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RESEARCH OPINIONS IN ANIMAL & VETERINARY SCIENCES

Comparison of enzyme-linked immunosorbent assay, fluorescence assay and indirect immunofluorescence assay in detection of Avian Leukosis Virus Subgroup J in DF1 cells

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Abstract

The levels of immunofluorescence assay (FA), indirect immunofluorescence assay (IFA), and enzyme-linked immunosorbent assay (ELISA) in the dynamic detection of the avian leukosis virus subgroup J (ALV-J) into DF1 cells were compared and evaluated. The rNX0101 strain of ALV-J was inoculated into DF-1 cells at three different concentrations (1×10^2, 1×10^3, 1×10^4 $TCID_{50}$). Results showed that with ELISA, the rNX0101 strain was first detected on the 3rd day at a concentration of 1×10^2 $TCID_{50}$ and on the first day at concentrations of 1×10^3 and 1×10^4 $TCID_{50}$. FA failed to detect the positive cells until the 3rd day after inoculation at a concentration of 1×10^2 $TCID_{50}$. IFA detected the positive cells in the culture at all concentrations from the 1st day to the 6th day, except for the 1st day, when used at a concentration of 1×10^2 $TCID_{50}$. The ratios of the positively infected cells highly conformed to the trend in inoculation concentration on the same days, and IFA exhibited higher sensitivity than did FA and ELISA in the dynamic detection of ALV-J.

Keywords: Avian Leukosis Virus Subgroup J; ELISA; FA; IFA

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Introduction

Avian leukosis virus (ALV), which belongs to the Alpharetrovirus genus of the family Retroviridae, can induce myelocytomatosis and nephromas in poultry (Lin et al., 2013). Cases of ALV infection and tumor in commercial layer chickens, broiler chickens, egg-type chickens (Xu et al., 2004; Lai et al., 2011; Min et al., 2011; Wu et al., 2011) and some local chickens have recently occurred in certain areas in China (Gao et al., 2010; Cheng et al., 2010; Pan et al., 2012). ALVs are classified into endogenous and exogenous viruses according to their cross-neutralization, host, range and viral interference patterns. Exogenous ALVs are further divided into subgroups A, B, C, D, and J (Li et al., 2012; Qin et al., 2013). Subgroup E is endogenous nonpathogenic and can be isolated from almost all chicken lines, whereas subgroups C and D are rarely observed in the field. Meanwhile, the exogenous pathogenic virus subgroups A, B, and J mainly induce lymphoid leukosis and myeloid leukosis in broiler chickens (Stedman and Brown, 2002). ALV-J exerted more serious effects than did other subgroups. ALV-J, which was first isolated from white-meat chickens in 1988 in Great Britain, was not isolated in China until 1999 (Cheng et al., 2010).

Subgroup J has recently been reported to cause myelocytomatosis in layer flocks, egg-type chickens

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(Min et al., 2011; Wu et al., 2011) and several Chinese local chicken breeds (Xu et al., 2004). The occurrence of these cases was most prevalent from 2008 to 2010 and led to significant economic loss (Cheng et al., 2010).

Persistent and extensive eradication efforts by major breeding companies can decrease or eliminate the subgroup J virus. ALV-J needs to be accurately detected and quickly eradicated, considering that ALV-J can be transmitted both vertically and horizontally. In this study, we compared the sensitivity of FA, IFA, and ELISA, as well as the combined IFA and ELISA, for accurate detection of ALV-J and the determination of efficient eradication programs.

Materials and Methods

Cells, virus and reagents

DF1 cells, which are susceptible only to exogenous ALVs, were procured from ATCC (USA). The basic medium used was Dulbecco's modified eagle medium (DMEM, pH = 7.3) (HyClone, USA) with 100 U/ml ampicillin and 100 µg /ml streptomycin. Subsequently, 10% foetal bovine serum (FBS) (HyClone, USA) was added into basic DMEM as growth medium and 1% FBS as maintenance medium. The DF1 cell lines were supplemented with 10% FBS and incubated at 37°C with 5% CO_2. The ALV-J rNX0101 strain was amplified, collected, and stored at -80°C. This strain was first isolated from broiler parent chickens in Ningxia Province, China in 2001.

The ALV-J test kit was purchased from IDEXX, Inc., (Westbrook, MA). The fluorescein isothiocyanate (FITC)-labeled antibody for FA detection was provided by Professor Cui (Shandong Agricultural University, Taian). The ALV-J-specific monoclonal antibody (mAb) JE9 was used forIFA test, in which the FITC-conjugated anti-mouse IgG (Sigma-Aldrich, USA) was used to test the primary antibody. All other chemicals used were analytical reagents. The tests were performed at the College of Veterinary Medicine, Shandong Agricultural University, China.

Samples with different concentrations

The DF1 cells were digested and inoculated into petri dishes. Supernates in each dish containing ALV-J rNX0101 were passed through 0.22 µm filters and inoculated into a DF-1 cell monolayer grown with different concentrations of rNX0101 at 1×10^2, 1×10^3, and 1×10^4 $TCID_{50}$. The inoculated DF1 cells were then seeded evenly to three 6-well tissue culture plates with each dose. The plates were marked A (A1, A2, A3), B(B1, B2, B3), and C(C1, C2, C3). Another set consisting of three plates marked D (D1, D2, D3) was uninfected with DF-1 cells to act as the negative control. Four hours later, the supernatant in each well was changed by DMEM with 1% FBS. Cover glasses were prepared in each well for detection by FA and IFA. Supernatant was collected every day for six days and then stored at -80°C. The cells on the cover glasses were fixed with acetone and ethanol (3:2) mixture and then stored at -20°C for FA and IFA.

Detection of different samples by ELISA, FA, and IFA

After a six-day collection, the supernatant samples were detected by ELISA using the ALV-J test kit according to the manufacturer's instructions (IDEXX, Inc., Westbrook, MA). The cover glasses of each well for each day were separately detected by IFA and FA. For the FA test, the FITC-labelled ALV-J special monoclonal antibody JE9 was used. The JE9 antibody was used as the primary antibody in IFA detection and tested by FITC-labelled goat anti-mouse IgG. Both FA and IFA-positive/negative samples were examined by fluorescence microscopy for repeatability and accuracy of results. All samples were simultaneously detected, with each sample detected thrice.

Results

Detection of ELISA based on the p27 antibody

According to the manufacturer's instruction of ELISA kit (IDEXX, Inc., Westbrook, MA), when the S/P ratio exceeds 0.6, the samples were evaluated as positive, indicating the presence of antibodies in ALV-J. Results showed that the rNX0101 strain was first detected at 1×10^2 $TCID_{50}$ on the 4^{th} day. The strains at both 1×10^3 $TCID_{50}$ and 1×10^4 $TCID_{50}$ were detected on the 3^{rd} day. The S/P ratio indicated that the virus demonstrated an increasing trend from the 1^{st} day to the 6^{th} day after inoculation.

Detection of FA and IFA special monoclonal antibody

The FA results with JE9 after inoculation with ALV-J rNX0101 at different concentrations revealed that the samples inoculated at 1×10^2 $TCID_{50}$ were negative on the first two days (Fig. 1, Table 1). IFA detection using the FITC-labelled anti-mouse IgG to test JE9 showed that only the samples at 1×10^2 $TCID_{50}$ were negative on the first day. In addition, the DF1 cells inoculated at concentrations of 1×10^3 and 1×10^4 $TCID_{50}$ from the 1^{st} day to the 6^{th} day showed positive for rNX0101 infection. The ratios of the positive cells increased gradually over time, especially the cells at 1×10^4 $TCID_{50}$ (Fig.2, Table 1).

Comparison of ELISA, FA, and IFA in the detection of ALV-J field strain rNX0101

The positive samples were first identified on the 4^{th} day at low concentrations and on the 3^{rd} day at high concentrations by ELISA. The positive samples were identified on the 3^{rd} day at lower concentration by FA. Meanwhile, IFA showed higher sensitivity compared with FA. IFA was able to identify the positively infected cells on the 2^{nd} day post-inoculation of the rNX0101 strain at

Table 1: Comparison of ELISA, FA and IFA in the detection of rNX0101 in DF1 cells

Inoculate	Detection	Days after inoculated by rNX0101					
Concentration	Method	1d	2d	3d	4d	5d	6d
	ELISA	-	-	-	-	-	-
Negative control	FA	-	-	-	-	-	-
	IFA	-	-	-	-	-	-
	ELISA	-	-	-	+	+	+
$1\times10^{2}TCID_{50}$	FA	-	-	+	+	+ +	+ + + +
	IFA	-	+	+ +	+ +	+ + +	+ + + +
	ELISA	-	-	+	+	+	+
$1\times10^{3}TCID_{50}$	FA	+	+	+ +	+ +	+ + +	+ + + +
	IFA	+	+ +	+ + +	+ + +	+ + + +	+ + + +
	ELISA	-	-	+	+	+	+
$1\times10^{4}TCID_{50}$	FA	+	+ +	+ +	+ + +	+ + +	+ + + +
	IFA	+ +	+ + +	+ + +	+ + + +	+ + + +	+ + + +

Note: "-"represents the detection result is negative; "+" represents the result is positive, and the number of "+" is related with the ratios of positive cells infected by rNX0101.

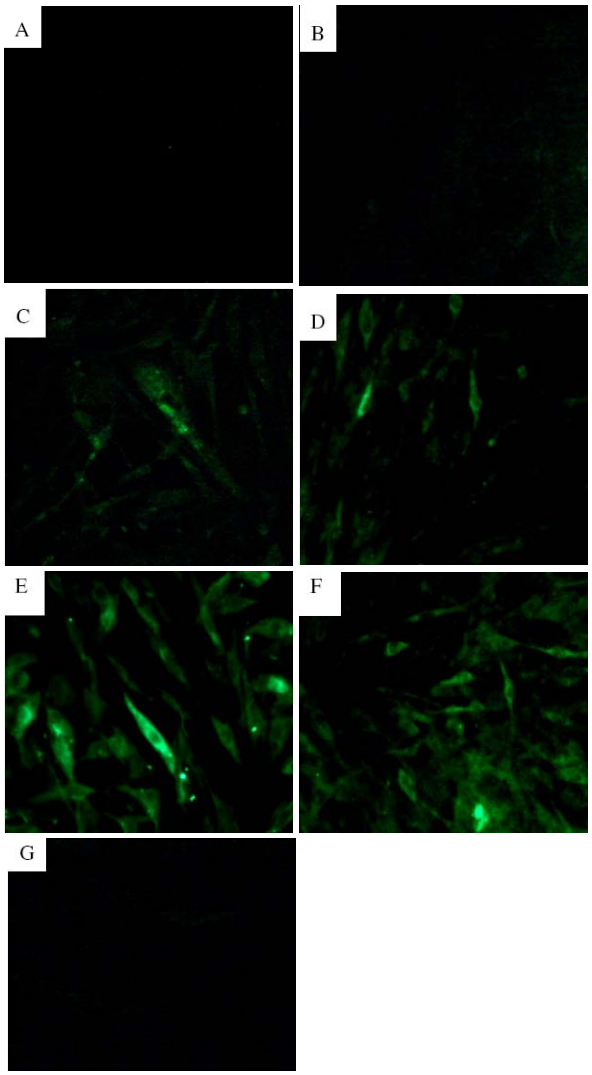

Fig. 1: Detection of FA with ALV-J special monoclonal antibody. Note: A-F. DF1 cells from the 1st day to the 6th day after inoculation with 1×10^{2} $TCID_{50}$ concentration of rNX0101; G. DF1 cells for the 3rd day without rNX0101 inoculated as negative control

low concentrations and on the 1st day at high concentrations. In addition, the ratios of positively infected cells highly conformed to the trend in inoculation concentration, although apoptosis occurred over time.

Discussion

Virus isolation involves complex procedures for cell culture and is time consuming. Thus virus isolation is unsuitable for the detection of ALV-J (Zhang et al., 2010).

In this study, the ELISA kit aimed at the p27 antigen detection of ALV-J virus. The detection results were expressed by the S/P ratio rather than the OD value, which reduced errors attributed to variations in experimental conditions. Despite the simplicity and efficiency of the ELISA, this method may induce a high rate of false-positive result because of the endogenous nonpathogenic virus subgroup E (Hang et al., 2011; Qin et al., 2001).

Detection by FA and IFA can identify samples that are positively infected with virus. Given their advantages, such as reduced costs, ease of operation, and high specificity, FA and IFA are deemed superior to other detection methods (Zhang et al., 2010). Despite the slightly lower sensitivity of IFA compared with that of RT-PCR, the former is widely used in field detection of ALV-J.

According to the results of the present study, FA was found to be more sensitive than ELISA because FA can identify infection at low concentrations. FA and IFA can identify and confirm infections within a short time. Moreover, IFA is a highly accurate assay, whereas ELISA can lead to high rates of false-positive results given that the findings of the present study were based on the ALV-J rNX0101 strain, the results are limited to the specific strain. Whether the same results would be obtained in for infections of other ALV-J strains or other subgroups of ALV needs further investigation. ELISA, FA, and IFA involve a simple procedure, but ELISA with the ALV-J

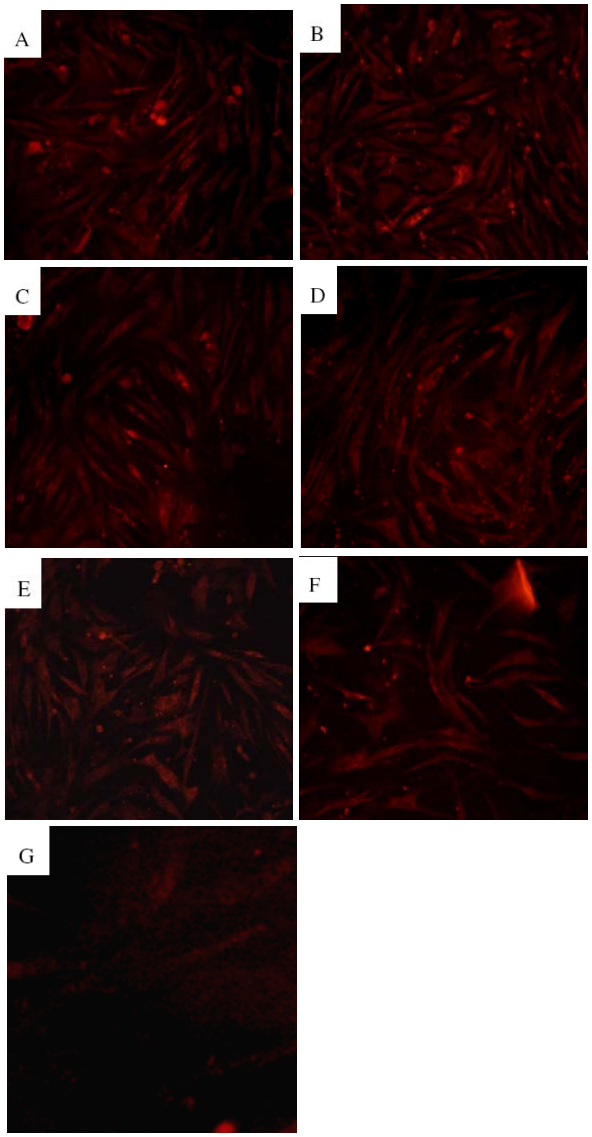

Fig 2: Detection of IFA with ALV-J special monoclonal antibody. Note: A-F. DF1 cells from the 1st day to the 6th day after inoculation with 1×10^4 $TCID_{50}$ concentration of rNX0101; G. DF1 cells for the 3rd day without rNX0101 inoculated as negative control

test kit is the simplest method. IFA performs more efficiently compared with FA. FA exhibits the highest sensitivity and is the most suitable method for rapid and accurate diagnosis. In some epidemiological investigations involving numerous samples, ELISA is the most convenient technique to use. Thus, if the three methods are combined, the sensitivity, accuracy, and speed of ALV-J detection could be significantly enhanced, thereby facilitating the reduction or elimination of infection.

In conclusion, in the dynamic detection of ALV-J, IFA exhibits the highest sensitivity, followed by FA and then ELISA.

Acknowledgments

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