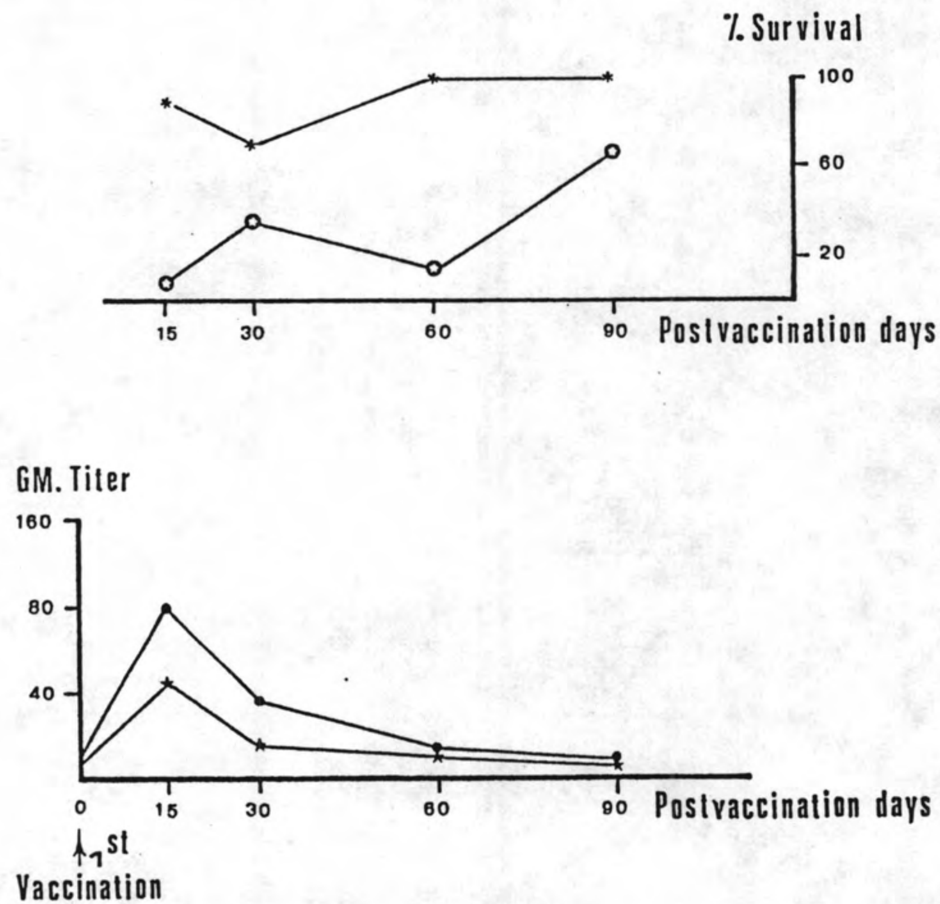


Fig. 4 The antibody titer and percentage of survival of ducks vaccinated once with corn - oil - adjuvanted vaccine (CDC) as measured by tube agglutination test and indirect hemagglutination test.

(●) IHA, (✱) TA, (✱) % survival of ducks vaccinated with CDC, (✪) % survival of control, (↑)represented the date of vaccination

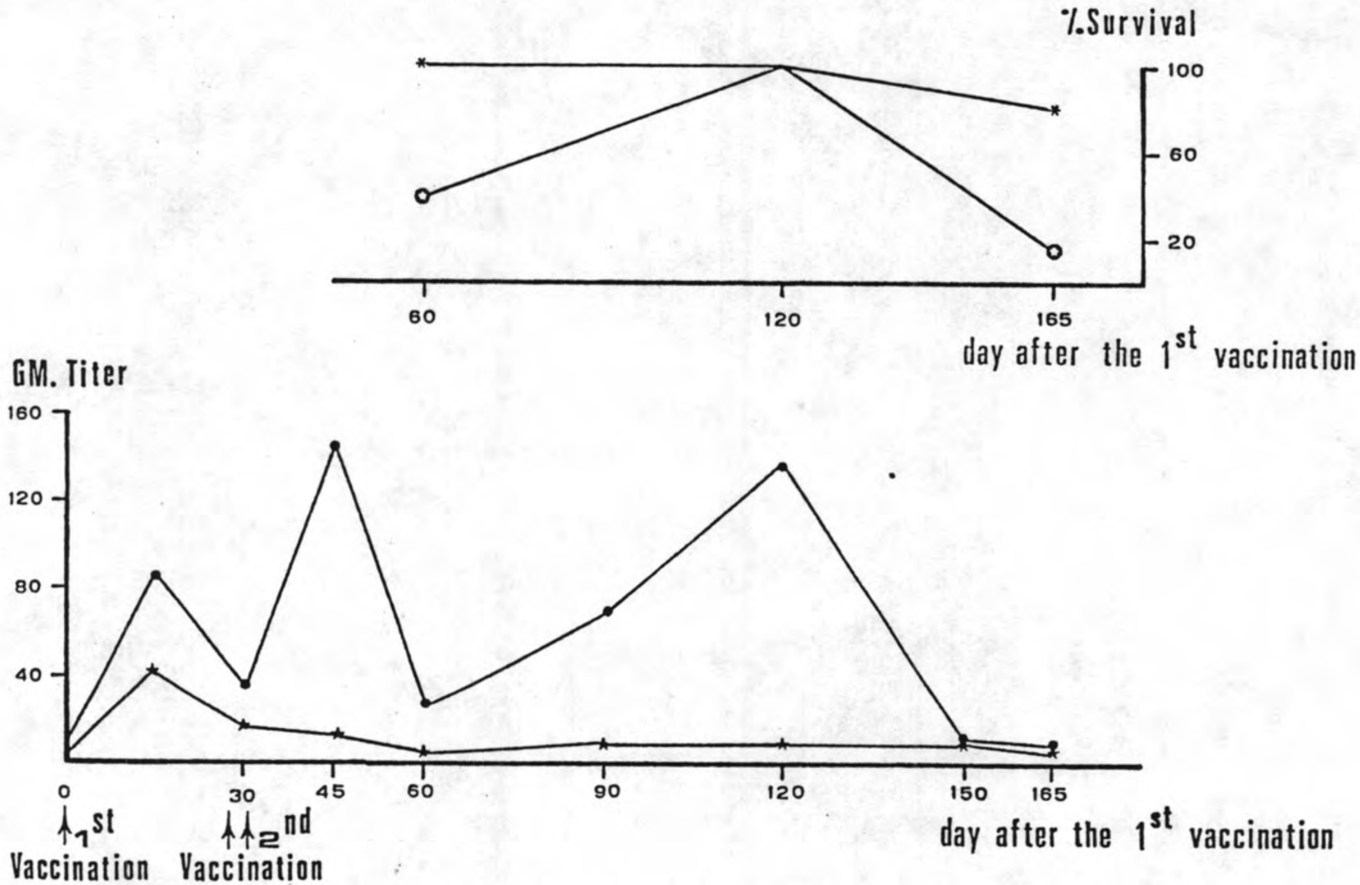
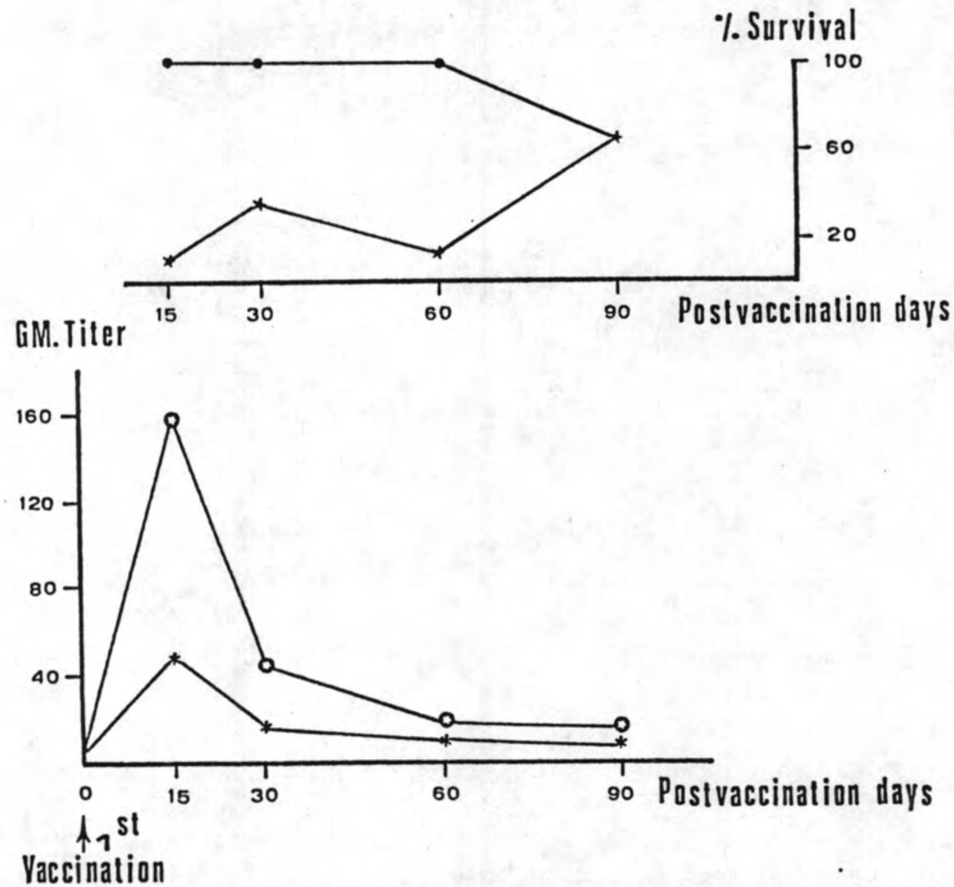


Fig. 5 The antibody titer and percentage of survival of ducks vaccinated twice with corn-oil-adjuvanted vaccine (CDC) as measured by tube agglutination test and indirect hemagglutination test. (●) IHA, (✶) TA, (*) % survival of ducks vaccinated with CDC, (✪) % survival of control, (↑) represented the Date of vaccination.



<u>Fig. 6</u> The antibody titer and percentage of survival of ducks vaccinated once with aluminium-hydroxide-absorbed vaccine as measured by tube agglutination test and Indirect hemagglutination test.

(✪) IHA, (✱) TA, (●) % survival of ducks vaccinated with DCL, (✶) % survival of control, (↑) represented the date of vaccination

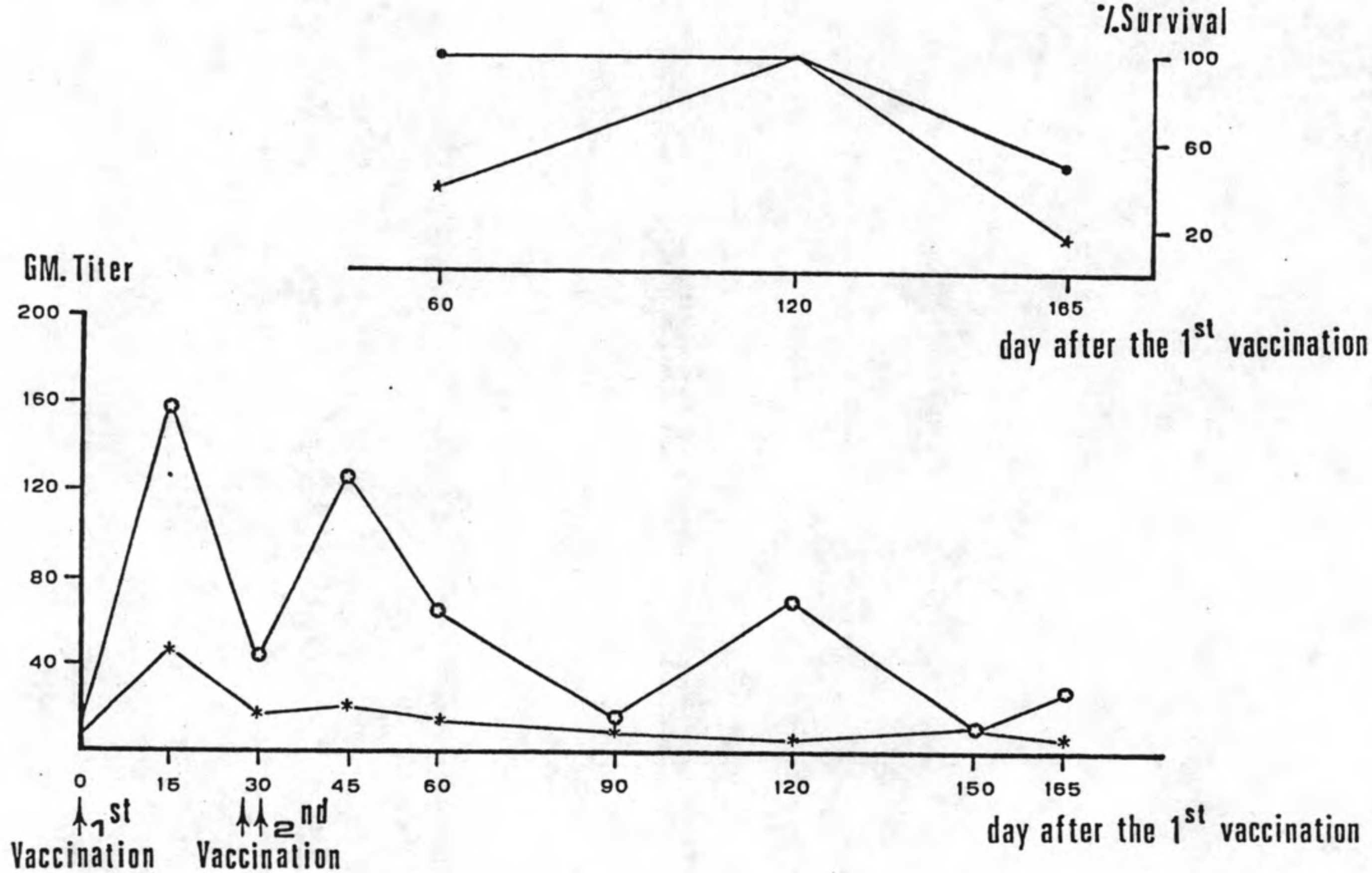
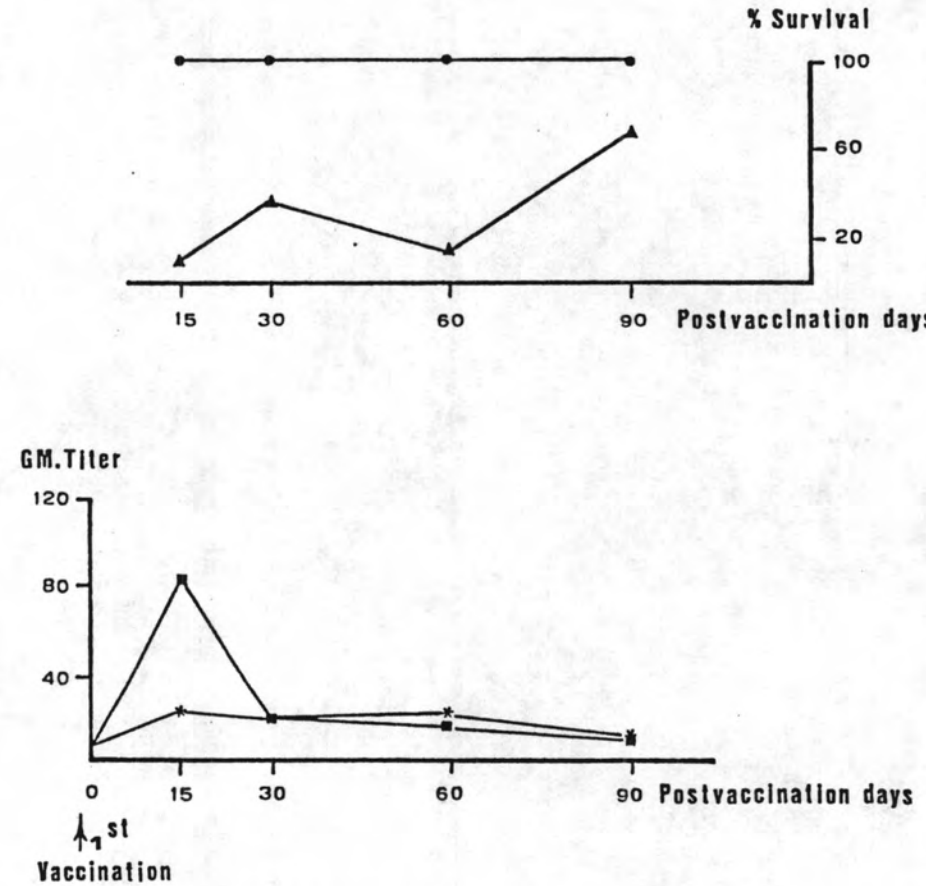


Fig. 7 The antibody titer and percentage of survival of ducks vaccinated twice with aluminium - hydroxide-absorbed vaccine (ADC) as measured by tube agglutination test and Indirect hemagglutination test.
(○) IHA, (*) TA, (●) % survival of ducks vaccinated with ADC, (*) % survival of control, (↑)represented the date of vaccination



<u>Fig. 8</u> The antibody titer and percentage of survival of ducks vaccinated once with formalin-killed whole-cell vaccine (FDC) as measured by tube agglutination test and indirect hemagglutination test.

(✱) IHA, (■) TA, (●) % survival of ducks vaccinated with FDC, (▲) % survival of control, (↑)represented the date of vaccination

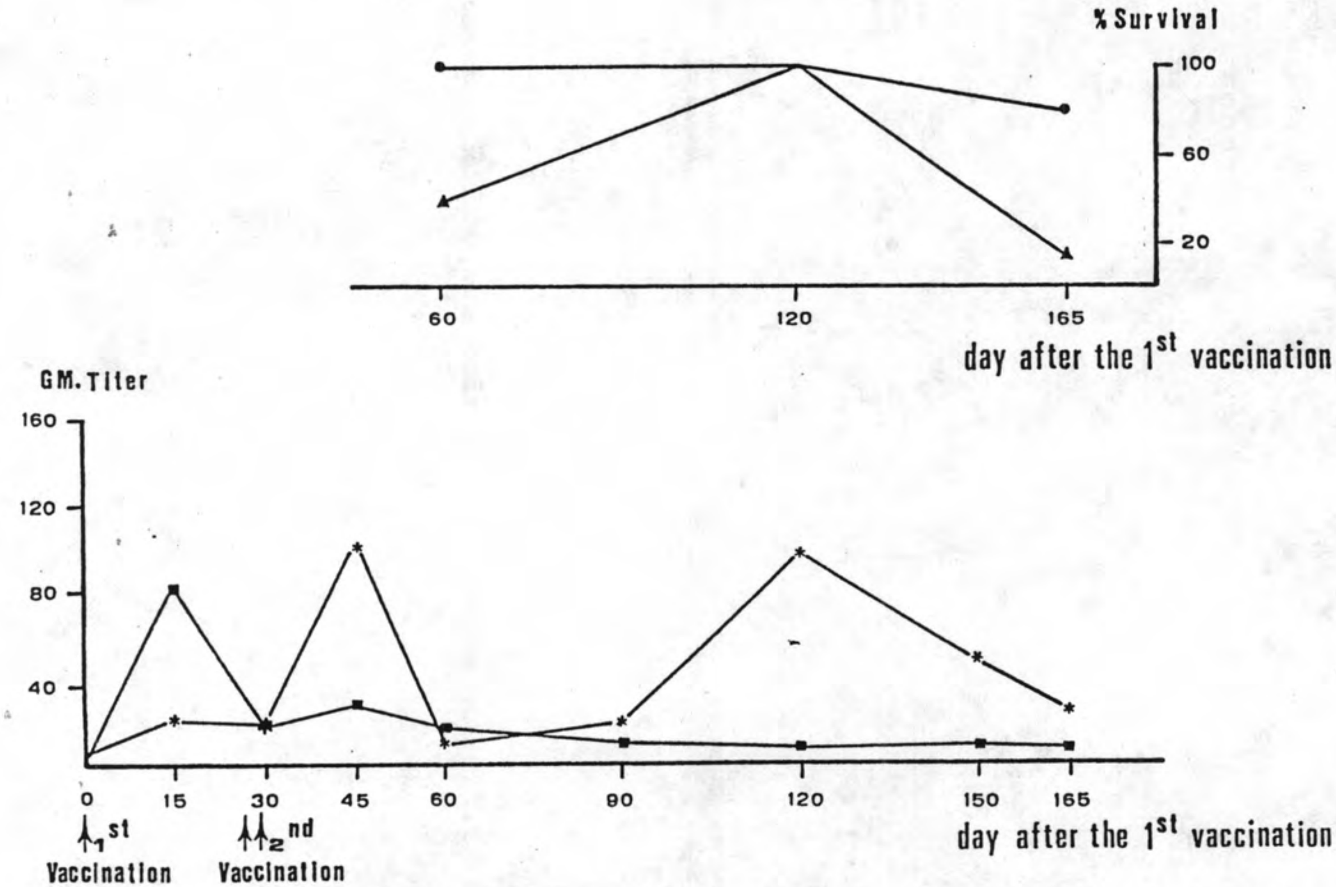
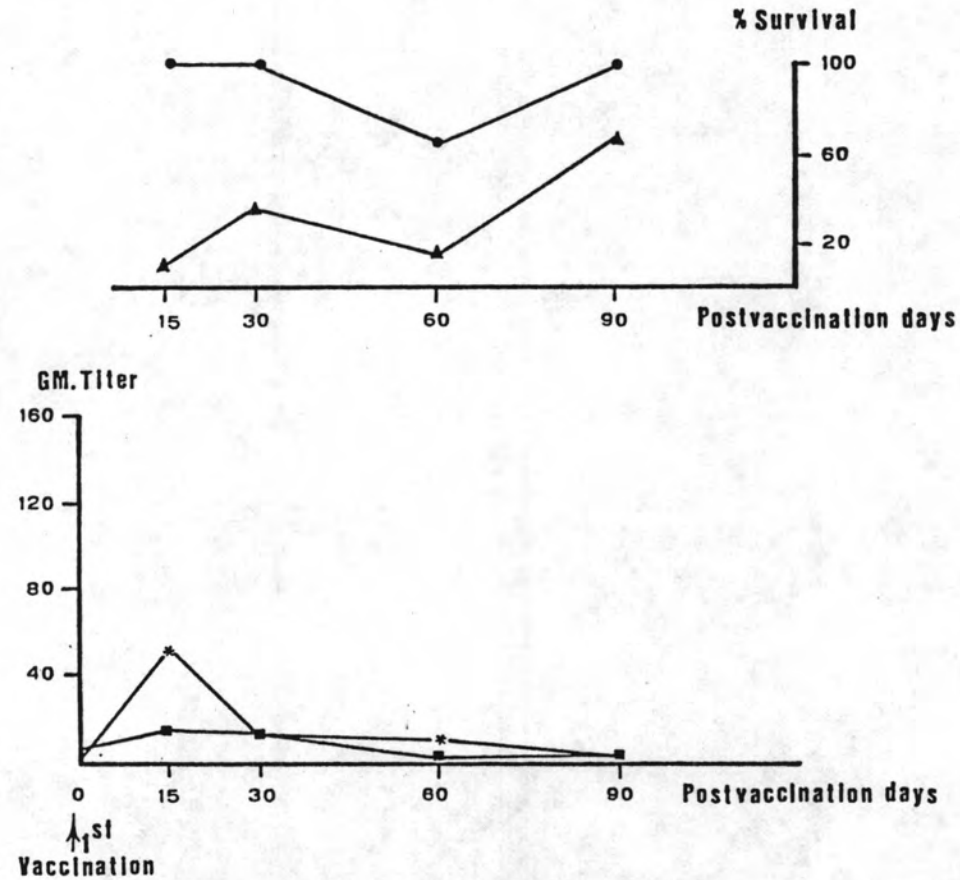
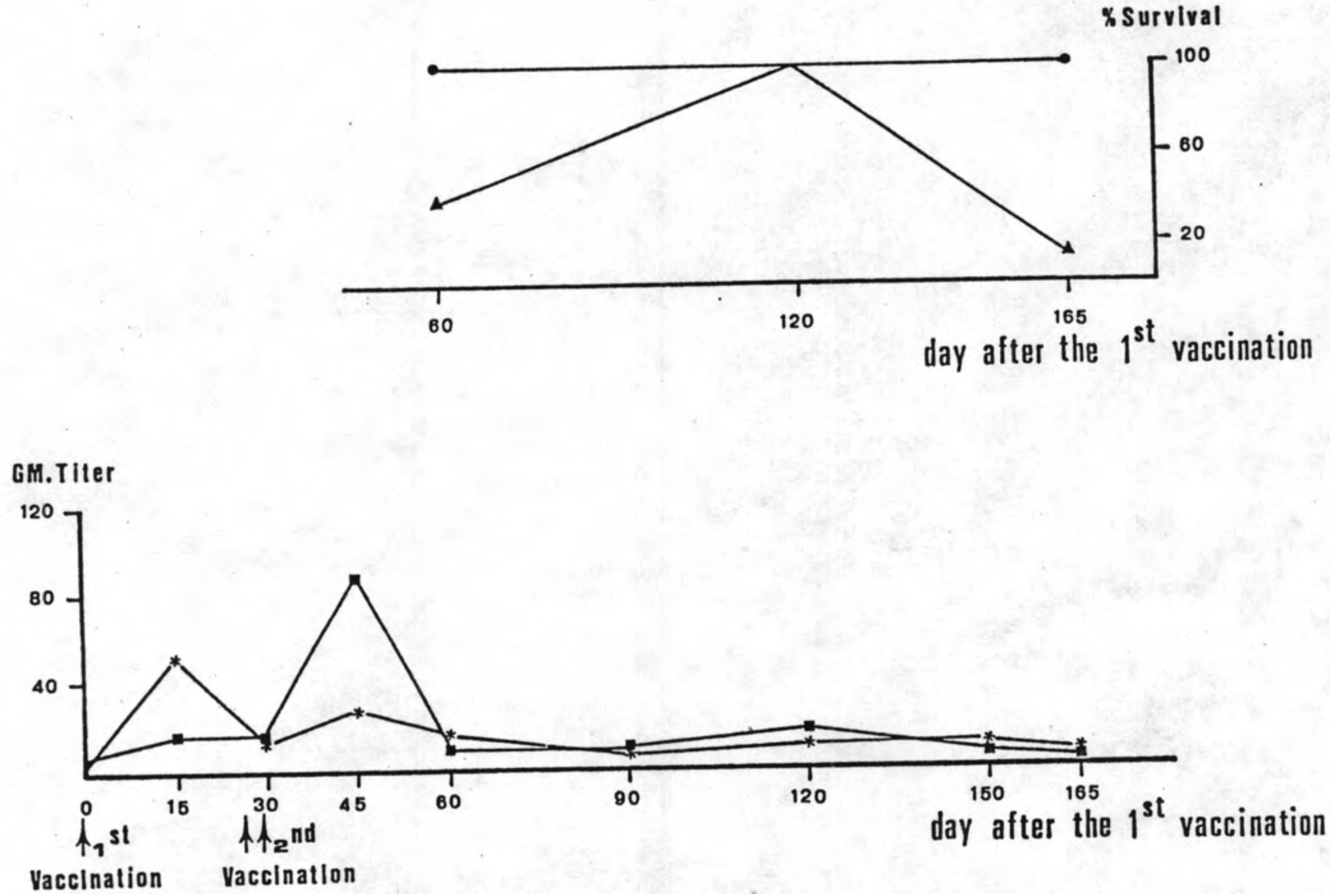


Fig. 9 The antibody titer and percentage of survival of ducks vaccinated twice with formalin-killed whole-cell vaccine (FDC) as measured by tube agglutination test and indirect hemagglutination test.

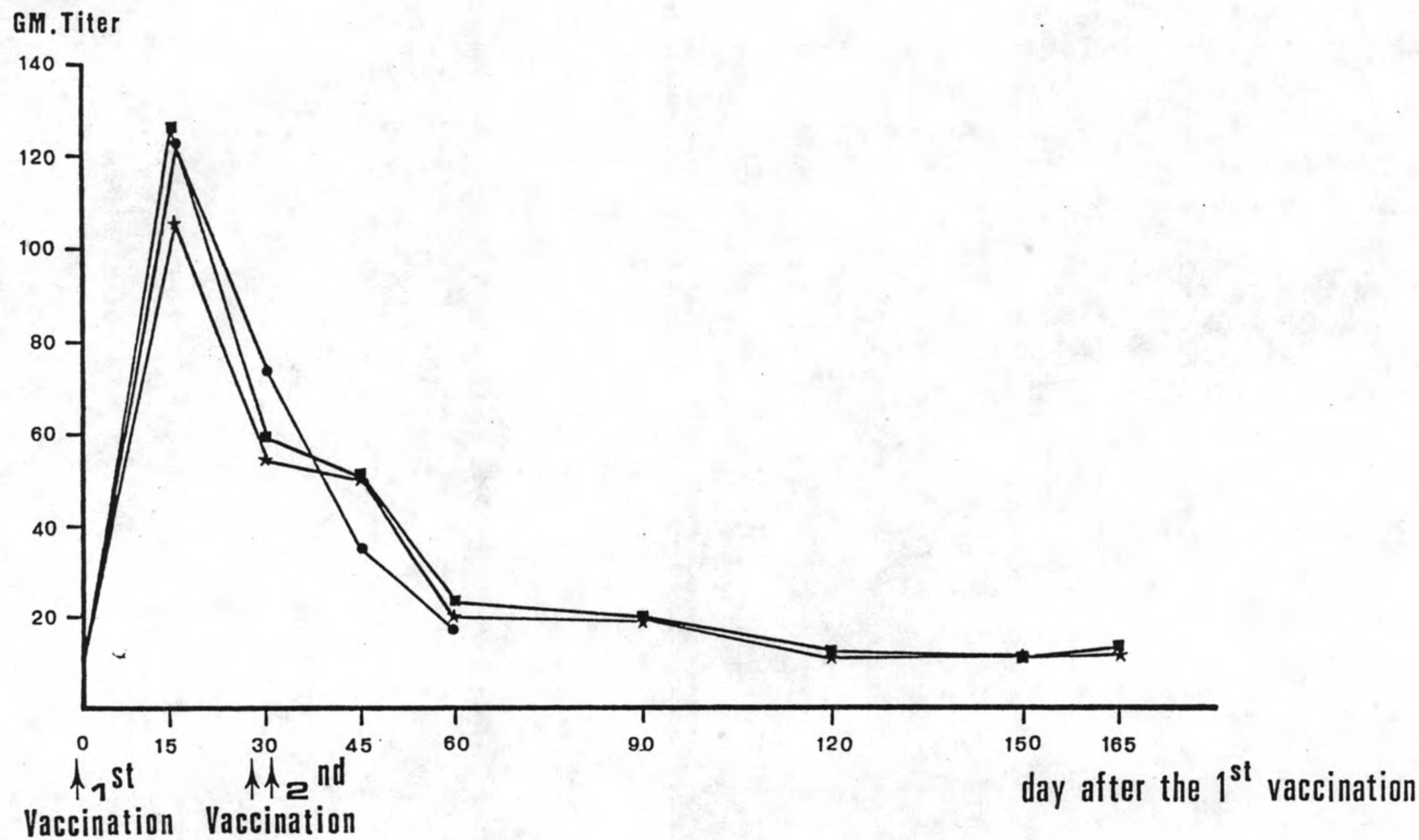
(✱) IHA, (■) TA, (●) % survival of ducks vaccinated with FDC, (▲) % survival of control, (↑)represented the date of vaccination



<u>Fig. 10</u> The antibody titer and percentage of survival of ducks vaccinated once with Livestock Department vaccine as measured by tube agglutination test and indirect hemagglutination test. (■) IHA, (*) TA, (●) % survival of ducks vaccinated with DCL, (▲) % survival of control. (↑)represented the date of vaccination



<u>Fig. 11</u> The antibody titer and percentage of survival of ducks vaccinated twice with Livestock Department vaccine as measured by tube agglutination test and indirect hemagglutination test. (■) IHA, (*) TA, (●) % survival of ducks vaccinated with DCL, (▲) % survival of control, (↑)represented the date of vaccination



<u>Fig. 12</u> Comparison of agglutinating antibody level of male and female ducks vaccinated twice with mineral-oil-adjuvanted vaccine (■) female and male, (✶) female, (●) male, (↑) represented the date of vaccination

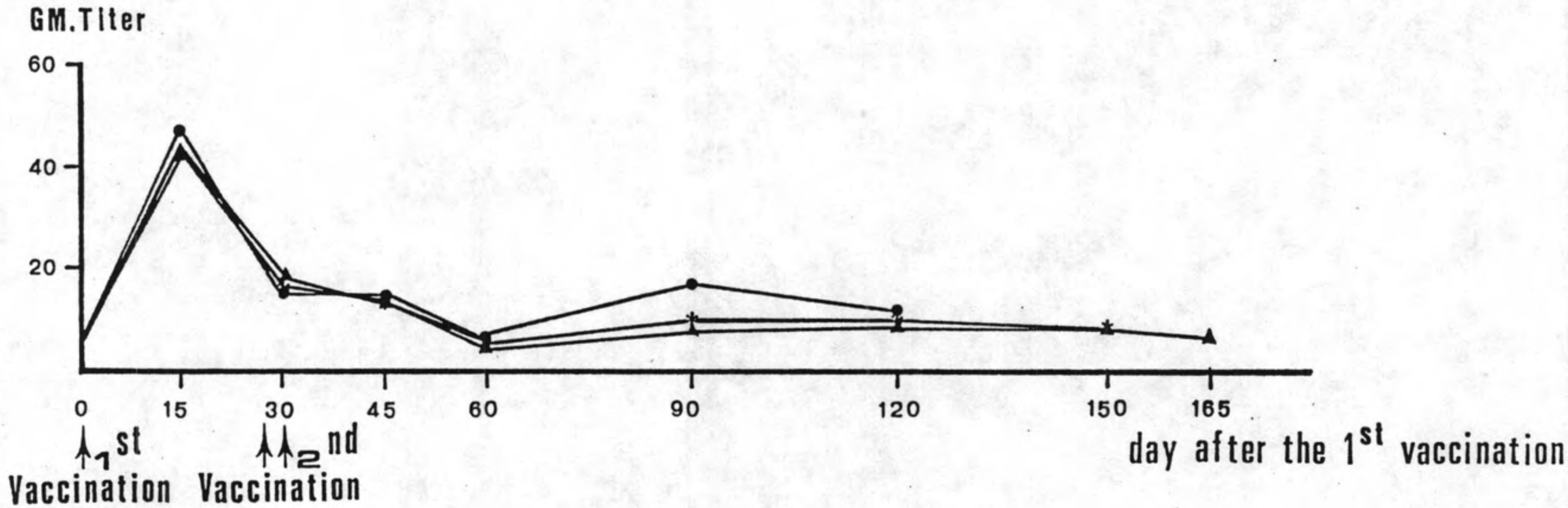


Fig. 13 Comparison of agglutinating antibody level of male and female ducks vaccinated twice with corn-oil-adjuvanted vaccine (*) female and male, (▲) female, (●) male, (↑) represented the date of vaccination