Egg Yolk Antibodies (IgY) in Routine Diagnostic Work

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Summary
According to Linscott's Directory of Immunological and Biological Reagents (1994/95) the commercial availability of egg-yolk antibodies (IgY) is extremely low.
For preparation, cleaning and detection of IgY it would be of advantage to have a „Protein Y" available analogous to protein A and protein G for mammalian antibodies. Until now, the search for „ProteinY" was unsuccessful.
IgY has been used for routine diagnostic work covering the following subjects:
1. Identification of the host species from abdominal blood of haematophageous insects;
2. IgY-anti-horse-Ig-PO conjugate for ELISA on dourine;
3. FITC-conjugated IgY-antirabies for diagnostic work on rabies;
4. FITC-conjugated IgY against avian virus diseases (Newcastle dis., Infectious bronchitis, Gumboro). In all cases satisfactory results have been achieved.

Keywords: IgY, commercial availability, „Protein Y", routine diagnostic work

Zusammenfassung: Dotterantikörper und ihr Einsatz in der Routinediagnostik.
Die kommerzielle Verfügbarkeit von Dotterantikörpern (IgY) ist entsprechend den Angaben aus Linscott's Directory of Immunological and Biological Reagents (1994/95) zur Zeit noch als ausgesprochen gering anzusehen.
Für die Präparation, Reinigung und Markierung von IgY wäre es vorteilhaft, ein „Protein Y" – analog dem Protein A und Protein G für mammäre Antikörper – verfügbar zu haben. Die Suche nach einem „Protein Y" führte bisher nicht zum Erfolg.
Untersuchungen mit aviären Antikörpern wurden auf folgenden Gebieten durchgeführt:
1. Identifizierung der Wirtstierart aus dem Abdominalblut hämatophager Insekten;
2. IgY-anti Pferd Ig-PO-Konjugat für den Einsatz in der Beschälseuchediagnostik;
3. IgY-anti-Tollwut-FITC-Konjugat für die Tollwutdiagnose;
4. IgY-anti-aviäre Virusinfektionen (Newcastle Disease, Inf. Bronchitis, Gumboro) konjugiert mit FITC. Mit den vorgestellten Präparationen wurden gute Ergebnisse erzielt.

1 Introduction

The Laboratory for Immunology and Diagnostics at the BgVV which is accommodating the project on Igy is concerned with development, production and distribution of reagents for the diagnosis of zoonoses and animal diseases. Additionally, a second project – the BgVV/GTZ sponsored Service-Laboratory – is attached to the Laboratory for Immunology and Diagnostics. Within the Service-Laboratory, serological techniques are adapted according to poor financial conditions of developing countries by selfproduction of serological reagents, cleaning and reuse of plastic material, a.s.o.

The IgY project which developed uncomplicated techniques for immunisation of hens, isolation of egg yolk antibodies, and their applicability in various test systems creates continuous considerations in regard of changing from mammalian derived antibodies to egg yolk antibodies for both, the National Laboratory for Immunology and Diagnostics and the internationally orientated Service Laboratory.

Regarding commercially available polyclonal antibodies being produced worldwide, these antibodies are listed in Linscott's Directory of Immunological and Biological Reagents (1994/95). In this directory, not only antigenic specificities are quoted but as well the donor animal species. Table 1 provides the number of polyclonal antibodies from chicken in relation to the total number of polyclonal antibodies recorded.

297 from almost 16.000 is less than 2% of polyclonal antibodies deriving from chicken, and only three from these have been prepared from egg yolk, the rest apparently from chicken sera. Nevertheless, chicken as donor animals occur in every group and it may be concluded that in general, chicken are potent antibody producers. Obviously, the IgY-technology needs further propagation.

Table 1: Linscott's Directory, polyclonal antibodies. Number of chicken derived antibodies from the total of polyclonal antibodies

Antibody Group	Total in Group	Derived fr. Chicken
anti-Immunoglobulin	6261	137
anti-C-Fractions	441	24
anti-Cells&Tissue Proteins	2950	75
anti-Miscellaneous	4455	43
anti-Infectious Agents	1892	18
Total	15999	297

Three subjects are presented :

1. Search for „Protein Y",
2. „Bloodmeal identification",
3. IgY for routine diagnostic work.

2 Search for „Protein Y"

Protein A from a specific *Staphylococcus aureus* strain and protein G from group G streptococci are heat stable cell wall proteins having the property of specific binding to the Fc portion of a wide range of immnunoglobulins (table 2).

Neither chicken IgG nor IgY are specifically bound by protein A or G. For affinity chromatography and for marker techniques, protein A and G has gained a very high importance for isolation, concentration and labelling of IgG isotypes from mammalian sera, and it can be expected that the same would be true for „protein Y" which remains still to be detected.

While searching for such a protein, 1000 staphylococcus and streptococcus strains have been screened by a specifically designed technique but till now without success.

3 Bloodmeal identification

In Africa, tsetse flies transmit trypanosoma infections causing sleeping sickness in humans and nagana in animals. It is estimated that roughly 10 Mio km^2 are inhabited by tsetse flies and the annual losses by death, emaciation, loss of meat, milk, and incapability to work as draught animals are calculated to amount to 5 billion US$ per annum. Additionally, trypanosoma infections may lead to immunosuppression causing secondary, mainly bacterial infections to flare up.

Good knowledge regarding the behaviour of tsetse flies are a prerequisite for successful campaigns against these parasitic insects. Tsetse flies have host preferences for certain animal species from which they take their bloodmeal. The knowledge about these preferences in given areas in connection with seasonal behaviour provides means for specific campaigns against tsetse flies.

Host preferences can be detected by analysing abdominal blood from flies for identification of the animal species from which this blood has been taken. The procedure includes an ELISA system with various anti-animal species antisera against which abdominal blood samples dried onto filter paper are tested after reconstitution. Typical reaction patterns of control antigens tested against homologous and heterologous antisera from rabbits are presented in table 3.

Table 2: Comparison of species and Ig-isotype reactivity of protein A and G

Immunoglobulin		Protein A	Protein G
Human	IgG1	++	++
	IgG2	++	++
	IgG3	–	++
	IgG4	++	++
Mouse	IgG1	+/–	+/–
	IgG2a	++	++
	IgG2b	++	++
	IgG3	++	++
Rat	IgG1	+/–	+/–
	IgG2a	–	++
	IgG2b	–	+/–
	IgG2c	++	++
Rabbit	IgG	++	++
Bovine	IgG1	–	++
	IgG2	++	++
Sheep	IgG1	–	++
	IgG2	++	++
Goat	IgG1	+/–	++
	IgG2	++	++
Horse	IgG	–	++
Chicken	IgG/IgY	–	–

If closely related species are to be identified, specific antisera have to be absorbed with antigen (serum) from competing species and the result of such absorption for the differentiation of domestic pig, warthog and bushpig is demonstrated in table 4.

When transferring the production of antibodies from rabbits to chicken it was observed that high titred IgY anti-animal species antibodies could be isolated from egg yolk of immunised hens. The principle of absorption with competing antigens was successfully applied though the phylogenetical distance from chicken to mammalian species seemed to require a more intense absorption procedure (Graph 1 and 2).

The bloodmeal identification service has been transferred to Burkina Faso, and it is planned to use IgY and not any more rabbit sera as a source for antibodies.

4 IgY for routine diagnostic work

The test prescribed by O.I.E. for serological diagnosis of *dourine* (*Trypanosoma equiperdum)* in solipeds is the complement-fixation test (CFT). The development of an ELISA test became possible by acquiring larger quantities of serum from infected horses. The conjugate used for this ELISA test is IgY-anti-horse Ig conjugated to POD. An application for the acceptance of this test as a prescribed or alternative test for international trade was directed to the O.I.E. A further advantage of ELISA in the sense of animal protection is a comparatively low consumption of antigen which is produced from

Table 3: Bloodmeal identification: Control set 1

Antigen	Antisera against							
	1	2	3	4	5	6	7	8
1 Dog	++	–	–	–	–	–	–	–
2 Elephant	–	+++	–	–	–	–	–	–
3 Cat	–	–	++	–	–	–	–	–
4 Chicken	–	–	–	+++	–	–	–	–
5 Hippopotamus–	–	–	–	–	+++	–	–	–
6 Crocodile	–	–	–	–	–	+++	–	–
7 Rat	–	–	–	–	–	–	+++	–
8 Lion	–	–	+	–	–	–	–	+++

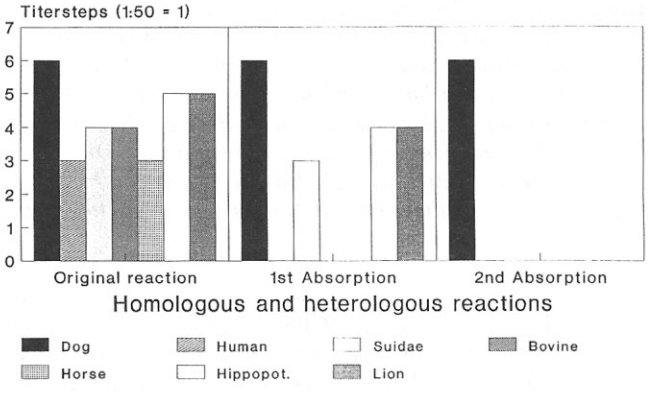
Cross–reaction between lion (8) and cat (3) indicate the relationship of *felidae*

Table 4: Bloodmeal identification: Control set 2

Antigen	Antisera against 1	2	3	4	5	6	7	8
1 Human	+++	–	–	–	–	–	–	–
2 Ruminant	–	+++	–	–	–	–	–	–
3 Monitor lizz.–	–	–	+++	–	–	–	–	–
4 *Suidae*	–	–	–	++	++	++	++	–
5 Domest.pig–	–	–	–	++	++	–	–	–
6 Warthog	–	–	–	+++	–	++	–	–
7 Bushpig	–	–	–	+++	–	–	++	–
8 Avian	–	–	–	–	–	–	–	++

Anti–*Suidae* serum (4) non–absorbed; anti–domest.pig (5), anti–warthog–(6), anti–bushpig–(7) sera absorbed with cross–reacting antigens to produce specific reactions.

Specific pathogen free (SPF) birds are not required in this context since affinity chromatography with appropriate ligands permits the isolation of specific antibodies out of a pool of specific and non-specific antibodies. Affinity chromatography has been adapted to a home-made system by using pulverized polystyrene material (microtitre plates) as a matrix material (Staak et al., 1996).



Antig.for absorpt.:1.Bovine;2.Lion/Hippo

Figure 1: IgY-anti dog: Original reaction and reaction after 1. und 2. absorption

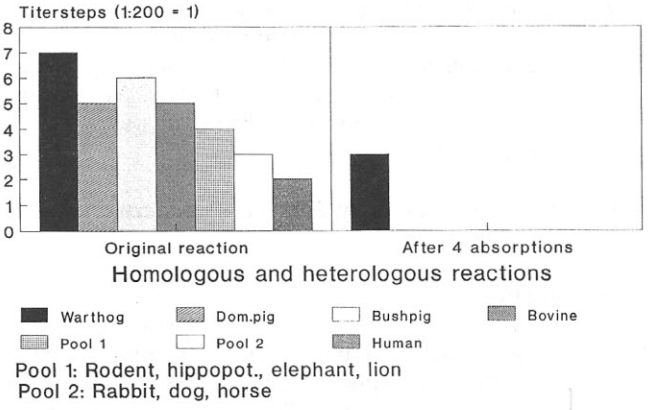


Figure 2: IgY-anti warthog: Original reaction and final reaction after 4 absorptions

artificially infected rats: The amount of antigen needed for conducting ELISA is 5% of the amount to be used in complement-fixation test.

For the identification of *rabies* in brain smears from suspected cases, fluorescent anti-rabies conjugates are frequently used. Commercially available conjugates are normally derived from rabbit sera.

Chicken vaccinated repeatedly with rabies vaccine (Rabipur, Behringwerke, Marburg) responded well with high IgY-anti-rabies antibodies which could be conjugated to FITC by the same procedure as used in the FITC-conjugation of mammalian antibodies. Resulting specific fluorescence was of the same quality as found in mammalian derived preparations.

IgY-FITC conjugates directed against *avian virus diseases* have been prepared as a joint project of the Jordan Centre for Veterinary Vaccines in Amman and the Service Laboratory attached to the Laboratory for Immunology and Diagnostics at the BgVV. Specific conjugates were directed against Newcastle disease (ND), infectious bronchitis (IB) and gumboro (IBDV). These conjugates were to be used in birds suspected to have died of one of the diseases mentioned by direct fluorescent staining of impression smears from affected organs. In artificially infected chicken, virus antigen could be specifically detected in all organs examined. High virus quantities were detected in the spleen, trachea and lungs following infection with NDV, in the trachea following infection with IBV and in the bursa of Fabricius following the infection with IBDV (Gervelmeyer, 1995).

The IgY technology seems to be well suited to meet many of the diagnostic requirements in European and developing countries. One of the advantages for developing countries is the availability of chicken everywhere.

References

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