鶏胚のトリレオウイルスに対する感受性に及ぼす移行抗体の影響

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Influence of Maternal Antibodies on Susceptibility of Embryonating Eggs to Avian Reovirus

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Avian reoviruses (ARVs), which are known to cause tenosynovitis in chickens, can be cultured in embryonating chicken eggs accompanied with death of embryos, and in primary cell cultures of chicken origin [6]. For isolation or propagation of the virus in embryonating eggs, yolk-sac route is preferred [5], although no direct comparisons have been made between the cultured cells and embryonating eggs.

Meanwhile, the possibility of egg-transmission in ARV infection has been suspected. Yamada *et al.* [9] and Jones *et al.* [3] isolated ARVs from dead-in-shell embryos derived from several commercial breeder flocks. ARVs were also detected in chicken embryo fibroblasts cultures prepared from embryonating eggs which were laid from experimentally infected broiler breeders [8]. Menendez *et al.* [4] and Hussain and Spradbrow [2] have been also proved experimentally that egg-transmission of ARV could occur.

However, the influence of maternal antibodies on the susceptibility of embryonating eggs to ARV has not been investigated. So, the authors tried to compare the susceptibility of embryonating eggs laid from a vaccinated breeder flock with that of embryonating eggs laid from a non-vaccinated breeder flock.

To obtain eggs with a high incidence of maternal antibodies against ARV, a specific pathogen free (SPF) breeder flock was vaccinated twice intramuscularly at 50 and 58 weeks of age with formalin inactivated ARV, strain 58–132, in aluminum hydroxide gel adjuvant. Strain 58–132, a highly virulent ARV, had been isolated from a 22-day-old broiler chicken with tenosynovitis [7]. During from 14th to 35th day after the second vaccination, eggs were collected. Yolk was sampled from 10 eggs and mixed with an equal volume of physiological buffered saline(PBS) and 10% chroloform. The mixture was shaked vigorously for 30 min at room temperature, then centrifuged at 3,000 rpm for 15 min. The supernatant was tested for neutralizing antibody to ARV, strain 58–132, by 90% plaque reduction method. Remaining eggs collected were incubated for 9 days and then inoculated with 0.1 m*l* of serial tenfold dilutions from $10^{-1.0}$ to $10^{6.0}$ plaque forming unit (PFU)/0.1 m*l* of the virus via the allantoic cavity or yolk-sac. For comparison, embryonating eggs laid from a non-vaccinated SPF breeder flock were inoculated with the virus in the same manner. For each virus dilution, 10 eggs were employed.

Records on mortaility were carefully maintained every day for 9 days. Death within the first 24 hours was considered non-specific.

The results were shown in Table 1. Median egg lethal doses (ELD_{50}) calculated from the mortality in eggs with maternal antibodies (a geometric mean antibody titer in yok was 9,200) were $10^{2.30}$ or $10^{2.35}$ PFU per egg, when the virus was inoculated via the allantoic cavity or the yolk-sac, respectively. On the other hand, those in eggs without maternal antibodies (antibody titers in yolk were all less than 10) were $10^{1.60}$ or $10^{1.30}$ PFU per egg, respectively.

Death times of embryonating eggs with maternal antibodies were slightly prolonged in comparison with those of eggs without antibodies, regardless of inoculation route. Recovery of the virus was tried from dead embryos pooled in each group that had been inoculated with $10^{3.0}$ PFU of the virus per egg. Virus was recovered from embryo pools with high virus titers of $10^{6.96}$ or $10^{7.52}$ PFU/g when the virus was inoculated into eggs with maternal antibodies via the allantoic cavity or the yolk-sac, respectively. These titers were as high as those ($10^{7.16}$ or $10^{7.57}$ PFU/g) of embryo pools which were derived from eggs without maternal antibodies.

These results reveal that the influence of maternal antibodies on the susceptibility of embryonating eggs to ARV infection is very low. And, they suggested that the eggs, even if which were laid from an ARV-exposed breeder flock, could be used for isolation or propagation of ARV, and also suggested that an egg-transmission of ARV may be scarcely influenced

Table 1. Susceptibility of chick embryos with or without maternal antibodies to avian reovirus, strain 58–132

Route of virus-inoculation	Maternal antibody in yolk	Median egg lethal dose (PFU/egg)	Virus-titer of inoculumn (PFU/egg)			
			$10^{6.0}$	$10^{5.0}$	$10^{4.0}$	$10^{3.0}$
Allantoic cavity	+(9,200)[1)]	$10^{2.30}$	4.6[2)]	5.8	5.8	6.2
	−(<10)	$10^{1.60}$	4.2	5.0	5.2	5.7
Yolk-sac	+(9,200)	$10^{2.35}$	5.0	4.8	5.2	5.8
	−(<10)	$10^{1.30}$	3.0	3.7	4.2	4.5

1) Geometric mean titer of neutralizing antibody to avian reovirus, strain 58–132, tested by 90% plaque reduction method.
2) Mean death time in days of embryos died.

by maternal antibodies in yolk. Furthermore, the results obtained here may explain the impossibility to estimate the immune condition of breeder flocks against ARV infection by challenge to embryonating eggs laid from them.

The data on ARV shown in this study were different from the data on infectious bursal disease virus (IBDV) reported by Hitchner [1] who described that IBDV could not propagate in embryonating eggs from an IBDV-exposed breeder flock.

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要　約

鶏胚のトリレオウイルスに対する感受性に及ぼす移行抗体の影響(短報)：高瀬公三・内村哲也・山元通孝(㈶化学及血清療法研究所)——トリレオウイルス(ARV)に対する移行抗体保有または非保有発育鶏卵の尿膜腔内あるいは卵黄嚢内にARVを接種し，胚の死亡を観察した．両発育鶏卵におけるARVの50%胚致死量および胚の平均死亡日数の差はいずれも小さく，移行抗体の影響は少ないものと考えられた．