Epidem. Inf. (1987), 99, 711–722
Printed in Great Britain

Detection of lymphoproliferative disease virus by an enzyme-linked immunosorbent assay

By J. R. PATEL* and R. W. SHILLETO*

Houghton Poultry Research Station, Houghton, Huntingdon, Cambridgeshire, PE17 2DA

(*Accepted 1 April 1987*)

SUMMARY

Hitherto, detection of lymphoproliferative disease virus (LPDV), a C-type retrovirus of turkeys, has proved difficult since no tissue culture or serological assay has been available. Development of serological tests has been hampered by the problems of raising virus-specific antisera. An indirect enzyme-linked immunosorbent assay (ELISA) is reported, using a viral antiserum raised with bromelain-digested virus.

The assay specifically detected purified virus at a concentration of 250 ng/ml or greater. In an experiment to detect virus in plasma from turkeys over a period of 4 weeks following LPDV infection, ELISA results correlated closely with the viral reverse transcriptase activity. Both assays were of similar sensitivity and detected small amounts of virus in high-speed pellets of turkey plasma. Evidence is presented indicating that LPDV-infected or hyperimmunized turkeys do not produce readily detectable circulating viral antibodies. In reciprocal ELISA tests, using antibodies to group-specific antigens of other avian retrovirus groups (avian sarcoma-leukosis (ASLV) and reticuloendotheliosis (REV)) no antigenic cross-reaction was found between LPDV, ASLV and REV.

INTRODUCTION

Lymphoproliferative disease virus (LPDV) is a relatively recent isolate of a C-type retrovirus which causes a leukosis in turkeys (Biggs *et al.* 1978; McDougall *et al.* 1978; Perk *et al.* 1979, Gazit *et al.* 1979, 1986; Yaniv *et al.* 1979). Biochemical (Gazit *et al.* 1986; Patel & Shilleto, 1987*b*) and limited genetic (Yaniv *et al.* 1979) data suggest that LPDV is distinct from avian retrovirus groups of sarcoma-leukosis (ASLV) and reticuloendotheliosis (REV). The prevalence of LPDV has not been well documented, and only histopathological data have been available recording LPD outbreaks in the U.K., continental Europe and Israel (see Biggs *et al.* 1978; Ianconescu *et al.* 1979). The paucity of epidemiological data on LPDV is attributed mainly to the lack of (i) a tissue culture system and (ii) virus-specific serological assays, because of problems in raising viral antisera (Patel & Shilleto, 1987*c*).

* Present address: Intervet Laboratories Ltd, The Elms, The Thicket, Houghton, Huntingdon, Cambridgeshire, PE17 2BQ.

Persistent viraemia is a feature of LPDV infection of turkeys (Ianconescu *et al.* personal communication; Gazit *et al.* 1982). An indirect enzyme-linked immunosorbent assay (ELISA) was therefore developed for the detection of LPDV in viraemic turkey plasma, using an antiserum raised against bromelain-digested virus. Data on the assay specificity, sensitivity and suitability for 'field' studies are reported. The limited evidence reported here shows that LPDV-infected or hyperimmunized turkeys do not produce readily detectable circulating viral antibodies. In reciprocal ELISAs using antibodies to group-specific antigens, no cross-reaction was found between LPDV and other avian retroviruses representing ASLV and REV groups.

METHODS

Viruses and their purification

LPDV from turkey plasma, strain HPRS-1 of the REV group grown in chick embryo fibroblasts, and strain RSV(RAV-1) of the ASLV group grown in transformed quail fibroblasts (QT) were purified by rate-zonal and equilibrium density-gradient centrifugation as described (Patel & Shilleto, 1987*b*).

Viral antisera

Rabbits were hyperimmunized with purified LPDV, REV (HPRS-1) and RSV (RAV-1). Prior to their use as immunogens, viruses were digested with bromelain and re-banded in sucrose gradients. For bromelain digestion, 2 mg (LPDV) or 4 mg (RSV-RAV-1, REV-HPRS-1) of purified virus in TNE buffer (10 mM-Tris-HCl, 100 mM-NaCl, 1 mM-EDTA, pH 7·4) was mixed with 2 mg of activated bromelain (10 mg/ml in TNE buffer, activated at 37 °C for 45 min, with 50 mM 2-mercaptoethanol and stored at −70 °C) (Calbiochem-Behring, La Jolla, CA) and incubated at 37 °C for 3 h (LPDV and REV) or 4 h (RSV-RAV-1) and then centrifuged for 3 h at 110000 *g* on linear 25–55% (w/v) sucrose gradients in TNE buffer. Bald virions, with 70–90% of the initial reverse transcriptase activity and banding at 1·13–1·14 g/ml (LPDV) and at 1·14–1·15 g/ml (RSV-RAV-1) and REV-HPRS-1), were used to immunize rabbits. For LPDV antiserum a rabbit was hyper-immunized intramuscularly and subcutaneously with an emulsion of 500–1000 μg of bromelain-digested virus in Freund's complete adjuvant (FCA), boosted similarly at 4 weeks and then 6 weeks later. Serum was collected 2 weeks later.

To prepare RSV (RAV-1) and REV (HPRS-1) antisera rabbits were injected subcutaneously (several sites) and intramuscularly (both hind legs) with about 600 μg of bromelain-digested virus emulsified in FCA. RSV (RAV-1) immune serum from one rabbit was collected 4 weeks later. The second RSV rabbit and two REV rabbits were boosted in the same manner 4 weeks after primary immunization and serum was collected 2 weeks later. The antisera were analysed for activity and specificity by immunodouble diffusion (IDD) and by radio-immunoprecipitation using ^{125}I-labelled viruses followed by SDS polyacrylamide gel electro-phoresis and autoradiography (RIP-SDS-PAGE). Turkey plasma-absorbed LPDV antiserum reacted with viral proteins p41, p32, p26, p13·5, p13 and p12. REV and RSV antisera reacted with the corresponding group-specific

proteins p29 and p27 respectively (see Dickson *et al.* 1982). A chicken was hyperimmunized with bromelain-digested LPDV while three adult female turkeys were hyperimmunized with whole purified virus as described elsewhere (Patel & Shilleto, 1987*c*).

Rabbit antisera to turkey IgG and turkey serum

Turkey IgG was purified using ammonium sulphate precipitation followed by chromatography on DEAE-cellulose using 20 mM phosphate buffer, pH 6·8, and a rabbit hyperimmunized subcutaneously and intramuscularly with 1 mg of the IgG in FCA. The animal was similarly boosted twice at 4-weekly intervals and serum collected 2 weeks after the last injection. The specificity of the serum was tested using immunoelectrophoresis and turkey serum as antigen (Hudson & Hay, 1976). Another rabbit was similarly immunized with a 1% solution of turkey plasma in PBS emulsified in FCA. The serum reacted to at least 12 turkey serum proteins by RIP–SDS–PAGE.

Reverse transcriptase (RT) assay

The method used was that standarized for LPDV by the Israeli group (Schwarzbard *et al.* 1980; Yaniv *et al.* 1979), using oligo $(dT)_{12\text{-}18}$-poly (rA) as primer-template. Tests with other natural and synthetic primer-templates, including oligo (dT)-poly (dA) showed, as the Israeli group had shown, that the assay measured virion-associated reverse transcriptase activity and not cellular DNA polymerase or deoxyribonucleotidyl terminal transferase. Incorporation of [^{3}H]dTTP was up to 1000-fold higher with oligo $(dT)_{12\text{-}18}$-poly (rA) (data not shown). The only modifications were (i) 10 μl of the sample (see plasma sample processing) were tested and (ii) 40 μg/ml of bee venom (Mellitin, Sigma Chemicals, Poole, Dorset) was used to disrupt virions instead of Nonidet P 40, as it gave two-fold higher activity. RT activity, picomoles of [^{3}H]dTTP incorporated in 1 h at 37 °C, is referred to in the text as units for convenience.

Experimental design

Details of experimental, control and field samples investigated are shown in Tables 1 and 2. Each sample was pelleted and assayed for reverse transcriptase activity and by the indirect ELISA.

Sample processing for ELISA and reverse transcriptase assays

Fresh clarified plasma (1·5 ml) was diluted to 10 ml with cold TNE and centrifuged at 60000 ***g*** for 1 h at 5 °C in a 24 × 14 ml MSE 21 angle rotor. Pellets were resuspended in 50 μl of TNE and stored at −70 °C until assayed. Aliquots (10 μl) of each suspension were assayed for reverse transcriptase (RT) activity and another 10 μl aliquot was diluted 100-, 200- and 400-fold in 15 mM carbonate–bicarbonate buffer, pH 9·5, containing 0·05% octyl glucoside (see materials) and assayed by the ELISA.

ELISA materials

Octyl glucoside, bee venom (Mellitin), alkaline phosphatase substrate tablets (cat. no. 104) and species Fab_2 anti-rabbit IgG alkaline phosphatase conjugate

https://doi.org/10.1017/S0950268800066577 Published online by Cambridge University Press

Table 1. *Experimental turkeys used in the study*

Group	Turkeys* No.	Strain	Virus inoculation	Tested post-inoculation
LPDV infected	24	1	LPDV at 2–3 days of age	1, 2 and 4 weeks
Hatchmate control	14	1	None	2 weeks of age
LPDV infected	20	2	LPDV at 2–3 days of age	1 2 and 4 weeks
Hatchmate control	14	2	None	2 weeks of age
Conventional control	24†	1 and 2	Marek's disease virus† at one day of age	1 year

* Infected and control groups were kept and maintained under disease isolation conditions (McDougall *et al.* 1978).

† Inocula consisted of 10000 plaque-forming units of the GA strain of Marek's disease virus.

Table 2. *Details of field turkey plasma samples collected from January to May 1986 and tested for LPDV by ELISA and reverse transcriptase tests*

Birds* No.	Strain	Age (days)	Sex
15	2	105	M
13	3	154	M, F
15	4	161	F
20	5	322–336	F
10	2	107	M
10	6	106	F
10	7	103	M
10	8	126	M
10	7	111	M
10	4	224	F
15	7	107	M
15	3	266	F

* Plasma samples were obtained from his flocks by a commercial breeder within 1 h of bleeding and were in transit for 16 h on ice.

(phosphatase conjugate) were obtained from the Sigma Chemical Company (Poole, Dorset). Flat-bottomed, 96-well polystyrene 129B ELISA plates were obtained from Dynatech (Billingshurst, Sussex). ELISA coating buffer, a × 10 concentrated solution of phosphate-buffered saline, pH 7·3 (PBS) with 0·5% Tween 20 and 0·1% NaN_3 (PBS-Tween) and ethanolamine substrate buffer, pH 9·5, were obtained from Don Whitley Scientific Ltd, Shipley, Yorkshire. All these solutions were found to be satisfactory after storage at +4 °C for periods up to 16 months.

Indirect ELISA

An indirect ELISA procedure similar to that of Voller, Bidwell & Bartlett (1976) was used. The wells of ELISA plates were coated with test antigen samples

or a reference preparation of purified LPDV followed by immune or pre-immune rabbit sera diluted in PBS-Tween. The diluent for the rabbit viral antiserum and its pre-immune serum also contained 2% (v/v) normal turkey plasma from RT-negative birds, to abolish almost completely any reaction between the viral immune serum with turkey plasma proteins on the solid phase. The phosphatase conjugate diluted in PBS-Tween was then added followed by phosphatase substrate (disodium *p*-nitrophenyl phosphate, 1 mg/ml in diethanolamine buffer, Voller *et al.* 1975). Antigen-coating and the reactions with the rabbit sera and the phosphatase conjugate were performed at 37 °C on an orbital shaker, while the substrate reaction was carried out at room temperature on the bench. The assay unit volume was 100 μl and, after each reaction step, the plates were washed 4 times with PBS-Tween.

The reaction times were 2 h for antigen coating, 1 h for the primary rabbit antibodies and respective pre-immune sera and 45 min for the phosphatase conjugate step. Optimal concentrations of the various reagents are given in the text. The assays were carried out in replicates of four unless otherwise stated.

Competition ELISA of LPDV antibodies in turkey area

The plates were coated as described above with purified LPDV at a concentration of 250 ng/ml, washed with PBS-Tween and then reacted with 50 μl unit volumes of dilutions of sera from (i) LPDV-infected or hyperimmunized turkeys, or (ii) RT-negative turkeys in PBS-Tween or (iii) with PBS-Tween alone (control) for 30 min at 37 °C. A further 50 μl of the rabbit LPDV-immune serum at a dilution of 1/2000 in PBS-Tween containing 2% normal turkey plasma was added to each well and the plates were re-incubated at 37 °C for 1 h, washed and then reacted for 45 min at 37 °C with the phosphatase conjugate at a dilution of 1/1000 in PBS-Tween. The plates were washed and the substrate reaction was carried out for 30 min and stopped with 3 N-NaOH as in the indirect ELISA. Absorbances were read at 405 nm with a Dynatech ELISA reader after zeroing against the substrate solution. A chicken LPDV antiserum (Patel & Shilleto, 1987*c*) was titrated in parallel as a positive control. The results are expressed as percentage inhibition caused by the dilutions of chicken and turkey sera with respect to the PBS-Tween control.

RESULTS

Assay parameters

Non-ionic detergents Nonidet p 40 (NP 40) and octyl glucoside, and a bee venom (Mellitin), were used to test whether virion disruption was advantageous. Table 3 illustrates typical results obtained with the reference stock of purified virus stored at −70 °C. About twofold higher absorbances were recorded after disruption with Mellitin or octyl glucoside. This enhancement was between 3- and 4-fold (data not shown) when virus pelleted from freshly collected plasma was used. In titrations, Mellitin at 2·5 μg/ml and octyl glucoside in the range of 0·2–0.05% were optimal, while NP 40 (0·2–0·05% was inhibitory. About 10% variation was recorded when the reference purified virus stock was titrated on 20 separate occasions (Table 4).

https://doi.org/10.1017/S0950268800066577 Published online by Cambridge University Press

Table 3. *Effect of membrane-disrupting agents on the reactivity of purified virus and high-speed pellet of plasma from RT-negative turkey (TPP)*

Treatment	Absorbance at 405 nm*	
	Purified virus	TPP
None	0·435, 0·503	0·018, 0·026
NP 40	0·015, 0·009	0·002, 0·001
Octyl glucoside	0·900, 0·952	0·002, 0·009
Bee venom (Mellitin)	0·812, 1·064	0·000, 0·000

* LPDV pre-immune rabbit serum gave absorbances of 0·019–0·05.

Table 4. *Reproducibility* of the indirect ELISA in 20 separate assays of reference purified virus stock*

Antigen	Absorbances at 405 nm approximate antigen concentration (ng/ml)		
	1500	500	170
LPDV	0·967±0·092†	0·502±0·051	0·312±0·048
Control antigen	0·016±0·005	0·010±0·004	0·006±0·005

* Collation of data from 20 separate ELISA of test samples in which the reference stock of purified virus and high-speed pellet of plasma from an RT-negative turkey were titrated in replicates of 4 using LPDV immune rabbit serum at a dilution of 1/2000. Absorbances were read 30–40 min after substrate addition.

† Mean of means±standard deviation.

Fig. 1 and Table 5 show that LPDV immune serum bound significantly to RT-free (< 0·01 units) turkey plasma (TP) or to high-speed pellets of TP (TPP), while it reacted with both purified virus and TPP from RT-positive turkeys. None of the plasma samples from 153 birds of seven different strains received from a commercial breeder (Table 2) during January to May 1986 was positive by either the ELISA or the RT assay. Pellets of the field plasma samples tested at a dilution of 1/100 by the ELISA gave absorbance values of 0·050–0·010 and had RT activity of 0·01–1·2 units (individual results not shown). Similarly, pre-immune serum was unreactive with purified virus (Fig. 1 and Table 5) or with crude viral pellets from experimentally virus-infected birds, tested at 1 and 4 weeks post-inoculation (Table 6). A conclusion from these tests and from the experiment reported in the next section (Table 6) was that the assay specifically detected LPDV. Taking a ratio (S/N ratio) between positive reactions and controls (RT-negative TP and TPP *vs* immune serum and virus *vs* pre-immune serum) of 2 as being significant, the assay readily detected purified virus at concentrations of 250 ng/ml or greater even when the immune serum was diluted 2000-fold (Fig. 1 and Table 5).

Detection of virus in test samples

The suitability of the assay for the detection of the virus in a situation comparable to a field outbreak was tested in an experiment in which groups of 2-

https://doi.org/10.1017/S0950268800066577 Published online by Cambridge University Press

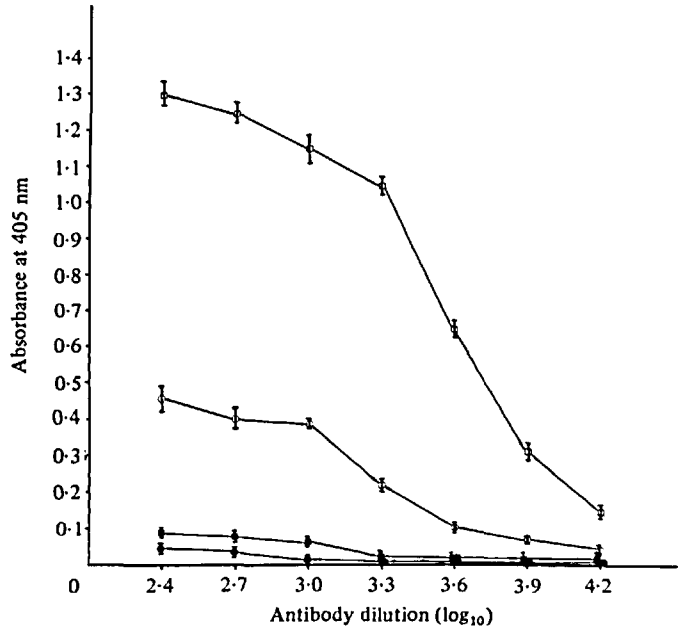

Fig. 1. Titration of viral antibody against purified virus (□, 2000 ng/ml: ○, 250 ng/ml) and high-speed pellet of normal turkey plasma (■, 4000 ng/ml: ●, 400 ng/ml).

Table 5. *Titration of reference purified LPDV, normal turkey plasma and high-speed pellets of normal turkey plasma in the indirect ELISA*

	Absorbance at 405 nm* Approximate antigen concentration (ng/ml)				
Antigen	2000	1000	500	250	125
LPDV	1·15 ±0·022	0·810±0·031	0·390±0·024	0·181±0·018	0·080±0·011
Normal plasma	0·030±0·009	0·018±0·011	0·008±0·003	0·009±0·004	0·010±0·00
Normal plasma pellets	0·040±0·010	0·028±0·008	0·016±0·004	0·011±0·003	0·009±0·00

* Means of 4 wells, 30 min after addition of substrate±standard deviation. Pre-immune serum gave values ranging between 0·008 and 0·001.

to 3-day-old turkey poults of two different strains were infected with LPDV and their high-speed plasma pellets tested for virus by the ELISA and the RT assay just before virus inoculation and at 1, 2 and 4 weeks post-inoculation. Other details are summarized in Table 1. RT activity in TPP from some 600 apparently normal turkeys ranged between 0·01 and 1·8 units (data not shown), and hence samples with an activity of 5 units were regarded positive but at the threshold of detection. In the ELISA, samples with absorbances of 0·120 or greater (S/N ratio > 2) were considered positive. A good correlation between the results of RT and ELISA tests was evident (Table 6). The relative detection rates in the ELISA and the RT tests respectively were 20/44 and 37/44 at week 1, 39/44 and 41/44 at week 2 and 100% in both tests at week 4 post-infection. Most of the ELISA-

https://doi.org/10.1017/S0950268800066577 Published online by Cambridge University Press

Table 6. *Detection of LPDV by ELISA and RT assay in high-speed plasma pellets following infection of 2-3-day-old turkey poults*

	No.+ve/no. tested*					
Weeks post inoculation...	1		2		4	
Birds	ELISA	RT	ELISA	RT	ELISA	RT
Virus-inoculated	20/44	37/44	39/44	41/44	44/44	44/44
Hatchmate controls†	NT	NT	0/28	0/28	NT	NT
MDV controls‡	0/24	0/24				

* High-speed pellets from 1·5 ml of plasma were resuspended in 50 μl of TNE, and 10 μl aliquots were tested by RT and ELISA. For ELISA, samples were tested in replicates of four dilutions of 1/100 and 1/400. Positive ELISA absorbances were 0·1–1·15 and RT assay values of 5–650 units.

† Absorbance values in ELISA were 0·08–0·020 and RT activities were between 0·001 and 0·8 units.

‡ Inocula consisted of 1000 plaque-forming units of the GA strain of Marek's disease virus and birds tested 1 year later.

Table 7. *Binding of purified LPDV by rabbit antisera to turkey IgG and to turkey serum*

	Absorbance at 405 nm*	
Rabbit antisera to	Purified virus	Turkey plasma pellet
Turkey IgG	0·964, 0·960	1·216, 1·182
Turkey serum	1·115, 1·191	1·236, 1·310

* Plates were coated with antigens diluted in octyl glucoside (0·05% w/v) containing coating buffer, followed by rabbit antisera at a dilution of 1/400 and then phosphatase conjugate at a dilution of 1/1000. Absorbances were read 30 min after substrate addition.

negative samples at 1 and 2 weeks post-infection were at the threshold of detection (5-7 units) in the RT assay. RT-negative samples were invariably negative in the ELISA, and 44 pre-infection, 28 hatchmate and 24 MDV-infected birds (Tables 1 and 6) were negative in both the assays.

Viral antibodies in turkey sera

By ELISA, rabbit antibodies to both turkey IgG and turkey serum were highly reactive with purified virus (Table 7). This has been explained by the fact that up to 20 bromelain-susceptible turkey plasma proteins remain adsorbed to the virion surface even after purification (Patel & Shilleto, 1987*b*). Therefore a competition ELISA (see methods) was used to test for viral antibodies in turkey sera. None of the sera from six LPDV viraemic turkeys (data not shown) bled 4 months after initial viral inoculation at the age of 4 weeks was positive ($\leqslant$ 10% inhibition, Fig. 2). Similarly, sera from three 10-week-old turkeys hyperimmunized with purified virus (Patel & Shilleto, 1987*c*) were negative. However, LPDV-immune chicken serum caused 50% inhibition in the binding of the immune rabbit serum at a dilution of 1/1000 (Fig. 2). The experiment suggested that, following an active

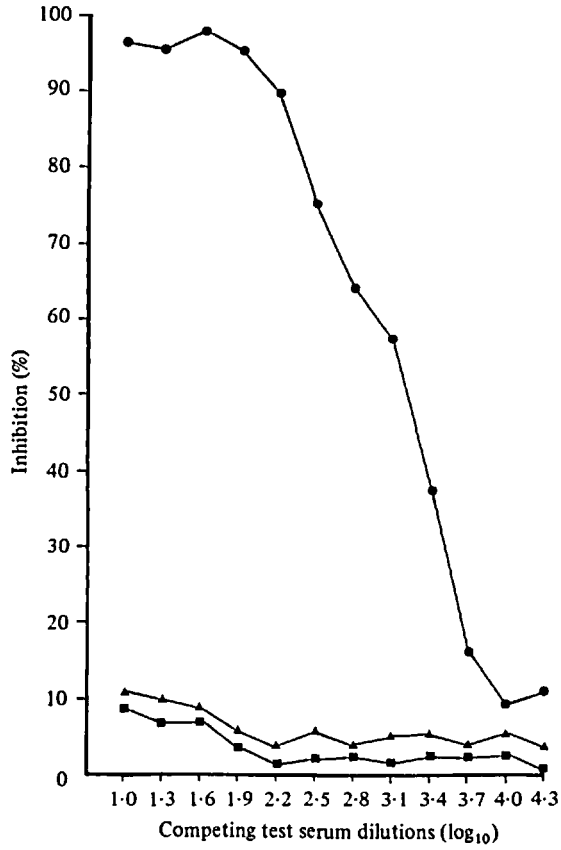

Fig. 2. Inhibition of binding of the rabbit viral antiserum by a hyperimmune chicken viral antiserum (●) and by sera from LPDV-convalescent (▲, average response from six birds) and LPDV-hyperimmunized (■, average response from three birds) adult turkeys in the competition ELISA.

LPDV infection or after hyperimmunization with purified virus, turkeys do not respond by producing significant levels of viral antibodies, as was also shown by RIP–SDS–PAGE analysis using ^{125}I-labelled LPDV (Patel & Shilleto, 1987*b*).

Antigenic cross-reactions with over avian retroviruses

Using rabbit antisera to group-specific proteins of REV (Maldonado & Bose, 1976) and ASLV (see Dickson *et al.* 1982) and to major structural polypeptides of LPDV (Patel & Shilleto, 1987*b*), no significant cross-reaction was recorded in reciprocal ELISA tests carried out with purified LPDV, REV (HPRS-1) and RSV (RAV-1) (Table 8). The tests included 2 antisera to REV, 2 to RSV (RAV-1) and one to LPDV. A rabbit antiserum to feline leukaemia virus, a kind gift from Prof. O. Jarrett, was also included in the test, and it reacted as expected with REV (Table 8). These results were also confirmed by RIP–SDS–PAGE tests with ^{125}I-labelled viruses and the rabbit viral antisera, as well as viral antisera raised in chickens (unpublished data). A conclusion from the experiments is that LPDV, REV and ASLV (RSV) are not antigenically related.

https://doi.org/10.1017/S0950268800066577 Published online by Cambridge University Press

Table 8. *Antigenic cross-reactions amongst LPDV, REV and ASLV*

	No. of hyperimmune rabbit antisera tested†					
Antigens*	LPDV	REV		Feline leukaemia virus‡	RSV (RAV-1)	
Purified viruses						
LPDV	1·036	0·007	0·018	0·008	0·079	0·063
	1·033	0·001	0·006	0·012	0·072	0·069
REV	0·042	0·635	0·811	0·351	0·010	0·018
	0·040	0·650	0·800	0·310	0·005	0·011
RSV (RAV-1)	0·079	0·008	0·021	0·006	0·760	0·811
	0·072	0·009	0·031	0·013	0·748	0·836
Control						
High speed pellet	0·038	0·004	0·008	0·012	0·018	0·021
of turkey plasma	0·058	0·000	0·013	0·014	0·017	0·023
Chick embryo	0·019	0·003	0·008	0·012	0·000	0·011
fibroblasts (CEF)	0·018	0·000	0·014	0·016	0·003	0·015
QT cell cultures (QT)	0·008	0·004	0·004	0·006	0·006	0·010
	0·004	0·004	0·009	0·011	0·000	0·009

* Plates were coated with octyl glucoside-distrupted viruses or materials from which viruses were purified, namely turkey plasma (LPDV), cultures of CEF (REV) or QT cells (ASLV) at a concentration of about 2000–4000 ng/ml.

† Results with immune sera diluted 1/2000 in PBS-tween containing 2% (v/v) turkey plasma and in cases of REV and RSV, also 2% frozen and thawed extract of corresponding control antigens. Corresponding pre-immune sera (LPDV, REV and RSV) gave absorbances of 0·020–0·002.

‡ Antiserum to feline leukaemia virus was a kind gift from Prof. O. Jarrett of the Veterinary School, University of Glasgow.

DISCUSSION

The aim of the present study was to develop a simple, rapid, sensitive and specific ELISA for the detection of LPDV suitable for use in epizootiological studies of the virus. The usefulness of the test is increased by the fact that (i) there is as yet no LPDV-susceptible cell culture system and (ii) turkeys appear to manifest, at best, a poor antibody response to the virus (Fig. 2). Therefore assays to measure antiviral antibodies in turkey sera will have little value in studies on LPDV prevalence.

However, it is particularly important here to validate the specificity of the RT assay, since the specificity of the ELISA test has been established in relation to the RT assay. The RT assay used was essentially that standardized for LPDV by Schwarzbard *et al.* (1980). This group has been working with the same isolate (Biggs *et al.* 1978; McDougall *et al.* 1978) of LPDV grown in turkeys as ourselves. Our evidence indicating that the assay measured particle-associated reverse transcriptase is shown below.

(1) NP40 or Mellitin treatment to disrupt virions was necessary for activity.

(2) Oligo (dT)-poly (dA) was about 1000-fold less active than oligo (dT)-poly (rA) as primer-template.

(3) Purified infectious bronchitis virus (1–2 mg/ml), and pelleted MDV did not incorporate [^{3}H]dTTP ($\leqslant$ 0·01 unit) in exogenous assays (unpublished data). On the other hand, other retroviruses (REV and ASLV) were active in the assay both as crude preparations and after purification.

(4) Peak RT activity in 60, 10 and 10 purifications in sucrose gradients respectively of LPDV, REV and RSV coincided with bands having a peak concentration of viral particles 100 to 110 nm in size by electron microscopy and also with peak detection of virus by SDS–PAGE analysis of entire gradients. By SDS–PAGE, LPDV, REV and RSV were found to have distinct polypeptide profiles (Patel & Shilleto, 1987*b*).

Because typical C-type particles were not readily and definitely identifiable in negatively stained crude pellets of plasma with low RT and ELISA activities, no attempt was made to correlate RT and ELISA results with viral detection by EM. Thin-sectioning of viral pellets was considered too laborious. Therefore the only evidence for the specificity of the ELISA was as follows. (1) Its correlation with results from the RT assay in that none of 252 RT-negative TPP samples (Tables 1, 2 & 6) was found to be positive by the ELISA, while the correlation between the assays was 100% once the concentration of virus was sufficiently high (Table 6). (2) Turkey plasma-absorbed LPDV immune serum was unreactive with (*a*) ^{125}I-labelled TP or TPP (from RT-negative birds) in RIP–SDS–PAGE tests, and (*b*) with tissues from RT-negative turkeys in indirect immunofluorescence test (IIF), while the serum detected virus-infected cells in spleen and thymus from RT-positive birds (Patel & Shilleto, 1987*a*).

As for future refinements of the assay, direct testing of test plasma for LPDV would be a considerable advantage, and could be provided by an indirect sandwich ELISA (Voller *et al.* 1976).

In conclusion we describe a rapid and simple indirect ELISA for the detection of LPDV in plasma of infected turkeys, and the results clearly demonstrate the specificity of the assay. As for the sensitivity and suitability of the assay for use in the field, our results show that the assay was capable of scoring all samples positive when they contained a minimum of 10 units of RT activity, and a limited field survey suggests that LPDV is probably not prevalent in the UK. Using a modification of the assay we have presented further, limited, evidence for the paucity of viral antibody responses in turkeys. Therefore assays measuring viral antibody will be, at present, not readily applicable to the study of LPDV prevalence and epizootiology. It should, however, be easy to differentiate between LPD and reticuloendotheliosis of turkeys, since no cross-reaction was found in reciprocal ELISAs using antisera to the group-specific antigen p29 of REV and to the major LPDV proteins. This has also been confirmed by reciprocal RIP–SDS–PAGE analysis using ^{125}I-labelled viruses and viral antisera raised in both rabbits and chickens (unpublished data). Similarly, LPDV is not antigenically related to the viruses of the avian sarcoma-leukosis group.

We thank Drs T. D. K. Brown (HPRS, Houghton) and J. R. Crowther (AVRI, Pirbright) for their advice on RT and ELISA tests respectively. Our grateful thanks go to Mrs Anita Ellis and Mrs Jennifer Precious for typing the manuscript.

REFERENCES

Biggs, P. M., McDougall, J. S., Frazier, J. A. & Milne, B. S. (1978). Lymphoproliferative disease of turkeys. *Avian Pathology* **7**, 131–139.

Dickson, C., Eisenmann, R., Fan, H., Hunter, E. & Teich, N. (1982). Protein biosynthesis and assembly. In *RNA Tumour Viruses* (ed. R. Weiss, N. Teich, H. Varums and J. Coffin), pp. 513–648. New York: Cold Spring Harbor Laboratory.

GAZIT, A., YANIV, A., IANCONESCU, M., PERK, K., AIZENBERG, B. & ZIMBER, A. (1979). Molecular evidence for a type C retrovirus eitology of the lymphoproliferative disease of turkeys. *Journal of Virology* **31**, 639–644.

GAZIT, A., SCHWARZBARD, Z., YANIV, A., IANCONESCU, M., PERK, K. & ZIMBER, A. (1982). Organotropism of the lymphoproliferative disease virus (LPDV) of turkeys. *International Journal of Cancer* **29**, 599–604.

GAZIT, A., BASRI, R., IANCONESCU, M., PERK, K., ZIMBER, A. & YANIV, A. (1986). Analysis of structural polypeptides of the lymphoproliferative disease virus (LPDV) of turkeys. *International Journal of Cancer* **37**, 241–245.

HUDSON, L. & HAY, F. C. (1976). *Practical Immunology*. Oxford: Blackwell Scientific Publications.

IANCONESCU, M., PERK, K., ZIMBER, A. & YANIV, A. (1979). Reticuloendotheliosis and lymphoproliferative disease: Pathology and differential diagnosis. *Refuah Veterinarit* **36**, 2–12.

MALDONADO, R. L. & BOSE, H. R. JR., (1976). Group-specific antigen shared by the members of the reticuloendotheliosis virus complex. *Journal of Virology* **17**, 983–990.

MCDOUGALL, J. S., BIGGS, P. M., SHILLETO, R. W. & MILNE, B. S. (1978). Lymphoproliferative disease of turkeys. II. Experimental transmission and aetiology. *Avian Pathology* **7**, 141–155.

PATEL, J. R. & SHILLETO, R. W. (1987*a*). Diagnosis of lymphoproliferative disease virus infection of turkeys by an indirect immunofluorescence test. *Avian Pathology* **16**, 367–376.

PATEL, J. R. & SHILLETO, R. W. (1987*b*). Characterisation of lymphoproliferative disease virus of turkeys. Structural polypeptides of the C-type particles. *Archives of Virology* **95**, 159–176.

PATEL, J. R. & SHILLETO, R. W. (1987*c*). Production of virus-specific antisera to lymphoproliferative disease virus of turkeys. *Avian Pathology*. In press.

PERK, K., IANCONESCU, M., YANIV, A. & ZIMBER, A. (1979). Morphologic characterisation of proliferative cells and virus particles in turkeys with lymphoproliferative disease. *Journal of the National Cancer Institute* **62**, 1483–1487.

SCHWARZBARD, A., YANIV, A., IANCONESCU, M., PERK, K. & ZIMBER, A. (1980). A reverse transcriptase assay for the diagnosis of lymphoproliferative disease (LPD) of turkeys. *Avian Pathology*, **9**, 481–487.

VOLLER, A., BIDWELL, D. & BARTLETT, A. (1975). Microplate enzyme immunoassays for the immunodiagnosis of viral infections. In *Manual of Clinical Immunology* (ed. N. R. Rose and H. Friedman), pp. 506–511. Washington: American Society of Microbiology.

YANIV, A., GAZIT, A., IANCONESCU, M., PERK, K. AIZENBERG, B. & ZIMBER, A. (1979). Biochemical characterisation of the type C retrovirus associated with lymphoproliferative disease of turkeys. *Journal of Virology* **30**, 351–357.