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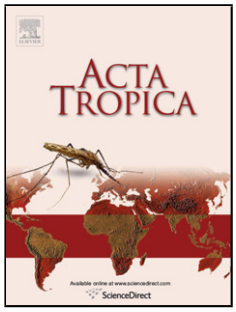

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Sero-prevalence of *Taenia* spp. infections in Cattle and Pigs in Rural Farming Communities in Free State and Gauteng Provinces, South Africa

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Abstract

The aim of this study was to determine sero-prevalence of bovine and porcine cysticercosis in cattle and pigs in rural farming communities in Free State and Gauteng Provinces, Republic of South Africa. Blood samples were collected for a period of twelve months from live cattle (n = 1315; 1159) and pigs (n = 436; 240) and the serum extracted and stored before analysis by a monoclonal antibody based (HP10) antigen detection ELISA. Results revealed a generally high sero-prevalence and wide distribution throughout the two provinces with Free State having a higher sero-prevalence in both cattle and pigs (23% and 34%) than Gauteng province (15% and 14%). Consumption of infected meat that is either not inspected/missed at meat inspection; poor livestock management practices and limited sanitation in rural communities might have

contributed to the occurrence of *Taenia* spp. infections in the two provinces. It is therefore, recommended that cysticercosis status of animals be established before slaughter. This would assist in ensuring that infected animals are not slaughtered for human consumption or zoonosis preventive measures are taken. Furthermore, public awareness programs on life cycles of *T. saginata*, *T. solium* and *T. hydatigena* and the use of more sensitive diagnostic tools are recommended as part of effective control strategies against taeniid infections.

Keywords: Cysticercosis; ; ; , HP10 AgELISA, Rural farming communities, South Africa

1. Introduction

Parasitic infections constitute an important problem in impoverished communities such as those living in rural areas. Among these parasitic infections are *Taenia saginata* and *T. solium*, the well-known parasites of medical, veterinary and economic importance causing cysticercosis in cattle and pigs (intermediate hosts) respectively, and taeniosis in humans (the definitive host). Although pigs are the main intermediate hosts of *T. solium*, humans can also act as intermediate hosts if they accidentally ingest eggs of this parasite causing cysticercosis in humans (Flisser, 1994; Yamasaki et al., 2002). Cattle and pigs may be directly infected from hands contaminated with *Taenia* eggs, but are more likely to be infected by ingesting eggs carried in drinking water or feed (Murrell, 2005). Feed may include scraps, garbage or excrement, which free roaming pigs may feed on. Contaminated water/feed can either derive directly from human faeces, via sewage plants after flooding or sewage sediment distributed on pastures.

Global economic impact studies (Carabin et al., 2006; Praet et al., 2010) have shown that *T. solium* infected pigs contribute 4.7% - 26.9% of overall costs of pig husbandry, resulting in total annual loss of €10million and US$18.6 to US$ 34.2 million respectively. However, this estimation gives only an indication rather than an accurate determination of economic loss (Bulaya et al., 2015). Although the economic losses due to cysticercosis in cattle and pigs can be substantial as a result of condemnation/treatment of infected carcasses (Yoder et al., 1994;

Ogunremi et al., 2010), this is not the major problem in the rural communities where not all animals slaughtered for human consumption are slaughtered in an abattoir or inspected for cysticercosis. The impact of *Taenia* infections in these communities is therefore, more of a public health problem. In fact, according to the Gauteng state veterinarian (E. Katanda, personal communication) cases of animals slaughtered in unregistered slaughter facilities (illegal slaughter) are rampant in Gauteng rural communities (Figure 2) but are not well documented.

Control measures for *T. saginata* and *T. solium* rely mainly on improved sanitation, anthelmintic treatment of the definitive host to kill tapeworms, and detection of infected animals at meat inspection where animals are slaughtered in registered abattoirs. However, these control programs are unlikely to be effective in South African rural communities due to lack of or improper use of toilet facilities and lack of registered slaughter facilities. Therefore, the aim of this study was to determine sero-prevalence of bovine and porcine cysticercosis in cattle and pigs in rural farming communities in Free State and Gauteng Provinces.

2. Materials and Methods

2. 1 Description of the study area

The study was conducted in selected rural farming locations in Free State and Gauteng Provinces whereby study sites were selected to be geographically representative of the two provinces. Geographical coordinates of each determined site were recorded and later used for mapping of areas where samples were collected. For Gauteng province, geographic areas are referred to as service regional centers, and for Free State province, districts are used.

2.2 Sample collection

Blood samples were collected from the coccygeal and jugular veins of cattle and pigs respectively in anticoagulant free vacutainers. These samples were stored in a cooler box and transported to the Helminthology laboratory at the Agricultural Research Council-Onderstepoort Veterinary Institute (ARC-OVI) where serum was processed, aliquoted and stored in labelled cryovials at -20°C until use.

2.3 Serological analysis

Serum was analysed using a slightly modified monoclonal antibody (HP10) based antigen ELISA (Harrison et al., 1989; 2005). The serum samples were used undiluted. The optical density (OD) of the reaction product was read at 450nm ELISA plate reader (Labsystems Multiscan RC Version 6, Helnsiki, Finland). ELISA plates were routinely set up to include five positives and negatives, six diluent control wells, and each test sample was run in duplicate. The mean sample ODs minus the mean diluent ODs were corrected for any day to day variation using a correction factor determined by the formula:

Correction Factor = Mean P^0 - Mean N^0/ Mean P^t - Mean N^t

where P = positive control, N = negative control, 0 is the reference day and t is the test day.

ELISA results were rejected if the correction factor for any particular plate varied more than 10% from the reference day. Samples were run with different reagents and positive and negative controls; hence, the negative cut off point was determined on a plate to plate basis using the formula: 2X + 3sd of negative controls, where X = the mean of the negative control and sd = standard deviation from the mean of the negative control.

2. 4 Statistical analysis

Data were transferred to spreadsheets using Microsoft© Excel (2001) and descriptive statistics were calculated and presented as tables and graphs. Contribution of each locality to the prevalence of each region was calculated as follows: Number of infected animals/Total number of animals tested in the region X 100. An XLSTAT 2014.4.06 program was used to analyse variance in prevalence among and between study sites in the two provinces.

3. Ethical considerations

The study was approved by both the Onderstepoort Veterinary Institute Animal Ethics Committee and Department of Agriculture, Forestry and Fisheries (DAFF).

4. Results

4. 1 Bovine and porcine cysticercosis sero-prevalence in Free State

Blood samples were collected from twenty-six localities (Figure 1) in the five districts of the Free State province. The overall sero-prevalence of cysticercosis was 23% (300/1315) and

34% (149/436) in cattle and pigs respectively. Sero-prevalence of bovine cysticercosis differed significantly ($p < 0.0001$) among various study sites in the province, whilst there was no significant difference ($p > 0.05$) in porcine cysticercosis sero-prevalence among various study study sites. Fezile Dabi district had the highest sero-prevalence (36%; 41%) of both bovine and porcine cysticercosis in the province, with the sero-prevalence range between 26 and 50% in cattle and 28 and 48% in pigs. The sero-prevalence of bovine cysticercosis also differed significantly ($p < 0.05$) within the Fezile Dabi district, but there was no significant difference ($p > 0.05$) in porcine cysticercosis sero-prevalence among various study sites in the district. On the other hand, Thabo Mofutsanyane district had the lowest sero-prevalence (15%; 28%) of bovine and porcine cysticercosis, but the highest number of examined cattle and pigs. There was also no significant difference ($p > 0.05$) in both bovine and porcine cysticercosis sero-prevalence between study sites in the district. Serological results of individual study sites and their contribution to the sero-prevalence of cysticercosis within the respective districts are depicted in Table 1.

4. 2 Bovine and porcine cysticercosis sero-prevalence in Gauteng

Blood samples were collected from twenty-eight localities in the Gauteng province (Figure 2). Results showed that 15% (174/1159) bovine and 14% (34/240) porcine blood samples collected from the province tested positive for *Taenia* infection. There were significant differences ($p < 0.05$) in bovine and porcine cysticercosis sero-prevalence between the Germiston, Pretoria and Randfontein.

Pretoria region had the highest sero-prevalence of both bovine 17% (100/578) and porcine cysticercosis 21% (14/67) and contributed the highest percentage (9%) towards the bovine cysticercosis sero-prevalence in the province, whilst Randfontein region contributed the most (6%) towards porcine cysticercosis sero-prevalence. Statistical analysis showed a significant difference ($p < 0.05$), but no significant difference ($p > 0.05$) in sero-prevalence of bovine and porcine cysticercosis respectively among the different areas in Pretoria. Serological results of

individual study sites and their contribution to the sero-prevalence of cysticercosis within the respective regional centres are depicted in Table 2.

Statistical analysis showed that there were significant differences ($p < 0.05$) in both bovine and porcine cysticercosis sero-prevalence between the two provinces.

5. Discussion and Conclusions

Both provinces had high sero-prevalence of cysticercosis in cattle and pigs, which is in contrast to the low prevalence previously reported in the country based on meat inspection. Surveys previously conducted in different South African areas reported prevalence of 0.5 - 2.07% (Viljoen, 1937) and 0 - 9.1% (Heinz and MacNab, 1978) based on meat inspection records. Qekwana et al. (2016) recently reported 0.70% (95% CI: 0.45, 0.95) prevalence of bovine cysticercosis in Gauteng. The results found in the current study were not surprising, as it has been reported repeatedly that the sensitivity of meat inspection is much lower than that of the AgELISAs (Harrison et al., 1989; Onyango-Abuje et al., 1996; Dorny et al., 2002; Kyvsgaard et al., 1990; Dorny et al., 2000; Rodriguez-Hidalgo et al., 2003; Asaava et al., 2009).

When comparing the two provinces, Free State had higher sero-prevalence of cysticercosis in both cattle and pigs. The close proximity of Fezile Dabi (in the Free State) to the Vaal River, where faecal contamination of the Vaal River Barrage was reported (Tempelhoff, 2009) may explain the high sero-prevalence obtained in the district. Sourcing of water from rivers and ponds has been reported as a risk factor for cysticercosis (Komba et al., 2013) and access by cattle to risky water sources with sewage treatment plant effluent in the proximity is a major risk factor for bovine cysticercosis (Calvo-Artavia et al., 2013). South Africa faces major challenges with regard to the provision of clean water and proper sanitation particularly in rural and peri-urban areas. Less than half of South African municipal sewage works are functional (Green Drop report). Furthermore, evidence of dangerously high level of faecal pollution was reported in the Vaal River Barrage, which is situated on the country's hardest working river

(Tempelhoff, 2009). This suggests that water contamination could have been the source of infection for cattle and pigs in the two studied provinces.

Sero-prevalence for bovine cysticercosis using the same HP10 AgELISA in Northern Turkana District, Kenya, was found to be 16.7% (13 - 20.9% CL) with the true prevalence figure calculated to be 20% (15 - 25%) (Asaava et al., 2009) using the Bayesian method. This figure compares with results found in the current study where Free State had 23% and Gauteng 15% bovine cysticercosis sero-prevalence, however it is lower than the 38% overall prevalence reported in Kenya (Onyango-Abuje, 1996). When compared to the 6.1% sero-prevalence of bovine cysticercosis in Zambia (Dorny et al., 2002), the current study obtained a higher sero-prevalence of bovine cysticercosis. Generally, sero-prevalence of bovine cysticercosis reported in this study compares closely with that reported in other parts of Africa where cattle are kept under more or less the same management and environmental conditions but was much higher than the prevalence in European countries where prevalence ranges between 0.07 and 6.8% (Boone et al., 2007)).

In sub-Saharan Africa, the prevalence of porcine cysticercosis ranges between 2.0 and 41.2% depending on the region and type of diagnostic test used to detect it (Assana et al., 2013). The 14.2% sero-prevalence of porcine cysticercosis currently obtained in Gauteng is lower than that reported in other parts of Africa using AgELISA. A 26.7% (8/30) sero-prevalence of porcine cysticercosis in Soutou, Senegal was reported in 2000 (Secka et al., 2010). In Mbozi and Mbeya districts in Tanzania the sero-prevalence of porcine cysticercosis was 32% and 30.7% respectively (Komba et al., 2013). However, the 34% porcine cysticercosis sero-prevalence obtained in Free State compares well with these results. On the other hand, (Krecek et al., 2008; 2011) reported 64.6% true prevalence of porcine cysticercosis in 21 villages in the Eastern Cape Province, South Africa using a Bayesian approach on the HP10 AgELISA in the absence of a gold standard.

Due to strong cross-reactivity to *T. solium*, the tests (B158/B60 and HP10 Ag-ELISAs) have shown good diagnostic characteristics in porcine cysticercosis based on Bayesian analysis

(Rogan, 1978). The antibodies are genus- and not species specific, and studies have shown that the B158/B60 Ag-ELISA has a problem of cross-reactions with *T. hydatigena* (Rodriguez-Hidalgo et al., 2003; Dorny et al., 2004), which is not generally believed to be common in cattle and pigs in the African context (Komba et al., 2013).

It was shown during the validation process of the HP10 AgELISA (Harrison et al., 1989) that this assay does not cross react with *T. hydatigena* and no studies have reported the *T. hydatigena* cross reaction in the HP10 AgELISA. However, authors (Kundu et al., 2016; Dorny et al., 2004; Cheng & Ko, 1991) have pointed out the need for caution when interpreting results based on serological assays, which are genus and not species specific. Thus because of assay limitations, the possibility that some of the infections recorded could be due to cross reactions with *T. hydatigena* cannot be ruled out, though likely to account for only a proportion of the sero-positive results, which are still indicative of a potential problem. Few prevalence studies have been carried out in Africa on *T. hydatigena* in small ruminants and even fewer in pigs. The only study conducted in Africa with a relative large sample size involving pigs was Nigeria with 1.7% prevalence based on 360 slaughtered pigs (Braae et al., 2015). Detailed information on the true prevalence of *T. hydatigena* in Africa is generally lacking and studies such as those conducted in Tanzania (Braae et al., 2015) are required over wider areas. Further studies and parasitological confirmation such as identification of lesions at slaughterhouse and PCR speciation are therefore recommended.

Meat inspection, being the currently used method of diagnosis for cysticercosis in order to control taeniosis/cysticercosis may have also contributed to the occurrence of cysticercosis in the provinces as lightly infected carcasses may have been missed during meat inspection and passed on for human consumption, thus perpetuating the parasitic life cycle. Furthermore, rural areas in South Africa have never been serviced properly in terms of meat inspection, commercial slaughtering was carried out only in urban areas (Veary and Manoto, 2008).

Serological results of this study indicate that *Taenia* spp. infections in cattle and pigs, which may include *T. solium* and *T. saginata* (and *T. hydatigena* to a lesser extent); parasites of

medical, veterinary and economic importance do occur in the two studied provinces of South Africa. Improvement in water and sanitation and programs on public awareness with regard to transmission and prevention of *Taenia* infections as well as a more detailed study that focuses on risk factors of taeniosis/cysticercosis in Free State and Gauteng Provinces are therefore highly recommended. Furthermore, future studies that include parasitological confirmation through slaughtering of animals, detailed meat inspection and speciation of the lesions by PCR for animals found positive with the HP10 AgELISA; and studies on prevalence of *T. hydatigena* in cattle and pigs in South Africa are recommended.

Cysticercosis is one of the neglected diseases and a zoonotic disease with important public health implications in South Africa. Effective control requires an integrated and holistic state intervention by all government stakeholders that include Departments of Water and Sanitation, Works, Human Settlement, Health and Agriculture to eliminate risk factors associated with high prevalence and transmission of the diseases. For effective control the disease should be declared a state controlled and notifiable disease under the Animal Disease Act, 1984 (Act No.35 of 1984) and the Animal Diseases Regulations; and National Health Act, 2003 (Act No. 61 of 2003). In addition to routine meat inspection at slaughter, use of more sensitive diagnostic tools is recommended to screen and identify infected animals before slaughter.

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7. Competing Interests

The authors have no conflict of interests on the writing and publishing of this manuscript.

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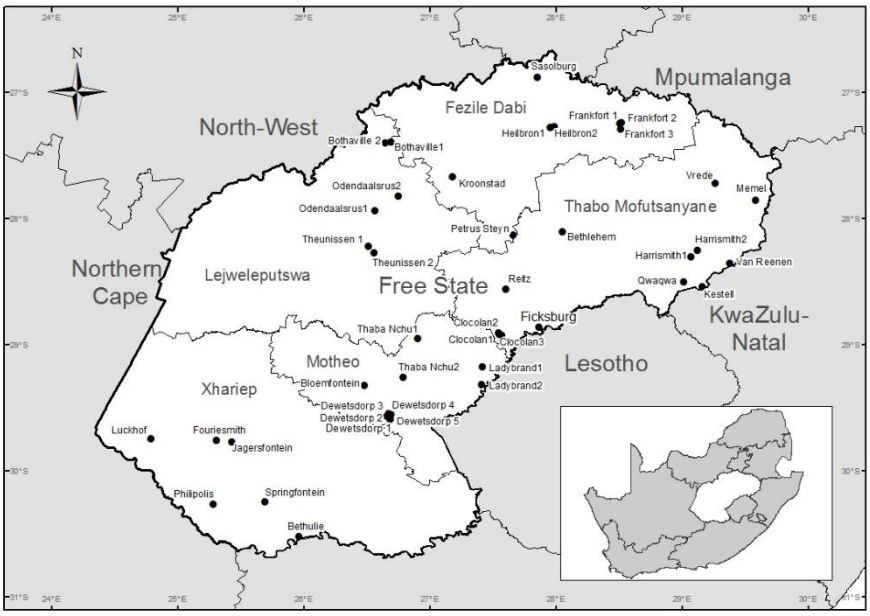

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Figure 1: A map showing various study sites in rural communities of the five districts (Fezile Dabi, Lejweleputswa, Motheo, Thabo Mofutsanyane and Xhariep) of the Free State Province where blood samples were collected for serological analysis. Free State is one of the nine provinces of South Africa as shown as the silhouette at the right bottom corner.

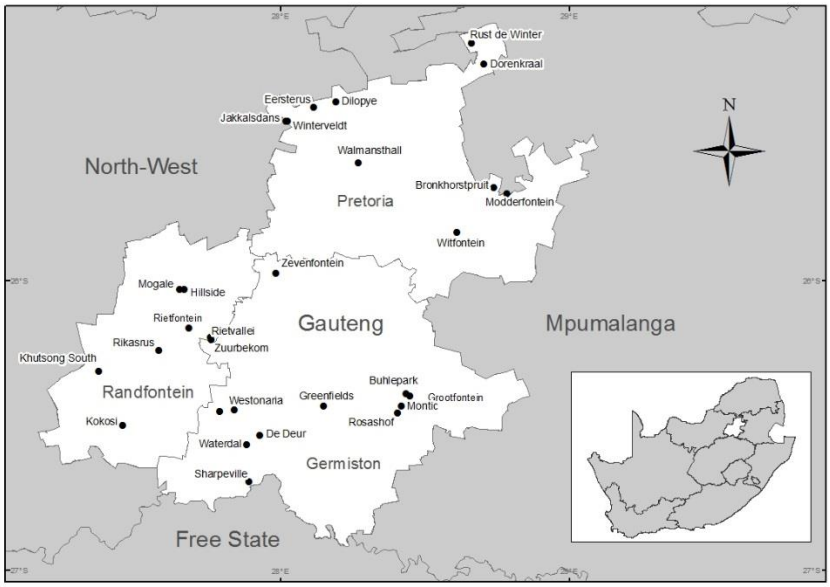

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Figure 2: A map showing various study sites in rural communities of three veterinary services'regional centres (Pretoria, Randfontein and Germiston) in Gauteng Province where blood samples were collected for serological analysis. Gauteng is one of the nine provinces of South Africa as shown as the silhouette at the right bottom corner.

Table 1: Sero-prevalence of *Taenia* cysticercosis, as determined by the HP10 Ag-ELISA, in cattle and pigs from farms in the Free State Province, South Africa. The number of positives (x) found out of those sample (y) and the percent is indicated in brackets (%) i.e x/y (%), where animals were not samples the cells are marked with a dash and contribution indicates number of infected animals/total number of examined animals in respective districts.

	Sampling site	Cattle	Contribution	Pigs	Contribution
Fezile Dabi					
	Frankfort	17/60 (28)	7%	11/32 (34)	9%
	Heilbron	14/53 (26)	6%	19/40 (48)	15%
	Kroonstad	24/60 (40)	10%	15/33 (45)	12%
	Sasolburg	30/60 (50)	13%	5/18 (28)	4%
Lejweleputswa					
	Bothaville	17/60 (28)	9%	12/30 (40)	13%
	Odendaalsrus	12/60 (20)	7%	9/30 (30)	10%
	Theunissen	15/59 (25)	8%	8/30 (27)	9%
Motheo					
	Bloemfontein	2/39 (5)	1%	-	-
	Dewetsdorp	14/66 (21)	6%	11/26 (42)	26%
	Ladybrand	10/60 (15)	4%	-	-
	Thaba Nchu	7/63 (11)	3%	6/17 (35)	14%
Thabo Mofutsanyane					
	Clocolan	11/60 (18)	3%	7/30 (23)	5%
	Ficksburg	13/49 (27)	3%	-	-
	Harrismith	10/51 (20)	2.5%	14/31 (45)	10%
	Kestell	-	-	0/4	0%
	Memel	1/11 (9)	0.25%	-	-
	Petrus Steyn	-	-	9/36 (25)	6%
	Qwaqwa	6/82 (7)	1.5%	6/25 (24)	1.5%
	Reitz	10/61 (16)	2.5%	1/6 (17)	1%
	Van Reenen	6/60 (10)	1.5%	-	-
	Vrede	2/23 (9)	0.5%	2/7 (29)	1%
Xhariep					
	Bethulie	18/61 (29)	6%	-	-
	Fouriesmith	-	-	7/22 (32)	17%
	Jagersfontein	10/30 (33)	4%	-	-
	Luckhof	24/66 (36)	9%	0/3 (0)	0%
	Philipolis	20/60 (33)	7%	7/16 (44)	17%
	Springfontein	7/61 (11)	3%	-	-

Table 2: Sero-prevalence of *Taenia* cysticercosis, as determined by the HP10 Ag-ELISA, in cattle and pigs from farms in Gauteng Province, South Africa. The number of positives (x) found out of those sample (y) and the percent is indicated in brackets (%) i.e x/y (%), where animals were not samples the cells are marked with a dash and contribution indicates number of infected animals/total number of examined animals in respective regional centres.

Region	Sampling site	Cattle	Contribution	Pigs	Contribution
Pretoria					
	Bronkhorstpruit	37/163 (23)	6%	-	-
	Dilopye	15/56 (27)	3%	2/5 (40)	3%
	Dorenkraal	-	-	1/4 (25)	2%
	Eersterus	6/21 (29)	1%	-	-
	Jakkalsdans	-	-	2/5 (40)	3%
	Modderfontein	3/34 (9)	1%	-	-
	Rust de Winter	31/254 (12)	4 %	-	-
	Walmansthall	-	-	2/13 (15)	3%
	Winterveldt	8/50 (16)	1%	4/19 (21)	6%
	Witfontein	-	-	3/21(14)	5%
Germiston					
	Buhlepark	6/65 (9)	2%	0/8 (0)	0%
	De Deur	-	-	2/12 (17)	2%
	Greenfields	12/102 (12)	3%	3/18 (17)	4%
	Grootfontein	7/45 (16)	2%	0/11 (0)	0%
	Montic	7/72 (10)	2%	-	-
	Rosashof	0%	0%	-	-
	Sharpeville	21/55 (38)	6%	0/28 (0)	0%
	Waterdal	-	-	0/7 (0)	0%
	Westonaria	3/23 (13)	1%	-	-
	Zevenfontein	2/7 (29)	0.5%	-	-
Randfontein					
	Hillside	1/26 (4)	0.5%	-	-
	Khutsong South	-	-	0/8 (0)	0%
	Kokosi	6/53 (11)	3%	-	-
	Mogale	-	-	1/7 (14)	1%
	Rietfontein	2/42 (5)	1%	-	-
	Rietvallei	5/53 (9)	2%	2/17 (12)	2%
	Rikasrus	2/38 (5)	1%	3/10 (30)	3%

	Zuurbekom	-	-	9/47 (19)	10%