

Cross-reactivity between the house dust mite *Dermatophagoides pteronyssinus* and the mange mites *Psoroptes cuniculi* and *P. ovis*

I. Demonstration of antibodies to the house dust mite allergen Dpt 12 in sera from *P. cuniculi*-infested rabbits

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The cross-reactivity of the mange mites Psoroptes cuniculi (PC) and Psoroptes ovis (PO) antigens with the house dust mite Dermatophagoides pteronyssinus (DP) antigens has been studied. Cross-reactivity between mange mite and house dust mite antigens was demonstrated by both ELISA and immunoelectrophoresis by use of a sheep anti-DP antiserum. Both PC and PO were demonstrated to contain eight cross-reacting antigens. Sera from rabbits infested with PC were demonstrated to produce antibodies to the homologous immunogen, to PO antigens, and to DP antigens. Of the seven sera from infested rabbits tested, four were demonstrated to produce a strong antibody response to a major DP antigen Dpt 12, and two were demonstrated to produce a weak response that was judged empirically by double-diffusion analysis. Two sera were judged to react with the DP lipoprotein, Dpt 4. Sera from control rabbits did not demonstrate reactivity with any extract tested. Despite the detection of anti-Dpt 12 antibodies, however, an antigen corresponding to Dpt 12 was not detected in either PC or PO extracts. The findings that mange mite-infested rabbits produce antibodies that recognize DP antigens probably explain previous observations in which it was demonstrated that commercially obtained rabbit anti-immunoglobulin antisera contain anti-DP antibodies, a finding that suggests caution in the use of such reagents in studies designed to measure antibody responses to DP allergens. (J ALLERGY CLIN IMMUNOL 78:293-9, 1986.)

Studies from this and other^{1, 2} laboratories have demonstrated by a variety of assays that commercial rabbit anti-immunoglobulin antisera contain antibodies to antigens seemingly derived from the house dust mite DP. In particular, it has been demonstrated that rabbit antihuman IgE, IgA, and IgG, and a rabbit antimouse IgG contained antibodies to a major house

Abbreviations used

DP: *Dermatophagoides pteronyssinus*
PC: *Psoroptes cuniculi*
PBS: Phosphate-buffered saline
IEP: Immunoelectrophoresis
SGM: Spent growth medium
DF: *Dermatophagoides farinae*

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dust mite-allergen Dpt 12,² a finding that may be of particular concern in assays with rabbit antihuman IgE in the study of allergic disease.

At least two possible explanations were offered for these results. First, it was possible, although it was considered unlikely, that rabbits used to raise antibodies to immunoglobulins had been actively immunized with the house dust mite. Second, it was possible that the antimite antibodies detected may have represented cross-reacting antibodies originally raised against different species of mites known to infest lab-

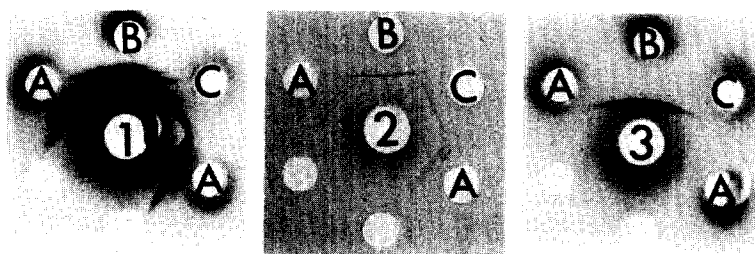


Fig. 1. Double-diffusion analyses of antigens (20 mg/ml) from PC (A), DP (B), and PO (C) against sheep anti-DP antiserum stained for protein (1) and lipid (2), and against rabbit anti-Dpt 12 antiserum (3).

oratory rabbits.³ In support of this view, studies have demonstrated that rabbits not only produce antibodies during infestation with mange mites such as PC^{4,5} but also that antigens from other rabbit-infesting mites such as *Sarcoptes scabiei* cross-react with antigens from DP.⁶

We examined, as presented in this article, sera from rabbits experimentally infested with PC for the presence of antibodies to antigens from DP and in particular to the allergen Dpt 12 in an attempt to explain the above mentioned observations.

MATERIAL AND METHODS

Preparation of mite extracts

DP. The house dust mite DP (free from growth medium) and SGM (free of whole mites) were kindly provided by Commonwealth Serum Laboratories (Parkville, Australia). The mites were extracted by grinding at 0° C in 0.05 mol/L of ammonium bicarbonate, pH 8.0, and the resulting extract was clarified by centrifugation at 15,000 × g for 15 minutes. The supernatant was removed, and the insoluble material was extracted twice more in a similar manner. The supernatants were combined and dialyzed exhaustively against 0.05 mol/L of ammonium bicarbonate, pH 8.0. The supernatant was then freeze-dried and stored desiccated at -20° C until required. SGM was processed in a similar manner. Details of the procedure used to culture mites and the nature of the growth medium were not provided by the manufacturer.

PO. PO antigen was prepared from mites obtained from the ears of infested rabbits. The mites were originally obtained from cattle and raised in the ears of successive rabbits. The mites were obtained from the tenth and eleventh passage collections and were isolated from freshly collected scabs. The scabs were placed in a jar, lid side down, on a warming plate.⁷ At various times the jar was inverted, and the mites adhering to the lid were tapped into a clean jar and stored at -18° C. When the mites were needed, they were thawed, pooled, washed in 0.01 mol/L of PBS, pH 7.2, containing 1% v/v of Tween 20 on a magnetic stirrer for 45 minutes, rinsed several times with PBS alone, dried at 38° C for about 2 hours, weighed, and ground on a Ten Breock tissue grinder in cold PBS at a ratio of 100 mg of mites to 1.75 ml of

PBS. The grinder was immersed periodically in an ice bath during the grinding process. The mixture was centrifuged at 48,200 × g for 1 hour at 4° C. The supernatant (antigen) was removed, exclusive of the lipid-like layer, and filtered successively through 0.45 μm and 0.20 μm filters.

PC. PC antigen was prepared from mites obtained from the ears of infested rabbits. The antigen was prepared in the same manner as the PO antigen except that the mites were dried for 1 hour at room temperature and processed as above at 4° C. Both PO and PC extracts were stored frozen at -18° C until required. The antigens were thawed and lyophilized before use in these studies. The antigens had been stored for 1 year before being lyophilized.

The protein concentration of each mite extract was determined by use of the Coomassie blue G 250 assay⁸ with bovine serum albumin as standard.

Antisera

Sheep anti-DP antiserum. The antiwhole DP extract antiserum was raised in sheep. Briefly, each of three sheep was immunized subcutaneously with 2 mg of whole DP extract dissolved in 1 ml of PBS and emulsified with an equal volume of Freund's complete adjuvant. A secondary injection of DP (2 mg) in Freund's incomplete adjuvant was administered 28 days later, and the sheep were bled 10 days after this and subsequent booster injections. Sera from each sheep were pooled and stored at -20° C until required. The sera from individual sheep did not detect DP antigens before immunization, and mange was not detected in any sheep within the holding facility before or during the course of this study.

Rabbit anti-DP antiserum. A rabbit antiwhole DP extract antiserum was prepared in a similar manner to that described for the sheep anti-DP antiserum. The rabbit anti-DP antiserum was pooled from five rabbits and stored at -20° C until required.

Anti-Dpt 12 antisera. The anti-Dpt 12 antisera were raised in both rabbits and sheep. Each of three rabbits was immunized subcutaneously with 500 μg of purified Dpt 12 in 0.5 ml of PBS emulsified with an equal volume of Freund's complete adjuvant. The rabbits received booster injections 26 days later with the same amount of material emulsified in Freund's incomplete adjuvant and bled 10 days after this and subsequent booster injections. Sera from each

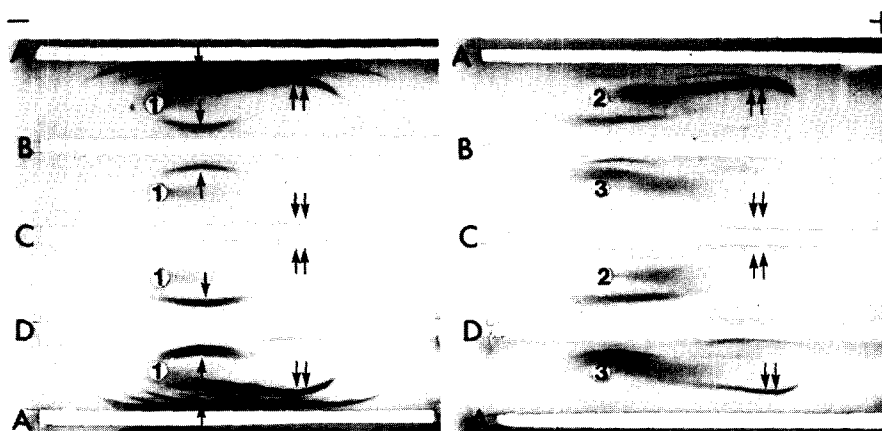


Fig. 2. Immunoelectrophoretic analysis of sheep anti-DP (A) and sera from three rabbits (B), 421; (C), 37; and (D), 23 infested with PC, and sheep anti-DP antiserum tested against antigens (20 mg/ml) from DP (1), PC (2), and PO (3). The arrows indicate reactivity with the allergen Dpt 12 (↑) and the lipoprotein (↑↑).

rabbit were pooled and stored at -20°C until required. The sheep antiserum was raised in a similar manner.

Rabbit antihuman IgE. The rabbit antihuman IgE antiserum was obtained commercially (name supplied on request) and represents the same antiserum used in a previous study,² the results of which demonstrated that it contained antibodies to the allergen Dpt 12.

Rabbit anti-PC. The serum samples were obtained from rabbits lightly to heavily infested with PC. Blood samples were collected from rabbits by cardiac puncture and were centrifuged. The serum was removed, lyophilized, and stored at -18°C . The degree of infestation in each ear was judged according to criteria previously described.⁹ Normal rabbit sera were obtained from rabbits with no known history of mange infestation housed in holding facilities in both Perth, Western Australia, and Kerrville, Texas.

Immunochemical methods

IEP and double-diffusion analysis were performed as described previously.¹⁰ Double-diffusion and IEP plates were stained for protein with Coomassie blue R 250 and for lipoprotein with Sudan black B.¹¹ Single radial immunodiffusion was performed with the rabbit anti-Dpt 12 antiserum as described previously.¹²

ELISA

The cross-reactivity of certain sera from mange mite-infested rabbits with antigens from the house dust mite was determined by ELISA.¹³ In the DP ELISA, plates (EIA, Flow Laboratories, McLean, Va.) were coated with DP at a concentration of 50 $\mu\text{g/ml}$ in alkaline carbonate buffer, pH 9.6. In the Dpt 12 ELISA, plates were coated with Dpt 12 at a concentration of 2 $\mu\text{g/ml}$. Plates were incubated with serial dilutions of sera at 4°C overnight. Plates were washed and incubated with a 1 in 1000 dilution of immunopurified sheep antirabbit IgG conjugated with alkaline phosphatase (grade 5, Sigma Chemical Co., St. Louis, Mo.)

at room temperature for 4 hours. After washing, plates were incubated with a 1 mg/ml solution of *p*-nitrophenol in diethanolamine buffer, pH 10, and the $\text{OD}_{405\text{ nm}}$ was recorded (Titertek multiscan, Flow Laboratories). Sera were analyzed in duplicate and on two separate occasions.

Preparation of Dpt 12

The allergen Dpt 12 was isolated by chromatofocusing from SGM in a manner similar to that described earlier.¹⁴ However, the ion exchange matrix and the buffer used in this study were the anion exchanger DE53 (Whatman Ltd, Maidstone, England) and the chromatofocusing buffer system described by Hearn and Lytle.¹⁵ A crude Dpt 12 allergen preparation was obtained from an aqueous solution of SGM by precipitation with 50% saturated ammonium sulfate. The Dpt 12 allergen was then partially purified by gel filtration on Sephacryl S-300 (Pharmacia South Seas Pty., Ltd., Sydney, Australia). Those fractions containing the allergen were pooled, concentrated, and dialyzed exhaustively against 0.005 mol/L of Tris buffer, pH 7.5. This preparation was then applied to a DE53 column, pre-equilibrated with the same buffer. After the removal of nonbinding material, the allergen was eluted with the chromatofocusing buffer adjusted to pH 5.0 with 1.0 mol/L of HCl. Those fractions containing the allergen (pI 6.9 to 5.8) were pooled, dialyzed against 0.05 mol/L of ammonium bicarbonate, pH 8.0, and freeze-dried. The allergen preparation was finally purified by gel filtration on a P-60 column (Bio Rad Laboratories Pty. Ltd., Sydney, Australia), equilibrated with 0.05 mol/L of ammonium bicarbonate, pH 8.0. The fractions containing the allergen were pooled and freeze-dried.

RESULTS

Relationship between DP and the mange mites PC and PO

Initial studies were designed to determine whether there was any immunochemical cross-reac-

TABLE I. Antibody responses to antigens from *Psoroptes cuniculi*, *P. ovis*, and *Dermatophagoides pteronyssinus* in sera from *P. cuniculi*-infested rabbits

Rabbit serum	Duration of infestation (mo)	Extent of ear canker* (left ear/ right ear)	No. of antigens detected by IEP			DP specific antibodies present†	
			PC	PO	DP	Anti-Dpt 12	Anti-Dpt 4
387	0	0/0	0	0	0	—	—
51‡	8	ND	7	8	3	+++ +	—
63	9	2/2	5	5	1	±	—
64	9	3/2	5	4	2	±	—
37	>12	6/6	4	4	1	—	+
23	>12	6/6	8	8	4	+++ +	—
421	>12	2.5/2.5	8	8	3	++	—
61	>12	4/4.5	12	13	5	+++ +	+

ND = not determined.

*Ear canker scoring system: 0 = scabs and mites absent to 6 = entire pinna filled with scabs and mites.

†The antibody response to Dpt 12 was judged empirically by double-diffusion and graded; — indicates no precipitate formation, whereas ± to ++++ indicates increasing size and staining intensity of precipitate. The antibody responses to Dpt 4 were detected by immunoelectrophoresis and are indicated as being present (+) or absent (—).

‡*Psoroptes cuniculi* used to infest rabbits was obtained from a goat/ibex cross.

tivity between the antigens of DP and PC and DP and PO.

The immunochemical relationship between the extracts was investigated by double diffusion and IEP analyses (Figs. 1 and 2). These studies demonstrated that PC and PO contained antigens that cross-reacted with DP as judged by their reactivity with a sheep anti-DP antiserum. Both PC and PO were demonstrated to contain at least eight antigens that reacted with the sheep anti-DP antiserum. The most prominent precipitin band in all three extracts was demonstrated to stain for lipid (Fig. 1, 1B) and has subsequently been identified as a lipoprotein.⁵

Double-diffusion studies were performed in order to try and identify a PC and PO antigen corresponding to the DP allergen Dpt 12 (Fig. 1, 1C). An antigen corresponding to Dpt 12 was not identified in extracts of either PC or PO by use of both rabbit (Fig. 1, 1C) and sheep anti-Dpt 12 antisera (data not presented). Single radial immunodiffusion studies with a Dpt 12 detection limit of 1 µg/ml were also performed (data not presented), but again an allergen corresponding to Dpt 12 was not identified.

Reactivity of sera from PC-infested rabbits with PC, PO, and DP

The reactivity of seven sera from PC-infested rabbits with extracts of DP, PC, and PO were studied by IEP. IEP studies (Fig. 2; Table I) demonstrated that all sera reacted with both PC and PO extracts. However, the number of antigens recognized by each serum varied. For example, the most potent serum (No. 61)

recognized 12 PC antigens and 13 PO antigens, whereas serum 37 recognized only four PC and PO antigens, respectively (Table I). The individual pattern of reactivity (i.e., the number of antigens detected, the intensity of staining, and electrophoretic mobility) with PC and PO extract was similar for each serum. Rabbit sera from animals that had not been infested with mange mites did not produce detectable precipitin bands to either DP, PO, or PC.

The sera were then tested for reactivity with the DP extract. IEP studies (Fig. 2; Table I) demonstrated that all sera from mite-infested rabbits reacted with DP, although the sera reacted with varying degrees of potency. The number of DP antigens detected ranged from one to five. Six out of the seven sera reacted with varying degrees of potency with a DP antigen similar in electrophoretic mobility to Dpt 12. A similar pattern of reactivity with DP was also observed when three selected sera (Nos. 23, 37, and 421) were examined by ELISA (Fig. 3). However, none of the sera from PC-infested rabbits tested was as potent as the rabbit anti-DP antiserum raised under laboratory conditions. On the basis of electrophoretic mobility, two sera (Nos. 37 and 61) were judged to have reacted with the mite lipoprotein Dpt 4.

Reactivity of sera from PC-infested rabbits with the allergen Dpt 12 from DP

The reactivity of the sera from PC-infested rabbits with the purified allergen Dpt 12 from DP was determined by double diffusion (Table I; Fig. 4). These studies demonstrated that six of the seven sera reacted

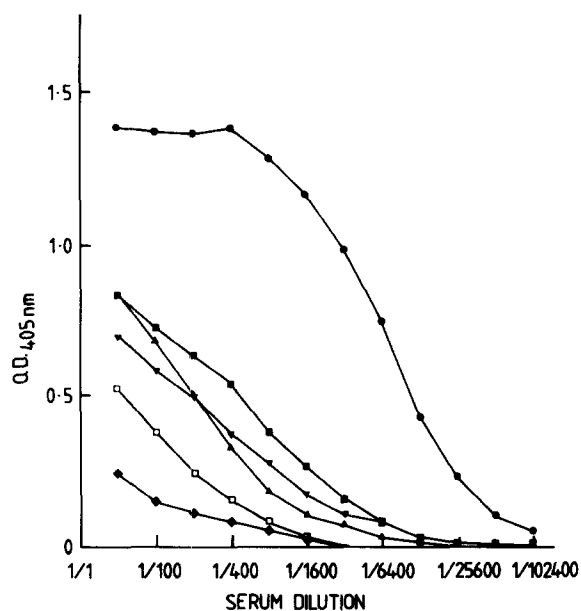


Fig. 3. ELISA of sera from rabbits infested with PC (Nos. 421, ▼; 37, ▲; and 23, ■), normal pooled rabbit serum (◆), rabbit anti-DP antiserum (●), and a commercial rabbit antihuman IgE antiserum (□). ELISA was performed with plates coated with whole DP extract.

specifically with Dpt 12, although the degree of reactivity varied with the serum studied. A line of identity between the rabbit anti-Dpt 12 antiserum and the sera from rabbits Nos. 23 and 421 with purified Dpt 12 was obtained in double-diffusion analysis (Fig. 4).

Reactivity of a commercial rabbit antihuman IgE with DP and Dpt 12

The reactivity of a commercial rabbit antihuman IgE antiserum with DP and Dpt 12 was determined by ELISA (Figs. 3 and 4). Both assays demonstrated that the antiserum reacted with DP and the allergen Dpt 12. Unfortunately, insufficient material remained to enable us to test the reactivity of this serum with either PO or PC extracts.

DISCUSSION

Extracts of the mange mites PC and PO (family, Psoroptidae) were demonstrated to cross-react with the house dust mite DP (family, Pyroglyphidae) with a sheep anti-DP antiserum, and at least eight cross-reacting antigens were detected in both PC and PO extracts. One of these cross-reacting antigens has been demonstrated to correspond to the lipoprotein allergen Dpt 4.¹⁶

The cross-reactivity between DP and the related Pyroglyphid mite DF and mites other than those from the family Psoroptidae has been previously reported. For example, studies have demonstrated that both DP

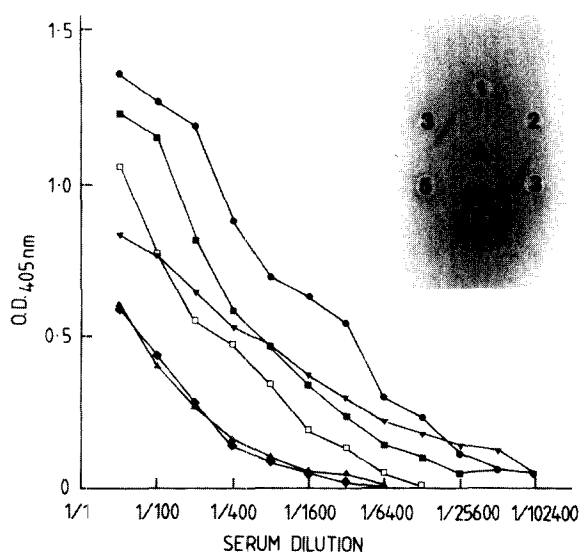


Fig. 4. ELISA of sera from rabbits infested with PC (Nos. 421, ▼; 37, ▲; and 23, ■), normal pooled rabbit serum (◆), rabbit anti-DP antiserum (●), and rabbit antihuman IgE antiserum (□). ELISA was performed with plates coated with the purified allergen Dpt 12. Inset is a double-diffusion analysis of sera from rabbits infested with PC (1, No. 421; 2, No. 37; and 4, No. 23), normal pooled rabbit serum (5), and rabbit anti-Dpt 12 antiserum (3) against Dpt 12 (A, 700 µg/ml).

and DF cross-react antigenically and allergenically with each other^{17, 18} and with the scabies mite *S. scabiei* (family, Sarcoptidae).^{6, 19} In the former studies,⁶ crossed IEP studies demonstrated that four antigens from *S. scabiei* were recognized by a rabbit anti-DF antiserum. Cross-reactivity between DF and DP with *Tyrophagus putrescentiae* (family, Acaridae) has also been reported. Crossed IEP and IEP studies have demonstrated that both anti-DF and anti-DP antisera recognized four antigens in extracts of *T. putrescentiae*.²⁰

Despite demonstrating cross-reactivity between PC, PO, and DP antigens, we were unable to identify an antigen corresponding to the DP antigen, Dpt 12, in the PC and PO extracts. Considering the reactivity of sera from rabbits infested with PC toward Dpt 12, these findings were unexpected. It is possible that the corresponding antigens were present in PC and PO extracts but at a concentration below the detection limits of the assay systems used. If PC and PO produce an antigen similar to Dpt 12, which in DP is primarily associated with mite feces,²¹ it is possible that the procedures used to isolate mange mites from scabs and tissue debris before extraction, particularly the washing and warming-plate treatments, may facilitate the removal of feces from the mites, thus accounting for the low allergen concentrations. In this regard, recent studies¹² on several DP extracts obtained from

a variety of sources indicate that the ratio of Dpt 12 to total protein can vary considerably from as low as 4.3% to as much as 75.4%. Although the precise manner in which some of these extracts were prepared is unknown, the data suggested that the DP extracts that demonstrated low levels of Dpt 12 were those that were prepared from isolated mite bodies in contrast to those prepared from combined mites and medium.

Other workers have also looked for Dpt 12 analogous antigens in other mites, particularly DF, *D. microceras*, *Acarus siro* and *T. putrescentiae*.^{18, 22-24} Such studies, with either polyclonal or monoclonal antibodies, demonstrated that extracts of DF and *D. microceras* but not *A. siro* or *T. putrescentiae* contained an allergen analogous to Dpt 12 (P₁). Further study demonstrated, however, that the corresponding antigens were not identical, since both species-specific and cross-reacting epitopes were detected. In the context of our failure to detect a corresponding antigen in PO and PC extracts, other observations described²⁴ are particularly interesting. Chapman et al.²⁴ demonstrated that, whereas polyclonal antisera raised in mice to P₁ demonstrated limited reactivity with DF Fr 1 (30% of sera reacting), mice immunized with Fr 1 demonstrated significant reactivity with P₁ (80% of sera reacting). Thus, it is possible that the anti-Dpt 12 antiserum used in our study recognized only species-specific epitopes, whereas the sera from PC-infested rabbits recognized both species-specific and cross-reacting epitopes. As yet, however, it is not known if any of the above suggestions are correct, and further studies are underway to determine the reasons for our findings.

Sera from rabbits infested with the mange mite PC but not sera from normal rabbits were demonstrated to have antibodies to the homologous immunogen in accord with previous observations.^{4, 5} A maximum of 12 antigens (13 antigens with the use of the PO extract) were detected by the most potent serum by IEP. In general, however, the number of antigens detected in the remaining sera (four to eight antigens) were similar to those reported in sera from infested sheep and rabbits.^{25, 26} The reactivity pattern of the rabbit sera suggested that extracts of PO and PC were antigenically very similar in accord with previous observations.²⁷

The sera from infested rabbits were also demonstrated to react with antigens from DP and, in particular, with Dpt 12 and to a lesser extent, Dpt 4. ELISA studies, in confirmation of previous observations,² demonstrated that a commercial rabbit antihuman IgE also reacted with Dpt 12. On the basis of these data, it is probable that the observed reactivity of commercial rabbit antihuman immunoglobulin antisera

with Dpt 12 arose because the rabbits used in their production had, at some stage, been infested with the mange mite PC, a very common ectoparasite infesting commercial rabbit colonies.³ The observation that sheep²⁶ as well as rabbits can be infested with PC and PO mites with the production of precipitating antibodies suggest that commercial antisera raised in these species must be carefully screened if they are to be used in studies designed to measure antibody responses to DP in clinical situations.

In conclusion, we have demonstrated that there is limited cross-reactivity between the house dust mite and the mange mites PC and PO. In addition, sera from rabbits infested with PC produce antibodies to the homologous immunogen and to DP antigens. Despite not being detected in the PC extract, rabbits infested with the mange mite PC produce a strong antibody response to the DP antigen Dpt 12. The allergenic cross-reactivity between DP and PC is currently being studied.

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