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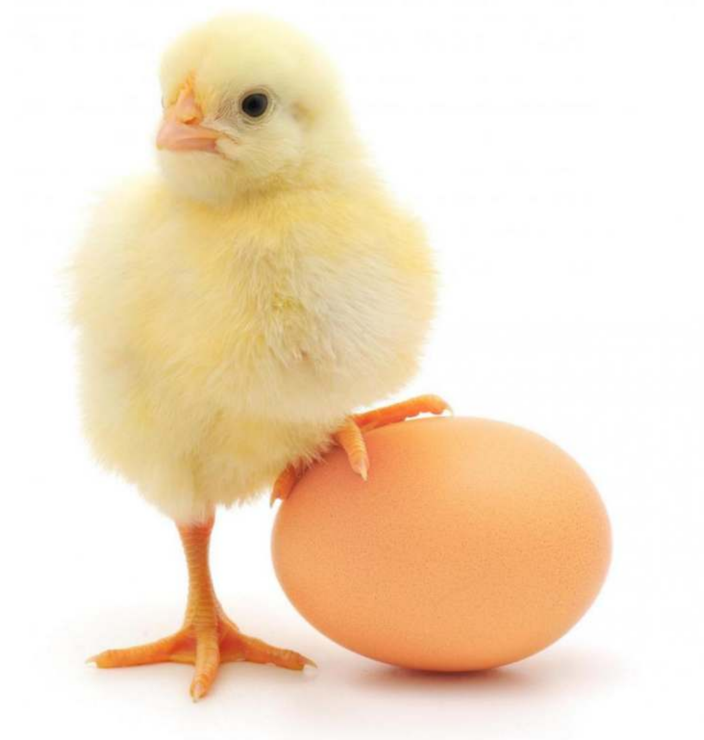

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Antibody Levels against Newcastle Disease Virus, Infectious Bursal Disease Virus and Avian Influenza Virus in Rural Chickens in Viet Nam

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Abstract: A serological survey on the prevalence of antibodies to Newcastle Disease (ND) virus, Infectious Bursal disease (IBD) virus and Avian Influenza (AI) virus was carried out in two communes of the Hue District, Middle Viet Nam. The villages in each commune and the households in each village included approximately 10% scavenging and 20% backyard chickens. The main objectives were to determine the prevalence of antibodies against ND virus at different seasons of the year, the prevalence of antibodies against IBD and AI virus, and a comparison of the situation of ND, IBD and AI in scavenging and backyard chickens. The results showed that there was no statistically significant difference in NDV titres between the seasons and between ND vaccinated and unvaccinated birds but there was a significant difference between the unvaccinated scavenging (33.9%) and the unvaccinated backyard birds (21.6%). Both ND vaccinated and unvaccinated birds in the 2- <6 months-old groups had a significantly lower NDV antibody titre than the $\geq$ 6 month-old age group. There was no overt correlation between NDV-HI- and IBDV-ELISA titres. A statistical analysis was precluded due to inadequate data sets. There were 5 reactors in the AI-ELISA but they were negative in the HI test with H_5 and H_7 HA antigens. This study showed that in the selected chicken population the level of protection of vaccinated birds was unsatisfactory. It further showed that there was no seasonal difference between rainy and dry season, that IBDV existed but did not appear to be associated with NDV. There were no specific antibodies against the H_5/H_7 subtypes of AIV.

Key Words: Newcastle Disease Virus, infectious Bursal Disease Virus, avian Influenza Virus,

Introduction

Rural poultry keeping is the dominant form of poultry production in the developing world. In Viet Nam (VN) scavenging and backyard chicken production systems are more important than the modern intensive poultry production. Backyard birds are fenced and kept in larger numbers than scavenging birds, which roam around the village in search of feed but go home for laying and for the night.

One of the main factors constraining poultry production is disease. In VN Newcastle disease (ND) is the most important cause of mortalities in chickens (Nguyen, 1992). Scavenging and backyard chickens appear to be affected throughout the year. The spread of ND in rural areas is normally via either newly introduced birds, selling or giving away sick birds, carrier birds and/or by roaming of chickens in search of feed. Tu *et al*. (1998) carried out experimental trials using a thermostable ND vaccine (strain I_2) and village chickens which were infected with velogenic ND virus isolated earlier by Hue *et al*. (1978). The results from these trials showed that this vaccine was suitable under the rural village conditions. However, its use has not been satisfactory in controlling or eradicating ND in VN.

Apart from ND, numerous other infectious diseases could affect rural chickens in Viet Nam. Infectious Bursal disease (IBD) occurs worldwide (Lukert and Saif, 1997). This disease was also noted in Viet Nam (Tripodi, pers. communication 2001) and IBD vaccines are used sporadically. Highly Pathogenic Avian Influenza (HPAI) is another disease of great concern, particularly after the severe outbreak in Italy (Capua *et al*., 2000) and several outbreaks in Pakistan (Naeem and Hussain, 1995). HPAI is also of growing concern in other parts of Asia. It was reported from neighbouring countries of Viet Nam, such as China (Chandler, 2001; Yu *et al*., 2001), Cambodia and Laos but not Viet Nam (OIE, 2002). Vaccination against AI is practised in some Asian countries, such as Pakistan (Qureshi, 2001) and China (Yu *et al*., 2001).

AI also poses a considerable public health risk because H_5N_1 viruses isolated from humans were identical to those isolated from poultry in Hongkong (Subbarao, 2001; Lin *et al*., 2000).

In view of this situation a cross-sectional survey was initiated in the Hue District, Thua Thien Hue Province, Middle Viet Nam, with the objectives of

- i establishing the prevalence of antibodies against ND virus at different seasons of the year,
- ii comparing the situation of ND, IBD and AI in scavenging and backyard chickens,
- iii comparing the situation of ND in two communes, Phu Thuong and Phu Mau communes.

Materials and Methods

The study area: The study was carried out in the Phu Thuong and the Phu Mau communes of Phu Vang Administrative District, Thua Thien Hue Province, 10-15 km south-east of Hue city. Phu Thuong comprises 8 and Phu Mau 9 villages. Each village has 100-400 households with 5-15 scavenging chickens per household. Additionally, in each village there are 10-30 owners of backyard chickens with approximately 40-50 birds. There are two seasons in the study area: dry and rainy seasons. The dry season lasts from April to August, the rainy season from September to March.

Sampling: Three out of 8 villages within Phu Thuong and 4 out of 9 villages within Phu Mau were randomly selected. In each village, households were randomly selected. Approximately 10% of the households per village for scavenging and 20% for backyard chickens were selected at random. Selection of birds was done haphazardly. Randomization was impossible, because birds could not be identified individually. The number of birds sampled was 1-2 scavenging chickens and 1-6 backyard birds per household. The study covered part of the rainy season (December-March) and of the dry season (April-June).

Blood sample collection and storage: A total of 800 blood samples were collected, 400 in the rainy and 400 in the dry season. For the NDV haemagglutination inhibition-(HI) test, the blood samples were allowed to clot, sera were separated and frozen at –15 ^{O}C until later use. The NDV HI test was performed at Hue University, Viet Nam while the serological tests for IBDV and AIV antibodies were performed at the Aulendorf Animal Health Laboratory, Germany. For the IBDV-ELISA, the clotted, non-haemolysed serum was absorbed onto paper strips (Lohmann Tierzucht GmbH, Cuxhaven, Germany) in Viet Nam. These strips were despatched by ordinary air mail to Germany. Preliminary ELISA testing using whole blood had been found unsuitable because the haemoglobin interfered with the ELISA reading, giving false-positive results. According to the manufacturer, the paper strip contains 0.1 ml blood or serum in our case. Each paper strip was pencil-marked at the serum-free end, dipped into an Eppendorf tube with 2.4 ml PBS and left for dilution over night with the stopper firmly pressed on. After 24 hrs, the 0.1 ml serum on the paper strip had diffused into the surrounding 2.4 ml PBS, giving a 1:25 serum dilution. The diluted serum was frozen at –20 ^{O}C until required for use.

Haemagglutination inhibition (HI) test for Newcastle disease: The serum samples were tested for NDV-HI antibodies, using the standard HI method as described by Allan and Gough (1974). The antigen used was reconstituted commercial NDV La Sota vaccine (TAD, Cuxhaven, Germany).

Infectious Bursal disease virus and Avian Influenza virus antibody ELISA: Sera from the dry season, each absorbed on paper strips and diluted 1:25 in PBS, was tested in Germany for antibodies using commercial ELISA kits (FlockChek IBD and FlockChek AI, IDEXX). Both assays were developed in the microtitre format with 96 well plates.

Avian Influenza haemagglutination inhibition (HI) test with H_5 and H_7 antigen: Since the AI-ELISA test is not subtype-specific, any ELISA-positive serum had to be retested for specificity using the standard HI test as described for ND but with specific commercial Avian Influenza HA antigen (PA 9009-H_5 and PA-9009-H_7 Central Veterinary Laboratory, England, supplied by cc pro GmbH, Neustadt, Germany) and commercial antiserum (HAR-INFH$_5$ and HAR-INFH$_7$, Gezondheidsdienst voor Dieren, Boxtel, The Netherlands) as controls.

Data management and analysis: Laboratory results of ND, IBD and AI serology were entered and managed using Microsoft Excel (Windows 1997, Duxbury Press). Descriptive statistics for the HI antibody titres were done using the same program. The Mann-Whitney rank test was used for the comparison of results between individual groups of chickens. Prevalences of HI antibody titres, < $\log_2 3$ and $\geq \log_2 3$ groups, were compared using the Chi-square test.

Results

Newcastle Disease: Overall sero-prevalence of antibodies to NDV in vaccinated and unvaccinated chickens in Thua Thien - Hue Province: In 170 vaccinated birds (Table 1) the prevalence of NDV-specific antibody titres ($\geq \log_2 3$) was 47.7% (n = 81). The prevalences of NDV-HI antibodies are shown in Tables 1 and 2. In 630 unvaccinated birds (Table 2), 28.4 % (n = 179) were positive and 71.6% (n = 451) were either negative or unspecific.

Newcastle Disease: Prevalence of antibodies to NDV in chickens according to seasons: The percentages of seropositive vaccinated birds (Table 1) were 47.4% for the dry and 48% for the rainy season. Tables 1 and 2 show the seasonal distribution of NDV antibody titres. In unvaccinated birds (Table 2) the percentages of sero-positive birds in the dry and in the rainy seasons were 27.5 and 29.2%, respectively. In both cases the differences were not significant (p > 0.05).

Newcastle Disease: Prevalence of antibodies to NDV in backyard and scavenging chickens: The prevalences for vaccinated birds were 55.6 % (n = 20) for scavenging and 45.5 % (n = 61) for backyard birds (Table 1). The difference was not significant (x^2 = 1.15, p = 0.29). Table 2 shows that 33.9 % (n = 118) of the

Table 1: Distribution of HI antibody titres in NDV vaccinated chickens in Thua Thien – Hue Province

Variable	HI titres < $log_2 3$		HI titres $\geq$ $log_2 3$	
	n	%	n	%
Vaccinated (overall)	89	52.6	81	47.7
Scavenging chickens	16	44.4	20	55. 6
Backyard chickens	73	54.5	61	45.5
Rainy season	39	52	36	48
Dry season	50	52.6	45	47.4
Chickens 2 - < 6 months	52	72.2	20	27.3
Chickens $\geq$ 6 months	37	37.8	61	62.2
Local breed	38	51	37	49
Exotic breed	51	53.7	44	46.3
Phu Thuong Commune	28	35.9	50	64.1
Phu Mau Commune	61	66.3	31	33.7

Table 2: Distribution of HI antibody titres in NDV unvaccinated chickens in Thua Thien – Hue Province

Variable	HI titres < $log_2 3$		HI titres $\geq$ $log_2 3$	
	n	%	n	%
Unvaccinated (overall)	451	71.6	179	28.4
Scavenging chickens	230	66.2	118	33.9
Backyard chickens	221	78.4	61	21.6
Rainy season	230	70.8	95	29.2
Dry season	221	72.5	84	27.5
Chickens 2 - < 6 months	225	81.2	52	18.8
Chickens $\geq$ 6 months	226	64.0	127	36.0
Local breed	348	69.1	156	30.9
Exotic breed	103	81.8	23	18.3
Phu Thuong Commune	213	69.2	95	30.8
Phu Mau Commune	238	73.9	84	26.1

unvaccinated scavenging and 21.63 % (n = 61) of the unvaccinated backyard birds were seropositive for NDV antibodies. The difference was significant (Chi-square = 11.54, p < 0.0001).

Newcastle Disease: Comparison of prevalence of antibodies to NDV in chickens between two communes (Phu Thuong and Phu Mau) : The proportions of seropositive vaccinated birds in Phu Thuong Commune and Phu Mau Commune were 64.1% and 33.7%, respectively (Table 1). The difference was significant (x^2 = 15.65, p < 0.0000). The corresponding figures for unvaccinated birds were 30.8% (n = 95) and 26.1% (n = 84) (Table 2). The difference was not significant (x^2=1.75, p = 0.19).

Newcastle Disease: Comparison of sero-prevalence of antibodies to NDV in chickens of different breeds ("local" vs. "exotic") : The sero-prevalences of local vs. exotic breeds are shown in Tables 1 and 2. In vaccinated birds the prevalence was 49.0% (n = 37) for local and 46.3% (n = 44) for exotic breeds not significant, p=0.70). The corresponding figures for unvaccinated birds were 30.9% (n = 156) in local breeds and 18.3% (n=23) in exotic breeds (significant, p=0.005).

Newcastle Disease: Comparison of prevalence of antibodies to NDV in chickens of different age groups (2- < 6 months vs $\geq$ 6 months) : The percentage of sero-positive birds for vaccinated birds (Table 1) was 27.8% (n = 20) in the 2 - < 6 month-old group and 62.2% (n=61) in birds $\geq$ 6 months. Table 2 shows the corresponding values in the unvaccinated group. In the 2 - < 6 month old group the percentage was 18.8% (n = 52), in birds $\geq$ 6 months it was 36% (n = 127). The differences are significant (x^2 = 22.59, p < 0.0000 for the unvaccinated 2 - < 6 month old group vs. birds $\geq$ 6 months; x^2 = 19.77, p < 0.0000 for the vaccinated 2 - < 6 month old group vs. birds $\geq$ 6 months).

Newcastle Disease: Distribution of NDV antibody titres (log_2) of samples tested : The distributions of NDV antibody titres for vaccinated and unvaccinated birds are shown in Tables 3 and 4. The mean titre was $log_2 2.6$ (n = 170) for vaccinated, $log_2 1.6$ (n = 630) for unvaccinated birds, and the titre ranges were 0 to 9 and 0 to 11, respectively. The difference was significant (p < 0.0000).

Newcastle Disease: Distribution of ND antibody titres (log_2) of samples according to seasons: The distributions of ND antibody titres (log_2) of samples according to seasons for vaccinated and unvaccinated birds are shown in Tables 3 and 4. The mean titres of vaccinated birds were $log_2 2.6$ (n=95) for the dry and

Table 3: Distribution of NDV HI Titres (log_2) in blood samples collected from NDV-vaccinated chickens in Thua Thien – Hue Province

Variable	n	Mean HI) titres(log_2	Standard deviation	Range Min.	Max.
Overall vaccinated	170	2.6	2.5	0	9
Rainy season	75	2.6	2.53	0	7
Dry season	95	2.6	2.48	0	9
Scavenging chickens	36	3	2.11	0	7
Backyard chickens	134	2.5	2.59	0	9

Table 4: Distribution of NDV HI titres (log_2) in blood samples collected from unvaccinated chickens in Thua Thien – Hue Province

Variable	n	Mean HI titres(log_2)	Standard deviation	Range Min.	Max.
Overall unvaccinated	630	1.6	2.45	0	11
Rainy season	325	1.6	2.55	0	11
Dry season	305	1.6	2.36	0	11
Scavenging chickens	348	1.9	2.60	0	11
Backyard chickens	282	1.3	2.23	0	11

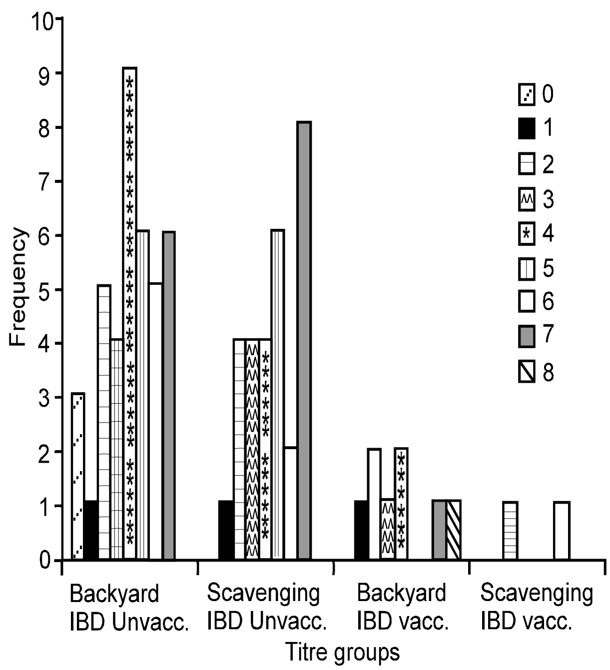

Fig. 1: Distribution of IBDV antibodies

$log_2 2.6$ (n = 75) for the rainy season. The respective values for unvaccinated birds were $log_2 1.6$ (n = 305) for the dry and $log_2 1.6$ (n = 325) for the rainy season. The differences were not significant .

Newcastle Disease: Distribution of ND antibody titres (log_2) of samples according to production systems: The distributions of ND antibody titres (log_2) for vaccinated and unvaccinated birds according to production systems are shown in Tables 3 and 4. The mean titres of vaccinated backyard birds was $log_2 2.5$ (n = 134) and of vaccinated scavenger birds was $log_2 3$ (n = 36). The respective figures for unvaccinated backyard birds were $log_2 1.3$ (n = 282) and $log_2 1.9$ (n = 348) for scavenger birds. The differences between the two production systems were statistically significant for unvaccinated birds ($p < 0.01$).

Infectious Bursal Disease: The distribution of IBDV-ELISA antibody titre groups are depicted in Fig. 1. This figure shows that most of the titre groups were recorded in the unvaccinated backyard chickens followed by the unvaccinated scavenging chickens. Low frequencies were observed in the backyard ND-vaccinated birds. Only two titre groups were obtained from the ND vaccinated scavenging chickens. Similar trends were observed in the modes of the titre groups.

Avian Influenza: There were 5 reactors in the AI ELISA test for NDV vaccinated and unvaccinated birds during the dry season. Further testing using H_5 and H_7 antigens in the HI test showed that all AI ELISA-positive test serum samples were negative in the H_5/H_7-specific HI test.

Discussion

A ND-HI titre of $log_2 3$ or above is generally accepted as indicative of specific immunity (Allan and Gough, 1974). Using these criteria in our work, about 52% of the vaccinated and 72% of the unvaccinated flocks showed no serological signs of specific immunity to ND. While there are no published data for the prevalence of NDV antibodies in rural chickens in Viet Nam, it is estimated (Pham Hong Son, personal communication) to range between 40-60%. This is higher than the 10% reported from Malaysia (Aini and Ibrahim, 1990) but lower than the sero-prevalence of 62.9-72% reported from Nigeria (Ezeokoli *et al*., 1984). There are several possible reasons for this low level of protection in vaccinated birds, such as poor vaccine quality, unsuitable vaccination schedule or vaccination technique, impaired immune-competence due to immune-suppressive substances in the feed or to immune-suppressive diseases. The immune-suppressive IBD seemed to play no role (Fig. 1) and the question of immune-suppressive substances in the feed could not be clarified but must always be considered, particularly under tropical climatic conditions. Poor vaccine quality is a common problem in developing countries and could be the result of poor manufacturing standards, lack of adequate storage facilities, application of expired vaccine batches, and faulty application. Previous experiments with the thermoresistant Australian I_2 vaccine strain of NDV (Tu *et al*., 1998), which maintains its potency even if not transported and stored under refrigeration, works well under Vietnamese village chicken conditions. Although the normal vaccination procedure (eye-drop at day 10 and day 20, revaccination every three months) were said to have been followed in vaccinating the chickens under investigation, failure to strict adherence to the above mentioned ND vaccination programme could not be ruled out completely. Another weak point might be the quality of the water used to dilute the vaccine before application. Since, according to Awan *et al*. (1994), low ND-HI antibody prevalence is suggestive of an interepidemic phase or early phase of infection, problems with ND outbreaks in the near future may have to be expected unless the vaccination practice is improved substantially.

There was no seasonal difference in the sero-prevalence of NDV antibodies in the Thua Thien – Hue Province (Tables 1 and 2). The issue of seasonal ND peaks has always been controversial and may vary according to the environmental, nutritional and socio-economic conditions, under which poultry is kept. Awan *et al.* (1994) reviewed the literature and found reports of ND peaks during (Asadullah, 1992; George, 1991; Mishra, 1992) and at the end of the dry season. Nguyen (1992) reported that in Viet Nam the ND peaks generally occur at the beginning of the rainy season (September-March). Martin (1992) in a review concluded that ND outbreaks are often associated with the change of seasons, specifically at the start of the wet season.

One of the objectives of this study was to compare the ND situation between scavenging and backyard chickens. In this study 33.9% of the unvaccinated scavenging and 21.6% of the unvaccinated backyard birds were sero-positive for NDV antibodies. This difference was significant. The corresponding figures for vaccinated birds were 55.6 % for scavenging and 45.5 % for backyard birds. This difference was not significant. Although there are no published reports about the differences of NDV antibodies between the two production systems in Viet Nam, the higher prevalence recorded in our study in unvaccinated scavenging birds can probably be explained by the more frequent contacts of this group of birds to wild and domesticated viral carriers.

Our results also show considerable differences between the two communities selected. In the Phu Thuong Commune 30.8 and 64.1% and in the Phu Mau Commune 26.1 and 33.7% of the unvaccinated and vaccinated birds, respectively, were seropositive for NDV antibodies. This much higher proportion of seropositive birds in the vaccinated group from Phu Thuong Commune can possibly be explained by the proximity of Phu Thuong Commune to Hue city where there is much easier access to properly stored vaccines. Furthermore, stock owners in this commune might be more interested in the health of their animals because they fetch higher prices in the city than in the remote production areas.

The question of breed susceptibility to ND is still controversial (Awan *et al*., 1994). The ND sero-prevalences of local and exotic unvaccinated birds were 30.9 and 18.3%, respectively. The corresponding figures for vaccinated birds were 49% for local and 46.3% for exotic breeds. While a clear difference appears to exist between the two breeds, interpretation of our results is difficult because often the distinction between local and exotic breeds is uncertain and some classification may have been arbitrary.

The percentages of sero-positive unvaccinated birds aged 2 - $\leq$ 6 months was 26.3% and that in birds aged $\geq$ 6 months was 36%. The corresponding figures for vaccinated birds were 27.8 and 62.2%. The differences were significant. This can be hypothesized to be due to more frequent exposure of older birds to field virus which might have survived the disease at an earlier age.

The means of NDV antibody titres for vaccinated and unvaccinated birds were $\log_2 2.6$ and $\log_2 1.6$, respectively. The $\log_2$ titres ranged from 0 to 9 for vaccinated and from 0 to 11 for unvaccinated birds. The difference between the mean titres was significant. The relatively low mean titre of vaccinated birds clearly constitutes unsatisfactory vaccination results which may be related to poor vaccine quality or an unsuitable vaccination regime. The wider range of NDV titres in unvaccinated birds may be due to natural infection which is known to produce higher antibody titres than vaccination (Luc *et al*., 1992).

The distribution of IBDV antibodies in the IBDV unvaccinated groups of scavenging and backyard birds during the dry season showed no obvious difference between the two groups. The low number of IBD vaccinated birds precluded a statistical comparison between IBDV unvaccinated and vaccinated birds.

There were 5 reactors in the AI ELISA test for NDV vaccinated and unvaccinated birds during the dry season. Further testing using commercial H_5 and H_7 antigens in the HI test showed that all AI ELISA-positive test serum samples were negative. Since there is no clinical evidence of AI either, it can be concluded that AI does not play a role in rural chickens in the selected study area in Viet Nam. This contrasts to recent reports from China (Chandler, 2001; Yu *et al*., 2001) and other neighbouring countries (OIE, 2002) where AI had been observed.

In conclusion, the level of protection of vaccinated birds was unsatisfactory, there were no seasonal differences between rainy and dry season, IBDV exists but does not appear to be associated with NDV. Also, there were no specific HPAI antibodies in the chicken blood samples tested.

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