Veterinary Serum and Vaccine Research Institute,
Abbasia, Cairo.

COMPARISON OF EGG YOLK AND SERUM SAMPLES FOR THE DETECTION OF ANTIBODIES TO PASTEURELLA MULTOCIDA AND NEWCASTLE DISEASE VIRUS

(With 2 Tables and 10 Figures)

By
H.A. HASSAN

(Received at 16/12/2000)

مقارنة عينات صفار البيض وعينات الدم في الكشف عن الأجسام المضادة للباستيريلا ملتوسيدا وفيروس مرض النيوكاسل

حسن عبد المقصود حسن

في تلك الدراسة تم تقييم مدى كفاءة استخدام صفار البيض بديلاً للسيرم لقياس الأجسام المضادة لفيروس النيوكاسل وبكتريا الباستيريلا ملتوسيدا في القطعان البياضة خلال فترة الإنتاج. وقد تبين لنا وجود ارتباط واضح وتماثل شبه تام بين مستويات المناعة لكل من عينات صفار البيض والسيرم. وبثبوت تلك العلاقة الوطيدة بين عينات السيرم وصفار البيض والتى جرى تجميعها معاً وفي نفس التوقيت، فإنه يمكن التوصية بمتابعة الحالة المناعية للقطعان البياضة بخصوص فيروس النيوكاسل وبكتريا الباستيريلا ملتوسيدا وذلك من خلال فحص عينات صفار البيض بديلاً عن عينات السيرم.

SUMMARY

Egg yolk was evaluated as an alternative source of antibodies for detection of the immunological status of laying flock to Newcastle disease virus (NDV) and Pasteurella multocida (PM) bacterin. Serum and corresponding egg samples were collected simultaneously from individual hens as well as at random from the tested flocks. Serum and chloroform-extracted yolk were assayed for antibody determination by the ELISA for NDV and PM and by haemagglutination inhibition (HI) test for NDV. Yolk prepared by chloroform extraction performed well in the serological tests used. A high correlation was consistently observed between serum and corresponding

yolk antibody titres to the antigens of NDV and PM. Results of this study, revealed that antibody monitoring of laying flocks to NDV and PM using egg yolk instead of serum is feasible and highly recommended.

Key words: *Egg Yolk, Antibodies.*

INTRODUCTION

High hen densities that currently exist in the poultry farms make disease management and control difficult. Knowledge of the immune status of layer and breeder hens throughout the laying period enables the flock owner to determine whether the hens possess adequate levels of antibody to disease they have been vaccinated against or not. Serological tests using sera are commonly used as screening tests for poultry health surveillance program. For serum collection, hens are handled and bled individually. Such procedure is time-consuming and costed. Moreover, birds may be stressed by handling and egg production may be adversely affected during blood sampling. (Donahoe et al., 1982). Bleeding crew may also spread disease from one farm to another (Coleman, 1982). All the above mentioned factors limit the frequency of flock antibody monitoring that can be done by avian practitioners.

Egg-yolk antibody levels have been reported to be closely related to serum levels (Brown et al., 1991; Rossi et al., 1966; Ling et al., 1998). So for the above mentioned factors the use of egg yolk instead of serum for monitoring laying flocks for infectious diseases became an attractive possibility (Piela et al., 1984 and Nicholas and Andrews, 1991).

The current study was designed to determine if yolk and serum antibody levels to Newcastle disease virus (NDV) and Pasteurella multocida (PM) are similar and consistent enough to warrant a large scale screening of flock in production by testing of egg yolk alone.

MATERIAL and METHODS

Individual bird sampling:

Twenty 30-week-old hens were randomly selected from a commercial breeder flock and caged individually to facilitate egg identification. Birds of that flock had been vaccinated three times with living attenuated NDV vaccine followed by inactivated one during the prelaying period. Moreover, the flock had also been vaccinated twice with

inactivated P. multocida vaccine during that period. As soon as an egg was laid, the hen was captured and a blood sample was taken from the brachial vein. Thus, both of yolk and serum samples had been collected from each hen and stored at 4°C for yolk extraction and serologic testing.

Flock sampling:

Sera and egg samples were randomly collected every month from the previous breeder hens. Such collections were done at the same day from different hens and at different sites of the bird house.

Egg yolk preparation:

NDV and P. multocida antibodies were extracted from the tested eggs using a chloroform polyethylene glycol procedure described by **Poslon (1990)**. In this procedure, the water soluble proteins which include chicken IgG (IgY) separated from the emulsion of chloroform and lipophilic substances. The IgY may then be separated from the associated water soluble proteins by precipitation with 12% polyethylene glycol. The uppermost clear liquid phase was collected and stored with its serum counterpart at -20°C until it was used for serologic testing.

Haemagglutination inhibition test:

The HI test for NDV was performed by the microtitre method (Alexander, 1989) using 4 haemagglutination (HA) units of antigen and 0.025 ml volumes. Sera and chloroform extracted yolks were screened at two-fold dilution starting with dilution of 1:5. Titres were expressed as the reciprocal of the highest serum or yolk dilution showing 100% inhibition of haemagglutination activity.

ELISA procedures:

Commercially available plate pre-coated with either ND virus or P. multocida antigen (x73-CU) were obtained from IDEXX (FlockCheck; Anti-NDV and Anti-PM; IDEXX Labs. Inc., Mainem USA). The kits included both negative and positive samples. The assays were conducted according to manufacturer's standard protocol. Each yolk sample extracts and serum samples were assayed at a final dilution of 1:500. The geometric mean antibody titres were calculated according to the IDEXX instruction provided with ELISA kits. FlockCheck data are presented in a histogram format. Titres are distributed into 18 groups on the histogram, so that the number of samples that fall in a particular titre group are determined.

Statistical analysis:

The nature and size of the relationship between serum titre and its corresponding yolk titre of the hen (correlation coefficient "r") was analyzed statistically. The results obtained in the present study were also subjected to statistical analysis of mean sera and yolk titres differences by the t-test method.

RESULTS

Newcastle disease virus:

For ND antibody detection, extracted yolks performed well in both HI test and ELISA. Although the yolk extract appeared slightly turbid, however, it gave the same sharply defined compact buttons of erythrocytes in a positive HI test.

Comparison of antibody titre of serum and corresponding yolk from 15 individual hens, are presented in Table (1) and Fig. (1, 2 and 3). The HI and ELISA titres of sera and egg yolk collected from the same hens on the same days were highly correlated with only two-fold difference in both tests. The correlation coefficient (r) was 0.91 in HI test and 0.88 in ELISA. There were no statistically significant differences between the geometric mean titres of all sera (384 in HI and 3499 in ELISA) and those of yolks (304 in HI and 3573 in ELISA).

Comparison on a flock basis was done with sera and eggs collected from breeder flock at the same day (Fig. 4, 5 and 6). A good correlation was frequently detected between mean antibody titre of sera and corresponding yolks regardless of the test used ($r_{HI} = 0.91$, $r_{ELISA} = 0.84$). Yolks gave ELISA and HI titres similar to those of sera, with a maximum difference of 1-2 two-fold. As the bird became older, serum and yolk titres declined similarly however, much slower rate was observed in ELISA than in HI test. Boostering of the flock with NDV LaSota strain was accompanied with a steady increase in ELISA titre in serum and corresponding yolk. While, corresponding HI titres were inconsistently fluctuated. By the 54th week of age and thereafter, antibody titres of sera and corresponding yolks, were dropped markedly in both HI test and ELISA.

Table 1: Newcastle disease (ND) ELISA and HI antibody titres in serum and corresponding egg samples collected from 15 individual hens.

Sample From hen No.	Antibody titre			
	Serum		Extracted yolk	
	HI	ELISA	HI	ELISA
1	320	3057	320	4250
2	320	3293	320	3803
3	320	4842	160	4077
4	320	3735	160	3701
5	320	3497	640	5757
6	320	2388	320	2988
7	320	3427	320	3871
8	640	4114	320	3427
9	640	3530	320	2554
10	640	6578	640	3360
11	320	4564	320	3701
12	160	3530	80	3837
13	640	2388	320	2554
14	320	2094	320	2522
15	640	3598	320	4527
Mean	416	3642	325	3662
GM	384	3499	304	3573
Min.	160	2094	80	2522
Max.	640	6578	640	5757

GM: Geometric Mean.

Pasteurella multocida:

Comparison of ELISA antibody titres from sera and corresponding yolks of 17 individual hens are shown in Table (2) and Figs. (7,8 and 9). Although the serum and corresponding yolk titres exhibited the lowest ELISA values in our study, however they still showed a high correlation between their titres ($r=0.84$). There was no statistically significant difference ($P > 0.05$) between their geometric mean titres (163 for serum and 274 for yolk).

Flock comparison data of serum and corresponding yolk titres as monitored every month, are shown in Figs. (10). The trend of the growth-

decline of egg yolk titre was basically in conformity with that of the serum titre (r = 0.84). Steady decline in both sera and yolk ELISA titres were began at 46-week old and thereafter reaching a minimum of 40 (mean titre) for serum and 77 (mean titre) for corresponding yolk.

Table 2: P. multocida (PM) ELISA antibody titres in serum and corresponding egg yolk samples collected from 17 individual hens.

Sample from	P. multocida antibody titre	
Hen No.	Serum	Extracted yolk
1	362	252
2	682	307
3	985	2062
4	67	144
5	118	252
6	118	223
7	93	170
8	43	118
9	170	252
10	118	223
11	653	534
12	170	252
13	279	390
14	223	196
15	170	223
16	43	118
17	144	223
Mean	223	378
GM	163	274
Min.	43	118
Max.	985	2062

GM : Geometric Mean.

DISCUSSION

Egg yolk as a source of antibody has been widely used for the diagnosis of a number of diseases (Yamaguchi et al., 1981; Kradel et al., 1984 and Nicholas and Andrews, 1991). The major deterrent for working with egg yolk is the necessity for removing the lipid before protein extraction (Vadehra et al., 1977).

Although a number of methods are available for extracting the yolk protein (containing immunoglobulins) from the lipid fraction, most of them do not provide an adequate yield of antibodies for detection by serological tests (Mester and White 1978). The only exception of this is the chloroform-extraction method suggested by Poslon (1990). Which showed that clear lipid-free protein extract (containing adequate level of immunoglobulins) has been obtained in a relatively short period of time.

The use of extracted yolk in the ELISA and HI test did not require any modifications of the assays, which were optimized using sera. Our findings are in complete agreement with the previous results of Piela andYates (1983) and Piela et al. (1984).

In general, there was a high correlation between the serum and yolk antibody titres for both assayed antigens (NDV and PM) in either individual or flock based comparison. There were no significant differences ($P>0.01$) between serum and corresponding yolk antibody titres for either NDV or PM, regardless of test used as suggested by Wang et al. (1989), and Ling et al. (1998). Similar findings were also reported for infectious bronchitis virus, infectious bursal disease virus, reovirus, adenovirus, and Mycoplasma gallisepticum (Piela et al., 1984 and Silim and Venne, 1989).

ELISA assay readily detected the specific immunoglobulin IgG for either NDV or PM in both sera and corresponding yolks. NDV ELISA titres exhibited a much higher levels than PM titres, such variation could be attributed to differences in vaccination programs for NDV and PM, type of immune response to vaccination (humoral or both humoral and cellular), and/or exposure to natural infection. All previous factors could not be fully verified in this limited study. For NDV, ELISA was more sensitive than the HI test, and higher titres were obtained consistently in both sera and yolks of the same bird. Observations of Orlans (1967), Rose and Orlans (1981) and Silim and Venne (1989) concluded that IgM

fraction is absent in the egg yolk and the predominant class of immunoglobulin in yolk is IgG. The HI test is known to be efficient in detecting IgM immunoglobulins and insensitive for IgG (Alexander, 1989) and (Yu Qingzhong et al., 1987) which readily detected in ELISA.

Because the chicks get their protection from the egg yolk, titration of the yolk rather than serum can prove to be a better indicator of maternal antibody in chicks for designing vaccination programs. It is an easier way to collect a larger number of egg samples than sera without stressing the flock or need for syringes, tubes and needles for blood collection and with easy identification of the egg with a pencil. Thus, we concluded that chloroform extracted yolk appears to be a convenient and suitable serum replacement for use in serological monitoring for Newcastle disease virus and P. multocida of laying flock.

REFERENCES

Alexander, D.J. (1989): Newcastle disease. In laboratory manual for the isolation and identification of avian pathogens. Third Ed., p. 114-120. The American Association of Avian Pathologists.

Brown, M.B.; Stoll, M.L.; Scasserra, A.E. and Butcher, G.D. (1991): Detection of antibodies to Mycoplasma gallisepticum in egg yolk versus serum samples. J. Clin. Microbiol., Dec. 1991, p. 2901-2903.

Coleman, M.A. (1982): A choice in titre testing-use cull hatching eggs instead of bleeding breeders. Poult. Dis., 41: 46-49.

Donahoe, J.P.; Coleman, M.A.; Mohan, R. and Young, M.H. (1982): Testing breeder flock immunity by yolk testing. Proc. 31st West. Poult. Dis. Conf. and 16th Poult. Health Symp., Coop. Ext., Univ. of Calif., Davis, Ca. pp. 26-28.

Kradel, D.C.; Fernando, W.W.D.; hultslander, F.L. and Miller, K.F. (1984): Practical surveillance testing in avian influenza. Proc. 27th Ann. Meet. Am. Assoc. Vet. Lab. Diag. pp. 73-86.

Ling, Y.S.; Guo, Y.J.; Yang, L.K.; Luo, Y.X.; Yu, S.X.; Zhen, L.Q.; Qiu, S.B. and Zhu, G.F. (1998): Serum and egg yolk IgG antibody titres from laying chickens vaccinated with Pasteurella multocida. Avian Dis., 42: 186-189.

Mester, H.W. and White, H.B. (1978): III. Preparation of lipid-free protein extracts of egg yolk. Anal. Biochem., 91: 75-81.

Nicholas, R.A.J. and Andrews, S.J. (1991): Detection of antibody to Salmonella enteritis and S. typhimurium in the yolk of hens' eggs. Vet. Rec., Feb. 2, 98-100.

Orlans, E. (1967): Fowl antibody. VIII. A comparison of natural, primary and secondary antibodies to erythrocytes in hen sera; their transmission to yolk and chick. Immunology, 12: 27-37.

Piela, T.H. and Yates, V.J. (1983): Comparison of enzyme linked immuno-sorbent assay with haemagglutination inhibition and immunodiffusion tests for detection of antibodies to a haemagglutinating duck adenovirus in chickens. Avian Dis., 27: 724-730.

Piela, T.H.; Gulka, C.M.; Yates, V.J. and Chang, P.W. (1984): Use of egg yolk in serological tests (ELISA and HI) to detect antibody to Newcastle disease, infectious bronchitis and Mycoplasma gallisepticum. Avian Dis., 28: 877-883.

Poslon, A. (1990): Isolation of IgY from the yolk of eggs by a chloroform polyethylene procedure. Immunological Investigations, 19 (3): 253-258.

Rose, E. and Orlans, E. (1981): Immunoglobulins in the egg, embryo and young chick. Dev. Comp. Immunol., 5: 15-20.

Rossi, V.R.; Gallardo, K.E. and Berkhoff, M.G. (1966): Determination of antibodies against Newcastle disease in yolk and serum of vaccinated, non-vaccineated and convalescent fowls. Zooiatria, 7: 4-12.

Silim, A. and Venne, D. (1989): comparison of egg-yolk and serum antibody titres to four avian viruses by enzyme linked immunosorbent assay using paired field sera. Avian Dis., 33: 643-648.

Vadehra, D.V.; Bair, J.M. and Burley, R.W. (1977): Extraction of protein from egg yolk. Biochem. J., 166: 619-624.

Wang Wenxiu; Wei, Jianping and Wang, Jinghe (1989): Comparison of anti-NDV antibody titre in egg yolk, egg white and blood serum in immunized hens. Chin. J. Vet. Med., 15 (6): 28-29.

Yamaguchi, S.; Imada, T.; Kawamura, H.; Taniguchi, T. and Kawakami, M. (1981): Pathogenicity and distribution of egg drop syndrome-1976 virus (JPA-1) in inoculated laying hens. Avian Dis., 25: 642-649.

Yu Qingzhong, Liu Wenzhou and Sun Zhongyu (1987): Study on methods of extracting IgG from egg yolk in immunized hens to NDV. Chin. Domest. Anim. Poult. Infect. Dis., 1: 44-46.

Fig (1): Newcastle disease (ND) ELISA antibody titres in serum and corresponding egg samples collected from 15 individual hens.

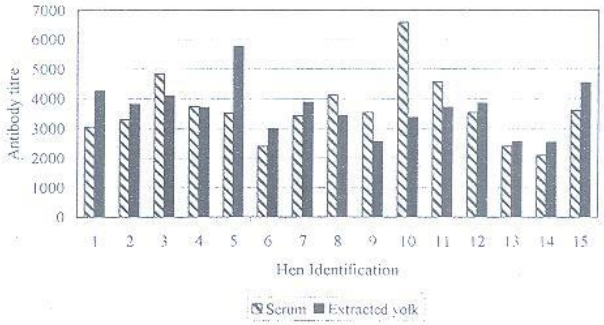
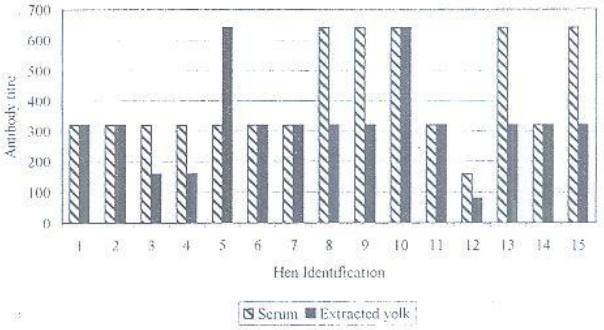
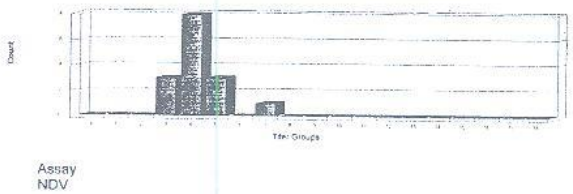


Fig (2): Newcastle disease (ND) HI antibody titres in serum and corresponding egg samples collected from 15 individual hens.



Serum Profile



Count: 15
Mean: 3642
GMean: 3499
%CV: 29.4
Min: 2094
Max: 6578

Assay
NDV

Yolk profile

Count: 15
Mean: 3662
GMean: 3573
%CV: 22.4
Min: 2522
Max: 5757

Assay
NDV

Fig. (3): A representative histogram formates of NDV ELISA antibody titres from matched sera and extracted yolks of 15 individual hens.

Fig. (4): Monitoring of NDV ELISA antibody titres in matched sera and extracted yolks obtained from commercial breeder flock at different ages during laying period.

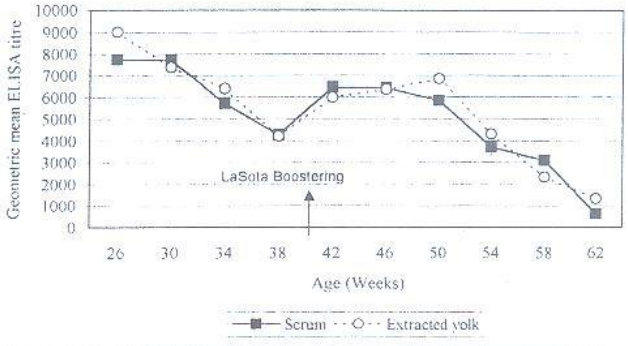
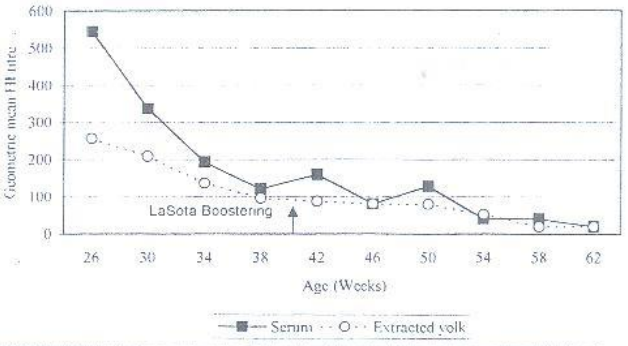


Fig. (5): Monitoring of NDV HI antibody titres in matched sera and extracted yolks obtained from commercial breeder flock at different ages during laying period.



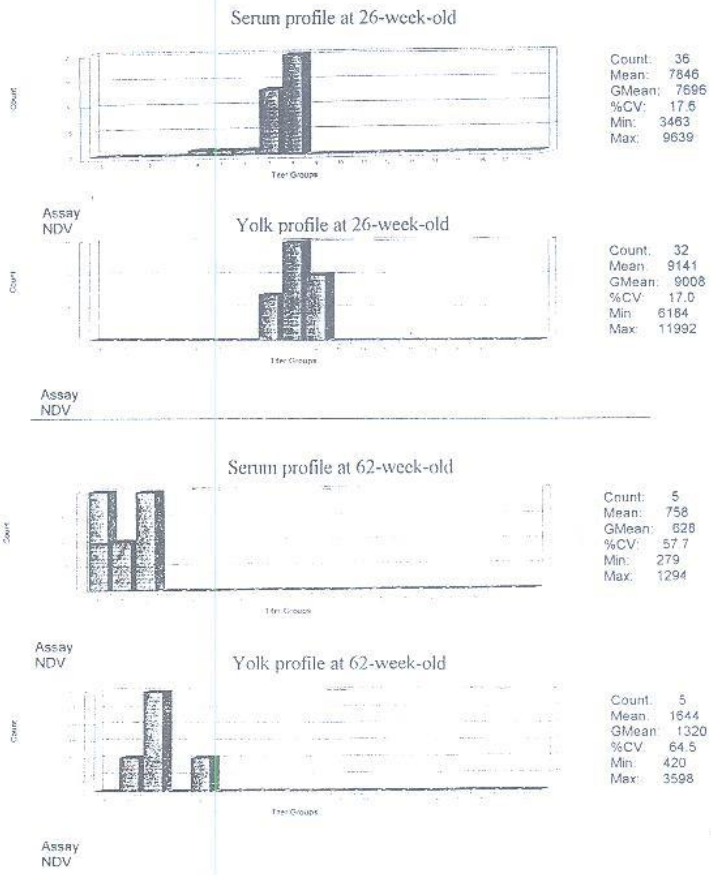
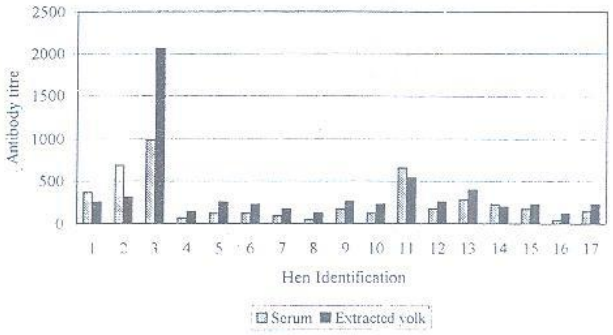


Fig. (6): Representative histogram formates of NDV antibody titres from matched sera and extracted yolks obtained from breeder flocks at two different ages during laying period.

Fig. (7): P. multocida (PM) ELISA antibody titres in serum and corresponding egg yolk samples collected from 17 individual hens



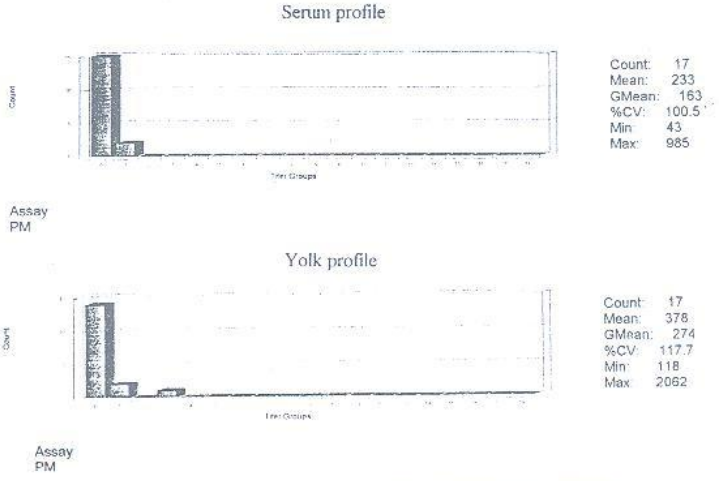


Fig. (8): Representative histogram formates of P. multocida ELISA antibody titres from sera and corresponding yolks obtained from 17 individual hens.

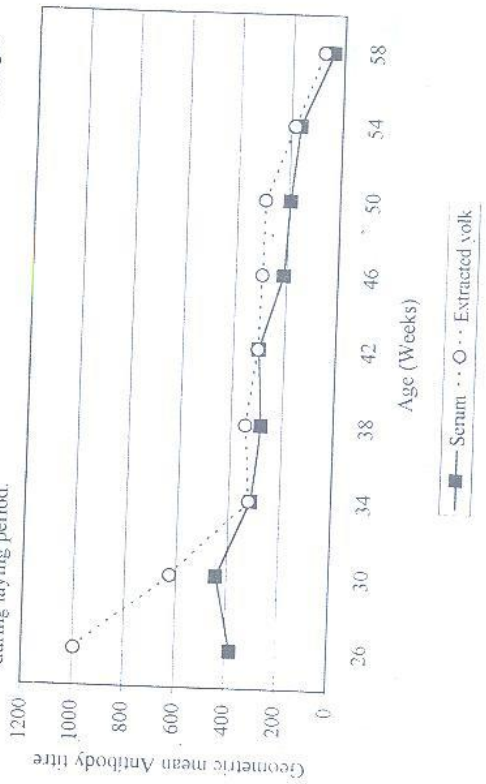


Fig. (9): Monitoring of P. multocida ELISA antibody titres in matched sera and extracted yolks obtained from breeder flock at different ages during laying period.

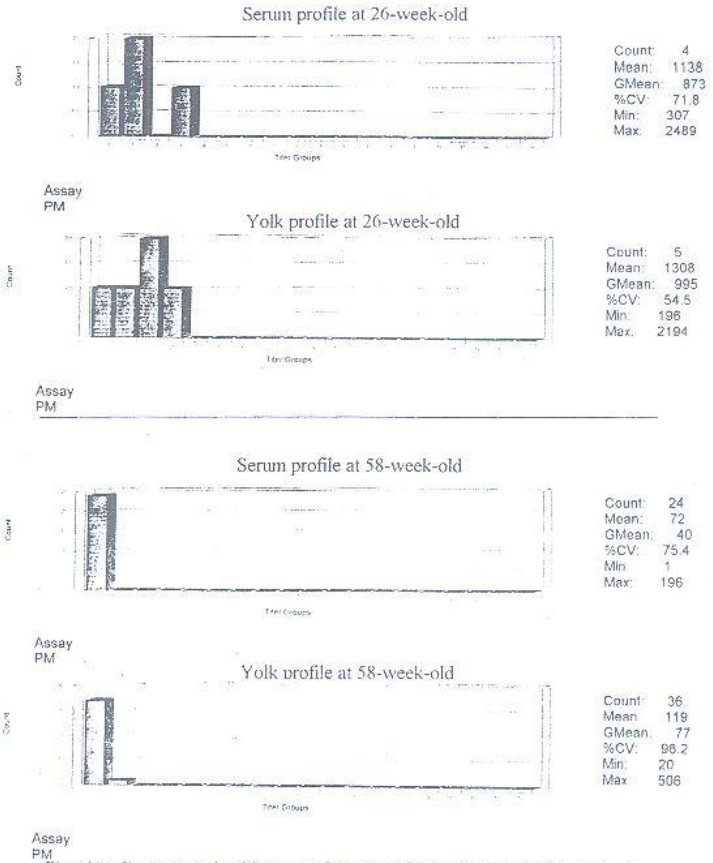


Fig. (10) Representative histogram formates of P. multocida ELISA antibody titres from sera and corresponding yolks obtained from breeder flock at two different ages during laying period.