Indian Journal of Animal Sciences **95** (10): 901–905, October 2025/Article
https://doi.org/10.56093/ijans.v95i10.158665

Prevalence of avian leukosis virus infection and its effect on growth and production performance in *Vanashree*, a slow growing chicken strain

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Received: 22 October 2024; Accepted: 27 January 2026

ABSTRACT

Avian leukosis is one of the important neoplastic viral diseases of poultry, which affects the economic traits negatively. A study was carried out to investigate the prevalence of avian leukosis virus (ALV) infection in male and female birds of *Vanashree*, a slow-growing native chicken strain. The prevalence of ALV infection over 11 generations was studied by screening for ALV shedding through the detection of p27 antigen from cloacal swab samples using an ELISA test. The growth and production performance of hens tested negative and positive for ALV shedding were compared using the data collected over the generations. A higher prevalence of ALV infection was observed in hens with an average of 19.91% as compared to the average of 1.65% infection in cocks. There was no significant effect of ALV infection on growth traits recorded at different ages up to 40 weeks. However, the ALV infection had a significant effect on egg production recorded up to 40 weeks. Egg production was significantly less (4.58 eggs) in ALV-positive hens when compared to ALV-negative hens. There was a 7.25% reduction in egg production in ALV-positive birds. However, there was no significant effect of ALV infection on age at first egg and egg weights. The study concluded that the sex of the birds seems to play an important role in the susceptibility to ALV infection in slow-growing chickens. Although ALV infection did not affect the growth, age at sexual maturity and egg weight traits, it reduced the egg production numbers in affected birds.

Key words Avian Leukosis, Performance, Slow-growing chicken, Sex

In India, higher demand for meat and eggs of native chickens is fuelling the growth of native chicken production in recent years. The population of native chickens in the country has been increasing rapidly from 99.23 million in 2016-17 to 138.26 million in 2022-23 with an average growth of 5.74% per year. The egg production of native chickens has also increased from 10.54 billion in 2016-17 to 14.85 billion in 2022-23 with an average growth of 5.93% (BAHS 2017, 2023). However, the contribution of native chickens to the total egg production in the country has come down from 11.96% (2016-17) to 10.73% (2022-23) in recent years. Although the population of native chicken in the country is increasing, egg and meat production is not increasing proportionately to commensurate with increasing consumer demand and hence, there is a rise of price of these commodities. This is mostly due to the slower growth and lesser egg production potential of native chickens. Therefore, there is a need for the improvement of native chickens through selective breeding for better growth and production performance.

Avian leukosis virus (ALV) belongs to the *Alpharetrovirus* genus of retroviridae family which causes lymphoid leukosis in different species of birds including domestic poultry. ALV infections have been reported worldwide to cause significant economic losses due to reduced immunity, growth and production performance, and liveability in high-producing White Leghorn lines (Gavora *et al.* 1980, Ignjatovic, 1986, Gaovra *et al.* 1991, Fulton *et al.* 2021). Besides other factors, the genotype of birds plays a significant role in the ALV infection (Freick *et al.* 2022, Mo *et al.* 2022). Several previous studies have reported the deleterious effect of ALV infection in fast-growing broilers or high-producing White Leghorn strains. Furthermore, the effect of ALV infection on economic or production traits was reported to be breed/line specific (Fulton *et al.* 2021). The prevalence of viral neoplastic diseases like avian leukosis virus (ALV) has already been reported to be higher in native chickens. A very high prevalence of ALV infections was observed in slow-growing chicken breeds indigenous to India like Aseel (76.5%), Kadaknath (76.3%), and Ghagus (75.3%) breeds, and *Vanashree*/ PD-4 (14.3%) strain (Kishore *et al.* 2022). *Vanashree*, an improved strain was evolved from a native chicken breed *(Aseel Peela)* through selective breeding for several generations for rearing in the backyard and free-range production systems. Therefore, the present study was carried out to investigate the prevalence of ALV

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infection in male and female birds and its effect on growth and production traits in the slow-growing *Vanashree* strain of chicken.

MATERIALS AND METHODS

Germplasm: Investigation on the effect of avian leukosis virus (ALV) shedding on the growth and production performance of *Vanashree* was carried out over eleven generations at the pure line farm of the ICAR-Directorate of Poultry Research, Hyderabad (17o 20' N, 78o 30' E), India. The work was duly approved by the Institute Animal Ethics Committee.

In each generation, about 900 to 1000 chicks were hatched in 2 to 3 hatches. Day-old chicks in each generation were wing banded, vaccinated against Marek's disease at hatchery and reared on deep litter for up to 20 weeks in an open-sided house. Mixed sex rearing was practised up to 14-16 weeks of age. Subsequently, male and female birds were segregated and reared separately. Around 20 weeks of age, pullets and cockerels selected for higher body weight were housed in individual cages and reared in open-sided houses.

The chicks were provided with *ad libitum* chick starter ration (2600 kcal/kg M.E. and 18% C.P. on calculated basis) up to eight weeks of age and grower ration (2500 kcal/kg M.E. and 16% C.P.) from 9 to 20 weeks of age, pre-layer ration (mix of grower and layer ration in equal proportion) up to the appearance of the first egg and subsequently layer ration (2600 kcal/kg M.E. and 16% C.P.). Feed ingredients used in ration formulations were maize, soybean meal, sunflower cake, de-oiled rice bran, salt and vitamin premix, lysine, DL-methionine, trace minerals and di-calcium phosphate. The layer ration was supplemented with extra shell grit to increase the calcium content to 3.5% of the ration. Adult male birds were provided with the same layer ration with 1.0% calcium in the diet.

Traits studied: Body weight at 0 day, 4, 8, 20 and 40 weeks of age and shank length at 8, 20 and 40 weeks of age were recorded, respectively to the nearest of 0.1g and 0.01mm accuracy. Sexual maturity (age at first egg), egg production up to 40 weeks of age and egg weights at 28, 32, 36 and 40 weeks of age were recorded. The weight of eggs produced consecutively for five days at each age was recorded using a digital balance (nearest to 0.01g accuracy). All these traits were recorded at the same age in each generation for eleven generations. Incidence of mortality during 21 to 40 weeks of age was recorded in all male and female birds.

Testing for ALV infection: All survived male (55 to 113 numbers in each generation) and female (160 to 290 numbers in each generation) birds housed for recording production traits were screened for ALV shedding after the completion of 40 weeks of age. Cloacal samples from all female and male birds of *Vanashree* were collected in micro-centrifuge tubes having ELISA sample diluent and were screened for shedding the ALV by detecting the p27 antigen. The p27 antigen was quantified by using a commercially available sandwich ELISA kit (IDEXX, USA) by following the manufacturer's instructions. The cut-off value of positive to negative control OD values (spectrophotometer readings) 0.2 (Sample to Positive Ratio) was considered and birds with 0.2 or above this value were categorized as positive for ALV infection and were removed from the breeding program, thus ALV positive birds were culled from the breeding population. Male and female birds tested negative for ALV shedding were used for pedigree mating to produce subsequent generations. Likewise, the screening for ALV shedding was done in 11 generations of the *Vanashree* strain.

Statistical analysis: Least square analysis was carried out using SPSS software (Version 12). A fixed effect model was used to study the effect of ALV shedding on different growth and production traits.

$$Yijkl=\mu + Ai + Bj + Ck + eijkl$$

Where Yijkl = value of the trait (BW/shank length/ASM/ Egg production/ Egg weight) of the ith individual of kth (positive or negative of ALV shedding) of the jth hatch of ith generation. μ = population mean; Ai = fixed effect of the ith generation (i=1 to 11); Bj = fixed effect of the jth hatch (j=1 to 3); Ck = fixed effect of ALV shedding (k=1 to 2); eijk = random error, assumed to be normally and independently distributed with mean zero and constant variance.

RESULTS AND DISCUSSION

ALV infection is a complex disease: The incidence and severity of ALV infections are influenced by the genotype of the bird, sex, age, rearing conditions, etc. The prevalence of avian leukosis virus (ALV) infection in *Vanashree* hens (Table 1) and roosters (Table 2) is relatively lesser than that observed in other slow-growing chicken breeds reared under similar management conditions at the same location (Anonymous 2011, Kishore *et al.* 2022). A very high prevalence of ALV infections (mostly ALV E and ALV J subtypes) was observed in slow-growing chicken breeds indigenous to India ranging from 73.5% in Ghagus to 76.5% in Aseel and 76.3% in Kadaknath breeds as compared to the *Vanashree* (14.3%) strain and other pure lines of chicken (Kishore *et al.* 2022). Previously also a high prevalence of ALV infection was observed in the Kadaknath (82.2%), a popular native chicken breed known for its fibromelanosis character as compared to that (9.4%) of *Vanashree* (Anonymous 2011). The genetics of the host and stains of the ALV are considered to play a major role in ALV shedding (Crittenden *et al.* (1984). Dong *et al.* (2015) have reported widespread prevalence of ALV (A, B or J subtypes) infections in the chickens indigenous to China. Genetic differences in susceptibility to ALV infections were reported in fancy chicken breeds from Germany (Freick *et al.* 2022). This indicates that the genotype of birds plays a significant role in the susceptibility of birds to ALV infections (Mo *et al.* 2022).

Occurrence of ALV infection was considerably less in cocks with an average prevalence of 1.65% as compared

Table 1. Incidence of ALV infection and liveability in females of *Vanashree* strain over the generations

Generation	Number of birds tested	Number of positive birds	Number of negative birds	ALV incidence (%)	Liveability of all females (%)
1	163	19	144	11.51	97.20
2	192	122	70	63.50	99.50
3	237	37	200	15.61	-
4	232	15	217	6.47	91.00
5	190	11	179	5.79	96.20
6	258	9	249	3.49	93.50
7	229	61	168	26.64	95.00
8	283	86	197	30.39	95.60
9	271	55	216	20.30	97.83
10	290	25	265	8.62	93.58
11	290	85	205	29.31	95.81
Total/Average	2635	525	2110	19.91	95.52

Table 2. Incidence of ALV infection and liveability in males of *Vanashree* strain over the generations

Generation	Number of birds tested	Number of Positive birds	Number of negative birds	ALV incidence (%)	Liveability of all males (%)
1	55	2	53	3.44	-
2	84	0	84	0.00	97.70
3	93	1	92	1.08	-
4	102	2	100	1.96	91.90
5	86	1	85	1.16	94.60
6	65	0	65	0.00	95.60
7	63	2	61	3.17	99.10
8	111	4	107	3.60	100.00
9	113	0	113	0.00	98.11
10	77	1	76	1.30	96.55
11	61	2	59	3.28	96.55
Total/ verage	910	15	895	1.65	96.68

to the average prevalence of 19.91% (ranging from 8.62 to 63.50%) in hens reared under the same housing and management conditions (Tables 1 and 2). There was no relationship/trend between male and female birds in the prevalence of ALV (ALV infection %) over the generations. This indicates that the sex of the birds plays an important role in the resistance or susceptibility of birds to ALV infection as well. Female birds seem to be more susceptible to ALV infection. The susceptibility of hens to ALV infection as compared to that of roosters can be explained by the fact that hens are heterogametic (ZW) and W chromosomes of hens carry 20 to 90% more endogenous retrovirus than males (Peona, *et al.* 2021). As a result, a high number of females might be shedding group-specific antigens or complete endogenous ALV (ALVE). The W chromosome also has higher expression of transposable elements due to reduced suppression of their expression (Warmuth *et al.* 2022). This might have resulted in reduced fitness in the female sex (hens) as compared to males (roosters) in bird species (Smith *et al.* 2022; Mo *et al.* 2022).

Differences in ALV infection among male and female birds are also reflected in the differences in the liveability of male and female birds during 21 to 40 weeks of age. Male birds had relatively higher liveability when compared to female birds during the period of 21 to 40 weeks of age as recorded over the generations with an average liveability of 96.68% in males and 95.52% in females (Tables 1 and 2). Part of this difference in mortality (liveability) may be attributed to the difference in the prevalence of ALV infection in male and female birds. Overall, the mortality was less in *Vanashree* birds and was comparable to the industrial standard of 1% mortality per month.

The chance of transmission of ALV infection through insemination of hens with the semen of ALV-infected

roosters appears to be rare or nil (Payne and Nair 2012). It was reported that hens infected horizontally with ALV J through the semen of roosters were able to vertically transmit the virus to their progenies (Li *et al.* 2017). However, this kind of transmission is considered to be rare as only 1 out of 34 chicks born to hen inseminated with ALV-contaminated semen was positive for ALVJ infection (Li *et al.* 2017). Thus, there is a rare chance of vertical transmission of ALV through the insemination of hens with ALV-positive cockerels. Furthermore, reduced prevalence of ALV infection in male birds in slow-growing chickens (*Vanashree*) has economic implications as reducing the cost of ALV testing by screening fewer male breeders for ALV infection or altogether not testing the male birds in the breeding population. The cost for testing for the detection of ALV infection is approximately ₹ 230.0 (US$ 2.77) per bird/sample. Therefore, this approach helps to reduce the cost of screening for ALV in slow-growing birds in resource-poor settings of developing countries where there is tremendous scope for native chicken production.

Based on the findings, it is evident that the prevalence of ALV infection in the *Vanashree* population was declining during the initial generations (Table 1). This shows that testing and removing ALV-positive birds and using only ALV-negative sires and dams for the production of the subsequent generations was effective in decreasing the ALV prevalence in the flock. However, the ALV prevalence increased in the later generations despite removing the ALV-positive birds before the regeneration of the flock. The higher prevalence might be because of the fact that these birds were artificially inseminated at every 4-5 days interval for the supply of chicks to the farmers in the later generations. Therefore, there was a higher chance of transmission of ALV from some positive hens during inseminations through fomites (semen cups, AI syringes) and farm workers (handling of birds). Hence, it might have resulted in a higher prevalence of ALV. This observation calls for the incorporation of additional screening of hens for ALV infection at the time of housing in cages, if artificial insemination is to be done for the purpose of supply.

The ALV infection also affects the growth and production traits in slow-growing birds like the *Vanashree* strain of chicken. The findings of the study revealed that there was no significant effect of ALV infection on growth traits such as body weight and shank length recorded at different ages up to 40 weeks (Table 3). However, the ALV infection had a significant effect on egg production recorded up to 40 weeks of age. Egg production was significantly less (4.58 eggs, 7.25%) in ALV-positive hens when compared to those of ALV-negative hens. However, there was no significant effect of ALV infection on age at sexual maturity (age at first egg). This indicates that ALV infection affects the persistency of egg production (number of eggs produced in the given period) in *Vanashree* hens. However, no significant effect of ALV infection on egg weights recorded at different ages. The severity of ALV infection, reflected through the SPR titer values did seem to influence the growth and production traits as there was no significant relationship between the SPR titers (ranged from 0.2 to 2.78 with the mean of 0.38±0.011) and production traits among ALV positive birds

ALV infections are known to have adverse effects on

Table 3. Effect of status of ALV infection on the growth and production performance of female birds of *Vanashree* strain (Mean±S.E.).

Trait	N	ALV positive	N	ALV negative	P value
		Body weight (g)			
0 day	478	33.57±0.25	1791	33.79±0.09	0.844
4 wks	277	174.2±3.22	1376	178.4±0.89	0.740
8 wks	400	487.4±6.04	1599	496.2±1.95	0.901
20 wks	278	1533±11.5	1135	1552±4.09	0.132
40 wks	416	1980±20.9	1898	1975±7.23	0.264
		Shank length (mm)			
8 wks	392	72.76±0.41	1454	73.49±0.13	0.795
20 wks	275	105.9±0.32	1040	106.1±0.13	0.412
40 wks	416	106.1±0.36	1897	106.2±0.12	0.890
		Production traits			
ASM (d)	485	170.1±1.11	1990	169.1±0.38	0.621
EP40 wks (Nos.)	485	58.58±1.49[b]	1990	63.16±0.50[a]	0.013
		Egg weight (g)			
28 wks	421	43.30±0.26	1593	43.46±0.09	0.759
32 wks	420	45.52±0.24	1639	45.43±0.09	0.235
36 wks	217	47.25±0.33	1221	47.03±0.10	0.084
40 wks	325	48.95±0.36	1495	48.51±0.10	0.462

[a, b] Figures bearing different superscripts row wise differ significantly, N: Number of birds

immunity, growth and production traits and often lead to low levels (1-2%) of mortality and sometimes, result in high (up to 20%) mortality (Elamurugan *et al.* 2015). The deleterious effect of ALV infection on egg production, age at first egg, and egg weights were reported previously (Gavora *et al.* 1980, Ignjatovic 1986). The ALV infection in high egg-producing White Leghorn lines affected egg production, egg weight and sexual maturity negatively (Gavora *et al.* 1980). Similarly, the shedding of endogenous ALV affected egg production and egg weight in the White Leghorn breed with no effect on age at first egg (Gaovra *et al.* 1991). Previous studies have also reported that the effect of endogenous ALV on production traits was breed/ line specific (Fulton *et al.* 2021). The lack or absence of significant effect on most of the production traits except egg number might be specific to this slow-growing strain of chicken. The slow growth of this particular strain may be the reason for not having significant effect of ALV shedding on the growth and egg weight traits. To the best of our knowledge this is perhaps the first study to report the effect of ALV infection on the growth and production traits in slow-growing and low producing strain of native chickens.

The present study concludes that ALV infection affects male and female birds differently in terms of the prevalence of infection. The sex of the birds besides genetics seems to play an important role in the susceptibility to ALV infection in slow-growing chickens as well. The study underscores the importance of screening the slow-growing native chickens, particularly hens for ALV infection as it affected the egg numbers significantly. The findings of the study might help in deciding the breeding strategies for the improvement of native chickens or the development of chicken strains for low-input backyard farming by focussing on detection and elimination of ALV shedding hens from the breeding population.

ACKNOWLEDGEMENTS

The assistance of in-charges of hatchery and farm in hatching and rearing of experimental birds is gratefully acknowledged. "Research was supported by the lndian Council of Agricultural Research, Department of Agricultural Research and Education, Government of lndia".

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