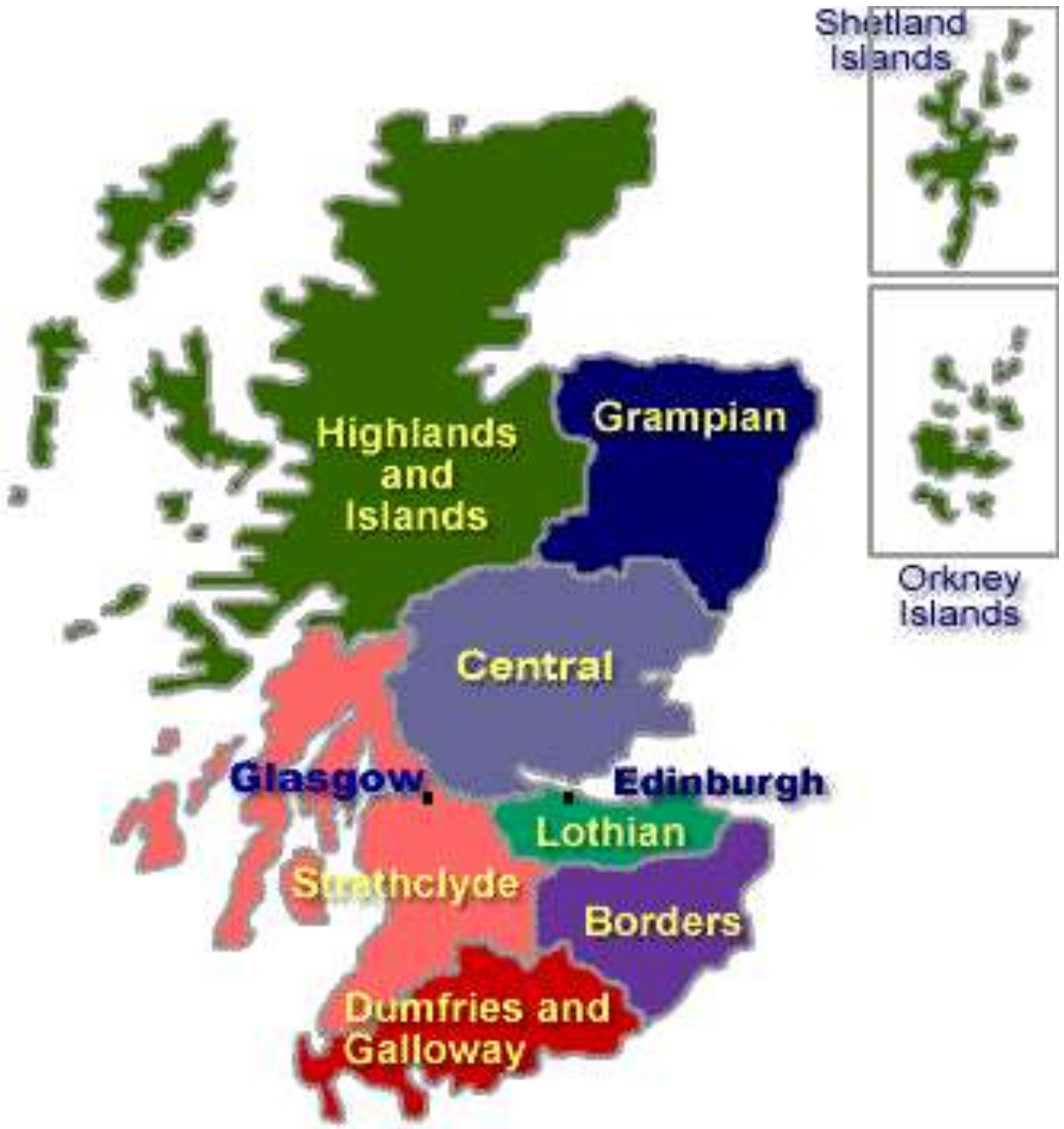
The impact on the routine laboratory of the introduction of an automated ELISA for the detection of Cryptosporidium and Giardia in stool samples

Nigel Stephenson
BMS 3
Department of Medical Microbiology
Aberdeen Royal Infirmary
NHS Grampian

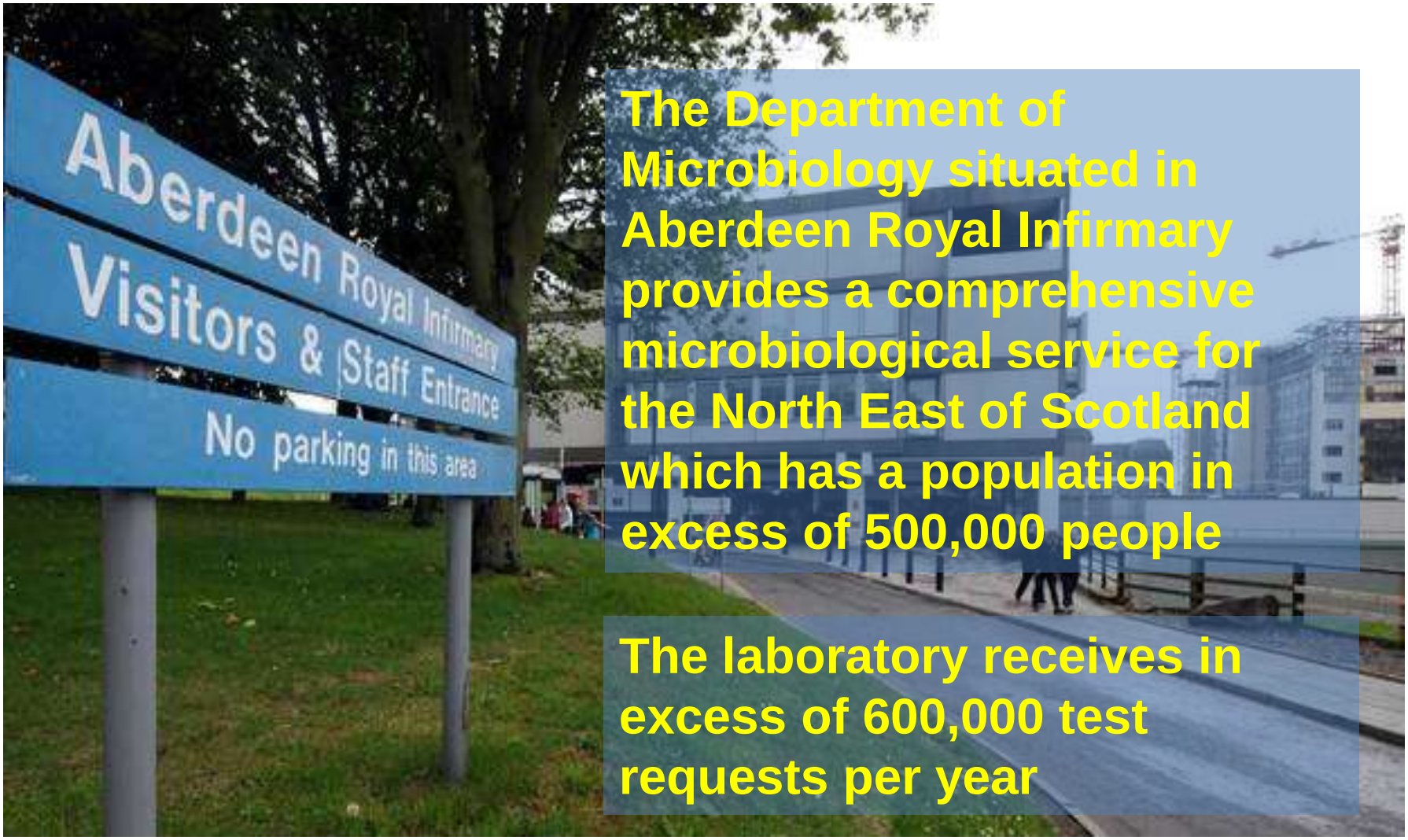
North East of Scotland region



Aberdeen Royal Infirmary

The Department of Microbiology situated in Aberdeen Royal Infirmary provides a comprehensive microbiological service for the North East of Scotland which has a population in excess of 500,000 people

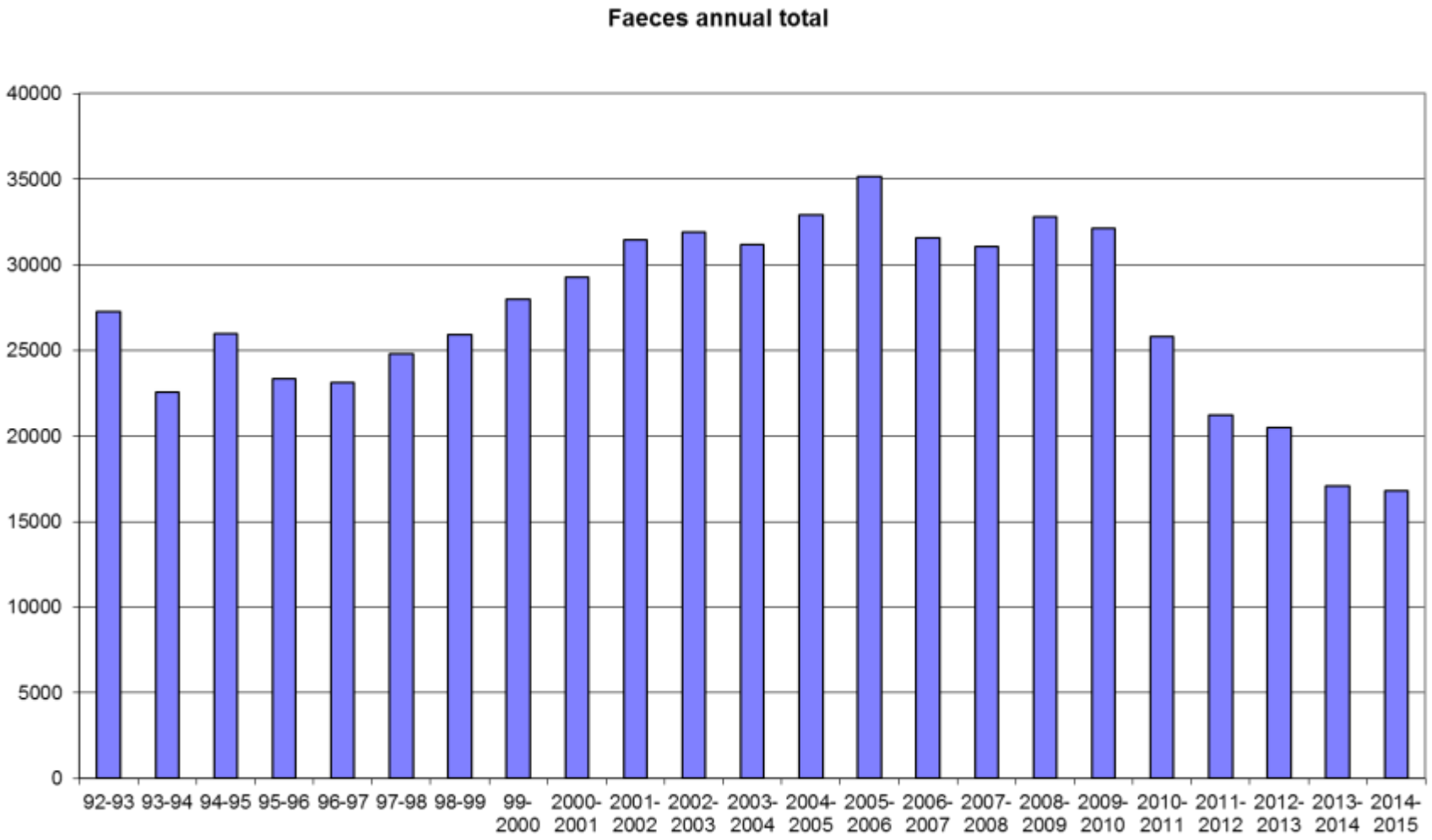
Aberdeen Royal Infirmary



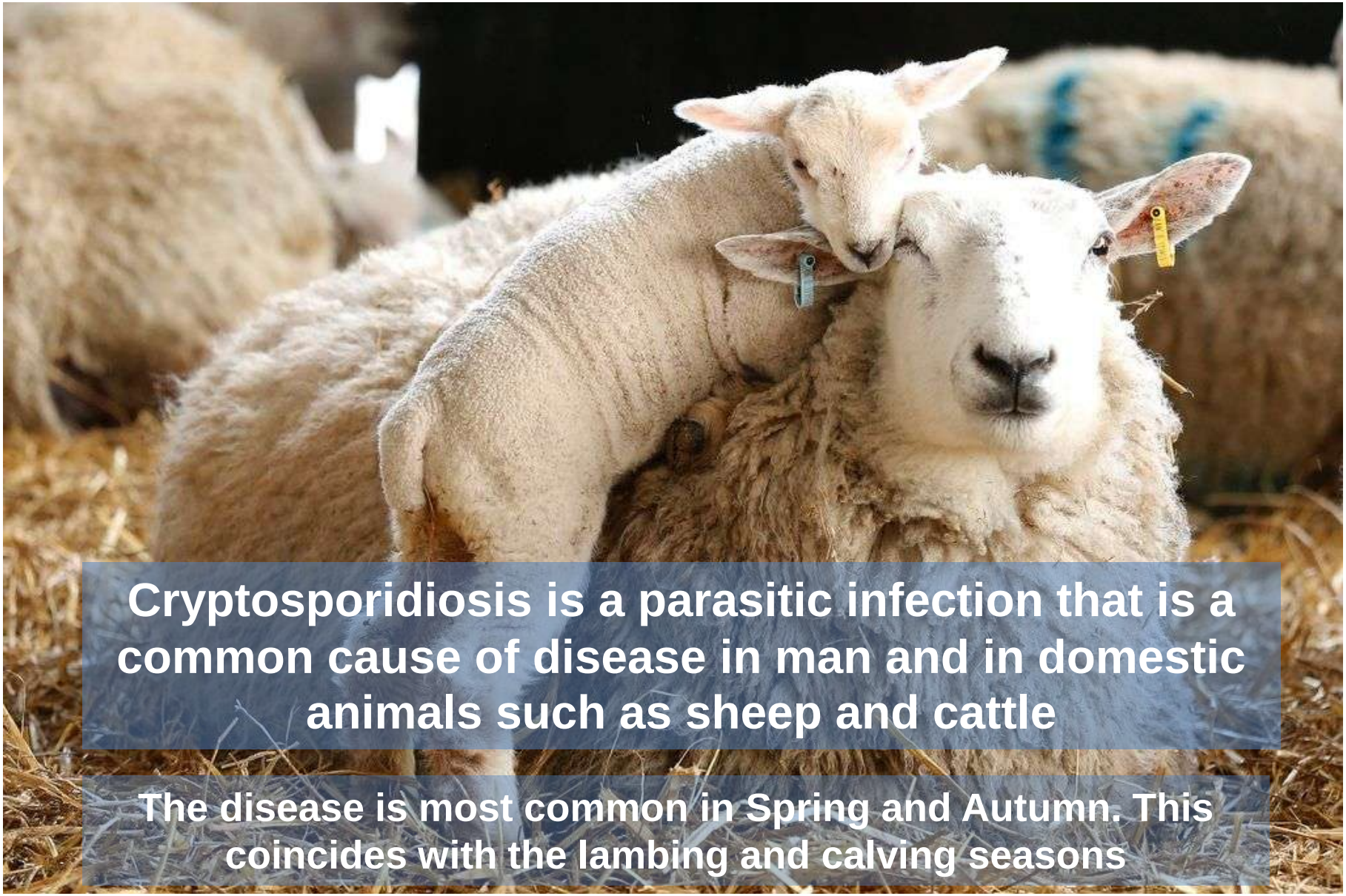
The Department of Microbiology situated in Aberdeen Royal Infirmary provides a comprehensive microbiological service for the North East of Scotland which has a population in excess of 500,000 people

The laboratory receives in excess of 600,000 test requests per year

Faecal sample requests for routine culture and/or C difficle



What is cryptosporidiosis?



Cryptosporidiosis is a parasitic infection that is a common cause of disease in man and in domestic animals such as sheep and cattle

The disease is most common in Spring and Autumn. This coincides with the lambing and calving seasons

How is cryptosporidiosis contracted?

Direct contact with infected farm animals e.g. farms visits where people do not wash their hands carefully before eating. The parasite can survive in the environment for months

How is cryptosporidiosis contracted?

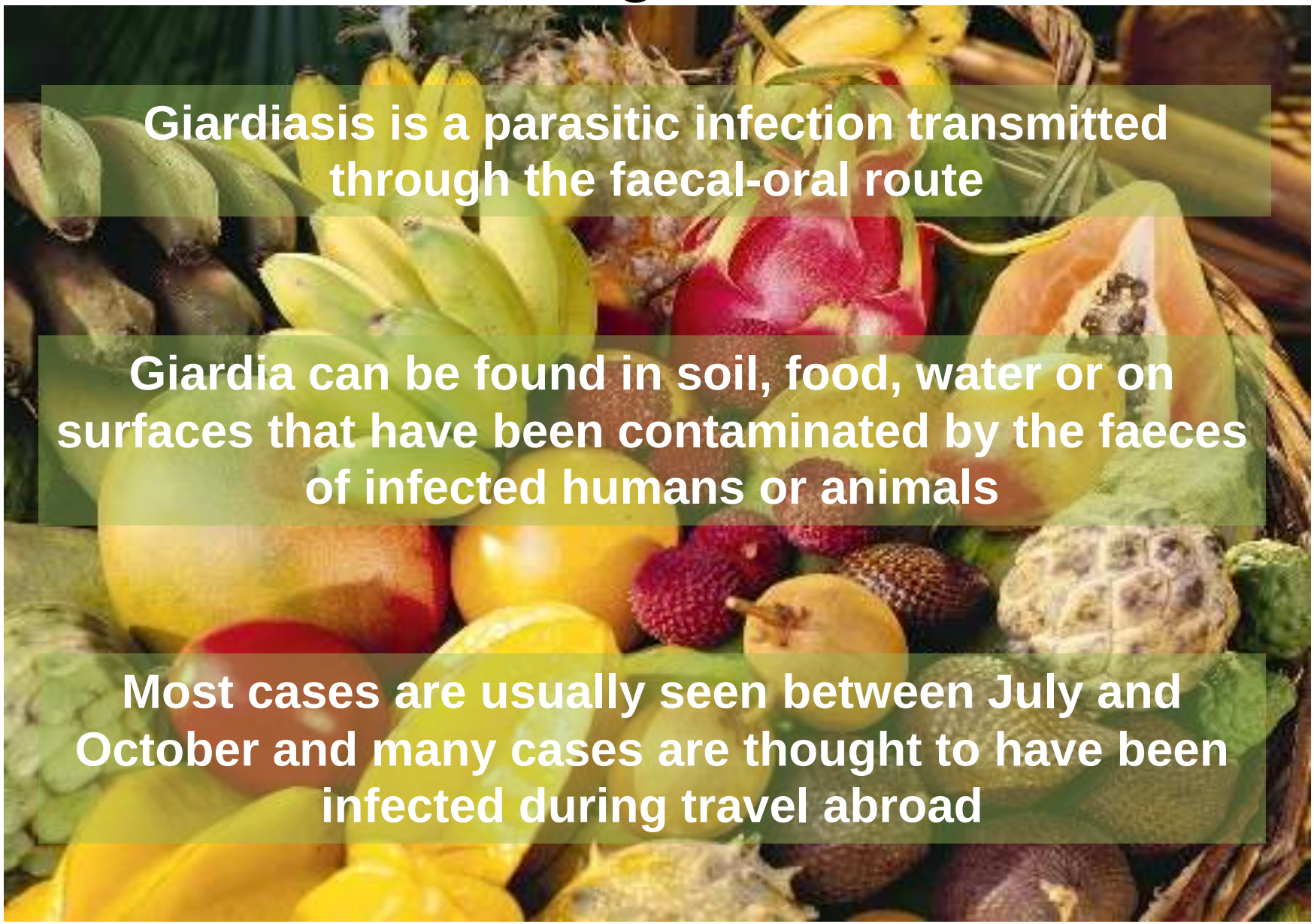
How is cryptosporidiosis contracted?

Infection has also been linked with swimming pools. This is usually as a result of accidental faecal contamination

HPS Briefing Note 26 Oct 2015
Cryptosporidium associated with Olympia swimming pool, Tayside

Three cases of Cryptosporidium from two NHS Health Boards with exposure to Olympia swimming pool, Dundee between 21st September – 3rd October. Initial investigations indicate that incubation period of all three cases is consistent with potential acquisition of infection from pool.

What is giardiasis?



Giardiasis is a parasitic infection transmitted through the faecal-oral route

Giardia can be found in soil, food, water or on surfaces that have been contaminated by the faeces of infected humans or animals

Most cases are usually seen between July and October and many cases are thought to have been infected during travel abroad

How is giardiasis contracted?

Drinking untreated water (raw water) from shallow wells, rivers, springs and ponds

Swallowing recreational water such as swimming pools or jacuzzis

Consumption of untreated ice or drinking water in countries where the water might be unsafe

Diagnosis

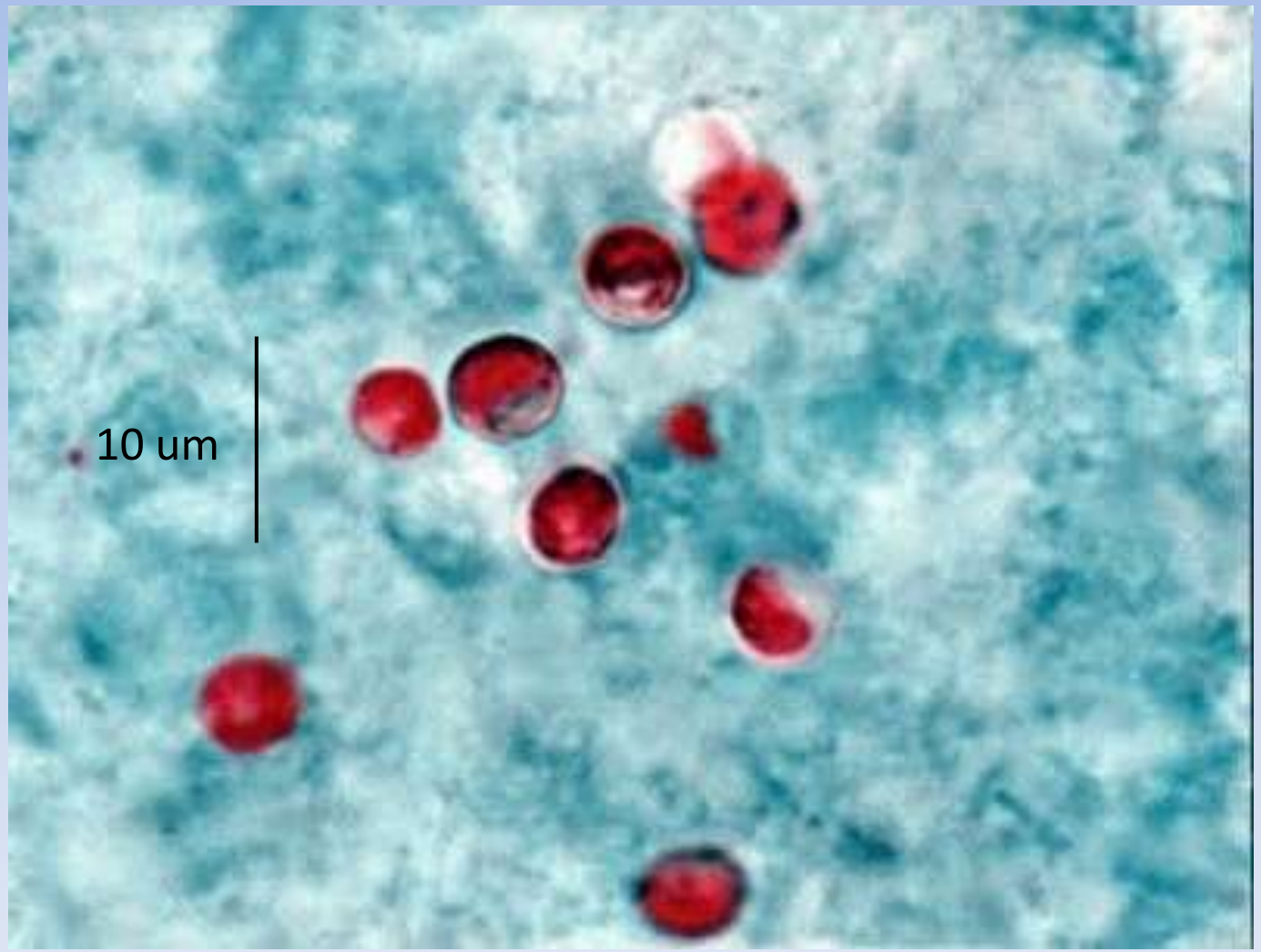
Primary laboratory diagnosis of Cryptosporidium is based on microscopical demonstration of the presence of oocysts in stool samples

UK SMI ‘Investigation of Specimens other than Blood for Parasites’ (Bacteriology/B31/Issue no: 4.1) recommends either auramine-phenol or modified Ziehl-Neelsen staining as a screening procedure

However the sensitivity of modified Ziehl-Neelsen microscopy has been shown to be significantly less than for other tests

(Chalmers, RM et al., ‘Comparison of diagnostic sensitivity and specificity of seven Cryptosporidium assays used in the UK’ J. Med Microbiol. 2011;60:1598-604)

Cryptosporidium oocysts in faecal smear after modified Zeihl Neelsen staining



Modified Zeihl Neelsen staining

Advantages:

- Inexpensive test
- Staining methdology is straightforward to perform
- No requirement for expensive equipment

Disadvantages:

- Experienced BMS’s are required to examine stained smears
- Examination of smears is time consuming
- Sensitivity has been shown to be significantly less than for other tests

Laboratory diagnosis of Giardia

Primary laboratory diagnosis of Giardia is based on microscopical demonstration of the presence of cysts or trophozoites in stool samples

Direct or concentrated wet preparations of faecal sample in saline with iodine are examined using light microscopy

Alternative methodologies include antigen detection and PCR.

Appearance of Giardia duodenalis in a wet prep following staining with iodine

Factors forcing the laboratory to examine alternative methodologies

- Decreased laboratory budget year on year
- Increased laboratory costs
- Increased wage costs associated with qualified BMS staff and associated incremental drift
- Loss of experienced staff seen in NHS Grampian due to retirement

Skilled laboratory personnel

A Workforce Planning Risk Assessment for Microbiology Laboratory Biomedical Scientists identified that:

'The current profile of qualified Band 6 staff and above shows that 37% of staff are >50 years old with a significant proportion of those staff predicted to retire within the next 10 years'.

(Risk Assessment Reference MMRA 171, 09/01/2013)

Alternative detection method

Antigen detection by enzyme immunoassay

Advantages:

- DS2 already in place for C difficle GDH and toxin AB assays
- Automated – walk away after sample preparation
- Interfaced – result transmission across LIMS
- Performed by lower staff grades (HCSW 3 or 4)
- Higher sensitivity and specificity relative to ZN microscopy
- Traceability of results – UKAS requirement
- Performance monitoring – determination of Measurement Uncertainty

Disadvantages:

- Requires investment to purchase an additional DS2 platform
- Requires purchase of kits which are more expensive than ZN stain

Dynex DS2 automated analyser

ZN microscopy versus Crypto/Giardia Combo EIA for detection for Cryptosporidium species

		ZN microscopy	
		POSITIVE	NEGATIVE
Crypto/Giardia Combo EIA	POSITIVE	23	28
	NEGATIVE	0	3717

RELATIVE SENSITIVITY = $\frac{\text{POSITIVE AGREEMENT}}{\text{Total microscopy +ve samples}}$ X 100 = 23 X 100 = **100%**

RELATIVE SPECIFICITY = $\frac{\text{NEGATIVE AGREEMENT}}{\text{Total microscopy –ve samples}}$ X 100 = $\frac{3717}{3745}$ X 100 = **99.2%**

Where the total number of samples tested = **3768**

Increase in isolation of Cryptosporidium species

MICROSCOPY

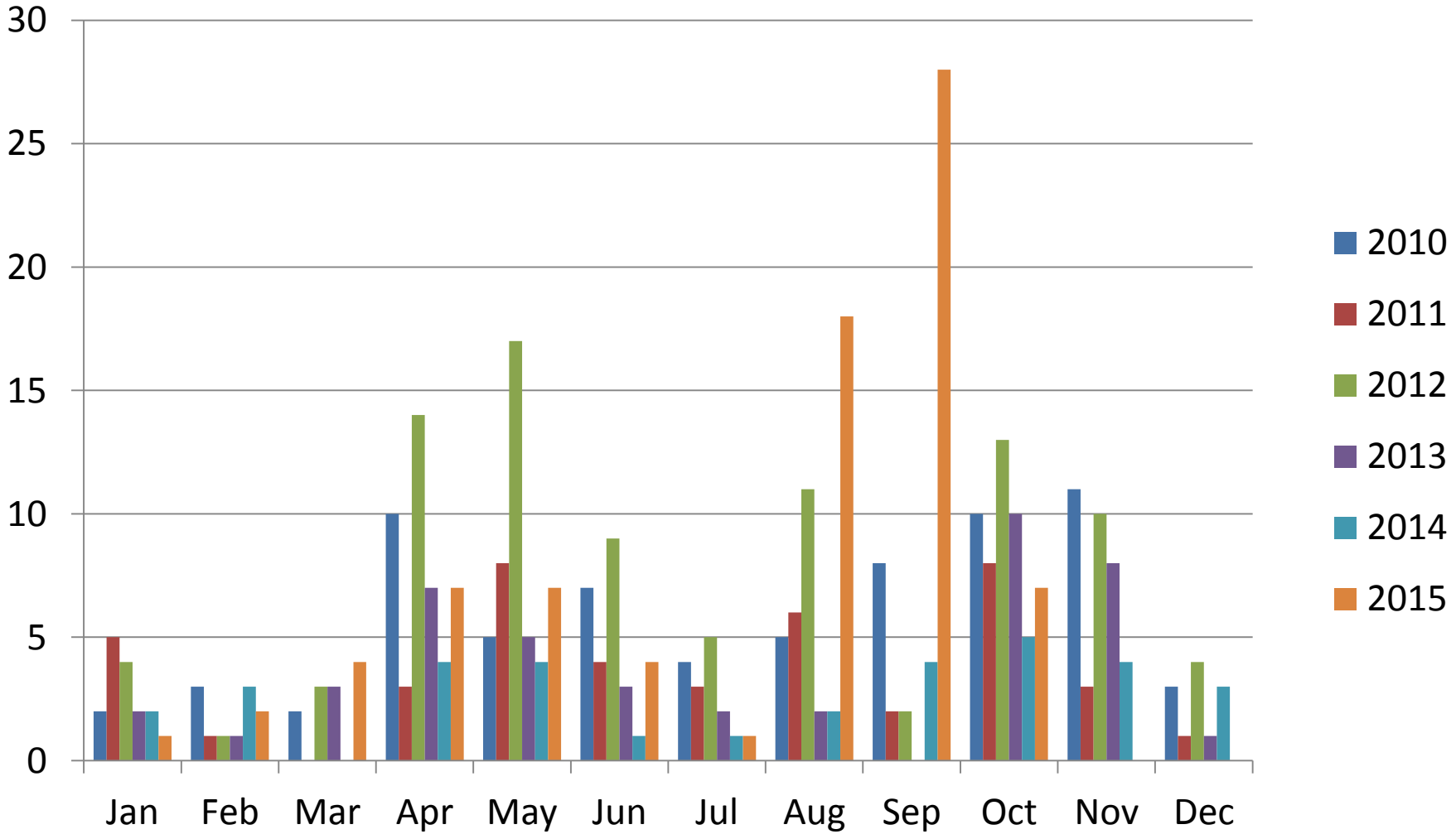
$$\frac{\text{ZN microscopy +ve samples}}{\text{Total number of samples}} \times 100 = \frac{23}{3768} \times 100 = 0.6\%$$

CRYPTO/GIARDIA COMBO EIA

$$\frac{\text{Crypto/Giardia Comba EIA +ve samples}}{\text{Total number of samples}} \times 100 = \frac{51}{3768} \times 100 = 1.35\%$$

AN INCREASE IN ISOLATION OF 122%

Incidence of Cryptosporidium species in NHS Grampian 2010 – 2015



Increase in isolation of Giardia duodenalis

MICROSCOPY

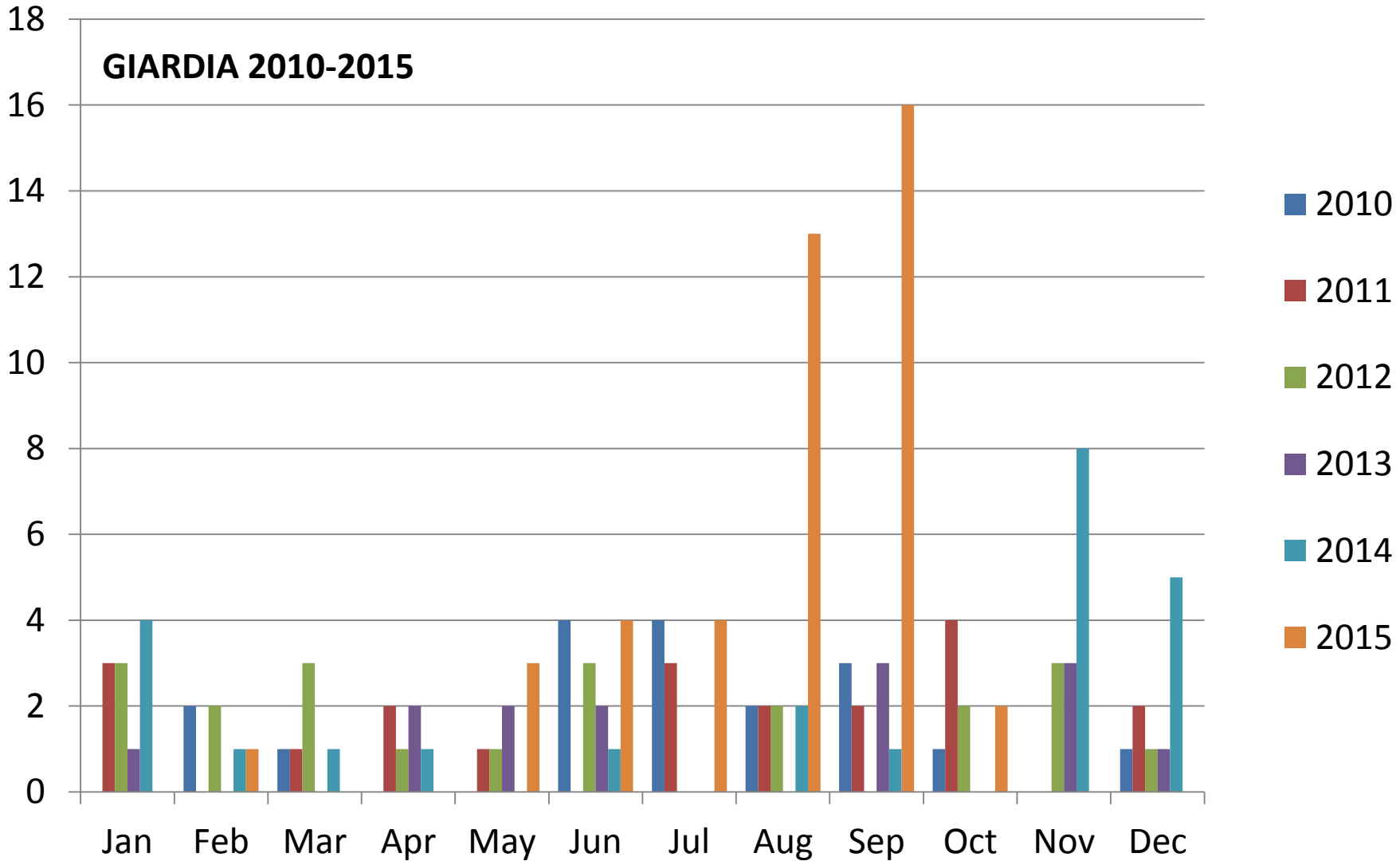
$$\frac{\text{Wet prep microscopy +ve samples}}{\text{Total number of samples}} \times 100 = \frac{3}{3768} \times 100 = 0.08\%$$

CRYPTO/GIARDIA COMBO EIA

$$\frac{\text{Crypto/Giardia Comba EIA +ve samples}}{\text{Total number of samples}} \times 100 = \frac{26}{3768} \times 100 = 0.7\%$$

AN INCREASE IN ISOLATION OF 866%

Incidence of Giardia duodenalis in NHS Grampian 2010 – 2015



Quality assurance

No current External Quality Assurance scheme is available for Cryptosporidium and Giardia detection by EIA:

UK NEQAS Parasitology organisers intend to commence a pre-pilot scheme for the non-microscopic detection of Cryptosporidium, Giardia and Entamoeba histolytica later this year

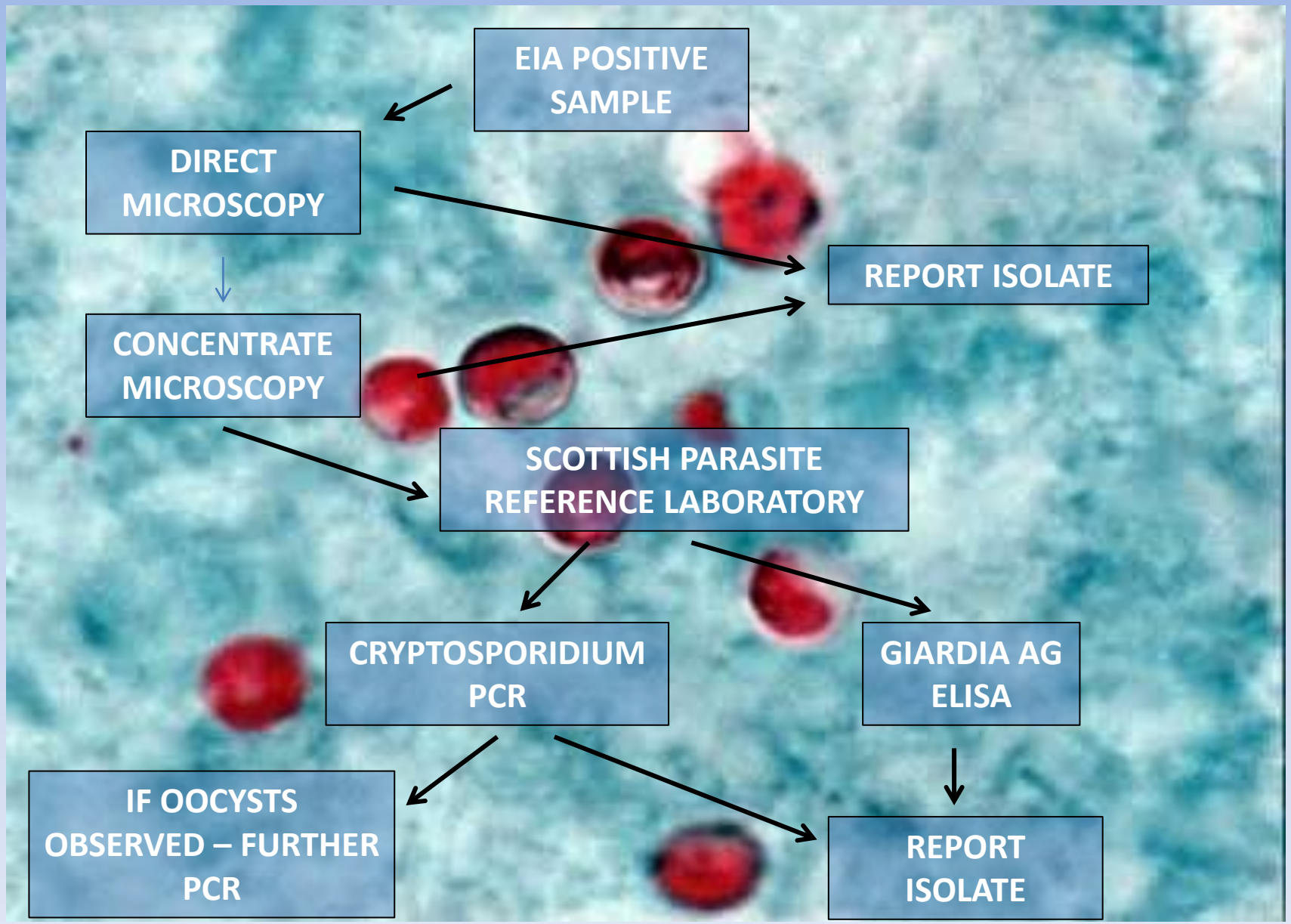
Quality assurance

No positive controls are available from a commerical supplier for use with EIA for Cryptosporidium and Giardia detection

Positive controls have been prepared from known positive samples:

- Cryptosporidium positive samples are diluted 1:20
- Giardia duodenalis positive samples are diluted 1:4000

Crypto/Giardia Combo EIA positive confirmation flow chart



Conclusion

- Introduction of Crypto/Giardia EIA has made a positive impact on the laboratory
- Increase in isolation of Cryptosporidium (122%) and Giardia (866%)
- EIA performed by lower staff grades enabling more efficient use of trained BMS's
- New methodology well received by staff
- Performance monitoring and result traceability will satisfy requirements of UKAS