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## Effects of certain stress factors on the re-excretion of infectious laryngotracheitis virus from latently infected carrier birds

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**Experiments were set up to assess the effects of 'natural' and 'artificial' stresses on the re-excretion of infectious laryngotracheitis (ILT) virus in latently infected chickens recovered from the acute phase of the disease. The stresses were rehousing with the addition of ILT-free contact birds, corticosteroid treatment and the onset of lay. The contact birds were also monitored for transmission of the virus from the carrier birds. Rehousing with unfamiliar birds induced ILT virus shedding in one of five birds and there was evidence of transmission from this bird to its mate. The onset of lay had a significant effect on the overall shedding rates of the carrier birds. Nine of 10 birds shed virus after onset of lay compared with only two in the three-and-a-half weeks before, and there was a highly significant increase ( $P < 0.001$ ) in the overall number of virus isolations during this period. Corticosteroid treatment did not affect virus shedding. These results may explain some of the apparently spontaneous outbreaks of ILT which occur in the field.**

INFECTIOUS laryngotracheitis (ILT) is an economically important respiratory disease of chickens caused by an  $\alpha$ -herpesvirus, Gallid herpesvirus 1. A carrier state has long been suspected in ILT and shown by Turner (1972) and Bagust (1985) in organ cultures derived from killed birds. More recently the carrier state was demonstrated in over 80 per cent of live clinically recovered birds by tracheal swabbing (Hughes and others 1987). As with other  $\alpha$ -herpesvirus infections, this carrier state was characterised by periods of latency interspersed with short, intermittent spontaneous episodes of virus shedding. No clinical signs of respiratory disease were observed during these episodes.

In  $\alpha$ -herpesvirus infections in some other species, such as the cat and the pigeon, it is known that certain 'natural' stress situations, for example, rehousing and the reproductive period, can induce episodes of virus shedding in carriers. These carriers may be of considerable epidemiological importance (Gaskell and Povey 1977, Vindevogel and others 1985). Shedding may also be induced artificially in such carriers by corticosteroid or cyclophosphamide treatment (Edington and others 1985, Gaskell and others 1985).

The purpose of the following experiments was to investigate the effects of certain stress factors on the ILT virus shedding patterns of carrier birds, and the possibility of transmission from shedding carriers to uninfected birds. Rehousing and onset of lay were selected as 'natural' stresses as these occur in commercial flocks. Corticosteroid treatment was also used in an attempt to induce shedding, cyclophosphamide having been ineffective in previous work (Hughes and others 1987).

The first experiment examined the combined effect of rehousing with the addition of unfamiliar, uninfected birds on virus shedding. The possibility of virus transmission to the uninfected birds was also investigated. Five specific pathogen free (SPF) cockerels (Jones and Jordan 1972), 22 months old, were used. They had been infected with field strain 216 of ILT virus at 12 weeks old and had shed virus for up to eight days during the acute phase (Hughes and others 1987). No virus was detected in any bird after this time until nine weeks after infection, when intermittent shedding was demonstrated in four of the five birds. These four were then considered as carriers (birds 1 to 4, Table 1). Tracheal swabs were taken daily from all birds 10 days before rehousing to ensure that virus shedding was not already occurring and to act as a control for the effect of swabbing. On day 11 the cockerels were bled and serum neutralisation titres ranged from 1/3 to >1/32 (Table 1). The birds were then removed separately from their communal cages, transferred to another building and put into individual cages (1 m<sup>2</sup>) within fibreglass isolators each containing an uninfected SPF hen of similar age. A control uninfected cockerel was treated similarly. All birds were swabbed daily for three weeks and then on alternate days until day 41. Attempted virus isolation from the swabs was carried out in chick embryo liver cells for up to three passages (Hughes and Jones 1988).

Five days after rehousing, on day 16, ILT virus was isolated from the trachea of bird 5 and continued to be recovered over the following five days (Table 1). None of the other four cockerels was shown to be shedding virus at any time up to day 41. On day 30, 19 days after rehousing and 10 days after the last detectable shedding episode of bird 5, virus was isolated from the trachea on one day only from the hen housed with it. However, this hen did not have any detectable serum neutralisation antibody to ILT virus when tested 30 days later.

The same group of birds was used to examine the effect of corticosteroid treatment on virus shedding. Starting on day 41, birds 1 to 4 and the uninfected control cockerel were injected intramuscularly on three alternate days with 0.3 ml of a mixture of 2.25 mg prednisolone and 0.75 mg dexamethazone pivalate (Opticortenol-S; Ciba-Geigy). These doses were derived from those used successfully in cats by Gaskell and Povey (1977). Bird 5 was treated similarly but with phosphate buffered saline. All birds were swabbed daily for 14 days then on alternate days until day 64. No virus was isolated from any of the birds during this period.

In the second experiment, the effect of the onset of lay on ILT virus shedding from recovered birds was studied in 10 SPF pullets infected via the trachea at 12.5 weeks old with 0.5 ml of ILT virus field strain 216 ( $10^{4.8}$  TCID<sub>50</sub> ml<sup>-1</sup>). The birds

TABLE 1: Infectious laryngotracheitis (ILT) virus shedding after individual rehousing with unfamiliar uninfected hens

Birds (σ <sup>a</sup> )	ILT carrier	SN titres*	Days										Transmission to contact ♀	
			1→10	11→15	16	17	18	19	20	21	22→41			
1	Yes	3	-	-	-	-	-	-	-	-	-	-	-	-
2	Yes	2	-	-	-	-	-	-	-	-	-	-	-	-
3	Yes	>32	-	-	-	-	-	-	-	-	-	-	-	-
4	Yes	8	-	-	-	-	-	-	-	-	-	-	-	-
5	?	24	-	-	+	+	+	+	+	+	+	-	+	†
Control	No	<2	-	-	-	-	-	-	-	-	-	-	-	-

↑ Rehousing

- + ILT virus isolation from tracheal swab
- No virus isolation
- \* Reciprocal of serum neutralisation titres
- † Positive on day 30 only

were housed in individual but adjoining cages within an isolation pen with absolute air filtration. Three control pullets in a separate isolation pen were sham-infected with cell culture medium. Over the following 10 days, during the acute infection, all infected birds but one showed mild respiratory signs and ILT virus was isolated from all tracheal swabs taken on day 2 after infection from the infected birds, confirming the presence of the virus.

Four weeks after the acute phase, when the birds were 18 weeks old, routine tracheal swabbing two to three times weekly was started, including the three controls, to monitor for virus shedding. The birds received 10 hours lighting per day at this time and from 19 weeks old this was increased by half an hour per week to a maximum of 14 hours. For the purposes of this experiment, onset of lay for the group of birds as a whole was taken to be the day the first egg was

produced. Swabbing was then increased to five times a week and continued until one week after all birds had come into lay.

Between week 18 and onset of lay (21.5 weeks), only two of the 10 birds and, taking the group as a whole, only two of 80 (2.5 per cent) of all swabs taken were positive for ILT virus (Table 2). From 21.5 weeks until one week after all the birds had come into lay (week 28), nine of the 10 birds shed virus and 57 of 280 (20.4 per cent) of all swabs taken were positive, a highly significant increase over the previous period (P<0.001). In keeping with earlier work, the majority of birds (nine of 10 [90 per cent]) appeared to be carriers (Hughes and others 1987). No virus was isolated at any time from the three control birds and they came into lay during the same period as the infected birds (Table 2).

It appears from these studies that the 'natural' stress of

TABLE 2: Infectious laryngotracheitis (ILT) virus shedding in recovered hens before and after onset of lay

Bird	Age (weeks)												
	13→18	19	20	21	22	23	24	25	26	27	28		
1	+				0								
2	+						+	+	+	+	+	+	
3	+					0							
4	+												
5	+		+				+						
6	+					+	+	+	+	+	+	0	
7	+							+	0				
8	+		+			+	+	+	+	+	+	+	
9	+					+	+	+	+	+	+	+	
10	+				0			+	+				
C1	-					0							
C2	-						0						
C3	-								0				
Swabs	2 or 3x weekly					5x weekly							
	↑	↑	↑	↑									
	Acute disease				Onset of lay								

- S Start of routine swabbing
- L Start of increasing lighting (0.5 hours per week)
- + ILT virus isolation from tracheal swab
- 0 First egg produced by each hen
- Birds 1 to 10 infected with ILT virus at 12.5 weeks of age
- Birds C1 to C3 sham infected with cell culture medium

rehousing and, or, the addition of a contact bird, and also the onset of lay may increase the shedding rate in the ILT virus carrier. In the rehousing experiment, although no virus shedding was detected in the 10 days before rehousing, one of the five birds started shedding five days after rehousing, and continued for a further five days. Although numbers were very small, both the re-excretion rate and pattern of shedding was similar to that seen with feline herpesvirus 1 (FHV 1) (Gaskell and Povey 1977, Goddard 1984). It was interesting that the bird that did shed was the only one that had not been detected as having shed previously since the acute disease. In this context, Gaskell and Povey (1977) noted that a refractory period appeared to exist after an episode of re-excretion of FHV 1, when a further episode is less likely to occur. In this study this may also be why corticosteroid at the dosage used appeared to be ineffective in inducing re-excretion, despite being highly effective in some mammalian herpesvirus infections, eg, in cattle, horses and cats (Sheffy and Davies 1972, Gaskell and Povey 1977, Edington and others 1985). However, it may be that the mechanism of either reactivation or its control is different in the domestic fowl.

The transmission studies were inconclusive. Only bird 5 appeared to transmit virus to its mate, and this bird was positive for one day only and had not developed detectable serum neutralising antibodies to ILT virus, on the single occasion tested. Subsequent testing by ELISA was also negative (Adair and others 1985). It is possible that the swabbing method was not sensitive enough to pick up very small virus concentrations on other days, or perhaps the infection was very transient and localised which may also explain the lack of detectable antibodies. Another explanation may be that the pairs of birds were in too large a cage; Gaskell and Povey (1982) found that FHV 1 transmission between cats did not occur unless they were maintained in very close contact.

The onset of lay appeared to have a significant effect on the overall shedding rates of the carrier birds. Nine of the 10 birds shed virus after onset of lay compared with only two in the three-and-a-half weeks before, and there was a highly significant increase in the overall number of virus isolations during this period. Although spontaneous, intermittent shedding was shown in latent ILT infection in cockerels in a previous experiment it was generally at a lower frequency (Hughes and others 1987). However, the birds in this previous study were also approaching sexual maturity and may have been subject to similar hormonal influences. The possibility of contact reinfection in the present study should also be considered as the birds were not totally isolated from each other.

The apparent influence on the virus shedding rate of physiological changes leading up to and during the reproductive period confirm the findings of the cat and pigeon herpesvirus work (Gaskell and Povey 1977, Vindeogel and others 1985). Point of lay has also been shown to influence the re-excretion of infectious bronchitis virus, a coronavirus

of hens (Jones and Ambali 1987). In evolutionary terms, the appearance of infectious virus at around this time may have had considerable biological significance with respect to transmission of virus to the next generation and perpetuation of the virus.

Although in all these studies the numbers used were small, if extrapolated to a commercial scale the numbers of birds shedding virus could be considerable — 20 per cent after rehousing and up to 90 per cent around point of lay. Re-excreted virus could be present in greater concentrations and for longer periods of time than in the experimental conditions used here and also the commercial birds would be housed at a higher density than the experimental birds. All these factors would undoubtedly enhance the possibility of transmission and the outbreak of disease and perhaps account for some of the unexplained outbreaks of ILT in the field.

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